Testing for hereditary mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene

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The technical information in this document is used by the Medical Services Advisory Committee (MSAC) to inform its deliberations. MSAC is an independent committee which has been established to provide advice to the Minister for Health on the strength of evidence available on new and existing medical technologies and procedures in terms of their safety, effectiveness and cost effectiveness. This advice will help to inform government decisions about which medical services should attract funding under Medicare.

MSAC's advice does not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

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EXECUTIVE SUMMARY

Testing for hereditary mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene

A.1 RATIONALE FOR ASSESSMENT

This contracted assessment examines the evidence to support the listing on the Medicare Benefits Schedule (MBS) of genetic testing for hereditary mutations in the *CFTR* gene. The service would be used for:

- the diagnosis of prenatal cystic fibrosis (CF)
- people suspected of CF or CFTR-related disorders
- partners of people with at least one known CFTR mutation—for the purpose of reproductive planning.

It is claimed in the application from the Royal College of Pathologists of Australasia (RCPA) that the MBS listing of CFTR testing in the target population and setting will create additional diagnostic surety for a lifelong, expensive and complex condition, affecting family planning options and the selection of treatment.

This assessment of CFTR mutation testing sought evidence on all the PICO[1] elements that were pre-specified in a research protocol that resulted from the application. The protocol was discussed and agreed by the Protocol Advisory Sub-Committee (PASC), and included input from Health Expert Standing Panel (HESP) members and the relevant policy area of the Department of Health (the Department). The research protocol was approved by the Department and can be found on the Medical Services Advisory Committee (MSAC) website.

A.2 PROPOSED MEDICAL SERVICE

Cystic fibrosis (CF) and other CFTR-related disorders are the most common autosomal recessive disorder in Caucasians, with a frequency of about 1 in 2,500–2,800 live births worldwide and a carrier frequency of 1 in 25 in Australia. The major cause of morbidity and mortality among young people with CF is progressive respiratory disease. Cystic fibrosis is usually clinically diagnosed with supporting evidence of a CFTR abnormality, either by sweat chloride measurement or through identification of mutations in the *CFTR* gene known to cause CF. Genetic testing occurs in three groups to provide information for reproductive planning: (1) individuals suspected of having CF or

[1] PICO = population, intervention (investigation/index test), comparator, outcomes

presenting with classic or non-classic CF symptoms (including men with congenital bilateral absence of the vas deferens (CBAVD)); (2) couples seeking prenatal diagnosis as a consequence of having had a previous child with CF or a CFTR-related disorder, or having been identified by other means to both be carriers of a CFTR mutation, or having a fetus with an echogenic bowel; and (3) a partner of someone with at least one known CFTR mutation. Most CF patients in Australia are currently diagnosed through national newborn screening (NBS) programs; all infants with elevated immunoreactive trypsinogen levels would be suspected of CF and tested. These infants would theoretically fall within group (1) mentioned above. Therefore, as this testing of newborns is already considered standard practice and funded by the states and territories (i.e. parents would not be paying for the test themselves), it was considered by PASC that testing of newborns would not need to be examined further in this assessment.

Different types of genetic tests are currently performed in Australia. Common mutation analyses are conducted in patients/parents/partners for whom familial mutations are not known, whereas total gene sequencing is undertaken if the clinical situation demands it and the common mutation analysis is unable to identify both CFTR mutations. In prenatal testing, if a mutation is known in both parents, a mutation analysis would be performed on a sample from the fetus, specifically targeting the parents' mutations. If the parents both carry the most common mutation, f508del, a single mutation test would be performed. However, if at least one of them carries a different mutation (investigated by the common mutation test), the fetus would be tested using the common mutation test.

Current funding arrangements

The RCPA Genetic Testing Survey 2011 identified 11 Australian diagnostic laboratories that were National Association of Testing Authorities (NATA)-accredited to perform more than 100 CFTR mutation tests in 2011. CFTR mutation testing is already practised in all states and territories, with funding derived from the sources listed in Table 8 on page 42.

A.3 PROPOSAL FOR PUBLIC FUNDING

The proposed MBS item descriptors are summarised in Table 7 on page 40. Fees were not proposed by the applicants.

A.4 COMPARATOR DETAILS

A full comparative assessment of the safety and clinical effectiveness of CFTR mutation testing was conducted for one of the population groups (group (2): prenatal diagnosis). Currently, parents receiving the test for prenatal diagnostic purposes would have to pay for CFTR mutation testing themselves (in the private system). If the test was not affordable, there would be no prenatal genetic testing and the diagnosis would be made after the child's birth through existing neonatal programs. The selected comparator for this assessment of population group (2) was 'no prenatal genetic testing'.

A.5 Clinical Use of the Test

The ways in which the tests are used in the three different population groups are shown in the clinical management algorithms in Figure 2, Figure 3 and Figure 4 on pages 44–46.

A.6 Key Differences in the Delivery of the Proposed Medical Service and the Main Comparator

In couples that are pregnant with a fetus that is identified to be at risk of CF, mutation testing of the *CFTR* gene provides knowledge regarding whether at birth the infant would have CF. This information may further inform a decision to terminate the pregnancy if the fetus is found to be affected. In couples planning to have a child, mutation testing allows carrier couples the option of conceiving through the use of in-vitro fertilisation (IVF) and preimplantation genetic diagnosis (PGD).

A.7 Clinical Claim

The applicant claimed that the identification of CFTR mutations is important for providing information at a molecular level about prognosis as a result of genotype–phenotype correlation. Prenatal testing could aid the decision whether to terminate a pregnancy. Furthermore, patients aged 6 years and older who are identified as having at least one G551D mutation are eligible for the drug ivacaftor (Kalydeco®) through the Pharmaceutical Benefits Scheme (PBS).

A.8–B.1 Approach Taken to the Evidence Assessment

The approach for the systematic review for the different population groups is shown in Table 9 on page 48 and in the PICO boxes in section A.8. Population groups (1) and (3) were assessed in terms of test accuracy alone because the health benefit would be assessed in the submission to the Pharmaceutical Benefits Advisory Committee (PBAC) for ivacaftor, and the benefit of testing for reproductive planning would be assessed in the contracted assessment of PGD (see MSAC application no. 1165). PASC agreed that this need not be re-examined specifically for CFTR. The medical literature was searched to identify relevant studies and systematic reviews, and a linked evidence approach was used to guide synthesis of the collated evidence. Additional searches were done to assess the safety of amniocentesis and chorionic villus sampling (CVS), and the health implications of possible management changes due to the absence of CF-specific studies.

B.2–B.5 Characteristics of the Evidence-base

No studies were identified that met the PICO criteria for directly assessing the clinical effectiveness or safety of (prenatal) CFTR mutation testing. Detailed characteristics of the studies linked through a chain of evidence to estimate the clinical effectiveness of CFTR mutation testing can be found in section B.4 and Appendix C, Appendix D and Appendix E. Section B.3 provides information on the risk of bias of the included studies.

A summary of the risk of bias in the test accuracy studies, determined using the QUADAS-2 tool, is given in section B.3.i and in Table 21 to Table 30. Summaries of the characteristics of the diagnostic accuracy studies are shown in Table 31 to Table 40. The key outcome assessed was clinical sensitivity, with some studies reporting yield or failure rates. As the majority of pregnancies with a fetus found to have two CFTR mutations were terminated, results based on a clinical diagnosis after birth could not be determined. Therefore, the specificity and false positive rate could not be reported.

Thirteen studies met the study eligibility criteria for assessing the accuracy of tests in patients with classical CF symptoms; they were mostly level III-2 studies of variable quality. Nine studies, all level III-2 and of variable quality, were included to assess the performance of CFTR mutation testing in men with CBAVD. No studies were identified to determine the accuracy of CFTR mutation testing in partners of CF carriers or parents with a fetus suspected of CF; however, 1 good-quality level III-2 study reported on the diagnostic accuracy of CFTR mutation testing in CF carriers with a known CFTR mutation. Four studies reported on prenatal testing of fetuses suspected of having CF but only 2 of these were comparative (i.e. level III-2 and of good quality). One of these studies also reported on the accuracy of CFTR mutation testing in fetuses with fetal echogenic bowel (FEB).

Testing failure rates were also investigated in section B.3.i; 5 studies (4 poor-quality non-comparative studies and 1 good-quality level III-2 study) were included.

Study quality for the change-in-management step of the linked analysis was evaluated using the IHE checklist for case series, as none of the studies were comparative (i.e. all level IV evidence). Five studies were of high quality and 4 of medium quality. Termination of pregnancy (TOP) rates and CF birth rates were the key outcomes extracted from every study.

B.6–B.8 RESULTS

Safety

Test adverse events

No studies on the safety of prenatal CFTR testing were identified. A separate search was conducted to investigate the safety of amniocentesis and CVS, both of which are used to retrieve fetal DNA for prenatal testing (discussed in section B.8.1). Evidence from systematic reviews was identified, comparing the fetal loss rates associated with amniocentesis and CVS with no invasive testing. The attributable risk of fetal loss due to amniocentesis was 0.1% according to the most recent systematic review (2015; k=7, each study N>1,000), whereas the only randomised controlled trial available, published in 1986, showed an increase of 1% in total fetal loss. For CVS a 2015 systematic review estimated an attributable risk of fetal loss of 0.22% (95%CI –0.71, 1.16, k=3, p=0.64).

Adverse events from change in management

Adverse events from TOP are shown in section B.8.1. There are a number of methods for TOP, both pharmaceutical and surgical, and the method selected often depends on the gestational age of the fetus, availability of these options, and physician or patient preference. Surgical TOP in the first trimester can lead to complications such as infection, cervical laceration (rare), incomplete evacuation, uterine perforation (rare), haemorrhage and problems with anaesthesia. Side effects and complications from pharmaceutical TOPs in the first trimester are bleeding (moderate to heavy), pain, nausea, vomiting and diarrhoea. No maternal deaths were reported from surgical or pharmaceutical TOP.

Second-trimester TOP can also be conducted by drug regimen or surgically. The incidence of combined major and minor complications was lower with the surgical method, and fewer adverse events were reported, compared with pharmaceutical TOP. However, side effects reported from pharmaceutical TOP were usually mild, except the need for surgical evacuation due to retained products of the placenta and heavy vaginal bleeding. It was concluded that there are safe and effective TOP methods available for use in both first and second trimesters.

Effectiveness

Direct effectiveness

No studies on the direct effectiveness of prenatal CFTR mutation testing were identified.

Effectiveness from linked evidence

1. Diagnostic accuracy

A summary of the diagnostic accuracy data can be found in Table 42 on page 74. DNA sequencing and clinical diagnosis were used as reference standards. Diagnostic accuracy was investigated for all population groups: patients suspected of CF (including men with CBAVD), parents of a fetus suspected of CF, and fetuses suspected of CF. No accuracy studies on partners of people with at least one known CFTR mutation were identified.

CFTR testing in patients with a high clinical suspicion of CF

The median sensitivity of CFTR mutation testing in CF patients, compared with DNA sequencing, was 85% (range 71–97; k=4) when all known mutations were included in the analysis, and 97% (range 90–100) when only those mutations designed to be detected by each test were included. This means that only 3% of samples were falsely negative, and the tests are highly accurate when compared with gene sequencing. Due to the reduced number of CFTR mutations detected by panel-based tests, the median sensitivity of panel-based CFTR tests compared with clinical diagnosis was only 80% (range 52–91; k=5), versus 91% (range 86–100; k=4) for DNA sequencing compared with clinical diagnosis. Meta-analysis could not be conducted to determine the accuracy of CFTR mutation testing in patients with a high clinical suspicion of CF. Only 1 study met the *a priori* inclusion criteria, and the

studies that met the broadened criteria compared CFTR mutation testing with either DNA sequencing methods in patients with known CFTR mutations, or with clinical diagnosis in patients definitively diagnosed with CF or CBVAD. As a consequence, only the sensitivity and false negative rate could be reported.

Panel-based CFTR mutation testing was compared with exon scanning CFTR mutation testing plus DNA sequencing and multiplex ligation-dependent probe amplification (MPLA) deletion detection in men with CBAVD in 1 study. The sensitivity to detect all mutations was 94% (95%CI 81, 99) when compared with DNA sequencing, and 89% (95%CI 75, 97) when compared with DNA sequencing plus MPLA. There were no false positive results. Panel-based testing compared with clinical diagnosis only had a sensitivity of 52% (range 45–72, k=4), due to the large proportion of patients and chromosomes for which a CFTR mutation could not be identified. Exon-scanning CFTR mutation testing plus DNA sequencing had a slightly higher sensitivity of 64% (range 47–88, k=5) when compared with clinical diagnosis.

CFTR testing in parents with a fetus suspected of CF

Only 1 study met the inclusion criteria to assess test performance in parents of a fetus suspected of having CF. The study compared the accuracy of four different panel-based tests to DGGE exon-scanning CFTR mutation testing plus DNA sequencing in 25 CFTR mutation carriers. The panel-based tests had a sensitivity of 100% for the mutations they were designed to detect, and 92% when all mutations were included in the analysis.

CFTR testing in fetuses

Four studies were included that reported on the accuracy of CFTR mutation tests in fetuses from carrier parents as compared with clinical diagnosis after birth, with samples collected through various methods (e.g. CVS or amniocentesis; see Table 86). No false negative results were reported in 2 of the studies (sensitivity = 100%), and the other studies only reported diagnostic yield (8%–22% with CF, 38%–55% for carriers, 24%–33% for normal). Of the fetuses with two identified CFTR mutations (from carrier parents), 95% are aborted (see 'change in patient management' below). The false positives of the test could not be determined as the presence of CF could not be clinically determined in those that were aborted.

The accuracy of CFTR mutation testing in fetuses with a fetal echogenic bowel (FEB) was only identified in 1 study. As no clinical outcomes were reported, the accuracy compared with clinical diagnosis could not be determined.

Test failure rates

The failure rates of seven different panel-based CFTR tests were reported in 5 studies (median 4.5%; range 0.0001–9), which suggests that around 4.5% of tests would need to be repeated in diagnostic laboratories.

Overall, the sensitivity of CFTR mutation tests is high when detecting mutations included in the mutation panel. Both panel-based and exon-scanning CFTR mutation tests, as well as DNA sequencing-based tests, cannot detect large deletion or insertion mutations, which occur in about 2% of CF patients worldwide. In the case of a negative test result, it is important for the diagnostic laboratory to explain the scope of the mutation testing undertaken and the likelihood of the person being truly negative, particularly when one of the consequences of testing may be TOP.

2. Change in patient management (prenatal testing group only)

Nine studies were included to assess the impact of prenatal testing on the management of patients. None of the studies included a comparison with pregnancy management when there was no prenatal CFTR testing; therefore, all 9 studies were non-comparative. Six studies reported on the rate of TOP in pregnancies affected by FEB, with TOP occurring in 65% (50/77) of the pregnancies where CFTR mutations were identified (range 0%–100%). In prospective parents who are known carriers, a positive CFTR test in the fetus led to TOP in 155/163 cases (95%, k=4). This shows that CFTR mutation testing does change management and that a positive test result predicts TOP. It is assumed that no TOP would occur in the absence of prenatal testing.

3. Treatment effectiveness

As outlined above, one key result of prenatal CFTR mutation testing is that fewer children with CF will be born.

No studies were identified on parental psychological health after proceeding with TOP, as a consequence of a CF-affected fetus, compared with the psychological health associated with proceeding with the pregnancy and raising a child with CF. Adverse events and complications due to TOP can be found in section B.8.1 and page xviii. A separate (non-systematic) search was conducted to identify studies investigating psychological outcomes after TOP was instigated following identification of various fetal anomalies (not specific to CF). Post-traumatic stress, grief, anger, guilt and depression were prevalent in this population. In the first few months after TOP, rates of post-traumatic stress (45.8%–67%), grief (36%–78%) and depression (around 30%) were high, but these rates decreased over time. Depression and anxiety rates reported in the first couple of months after TOP were similar to the rates among mothers of children suffering from CF, with 20%–34% and 48% of these women scoring above the threshold for depression and anxiety, respectively. Psychological health improved over time in women who underwent TOP, but it is not fully known whether this is also the case for women with children suffering from CF. One study indicated that in this group, severe depression and anxiety were associated with a younger age of the child. Women showed

more (psychological) symptoms than men, both in the post-TOP group and the group with CF-affected children.

Conclusion

On the basis of the benefits and harms reported in the evidence-base (summarised above), it is suggested that, relative to no genetic testing and subsequent clinical diagnosis after birth, prenatal CFTR mutation testing is likely to correctly identify most CFTR mutations if the appropriate test is used, and will result in the termination of a majority of the affected pregnancies. CFTR mutation testing reduces the frequency of people being born with CF. The test has inferior safety compared with clinical diagnosis after birth, due to the risk of miscarriage associated with prenatal sampling procedures and the adverse events associated with TOP. There was insufficient evidence to make a direct comparison between the psychological impact of TOP and the impact of raising a child with CF, although in the short term the rates of depression appear to be similar.

C. TRANSLATION ISSUES

A full economic evaluation was done only for prenatal testing; that is, populations 2a and 2b. The key issues identified in translating the systematic review of the clinical evidence to the base-case economic evaluations were:

- What is the analytical validity of CFTR mutation testing in the prenatal population?
- What is the clinical sensitivity (detection rate) of the CFTR mutation test?
- What is the incidence of CF in fetuses diagnosed with echogenic bowel?
- What is the risk of miscarriage associated with invasive testing?
- What is the uptake rate of termination of pregnancy?

Pre-modelling studies were conducted to address these issues. A summary of the findings of each pre-modelling study and its implications to the economic evaluation can be found in Table 53 on page 100.

D. ECONOMIC EVALUATION

An analysis is presented to investigate the cost-effectiveness of prenatal genetic CFTR testing in pregnancies where fetuses are assessed as high risk of having CF (populations 2a and 2b) compared with the current situation of no prenatal genetic testing and CF diagnosis through NBS after birth. A summary of the key characteristics of the economic model is presented in Table 1.

Table 1 Summary of the economic evaluation

Perspective	Australian healthcare
Comparator	No prenatal diagnosis for cystic fibrosis (CF)
Type of economic evaluation	Cost-effectiveness
Sources of evidence	Systematic review
Time horizon	Pregnancy to newborn screening
Outcomes	Costs per prenatal CF case detected, costs per CF birth averted, costs per pre-informed CF birth
Costs	Australian dollars, 2014 prices
Methods used to generate results	Decision tree analysis
Discount rate	Not applicable
Software packages used	TreeAge Pro Software 2015, MS Excel 2010

Two economic models are presented for populations 2a and 2b:

- **Model 1 (population 2a):** Fetus at 1:4 risk of CF due to parents being known carriers or having a previous child clinically diagnosed with CF
- **Model 2 (population 2b):** Fetus at risk of CF due to diagnosis of echogenic bowel on the second-trimester ultrasound

Key structural assumptions of the models are:

- Prenatal diagnostic CFTR testing is done within a valid timeframe to allow for TOP if CF is diagnosed.
- The parents accepting the test are prepared to take the risk of invasive testing.
- Consistent with the limited available evidence-base, mutation tests for this population group are assumed to be 100% sensitive and it is assumed that the tests used are appropriate for the mutations being identified. In the absence of any evidence, it is assumed that the tests are also 100% specific. The analytical validity of the diagnostic tests is, therefore, considered 100%; that is, the mutation tests are assumed to accurately detect the presence or absence of specific mutations. The impact of varying the test accuracy was not assessed in the sensitivity analyses.
- Parenting partnerships are considered stable and transparent (i.e. parents with a previous CF child between them are both assumed to be carriers).

The proposed MBS listing for the common mutation test suggests the inclusion of a minimum of 10 mutations in the panel. This does not appear to reflect current clinical practice as a larger number of mutations are generally included in the common mutation panel. As a consequence, four scenarios were considered in the economic evaluation, varying according to the number of mutations included in the panel and by the clinical sensitivity of the common mutation test. A summary of the scenario analyses is presented in Table 2.

Table 2 Scenarios analysed in economic evaluation

Number of common mutations included in the panel	Clinical sensitivity	Cost	Model usage
10 (PASC-recommended minimum)	80%	$135	Base-case analysis
17 (HGSA-recommended)	83.5%	$150	Additional scenario 1
23 (ACOG-recommended)	88%	$170	Additional scenario 2
32 (clinical evidence; see section B.6)	92%	$200	Additional scenario 3

ACOG = American College of Obstetricians and Gynaecologists; HGSA = Human Genetics Society of Australasia; PASC = Protocol Advisory Sub-Committee

Under baseline assumptions (10-mutation panel with 80% test sensitivity and cost of $135), the incremental costs per CF birth averted were $1,898 and $23,254 for model 1 and model 2, respectively. Although the mutation panels (additional scenarios) with higher sensitivity result in higher effectiveness, this is offset by the higher costs of the mutation test. The incremental costs per CF birth averted were observed to be driven largely by the cost of the diagnostic tests (common mutation test and whole gene sequencing).

The summary results of the economic evaluation are presented in the Table 3. An alternative presentation of the economic model calculating benefits and harms resulting from a given expenditure associated with the proposed testing is presented in section D.5 on page 121.

Table 3 The incremental cost-effectiveness of prenatal CFTR mutation testing for all scenarios explored in the economic evaluation

Strategy	Base-case scenario 10-mutation panel	Alternate scenario 1 17-mutation panel	Alternate scenario 2 23-mutation panel	Alternate scenario 3 32-mutation panel
Model 1 – *Parents are known CF carriers*				
Incremental cost per prenatal CF diagnosed	$1,804	$1,816	$1,840	$1,977
Incremental cost per CF birth averted	$1,898	$1,910	$1,935	$2,079
Model 2 – *Fetus has FEB*				
Incremental cost per prenatal CF diagnosed	$15,182	$15,331	$15,537	$16,304
Incremental cost per CF birth averted	$23,254	$23,480	$23,794	$24,972

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; FEB = fetal echogenic bowel; ICER = incremental cost-effectiveness ratio

In model 1 (both parents are known CF carriers) the results were most sensitive to the uptake rate of terminations of CF-affected pregnancies, the cost of NBS in infants tested prenatally, the sensitivity of the common mutation panel and the cost of whole gene sequencing. In model 2 the ICER was most sensitive to changes in the incidence of CF in fetuses with FEB, as well as TOP uptake rates in the tested population (Table 4).

Table 4 Key drivers of the economic models

Description	Values assessed in sensitivity analysis	Impact
Model 1 – Parents are known CF carriers		
Uptake of termination of affected pregnancy	Varied between 80% and 100%	High; lower termination uptake rates favour the comparator
Cost of NBS in infants tested prenatally	Varied between $5 and $250	High; retesting neonates for CFTR mutations during NBS favours the comparator
Clinical sensitivity of common mutation test	Varied between 80% and 98%	High; higher clinical sensitivity favours the intervention
Cost of whole gene sequencing	Varied between $500 and $1,005	High; lowering the cost favours the intervention
Model 2 – Fetus has FEB		
Uptake of termination of affected pregnancy	Varied between 30% and 100%	High; lower termination uptake rates favour the comparator
Incidence of CF in FEB	Varied between 2% and 13%	High; higher incidence of CF in FEB favours the intervention

CF = cystic fibrosis; FEB = fetal echogenic bowel; NBS = newborn screening

Note: Intervention is CFTR mutation common panel test (base-case scenario) and comparator is no genetic testing.

E. ESTIMATED EXTENT OF USE AND FINANCIAL IMPLICATIONS

A market-based approach has been used to estimate the financial implications of listing CFTR mutation testing on the MBS. The estimate has been based on extrapolations from data collected in the RCPA Genetic Testing Survey conducted in 2011. While the survey recorded the number of CFTR tests by broad reason, data could not be distinguished by patient indication. As the proposed descriptor for the MBS item does not include all current indications for testing (i.e. newborn or general population screening are not proposed), the proportion of current CFTR tests that would be eligible for use with the MBS item was sought from the literature and expert opinion.

CFTR mutation testing is currently provided by the states/territories or is privately funded by the populations eligible for MBS funding. Should listing on the MBS be recommended, it is expected that the cost of testing will shift from the states/territories or patients to the MBS (with no cost-offsets to the MBS anticipated).

The financial implications to the MBS resulting from the proposed listing of CFTR mutation testing are summarised in Table 5.

Table 5 Total costs to the MBS associated with CFTR mutation testing

	2015	2016	2017	2018	2019
CFTR mutation tests					
Single mutation analyses [a]	720	828	953	1,096	1,260
Common mutation panels	6,608	7,599	8,739	10,049	11,557
Whole gene screens	1,176	1,353	1,555	1,789	2,057
Total number of tests	8,504	9,780	11,247	12,934	14,874
Cost per test					
Single mutation analyses [a]	$80	$80	$80	$80	$80
Common mutation panels	$135	$135	$135	$135	$135
Whole gene screens	$1,000	$1,000	$1,000	$1,000	$1,000
Cost of testing, by type					
Single mutation analyses [a]	$57,625	$66,269	$76,209	$87,640	$100,786
Common mutation panels	$892,040	$1,025,846	$1,179,723	$1,356,681	$1,560,183
Whole gene screens	$1,176,131	$1,352,551	$1,555,434	$1,788,749	$2,057,061
Total cost of CFTR testing	$2,125,796	$2,444,665	$2,811,365	$3,233,070	$3,718,030
MBS rebate (85%)	$1,806,926	$2,077,965	$2,389,660	$2,748,109	$3,160,326
Patient contribution	$318,869	$366,700	$421,705	$484,960	$557,705

[a] Single mutation analyses are performed to identify one or two F508del mutations.
CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule

As the proposed listing does not attempt to change existing clinical practice, the net cost to the Australian health system should be zero for an MBS listing for CFTR mutation testing. If, however, there is growth in the market, the net overall cost of CFTR mutation testing will be positive, but the effect may be small as it is likely that the majority of patients eligible for MBS funding would continue to receive testing funded by the states/territories.

The analyses were most sensitive to changes that increased the number of common mutation screening tests, such as increasing the proportion of current eligible screening tests, which increased the cost implication to the MBS by up to 25%. Reducing the market growth rate, improving the clinical sensitivity of the common mutation panel and reducing the cost of the whole gene screen would result in a substantial reduction in costs to the MBS (25%–50%).

F. OTHER RELEVANT CONSIDERATIONS

Ethical considerations

The goal of prenatal testing is to help couples make an informed choice of whether to terminate their pregnancy. As genetic tests pose their own specific considerations, the relevant literature on ethical theory, genetic testing and prenatal testing was reviewed and the balance of arguments was assessed. The perspective of the fetus was not assessed in this analysis, as the difference between 'existing' and 'not existing' is beyond comparison here.

Ethical framework

The four-principles approach, also called 'principlism', was adopted in this assessment. It comprises respect for autonomy, non-maleficence, beneficence and justice—which are used to analyse and assess the ethical considerations relating to the provision of healthcare.

Ethical issues specific to prenatal testing

The main ethical issues for prenatal CFTR testing and their most relevant principles can be found in Table 85 on page 143.

Informed choice and counselling: Providing non-directive, balanced, complete information and counselling is important to give unbiased options for meaningful reproductive choice. Without the provision of informed choice, genetic testing and offering the option for TOP means that there is an initial pressure on couples to consider both. The more suggestive the offer and the less clear the opportunity to freely decline it, the greater the pressure.

Disability rights critique: The disability rights critique gives a critical view of selective TOP as a means to avoid the birth of children with a disorder or disability. This argument is less convincing, however, if informed decisions (rather than TOP rates) are taken as a measure of success in prenatal testing. There is a concern that prenatal testing will lead to a degradation of society's willingness to accept and care for children deemed 'abnormal', while at the same time enlarging the category of unacceptable 'abnormality' and narrowing the range of acceptable 'normality'. It would also diminish the importance of developing cures for 'preventable' disorders.

Access to testing and TOP: Access to medical services in Australia is generally adequate and equitable, although access problems among rural populations are well known. In most cases CFTR testing is paid for by the states/territories.

Privacy and confidentiality: Because CFTR mutations affect both individuals and their families, ethical dilemmas can occur when a clinician has to balance the competing needs of maintaining the confidentiality of test results and informing family members of their own risk of having a child with CF. In the case of CF-carriers, genetic counsellors may seek to persuade patients to disclose their carrier-status to siblings, parents and/or children instead of providing non-directive counselling; or they may ask the patients to allow them to disclose this information.

Weighing risks and benefits: The principles of non-maleficence and beneficence could be taken to entail that the risks of harm should be outweighed (perhaps substantially) by the probable benefits before a genetic test is accepted into general practice. Thus, relevant factors include the predictive value of the test, the benefits and harms provided by interventions associated with the test (amniocentesis or CVS) and with a positive test result (TOP), and the availability and acceptability of those interventions.

Ethical analysis conclusions: Prenatal genetic CFTR mutation testing should only be offered on the MBS in conjunction with non-directive, pre- and post-test genetic counselling from accredited counsellors.

Non-invasive prenatal diagnosis (NIPD)

It is anticipated that non-invasive prenatal tests for CF using maternal blood will be available in the near future. The advantages of NIPD are that there is no risk of miscarriage associated with the test, testing can occur at an earlier gestational age (7–9 weeks), and NIPD is less invasive than amniocentesis and CVS. NIPD is mostly seen as a positive development; however, the following ethical concerns regarding it have been raised

- NIPD must be accurate.
- 'Routinisation' of the test due to its non-invasiveness may lead to an erosion of informed decision-making, emphasising the importance of counselling.
- The decreased risks of testing may increase pressure on couples to undertake prenatal testing.
- With the routinisation of NIPD, the expectation is that more gene disorders will be able to be identified, which will widen the scope of testing and could lead to further difficulties in ensuring informed consent.

Legal implications

TOP is regulated by the states/territories. Therefore, whether TOP is lawful when CF is prenatally diagnosed could differ between states/territories. This might need to be considered in the decision-making process.

GLOSSARY AND ABBREVIATIONS

Acronym or term	Description
ACFDR	Australian Cystic Fibrosis Data Registry
ACMG	American College of Medical Genetics
ACOG	American College of Obstetricians and Gynaecologists
AHTA	Adelaide Health Technology Assessment
ARMS	amplification refractory mutation system
ASOH	allele-specific oligonucleotide hybridisation
BDI	Beck Depression Inventory
CAVD	congenital absence of the vas deference
CBAVD	congenital bilateral absence of the vas deferens
CES-D	Center for Epidemiologic Studies – Depression scale
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CI	confidence interval
CVS	chorionic villus sampling
DGGE	denaturing gradient gel electrophoresis
DHPLC	denaturing high performance liquid chromatography
DNA	deoxyribonucleic acid
eMAP	elongation-mediated multiplexed analysis of polymorphisms
EPDS	Edinburgh Postnatal Depression Scale
F508del	a three base-pair deletion that results in the loss of phenylalanine at position 508
FEB	fetal echogenic bowel
HADS	Hospital Anxiety and Depression Scale

Acronym or term	Description
HESP	Health Expert Standing Panel
HGSA	Human Genetics Society of Australia
HTA	health technology assessment
ICER	incremental cost-effectiveness ratio
IES	impact of event scale
IES-R	impact of event scale revised
IRT	immunoreactive trypsinogen
IVF	in-vitro fertilisation
MBS	Medicare Benefits Schedule
MLPA	multiplex ligation-dependent probe amplification
MSAC	Medical Services Advisory Committee
mTTGE	modified temporal temperature gradient electrophoresis
NBS	newborn screening
NIPD	non-invasive prenatal diagnosis
OLA	oligonucleotide ligation assay
OR	odds ratio
PASC	Protocol Advisory Sub-Committee
PBS	Pharmaceutical Benefits Scheme
PCR	polymerase chain reaction
PGD	pre-implantation genetic diagnosis
PICO	population, intervention (investigation/index test), comparator, outcomes
PND	prenatal diagnosis
QAP	quality assurance program
RCPA	The Royal College of Pathologists of Australasia
RCT	randomised controlled trial

Acronym or term	Description
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RR	relative risk
SD	standard deviation
SSCP	single-stranded conformation polymorphism
STR	short tandem repeats
TOP	termination of pregnancy

SECTION A CONTEXT

This contracted assessment of testing for hereditary mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene is intended for the Medical Services Advisory Committee (MSAC). It examines the evidence to support listing on the Medicare Benefits Schedule (MBS) of diagnostic testing for hereditary mutations on the CFTR gene. MSAC evaluates new and existing health technologies and procedures for which funding is sought under the MBS in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. MSAC adopts an evidence-based approach to its assessments, based on reviews of the scientific literature and other information sources, including clinical expertise.

Adelaide Health Technology Assessment (AHTA) has been commissioned by the Australian Government Department of Health (the Department) to conduct a systematic literature review and economic evaluation of CFTR mutation testing. This assessment has been undertaken in order to inform MSAC's decision-making regarding whether the proposed medical service should be publicly funded.

Appendix A provides a list of the people involved in the development of this assessment report, including clinical expertise sourced from the Health Expert Standing Panel (HESP). HESP is a pool of experts collated from various medical fields who have been nominated by their associated professional body or by applicants. HESP members are a panel of MSAC and are engaged to provide practical, professional advice that directly relates to each application and the service being proposed for the MBS. HESP members are not members of either MSAC or its subcommittees. Their role is limited to providing input and guidance to the assessment groups to ensure that the pathway is clinically relevant and takes into account consumer interests. HESP members' advice is used to inform the deliberations that MSAC presents to the Federal Minister for Health.

The proposed use of diagnostic testing for hereditary mutations in the *CFTR* gene in Australian clinical practice was outlined in a protocol that was presented to the Protocol Advisory Sub-Committee (PASC). The protocol was released for public comment in February 2014. A separate research protocol based on the PASC Protocol, input from HESP members and a teleconference with the Department of Health was approved by the Department and is accessible through the MSAC website.

CYSTIC FIBROSIS AND BURDEN OF DISEASE

Cystic fibrosis (CF) and other CFTR-related disorders are the most common autosomal recessive disorder in Caucasians, with a frequency of about 1 in 2,500–2,800 live births worldwide and a carrier frequency of 1 in 25 in Australia (Bell et al. 2011; Ratjen & Doring 2003). The major cause of morbidity and mortality among young people with CF is progressive respiratory disease. On 31

December 2012 the Australian Cystic Fibrosis Data Registry (ACFDR) held records of 3,156 people with CF (Cystic Fibrosis Australia 2013). The actual numbers of people suffering from CF is slightly higher, as it is estimated that only 90% of the people with CF are registered on the database (Cystic Fibrosis Australia 2013). Based on a population of 22.7 million Australians (2012), 1 in 7,193 people in Australia had been diagnosed with CF and were registered with ACFDR. In the same year 63 (out of 309,582 births) CF cases were identified through NBS; that is, 1 in 4,914 newborns received a CF diagnosis and were registered with ACFDR. Over 80% of infant diagnoses are completed by 3 months of age and are aided by neonatal screening programs, but some individuals are diagnosed later—ranging from early childhood to 35 years and older, depending on disease severity. Early diagnosis is expected to be associated with improved health outcomes but may have adverse social and psychological outcomes.

In Australia, between 1979 and 2005, the mean life expectancy of people with CF increased from 12.2 to 27.9 years for males and from 14.8 to 25.3 years for females (Reid et al. 2011). The mean age of the registry population at the end of 2012 was 19.5 years, which is higher than in previous years (19.1 in 2011, 18.8 in both 2010 and 2009). The proportion of patients who were adults (i.e. ≥18 years of age) was 49.3% in 2012, compared with 35% in 2001, demonstrating improved life expectancy. Increases in life expectancy have had a progressive impact on healthcare utilisation. CF in adulthood is associated with severe lung disease, poor nutritional status and CF-related complications, leading to a high burden of disease.

THE *CFTR* GENE

CF and CFTR-related disorders are caused by mutations in the 230-kb *CFTR* gene on chromosome 7, encoding a polypeptide that is 1,480 amino acids long (Ratjen & Doring 2003). CFTR belongs to a family of transmembrane proteins called ATP-binding cassette (ABC) transporters that function as a chloride channel in epithelial membranes (Ratjen & Tullis 2008). Disease expression varies by class of CFTR mutation, along with genetic modifiers and environmental factors (Moskowitz et al. 2008). Development of classic CF requires two loss-of-function mutations in the *CFTR* gene. The disorder is characterised by chronic bacterial infection of the airways and sinuses, fat maldigestion due to pancreatic exocrine insufficiency, infertility in males due to congenital bilateral absence of the vas deferens (CBAVD), and high concentrations of chloride in sweat (Knowles & Durie 2002). The most common changes are seen in the airways, where classic CF causes chronic pulmonary infections. Non-classic CF develops when there is at least one 'mild' mutation that results in partial functionality of the CFTR protein. Some of these mutations are linked to diseases of one organ, such as late onset pulmonary disease, CBAVD or idiopathic pancreatitis (Knowles & Durie 2002). These patients are usually pancreatic sufficient, have chloride values that are close to normal and are typically diagnosed at an older age (Ratjen & Tullis 2008). The best correlation between genotype and phenotype in CF is seen in the context of pancreatic function. Prognosis in classical CF largely

depends on whether the affected individual is pancreatic sufficient or insufficient (most are insufficient). Different genotype–phenotype correlations are shown in Table 6 (page 36).

CFTR mutations can be grouped into six classes (Rowe, Miller & Sorscher 2005):

Class I–III mutations are associated with classic CF—pancreatic exocrine insufficiency and progressive lung disease, exhibiting:

I. the absence of synthesis of the CFTR protein
II. defective protein maturation and premature degradation
III. disordered regulation, such as diminished ATP binding and hydrolysis

Class IV–VI mutations give a broader phenotype (and less severe disease) due to some functionality of the CFTR protein, usually without pancreatic insufficiency, showing:

IV. defective chloride conductance or channel gating, leading to partial channel activity
V. reduced number of CFTR transcripts due to a promotor or splicing abnormality
VI. accelerated turnover from the cell surface due to defective stability of the CFTR protein.

To date, 1,993 mutations in the *CFTR* gene are listed on the Cystic Fibrosis Mutation Database[2]. Most are either point mutations or small deletion/insertion mutations that can be detected by DNA sequencing, exon-scanning-based CFTR mutation tests, or panel-based CFTR mutation tests if the mutation is included in the test's mutation panel (Moskowitz et al. 2008). Large deletion or insertion mutations account for approximately 1–3% of all CFTR mutations and occur in less than 2% of all CF patients worldwide; however, they cannot be detected by any of these tests (Castellani et al. 2008). They can only be detected using methods such as multiplex ligation-dependent probe amplification (MLPA) or quantitative fluorescent multiplex polymerase chain reaction (PCR). Additionally, even the most extensive tests fail to detect all CF alleles, as 1–5% of CF alleles have not yet been identified (Castellani et al. 2008).

Worldwide, the most common mutation in the *CFTR* gene is a Class II mutation, caused by a three-base-pair deletion that results in the loss of phenylalanine at position 508 (F508del). It accounts for approximately 70% of CFTR mutations globally but its frequency varies between different ethnic groups. For example, F508del accounts for 82% of mutations in CF patients in Denmark but only 32% in Turkey (Ratjen & Doring 2003). With this mutation, CFTR is misfolded and stays trapped in the endoplasmic reticulum, where it eventually gets degraded. The most common mutations in the *CFTR* gene in Australia are shown in Figure 1 (page 35). Approximately 85% of CF patients have at least one copy of the F508del mutation, and 52% have two copies.

[2] Available from <http://www.genet.sickkids.on.ca/StatisticsPage.html> (accessed 27 January 2015)

The pathogenicity of some mutations may be influenced by other variants within the *CFTR* gene. For example, the poly-T mutations are abbreviated tracts of a number of thymidines in intron 8 (IVS8), a splicing mutation that confers a mild phenotype by reducing the levels of functional CFTR protein (Groman et al. 2004). The R117H mutation can result in either CF or CBAVD and is associated with either a T5 or T7 allele. The more severe R117H/T5 haplotype is only found in CF patients, although the milder R117H/T7 haplotype can be found in both CF and CBAVD patients (Cuppens & Cassiman 2004).

In fact, the mutation profiles of patients with CF and men with CBAVD differ significantly. A mutation on both *CFTR* genes can only be detected in approximately 70% of CBAVD patients, and about 15% of patients have only one detectable CFTR mutation (Cuppens & Cassiman 2004). The most frequent CFTR mutation found in CBAVD patients is the IVS8-T5 polymorphism, which is found on about 30% of the *CFTR* genes derived from CBAVD patients. The frequency of F508del, the most common CFTR mutation found in CF patients, decreases from approximately 70% to 20% in CBAVD patients. Conversely, the frequency of the rare R117H mutation increases to approximately 8% in CBAVD patients (Cuppens & Cassiman 2004). Among CBAVD patients with two CFTR mutations, 88% have one classic and one mild CFTR mutation, with the most common genotypes being F508del/5T and F508del/R117H/7T. The remaining 12% have mild mutations on both *CFTR* genes (Claustres et al. 2000). This is in contrast to CF patients, where about 88% have classic mutations on both *CFTR* genes, and 12% have one classic and one mild CFTR mutation (Cuppens & Cassiman 2004).

	Mutation 1						
	F508del	G551D	G542X	R553X	R117H	Other	Total
Mutation 2							
F508del	51.8%						**51.8%**
G551D	6.2%	0.2%					**6.4%**
G542X	2.1%	0.2%	0.1%				**2.4%**
R553X	0.5%	0.0%	0.0%	0.0%			**0.5%**
R117H	2.8%	0.1%	0.0%	0.0%	0.1%		**3.0%**
Other	7.2%	0.2%	0.2%	0.0%	0.1%	11.3%	**19.0%**
Unknown / missing	13.0%	0.5%	0.3%	0.2%	0.2%	0.8%	**15.0%**
Missing	1.7%	0.0%	0.0%	0.0%	0.0%	0.3%	**2.0%**
	85.3%	**1.2%**	**0.6%**	**0.2%**	**0.4%**	**12.4%**	**100.1%[b]**

[a] Patients with missing genotype data for both alleles were excluded from analysis
[b] Total does not add up to exactly 100%due to rounding

Figure 1 Genotypes of CFTR mutations in Australia, 2012 [a]

Source: Cystic Fibrosis Australia (2013)

Table 6 CFTR genotype–phenotype correlations

Allele 1	Allele 2	Range of phenotypes
Classic [a]	Classic	Classic >> non-classic
Mild [b]	Classic or mild	Non-classic > classic
R117H/5T	Classic or mild	Non-classic > classic
R117H/7T	Classic or mild	Asymptomatic female or CBAVD > non-classic CF
5T/TG11	Classic or mild	Asymptomatic > CBAVD
7T or 9T	Classic or mild	Asymptomatic
7T or 9T	7T or 9T	Asymptomatic

Source: Moskowitz et al. (2008)
[a] Classic refers to Class I, II and III mutations.
[b] Mild refers to Class IV, V and VI mutations, exclusive of R117H and 5T alleles.
CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis

A.1. RATIONALE FOR ASSESSMENT

This contracted assessment examines the evidence to support the listing of diagnostic testing for hereditary mutations on the *CFTR* gene on the MBS. The service would be used for prenatal CF diagnosis in patients suspected of CF or CFTR-related disorders, and in partners of people with known CFTR mutations for the purpose of reproductive planning. It is claimed that successful listing of the technology in the target population will lead to additional diagnostic surety for a lifelong, expensive and complex condition, changed family planning options, and more treatment options—there are therapies available that are tailored to a specific *CFTR* gene mutation, for example ivacaftor for the G551D mutation.

A.2. PROPOSED MEDICAL SERVICE

A.2.1. INTENDED PURPOSE

CF is usually clinically diagnosed with supporting evidence of a CFTR abnormality, either by sweat chloride measurement or identification of mutations in the *CFTR* gene known to cause CF. An elevated immunoreactive trypsinogen (IRT) level during screening can replace clinical features as a diagnostic criterion in newborns (see page 38). Diagnosis is usually simple following NBS or clinical presentation with an elevated sweat chloride level, but in some situations the combined information makes the diagnosis difficult, for example mild symptoms, a (borderline) positive sweat test, or a new CFTR sequence variation of unknown significance (Farrell et al. 2008).

As stated previously, diagnostic testing for hereditary mutations in the *CFTR* gene occurs in three distinct groups/indications:

1. in people with a high clinical suspicion of CF
2. for prenatal CF diagnosis
3. in partners of people with known CFTR mutations for the purpose of reproductive planning.

The first group includes individuals presenting with classic or non-classic CF symptoms (including men with CBAVD). Prenatal diagnosis would be indicated for couples who have had a previous child with CF or a CFTR-related disorder, or who have been identified by other means to both be carriers of a CFTR mutation. It could also be used in cases where the fetus is found to have an 'echogenic gut'. In this scenario the fetus's parents would undergo CFTR mutation testing to determine if they are carriers, prior to the fetus being tested (if both parents are found to be carriers). The third group includes the testing of a partner of someone with at least one known CFTR mutation, which would influence their reproductive planning to allow an additional option of pre-implantation genetic diagnosis (PGD) if both partners have at least one CFTR mutation.

Each person or fetus being tested for hereditary mutations in the *CFTR* gene would only need to be tested once in their lifetime. Most CF patients would be diagnosed through NBS (see page 38), and the ultrasound examination showing 'echogenic gut' leads to approximately 11% of CF diagnoses (Scotet et al. 2002). A small percentage of patients are diagnosed when older, as they often have a milder form of the disease (such as men with CBAVD).

Different gene tests

The common CFTR tests are:

- F508del mutation
- single mutation
- common mutation
- poly-T (for mild disease and infertility)
- total gene and rare mutation
- prenatal.

Different *CFTR* gene tests would be conducted according to the different groups:

Group 1 (High clinical suspicion of CF): Common mutation analyses are conducted in symptomatic patients, followed by an expanded mutation panel and then total gene sequencing if the clinical situation demands and the common mutation analysis is unable to identify both mutations. In men with CBAVD (group 1d), common mutation analysis is done with the addition of R117H and intron 8 plus possibly poly-T testing. Neonates with a positive sweat test after having one mutation identified through NBS would have total gene sequencing, as they have already been tested for the most common mutations (Figure 2 on page 44).

Group 2 (Prenatal diagnosis): In the parents of a fetus where no familial mutations are known, common mutation analysis would be done. If a mutation is found or known in both parents, a single mutation analysis in the fetus would be performed, as the test would specifically target the previously identified parents' known mutations. In some cases, if the parents have been tested for common mutations and none have been identified, it may be warranted for a whole gene screen to be done in the fetus (Figure 3 on page 45).

Group 3 (Reproductive planning): If a person has at least one CFTR mutation identified and is planning on having children, their partner would undergo common mutation analysis for carrier screening. No additional testing would be done in this group if the initial tests are negative (Figure 4 on page46).

A.2.2. OTHER INDICATIONS

Newborn screening

As stated in section A.2, most CF patients are currently diagnosed through Australia-wide NBS. The first step in screening of neonates for CF is the test for IRT, which is an indirect measure of pancreatic injury that is present at birth in most newborns who have CF. If elevated IRT levels are found in the 48–72-hour dried blood sample (taken through a heel prick), the second step is to do a common mutation analysis to test for common CFTR mutations (NHMRC 2007). As NBS is state/territory regulated and funded, the panel of mutations included in the common mutation test varies across states and territories. If no mutations are found, CF is not indicated and no further tests would be done. Those with heterozygous DNA results (e.g. one identified mutation) would be referred to a sweat test. If this test is positive, the neonate would be diagnosed and referred to a CF clinic. If two mutations are detected in the common mutation test, the newborn would be diagnosed and directly referred to a CF clinic (and in some cases a sweat test would be done).

Within the current public health system, all neonates identified as being at risk for CF due to having one CFTR mutation detected at NBS would be further investigated through additional genetic tests. This is currently funded by the states/territories. As this testing is already considered standard practice, and parents would not be funding the tests themselves, it was considered by PASC that this indication would not need to be examined further in this assessment (MSAC 1216).

Mutation-specific drugs for CF are currently under development, and the first one has been approved for listing on the Pharmaceutical Benefits Scheme (PBS) since the application for CFTR testing was received. When newborns are diagnosed with at least one G551D mutation, they have to wait until they turn 6 years of age to be eligible to access this drug, Kalydeco® (containing ivacaftor), through the PBS.

A.2.3. CURRENT CFTR MUTATION TESTING IN AUSTRALIA

The Royal College of Pathologists of Australasia (RCPA) Genetic Testing Survey 2011 identified 11 National Association of Testing Authorities (NATA)-accredited Australian diagnostic laboratories that performed more than 100 CFTR mutation tests in 2011[3]. Most (66%) of the tests were conducted as

[3] Statistics extrapolated from RCPA Genetic Testing Survey 2011 raw data. Received via email on 19 November 2014.

part of the NBS program, 20% were diagnostic (to determine the genetic basis of an affected patient), 8% were to determine carrier status and 5% were prenatal testing of a fetus.

Only eight of these laboratories are listed on the RCPA 'Catalogue of genetic tests and laboratories' website[4] and all offer panel-based CFTR mutation testing for 10–32 of the most common mutations, which detect up to 85% of mutations in the Australian Caucasian population. Only two of these laboratories offer comprehensive mutation scanning—both offer DNA sequencing of the entire coding region of CFTR plus the splice sites, but only one offers multiple MLPA to detect large deletion or insertion mutations that cannot be detected by DNA sequencing.

In Australia there are no mandates on how many mutations should be included in a 'common' mutation panel. The best guideline on what should be included as a minimum comes from the Human Genetics Society of Australia (HGSA) position statement (October 2013), recommending that all mutations with a frequency greater than 0.1% in individuals with clinically diagnosed CF in Australasia be included. This would identify a minimum of 80% of carriers in Australasia and equates to about 650,000 individuals. These mutations (17 in total) are:

- p.Phe508del
- p.Gly551Asp
- p.Gly542X
- p.Asn1303Lys
- c.489+1G>T
- c.1585-1G>A
- p.Trp1282X
- p.Arg553X
- p.Ile507del
- c.3717+12191C>T (3849+10kb)
- p.Arg560Thr
- p.Val520Phe
- p.Arg1162X
- c.3528delC
- p.Asp1152His
- c.1766+1G>A
- p.Gln493X.

[4] Available from <http://genetictesting.rcpa.edu.au/> (accessed 3 February 2015).

All 11 Australian laboratories participated in an external European quality assurance program (QAP) for CF in 2014[5]. The QAP involved testing of DNA samples from three individuals, a CF patient with the common F508del mutation and a rare CFTRdele2,3 mutation, a CF carrier with a D507del mutation, and a normal patient with no CFTR mutations. All laboratories correctly identified the three chromosomes without mutations, and the two mutations included in the mutation panels used for testing. However, the rare CFTRdele2,3 mutation (worldwide frequency is 0.4%)[6] was not tested for by 10 of the laboratories—two provided wrong or insufficient interpretation of this result and one made an error in risk calculation.

The results of the QAP suggest that the 85% of CFTR mutations detectable by panel-based testing in Australian laboratories would be correctly identified. However, 15% of people with a CFTR mutation would receive a false negative diagnosis, requiring further testing to determine their true status. It is important that the requesting clinician receives a correct interpretation from the laboratory of the risk of their patient having an unidentified CFTR mutation.

A.3. PROPOSAL FOR PUBLIC FUNDING

The proposed MBS item descriptors are summarised in Table 7. Fees were not proposed by the applicants. Costs associated with CFTR mutation testing in Australia can be found in Table 102 (Appendix I), and vary between $135 and $500 for a mutation panel-based test (10–145 mutations), between $50 and $160 for a single mutation test, and around $1,000 for whole gene sequencing, depending on the laboratory, the testing method used and the number of mutations tested. These costs are laboratory costs and do not include counselling and other fees. Counselling is a requirement for tier 2 genetic testing (NPAAC 2013).

Table 7 Proposed MBS item descriptors

Category 6 – Pathology services
MBS [proposed MBS item number 1] Detection of the maternal and paternal known genetic mutation(s) of the *CFTR* gene in sample of blood or other fluid or tissue, in the following situation: Pregnant woman whose fetus is at 25% or more risk of CF Fee: To be advised Prior to ordering these tests the ordering practitioner should ensure the patient has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient either by the treating practitioner, a genetic counselling service or by a clinical geneticist on referral.

[5] CF network, Biomedical Quality Assurance Research Unit, Department of Public Health and Primary Care, Catholic University Leuven, Leuven, Belgium. Pers. comm. via email on 31 January 2015.

[6] Available from <http://www.CFTR2.org/files/CFTR2_22July2013.pdf> (accessed 4 February 2015).

Category 6 – Pathology services
Further counselling may be necessary upon receipt of the test results.
MBS [proposed MBS item number 2] Simultaneous detection of multiple common mutations (Level 1 testing, minimum of 10 mutations) in the *CFTR* gene in blood or other fluid / tissue sample in a: (a) Patient suspected of cystic fibrosis or a CFTR related disorder (with the exception of newborns suspected of cystic fibrosis through newborn screening); (b) Man with congenital bilateral absence of the vas deferens (CBAVD) Fee: To be advised Prior to ordering these tests the ordering practitioner should ensure the patient (or their parent/guardian in the case of children) has given informed consent. For b: testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient either by the treating practitioner, a genetic counselling service or by a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.
MBS [proposed MBS item number 3] Simultaneous detection of multiple common mutations (Level 2 testing, minimum of 10 mutations) in the *CFTR* gene in blood or other fluid / tissue sample in a: Prospective parent whose fetus is suspected of having a CFTR related disorder Partner of someone with a known CFTR mutation, for reproductive planning purposes Fee: $ SAME AS PROPOSED MBS ITEM NUMBER 2 Prior to ordering these tests the ordering practitioner should ensure the patient (or their parent/guardian in the case of children) has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient either by the treating practitioner, a genetic counselling service or by a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.
MBS [proposed MBS item number 4] Sequencing analysis of the entire *CFTR* gene (Level 1 testing) for constitutional genetic abnormalities causing CFTR-related disorders, where the results in item [proposed MBS item number 2 or newborn screening common mutation analysis (funded by the State / Territory) are inconclusive, either as: Diagnostic studies of a CF affected person; or Identification of the second mutation in a newborn with one identified mutation and a positive or indeterminate result from item 66686 (sweat test) Fee: To be advised Prior to ordering these tests the ordering practitioner should ensure the patient (or their parent/guardian in the case of children) has given informed consent.
MBS [proposed MBS item number 5] Sequencing analysis of the entire *CFTR* gene (Level 2 testing) for constitutional genetic abnormalities causing CFTR related disorders in the following situations: A negative common mutation test [proposed MBS item number 3] in a parent who previously had a child diagnosed with a CFTR related disorder A negative common mutation test [proposed MBS item number 3] in a prospective parent whose fetus is suspected of having a CFTR related disorder Fee: To be advised

Category 6 – Pathology services
Prior to ordering these tests the ordering practitioner should ensure the patient (or their parent/guardian in the case of children) has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient either by the treating practitioner, a genetic counselling service or by a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.

A.3.1. CURRENT FUNDING ARRANGEMENTS

CFTR mutation testing is already current practice in all states and territories; the funding sources for CFTR mutation tests performed in 2011 in Australia are shown in Table 8[7].

Table 8 Funding sources of CFTR mutation tests performed in 2011

FUNDING SOURCES	Number of tests	% breakdown
Federal	0	0%
State/territory	6,609	43%
Grant/research	0	0%
Patient	7,879	51%
Not provided	874	6%
TOTAL tests performed	15,362	100%

CFTR = cystic fibrosis transmembrane conductance regulator

A.4. COMPARATOR DETAILS

A full assessment of the safety and effectiveness of CFTR mutation testing was only conducted for one population group (group 2: prenatal diagnosis); see section A.8 for the approach taken for the different populations.

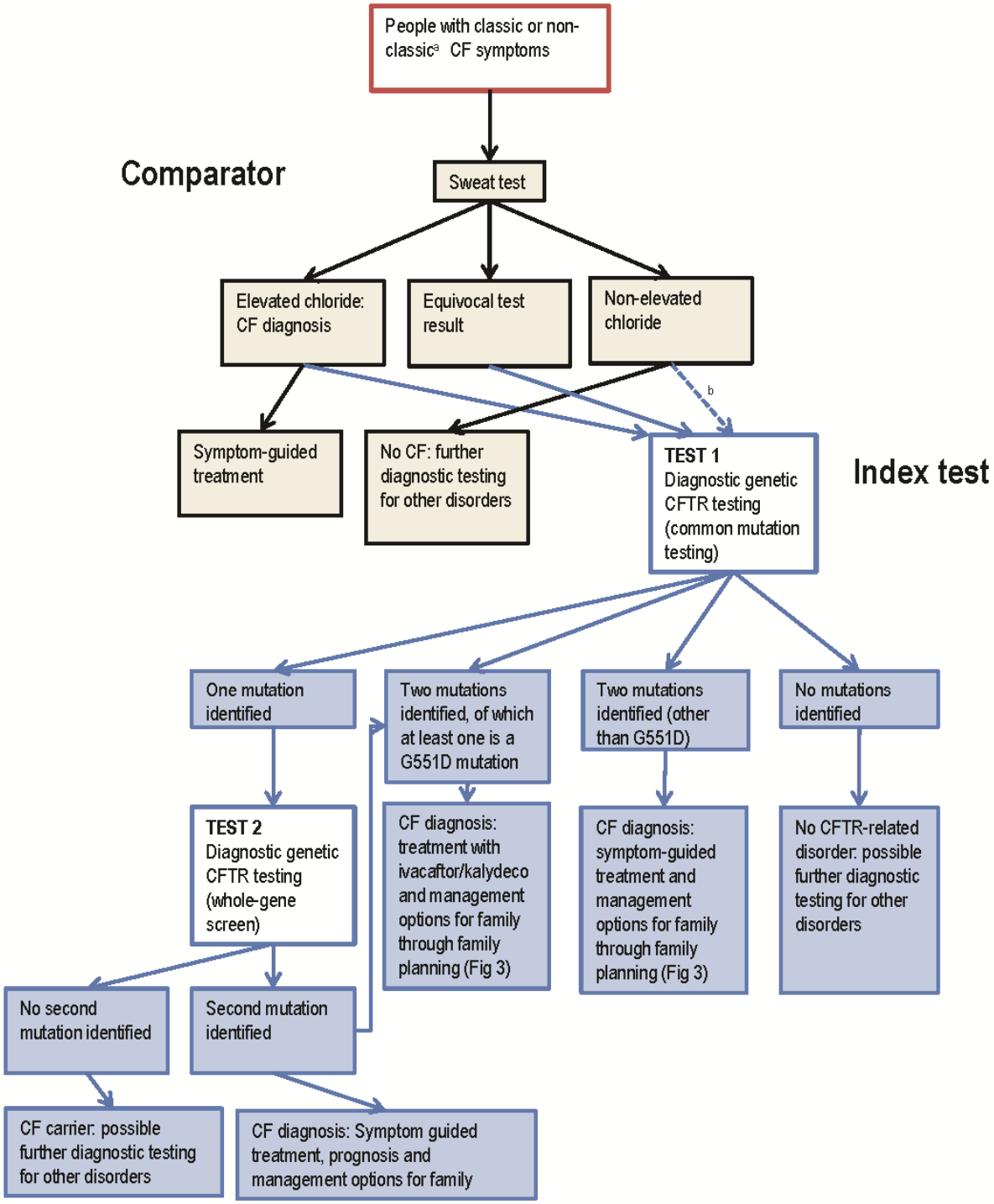
Comparators are normally chosen based on what the proposed technology is likely to replace. CFTR mutation testing in the parents of a fetus at risk of having CF would be self-funded, and testing in fetuses where the parents are both carriers is currently standard practice and would be funded by (some) states and/or territories. However, there is a sizeable proportion of the population who would be receiving their prenatal care in the private health system, and would currently be paying for the fetal CFTR mutation tests themselves. Even though most parents would decide to pay for (parental) CFTR mutation testing, the comparator would be 'no prenatal genetic testing' and diagnosis of the child after birth.

[7] Statistics extrapolated from RCPA Genetic Testing Survey 2011 Raw Data. Received via email on 19 November 2014

A.5. CLINICAL USE OF THE TEST

How the tests are used in the three different population groups is shown in the clinical management algorithms in Figure 2, Figure 3 and Figure 4. The blue boxes show the pathway related to intervention (which is current clinical practice), and the grey boxes show clinical practice in the absence of the intervention, which is the comparator pathway or the historical clinical pathway.

A.5.1. CLINICAL MANAGEMENT ALGORITHMS

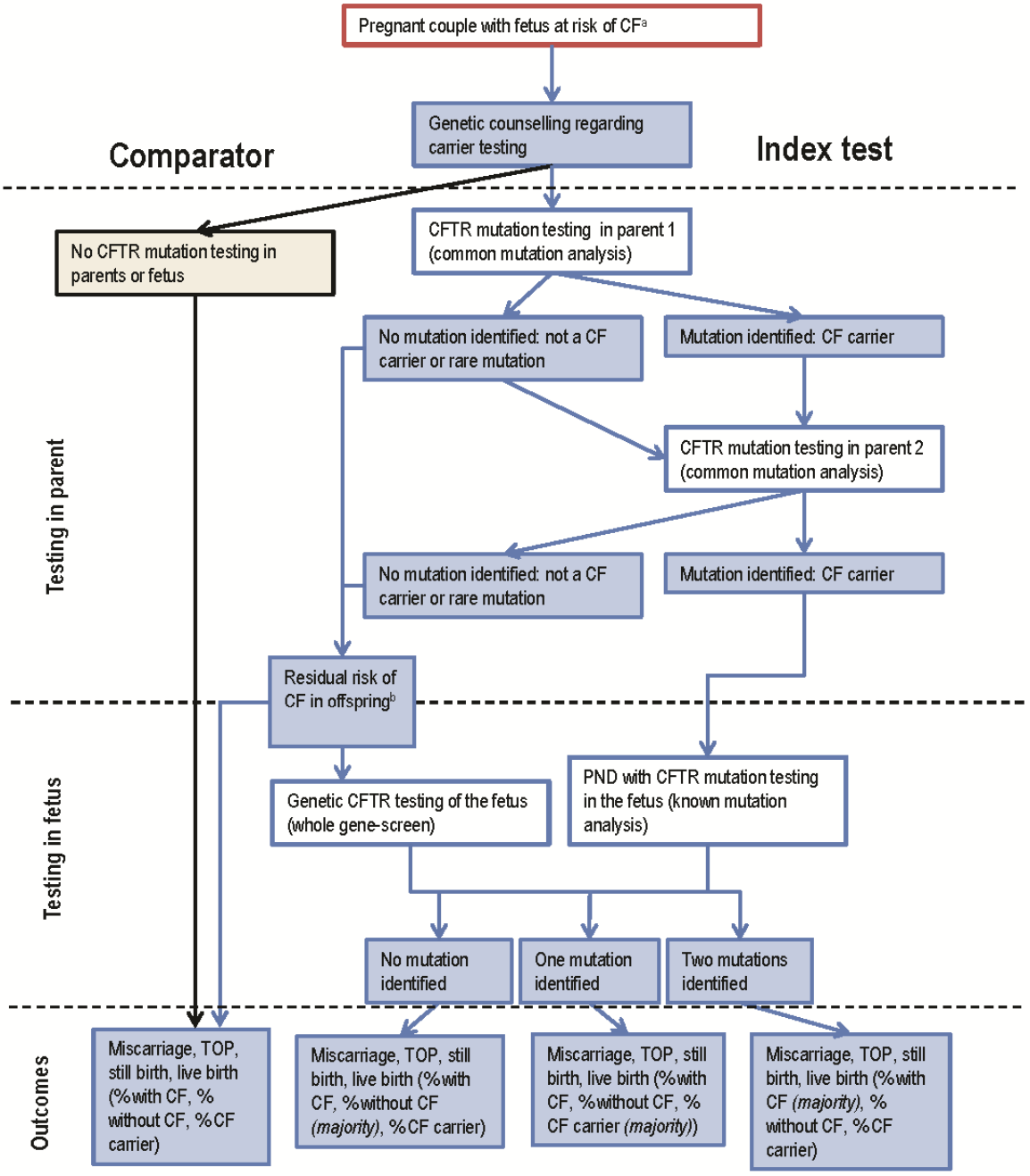


CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator

[a] non classic CF symptoms include CBAVD, bronchitis/bronchiectasis, chronic pancreatitis, salt-losing syndromes etc.

[b] If clinical symptoms are suggestive of CF or a CFTR related disorder, genetic testing may be warranted in some cases despite a negative sweat test, as some mutations result in normal sweat chloride concentrations.

Figure 2 Clinical pathway for use of a genetic CFTR test to identify mutations in people with a high clinical suspicion of CF

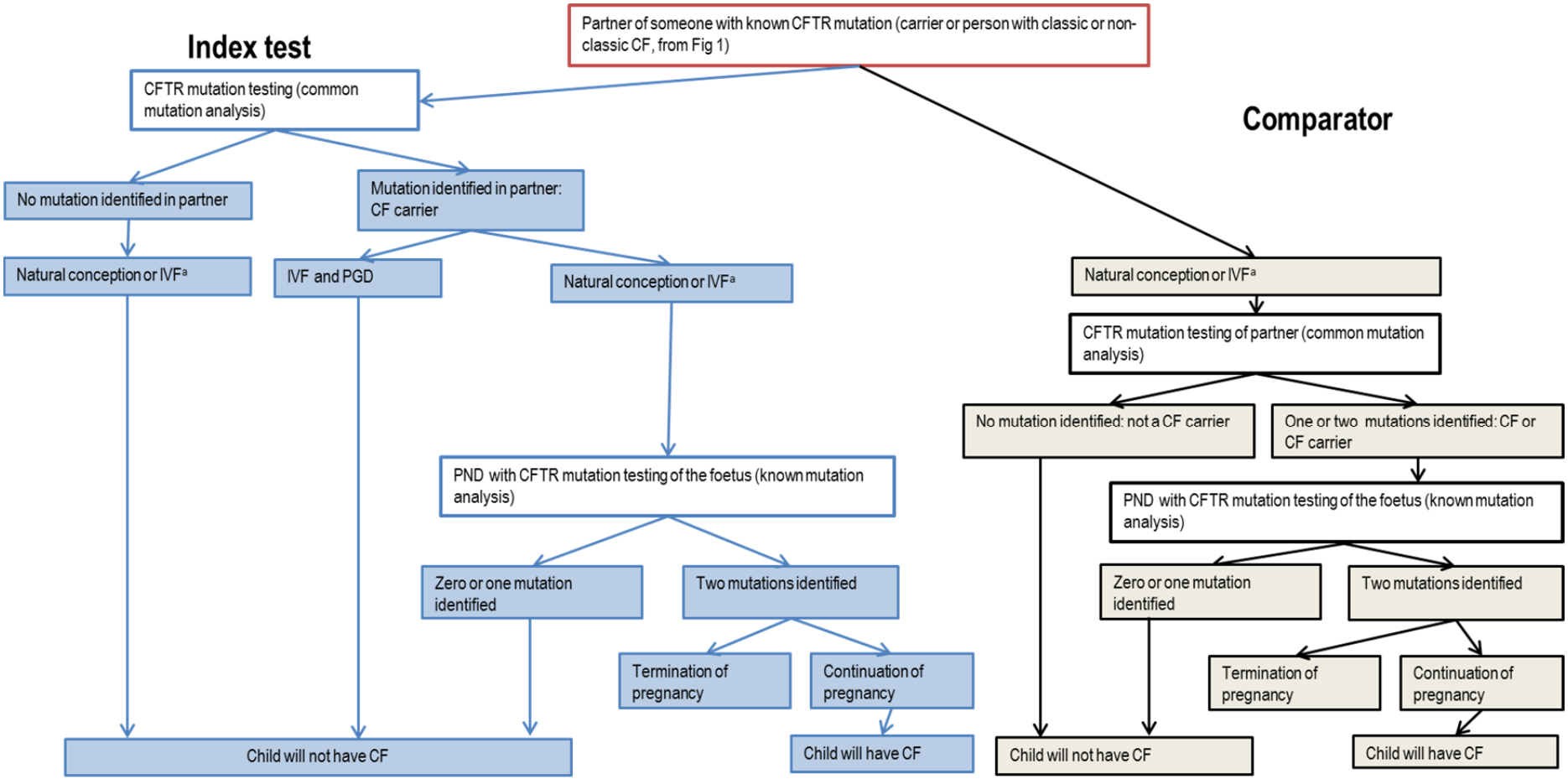


CF = cystic fibrosis; PND = prenatal diagnosis; TOP = termination of pregnancy

[a] This includes parents whose fetus has been diagnosed with echogenic gut or at risk of CF due to a previous child being clinically diagnosed with CF (unknown mutations). In cases where the parents are already diagnosed with (known) CFTR mutations (e.g. in tests during a previous child being diagnosed with CF), only tests in the fetus will be conducted (known mutation analysis).

[b] If someone has a rare CFTR mutation that does not get picked up by common mutation analysis, there is still a chance of CF or CFTR-related disorders in offspring. For parents with a previous child with CF, the probability of a rare mutation being present would be significant (almost 100%), assuming paternity is accurate and there has not been a change of partner. The risk of the fetus having CF would still be around 25%, regardless of the result of the screening test. Similarly, if a fetus has echogenic gut and one parent is found to be a carrier, the risk of an affected foetus is still approximately 27% (based on a test with 80% sensitivity).

Figure 3 Clinical pathway for use of a genetic CFTR test in pregnant couples to determine the CF status of the fetus



CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; IVF = in-vitro fertilisation; PGD = preimplantation genetic diagnosis; PND = prenatal diagnosis

[a] Men with CBAVD are infertile and can therefore only conceive through IVF. CF carriers and female CF patients are able to conceive naturally.

Figure 4 Clinical pathway for use of a genetic CFTR test to inform reproductive planning, prior to conception (plus PGD or prenatal CFTR testing), versus prenatal CFTR testing

A.6. KEY DIFFERENCES IN THE DELIVERY OF THE PROPOSED MEDICAL SERVICE AND THE MAIN COMPARATOR

In patients with a high clinical suspicion of having CF, the key difference that may occur through the use of genetic testing is the ability for those with at least one G551D mutation to potentially receive treatment with ivacaftor.

In couples with a fetus at risk of CF, mutation testing of the *CFTR* gene provides, with reasonable certainty, knowledge regarding whether the potential child would have CF, and gives the option to terminate the pregnancy if the fetus is affected.

In couples planning to have a child, mutation testing of the *CFTR* gene prior to conception allows them the option of conceiving through the use of IVF and PGD, and selection of an unaffected embryo.

A.7. CLINICAL CLAIM

The applicant claims that identification of CFTR mutations is important for providing information at a molecular level about prognosis as a result of genotype–phenotype correlation. Furthermore, identification of CFTR mutations in an individual with CF or another CFTR-related disorder is essential if prenatal diagnosis or PGD is to be offered to prospective parents within their extended family. Knowledge of CFTR mutations in a fetus could aid the decision regarding whether to terminate the pregnancy.

No clinical claim regarding CFTR testing for reproductive planning was made by the applicant. However, according to HGSA, pre-pregnancy testing is preferable because it allows more options for carrier couples, including PGD, donor gamete/embryo and prenatal diagnosis with the option of terminating the pregnancy, leading to a decreased incidence of CF.

Since the application for CFTR testing was received, there has also been a change in accessible CF drugs. Patients aged 6 years or older who are identified as having the G551D mutation can now access the drug Kalydeco® (containing ivacaftor) through the PBS[8].

[8] Available from <http://www.health.gov.au/internet/main/publishing.nsf/Content/MC14-000305-pharmaceutical-benefits-scheme-listing-of-kalydeco> (accessed 3 February 2015).

A.8. SCOPE OF THE CLINICAL EVALUATION

The guiding framework of a protocol is recommended by MSAC for each assessment. The protocol describes current clinical practice and reflects likely future practice with the proposed medical service.

Determination of the clinical effectiveness of an investigative test requires either:

- evidence of the effectiveness of CFTR mutation testing from high-quality comparative studies evaluating the use of the testing and subsequent treatment compared with no genetic testing and treatment (direct evidence). Randomised controlled trials (RCTs) provide the highest quality evidence for this comparison. Or, if this is not available:
- evidence of treatment effectiveness from high-quality comparative studies evaluating the treatment for CF, linked with applicable and high-quality evidence of the analytical validity of CFTR mutation testing to diagnose CF compared with no genetic testing. This is called 'linked evidence'.

Outlined below is the approach formulated according to the information provided in the application from RCPA; discussions of PASC; and communication between the contracted assessment group, the MSAC Secretariat and the relevant policy area from the Department of Health (Table 9).

Table 9 Overview of approach taken in assessing the benefit of CFTR testing for the different populations

Population requested to be assessed in application	Clinical pathway	How this has been assessed	Summary of approach (PICO boxes)
1a. Newborns found to have one CFTR mutation on NBS and who had a positive sweat test	-	PASC suggested that all neonates currently identified as having one CFTR mutation from NBS would be further investigated (i.e. receive additional genetic tests) within the public health system, funded by the states and territories. As this testing is already considered standard practice, and parents would currently not be funding the testing themselves, it was considered that this indication would not need to be examined. A discussion was provided on CFTR testing within this population, but a systematic review was not performed.	Discussion
1b. Patients with symptoms of classic CF	Figure 2 and, if using the information for reproductive planning, Figure 4	Within this population, information on CFTR mutations may assist: (1) in determining eligibility for ivacaftor or (2) for reproductive planning. The health benefit of testing for (1) above has already been examined in the submission to the Pharmaceutical Benefits Advisory Committee (PBAC) for ivacaftor. Therefore, only the accuracy of CFTR testing for this indication was examined in the MSAC assessment. The benefit of testing this population for reproductive planning will be examined in the contracted assessment of pre-implantation genetic diagnosis (MSAC 1165). PASC agreed that it need not be re-	Accuracy (see Table 10) Financial impact

Population requested to be assessed in application	Clinical pathway	How this has been assessed	Summary of approach (PICO boxes)
		examined specifically for CFTR. Therefore, only the accuracy of CFTR testing for this indication was assessed. The financial implications associated with the genetic testing of patients with symptoms of classic CF were evaluated.	
1c. Patients with chronic symptoms of non-classic CF	Figure 2 and, if using the information for reproductive planning, Figure 4	As per population 2 above.	Accuracy (see Table 10) Financial impact
1d. Men with CAVD	Figure 2 and Figure 4	Considered to have symptoms of non-classic CF. The key benefit of testing within this population would be to inform reproductive planning. The benefit of testing for this purpose will be examined in the contracted assessment of pre-implantation genetic diagnosis (MSAC 1165). PASC agreed that this need not be re-examined specifically for CFTR. Therefore, only the accuracy of testing for this indication was assessed and the financial implications of providing this testing were estimated.	Accuracy (see Table 10) Financial impact
2a. Prenatal diagnosis of couples who have a previous child with CF or CFTR-related disorder, or who are found to be carriers of a CFTR mutation	Figure 3	This population has not been assessed elsewhere. Therefore, a systematic review was performed assessing the safety and effectiveness of prenatal testing of couples, and, if they are found to be carriers, genetic testing of the fetus and possible TOP. A discussion was provided on the psychological impact of TOP and of caring for a child with CF. Cost-effectiveness was determined by cost per case avoided, with a discussion on the lifetime cost of treating a person with CF.	Safety, effectiveness and cost-effectiveness (seeCF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator Table 11 and Table 12) Linked evidence analysis: Accuracy (see Table 13 and Table 14) Change in management (see Table 15 and Table 16) Impact of change in management (see Table 17) Financial impact
2b. Fetuses with an echogenic gut	Figure 3	As per population 5 above.	As above
Additional population accepted by PASC	Clinical pathway	How this has been assessed	Summary of approach
3. Partners of someone who is known to have CF or be a carrier of a CFTR mutation	Figure 4	The benefit of testing to inform reproductive planning will be examined in the contracted assessment of pre-implantation genetic diagnosis (MSAC 1165), and PASC agreed it need not be re-examined	Accuracy (see Table 10) Financial impact

Population requested to be assessed in application	Clinical pathway	How this has been assessed	Summary of approach (PICO boxes)
		specifically for CFTR. Therefore, only the accuracy of testing for this indication was assessed and the financial implications of providing this testing were estimated.	

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; MSAC = Medical Services Advisory Committee; NBS = newborn screening; PASC = Protocol Advisory Sub-Committee; PICO = population, intervention (investigation/index test), comparator, outcomes; TOP = termination of pregnancy

The population, intervention (investigation/index test), comparator and outcomes (PICO) that were pre-specified to guide the systematic literature review for a direct evidence approach, along with additional criteria for selecting studies for the evidence-base, are presented in Table 10 to Table 12. These criteria were defined *a priori* to minimise any bias associated with study selection in the systematic literature review.

Table 10 Selection criteria for the diagnostic accuracy of CFTR mutation testing in patients with a high clinical suspicion of CF and partners of CF carriers (diagnostic accuracy only)

Population	Patients with classical CF symptoms Patients with non-classic CF symptoms (CBAVD, bronchitis bronchiectasis, chronic pancreatitis, salt-losing syndromes etc.) Partners of CF carriers
Intervention	Diagnostic CFTR mutation testing (common mutation analysis, if necessary followed by whole gene screen)
Evidentiary standard	Whole gene sequencing (in association with copy number analysis to include whole gene deletions or partial gene deletions and duplications
Outcomes	Analytic validity: test–retest reliability, invalid/uninterpretable test results Clinical validity: sensitivity, specificity, false positive rate, false negative rate, negative predictive value, positive predictive value (by reference to the evidentiary standard)
Study design	Level I to level III-3 diagnostic study designs in Table 20
Search period	As the first CFTR mutations were identified around 1989, the search period was 1/1989 – 10/2014
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base
Research questions	What is the diagnostic accuracy of CFTR mutation testing in patients with a high clinical suspicion of CF? What is the diagnostic accuracy of CFTR mutation testing in partners of CF carriers?

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator

Table 11 Selection criteria for evidence assessing the safety and effectiveness of CFTR mutation testing in parents with a fetus suspected of CF

Population	Parent with a fetus showing echogenic gut on second-trimester ultrasound Parent with a fetus at risk of CF due to a previous child being clinically diagnosed with CF (unknown mutations)
Intervention	CFTR mutation testing (common mutation analysis) in the parents, and in some cases prenatal diagnosis (PND) + CFTR mutation testing (known mutation analysis or whole gene screen) in the fetus
Comparators	No prenatal CFTR mutation testing, and diagnosis of the child after the birth
Outcomes	Miscarriage rate, rate of TOP, reason for TOP *(if applicable)*, rate of stillbirths, rate of live births, % change in patients proceeding to PND % change in method of CF diagnosis in child/fetus, parental psychological health, parental quality of life
Study design	Randomised or non-randomised controlled trials, cohort studies, case series, or systematic reviews of these study designs
Search period	As the first CFTR mutations were identified around 1989, the search period was 1/1989 – 10/2014
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base
Research question	What is the safety, effectiveness and cost-effectiveness of prenatal CFTR mutation testing of couples carrying a fetus with a high clinical suspicion of CF, compared with determining the diagnosis of the child after the birth?

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; PND = prenatal diagnosis; TOP = termination of pregnancy

Table 12 Selection criteria for evidence assessing the safety and effectiveness of CFTR mutation testing of a fetus conceived by parents that are both CF carriers

Population	Fetuses where both parents have been identified as CF carriers (parents identified due to: signs/symptoms of CF in themselves, a previous child with CF, or due to investigations following an echogenic gut in the fetus)
Intervention	PND followed by CFTR mutation testing (whole gene screen in fetuses with echogenic gut where common mutations are not identified in parents, and known mutation analysis and possible whole gene screen for fetuses whose parents are carriers) with the option of TOP if the fetus is affected
Comparators	No prenatal CFTR mutation testing, and diagnosis of the child after the birth
Outcomes [a]	Physical harms directly associated with testing procedure: % with CF, % without CF, % CF carrier
Study design	Randomised or non-randomised controlled trials, cohort studies, case series or systematic reviews of these study designs
Search period	As the first CFTR mutations were identified around 1989, the search period was 1/1989 – 10/2014
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base
Research question	What is the safety, effectiveness and cost-effectiveness of CFTR mutation testing of a fetus conceived by parents that are both CF carriers, compared with determining the diagnosis of the child after the birth?

[a] Note: This assessment will not be formally assessing the impact of CFTR testing on the life expectancy, morbidity, quality of life or functional status of children with CF, as the expected disease course is known and the test is unable to affect the course of the disease (except with regard to a parental decision of TOP—addressed in CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator

Table 11).

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; PND = prenatal diagnosis; TOP = termination of pregnancy

A full linked evidence approach was necessary to answer the research questions, and the PICO that were pre-specified to guide the systematic literature review for a linked evidence approach are presented in Table 13 to Table 17.

A.8.1 DIAGNOSTIC ACCURACY

Table 13 Selection criteria for the accuracy of CFTR mutation testing in parents with a fetus suspected of CF

Population	Parent with a fetus showing echogenic gut on second-trimester ultrasound Parent with a fetus at risk of CF due to a previous child being clinically diagnosed with CF (unknown mutations)
Intervention	CFTR mutation testing (common mutation analysis) in the parents, and in some cases PND + CFTR mutation testing (known mutation analysis or whole gene screen) in the fetus
Evidentiary standard	Clinical diagnosis (NBS + symptoms) after the birth
Outcomes	Analytic validity: test–retest reliability, invalid/uninterpretable test results Clinical validity: sensitivity, specificity, false positive rate, false negative rate, negative predictive value, positive predictive value (by reference to the evidentiary standard)
Study design	Level I to level III-3 diagnostic study designs in Table 20
Search period	As the first CFTR mutations were identified around 1989, the search period was 1/1989 – 10/2014
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base
Research question	What is the diagnostic accuracy of CFTR mutation testing in parents of a fetus suspected of CF?

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; PND = prenatal diagnosis

Table 14 Selection criteria for the accuracy of CFTR mutation testing in fetuses where both parents are CF carriers

Population	Fetuses where both parents have been identified as CF carriers (parents identified due to: signs/symptoms of CF in themselves, a previous child with CF, or due to investigations following an echogenic gut in the fetus)
Intervention	PND followed by CFTR mutation testing (whole gene screen in fetuses with echogenic gut where common mutations are not identified in parents, and known mutation analysis and possible whole gene screen for fetuses whose parents are carriers), with the option of TOP if the fetus is affected
Evidentiary standard	Clinical diagnosis (NBS+ symptoms) after the birth
Outcomes	Analytic validity: test–retest reliability, invalid/uninterpretable test results Clinical validity: sensitivity, specificity, false positive rate, false negative rate, negative predictive value, positive predictive value (by reference to the evidentiary standard)
Study design	Level I to level III-3 diagnostic study designs in Table 20
Search period	As the first CFTR mutations were identified around 1989, the search period was 1/1989 – 10/2014
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base
Research question	What is the diagnostic accuracy of CFTR mutation testing in fetuses where both parents are CF carriers?

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; PND = prenatal diagnosis; TOP = termination of pregnancy

A.8.2 CHANGE IN MANAGEMENT

Table 15 Selection criteria to determine the impact of testing on the clinical management of pregnancies where the fetus has suspected CF

Population	Parent with a fetus showing echogenic gut on second-trimester ultrasound Parent with a fetus at risk of CF due to a previous child being clinically diagnosed with CF (unknown mutations)
Intervention	CFTR mutation testing (common mutation analysis) in the parents, and in some cases PND + CFTR mutation testing (known mutation analysis or whole gene screen) in the fetus
Comparators	No prenatal CFTR mutation testing, and diagnosis of the child after the birth
Outcomes	% change in patients proceeding to PND % change in method of CF diagnosis in child/fetus
Study design	Randomised trials, cohort studies, case series or systematic reviews of these study designs
Search period	As the first CFTR mutations were identified around 1989, the search period was 1/1989 – 10/2014
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base
Research question	Does prenatal CFTR mutation testing (common mutation analysis) affect the clinical management of a pregnancy where the fetus is suspected of having CF, compared with determining the diagnosis of the child after the birth?

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; PND = prenatal diagnosis

Table 16 Selection criteria to determine the impact of testing on the clinical management of pregnancies where both parents are CF carriers

Population	Fetuses where both parents have been identified as CF carriers (parents identified due to: signs/symptoms of CF in themselves, a previous child with CF, or due to investigations following an echogenic gut in the fetus)
Intervention	PND followed by CFTR mutation testing (whole gene screen in fetuses with echogenic gut where common mutations are not identified in parents, and known mutation analysis and possible whole gene screen for fetuses whose parents are carriers), with the option of TOP if the fetus is affected
Comparators	No prenatal CFTR mutation testing (and diagnosis of the child after the birth, *where relevant*)
Outcomes	% change in termination of pregnancy rate; live births: % with CF, % without CF, % CF carrier
Study design	Randomised trials, cohort studies, case series or systematic reviews of these study designs
Search period	As the first CFTR mutations were identified around 1989, the search period was 1/1989 – 10/2014
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base
Research question	Does CFTR mutation testing of a fetus conceived by parents that are both CF carriers affect the clinical management of the pregnancy, compared with determining the diagnosis of the child after the birth?

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; PND = prenatal diagnosis; TOP = termination of pregnancy

A.8.3. EFFECTIVENESS OF CHANGE IN MANAGEMENT

Table 17 Selection criteria to determine the impact of change in management in parents with a fetus suspected of CF

Population	Parent with a fetus showing echogenic gut on second-trimester ultrasound Parent with a fetus at risk of CF due to a previous child being clinically diagnosed with CF (unknown mutations)
Intervention	TOP
Comparators	No TOP; caring for a child with CF
Outcomes	Parental psychological health, parental quality of life
Study design	Randomised trials, cohort studies, case series or systematic reviews of these study designs
Search period	As the first CFTR mutations were identified around 1989, the search period was 1/1989 – 10/2014
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base
Research question	If there are alterations in clinical management (e.g. TOP) and treatment options available to parents of a fetus suspected of CF, does this have an impact on the health outcomes of the parents?

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; TOP = termination of pregnancy

This contracted assessment of CFTR mutation testing addresses the PICO elements that were pre-specified in the protocol.

SECTION B CLINICAL EVALUATION

B.1. LITERATURE SOURCES AND SEARCH STRATEGIES

The medical literature was searched on 13 October 2014 to identify relevant studies and systematic reviews published during the period 1 January 1989 to 13 October 2014. Searches were conducted using the databases and sources described in Appendix B. Search terms are described in Table 18.

Additional non-systematic searches were undertaken to assess the safety of amniocentesis and CVS and the health implications of management changes (last step of the linked analysis), as no studies specific to CF and prenatal CFTR testing were identified.

Table 18 Search terms used (PubMed/Medline search platform)

Element of clinical question	Search terms
Population & Intervention	((CFTR OR cystic fibrosis conductance transmembrane regulator) OR ((cystic fibrosis OR cystic fibrosis [MeSH]) AND (gene OR gene* OR carrier* OR prenatal OR antenatal OR fetus* OR foetus* OR fetal OR foetal))) AND ((screen* OR test* OR diagnos*) OR ("Cystic Fibrosis Transmembrane Conductance Regulator/diagnostic use"[Mesh] OR "Cystic Fibrosis/prevention and control"[Mesh] OR ("Cystic Fibrosis/diagnosis"[Mesh] AND "Cystic Fibrosis/genetics"[Mesh]))) OR (“cystic fibrosis”[Text Word] AND ("genetic testing"[MeSH Terms] OR genetic screening[Text Word])
Comparator (if applicable)	-
Outcomes (if applicable)	-
Limits	Publication date from 01/01/1989 to 10/2014, NOT (Animals NOT (Animals + humans))

MeSH = Medical Subject Heading, based on a Medline/PubMed platform

B.2. RESULTS OF LITERATURE SEARCH

The PRISMA flowchart shown in Figure 5 provides a graphic depiction of the results of the literature search and the application of the study selection (Liberati et al. 2009). Studies were selected by a single reviewer. Studies that could not be retrieved or that met the inclusion criteria but contained insufficient or inadequate data for inclusion are listed as Excluded Studies in Appendix D. All other studies that met the inclusion criteria are listed in Appendix C, where a profile of each included study is given. Study profiles describe the authors, study ID, publication year, study design and quality (i.e. level of evidence and risk of bias), study location, setting, length of follow-up of patients, study population characteristics, description of the test and associated interventions, description of the comparator and relevant outcomes assessed.

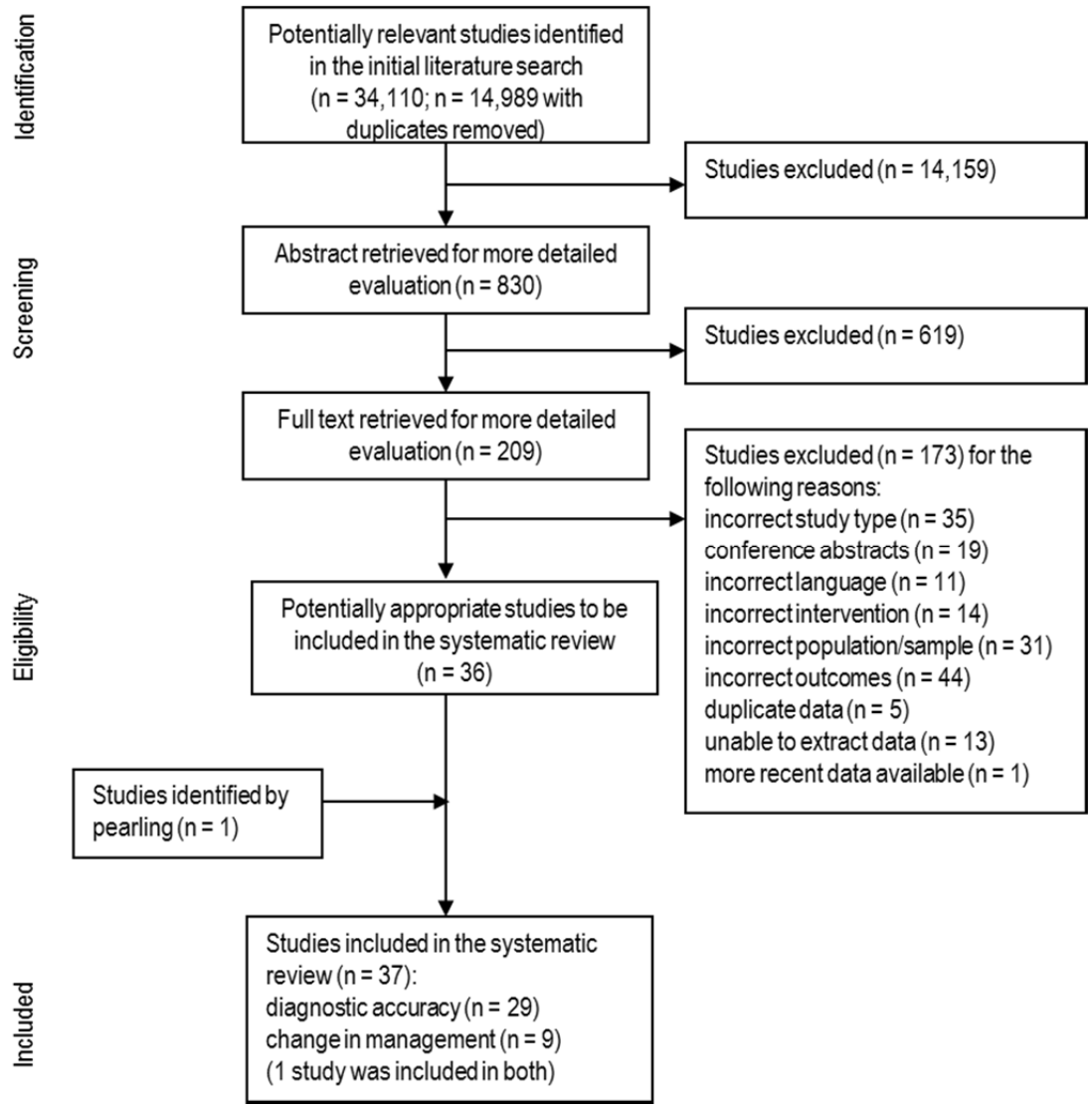


Figure 5 Summary of the process used to identify and select studies for the assessment

B.2.1. DIRECT EFFECTIVENESS

B.2.1.1. Safety and direct effectiveness

No studies that met the PICO criteria defined *a priori* for the direct effectiveness of CFTR testing or the safety of prenatal CFTR testing or downstream consequences were identified.

Due to the lack of evidence, a separate search on the safety of amniocentesis and CVS was done, as discussed in section B.8.1.

B.2.2. LINKED EVIDENCE APPROACH

B.2.2.1. Diagnostic accuracy

Only 1 study was identified that met the inclusion criteria determined *a priori* to assess the diagnostic accuracy of CFTR mutation testing in patients with a high clinical suspicion of CF, as outlined in Table 10. Two studies were identified that met the inclusion criteria to assess the diagnostic accuracy of CFTR mutation testing compared with clinical diagnosis after birth in fetuses where both parents are CF carriers, as outlined in Table 14. No studies met the inclusion criteria to inform on the accuracy of CFTR mutation testing in parents with a fetus suspected of CF, as outlined in Table 13. Due to the lack of evidence, the inclusion criteria were broadened and a further 26 studies that met the broadened criteria were included.

To assess the accuracy of CFTR mutation testing in patients with either classical or non-classical CF symptoms, the criteria for the evidentiary standard were broadened to include either DNA sequencing alone or clinical diagnosis. As a result of the selection process, 4 studies compared a CFTR mutation test with DNA sequencing in patients with CF, 4 studies compared DNA sequencing with clinical diagnosis and 5 studies compared a CFTR mutation test with clinical diagnosis.

The only studies that included patients with non-classical CF were conducted in those diagnosed with CBAVD. One study compared CFTR mutation testing with the evidentiary standard in this patient group, 3 studies compared CFTR mutation testing with clinical diagnosis and 5 studies compared DNA sequencing with clinical diagnosis.

A further 4 non-comparative studies that reported on the failure rates of various CFTR mutation tests in CF patients were also included to assess test reliability.

No studies were identified that could inform on the accuracy of CFTR mutation testing in the parents of a fetus suspected of having CF, as outlined in Table 13. However, 1 study reported on the diagnostic accuracy of CFTR mutation testing compared with DNA sequencing in CF carriers with a known CFTR mutation, and was included to provide surrogate data.

To assess the accuracy of prenatal CFTR mutation testing, 4 studies that reported on CFTR mutation testing in fetuses were included, but only 2 of these included a reference standard.

B.2.2.2 Change in management

Nine studies were identified that met the PICO criteria outlined *a priori*; however, none of these studies had a comparator (e.g. pregnancy management after no prenatal genetic testing). Therefore, the assumption was made that no TOP would occur in the absence of testing.

Six articles were included that reported change-in-management outcomes where fetal echogenic bowel (FEB) was detected, of which 1 study was Australian (Table 44). Four studies were detected

that included women with a 1:4 risk of having a child with CF (Table 45), and 1 of these included both pregnant women with FEB and a 1:4 risk.

B.2.2.3 Impact of change in management

No studies were identified on parental psychological health after TOP due to a CF-affected fetus compared with no TOP and raising a child with CF. Therefore, an additional broader (non-systematic) search was conducted in PubMed on parental psychological outcomes after the diagnosis of fetal anomalies (non-comparative). The evidence is narratively discussed in section B.8.1 and provided in Appendix E (Wool 2011), to show the consequences of change in management (i.e. TOP) in affected pregnancies.

B.2.3. APPRAISAL OF THE EVIDENCE

Appraisal of the evidence was conducted in three stages (Table 19):

Stage 1: Appraisal of the applicability and quality of individual studies (or systematic reviews) included in the review (strength of the evidence) (see section B.3, Table 20).

Stage 2: Appraisal of the precision, size of effect and clinical importance of the results reported in the evidence-base as they relate to the pre-specified primary outcomes for this assessment (see section B.5).

Stage 3: Integration of this evidence for conclusions about the net clinical benefit of the test and associated interventions in the context of Australian clinical practice (see sections B.6–8).

Table 19 Dimensions of evidence

Type of evidence	Definition
Strength of the evidence:	
Level	The study design used, as an indicator of the degree to which bias has been eliminated by design [a]
Quality	The methods used by investigators to minimise bias within a study design
Statistical precision	The p-value or, alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect
Size of effect	The distance of the study estimate from the 'null' value and the inclusion of only clinically important effects in the confidence interval
Relevance of evidence	The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used

[a] See Table 20 (designations of levels of evidence according to type of research question)

Table 20 Designations of levels of evidence according to type of research question

Level	Intervention [a]	Diagnostic accuracy [b]
I [c]	A systematic review of level II studies	A systematic review of level II studies

Level	Intervention [a]	Diagnostic accuracy [b]
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard [d], among consecutive persons with a defined clinical presentation [e]
III-1	A pseudo-randomised controlled trial (i.e. alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard [d], among non-consecutive persons with a defined clinical presentation [e]
III-2	A comparative study with concurrent controls: • non-randomised, experimental trial [f] • cohort study • case-control study • interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for levels II and III-1 evidence
III-3	A comparative study without concurrent controls: • historical control study • two or more single-arm studies [g] • interrupted time series without a parallel control group	Diagnostic case-control study [e]
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard) [h]

Source: Merlin, Weston & Tooher (2009)

Explanatory notes:

[a] Definitions of these study designs are provided in NHMRC (2000; pp. 7–8) and in the accompanying Glossary.

[b] These levels of evidence apply only to studies assessing the accuracy of diagnostic or screening tests. To assess the overall effectiveness of a diagnostic test there also needs to be a consideration of the impact of the test on patient management and health outcomes (MSAC 2005; Sackett & Haynes 2002). The evidence hierarchy given in the 'Intervention' column should be used when assessing the impact of a diagnostic test on health outcomes relative to an existing method of diagnosis/comparator test(s).

[c] A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence. Systematic reviews of level II evidence provide more data than the individual studies and any meta-analyses will increase the precision of the overall results, reducing the likelihood that the results are affected by chance. Systematic reviews of lower level evidence present results of likely poor internal validity and thus are rated on the likelihood that the results have been affected by bias, rather than whether the systematic review itself is of good quality. Systematic review quality should be assessed separately. A systematic review should consist of at least two studies. In systematic reviews that include different study designs, the overall level of evidence should relate to each individual outcome/result, as different studies and study designs might contribute to each different outcome.

[d] The validity of the reference standard should be determined in the context of the disease under review. Criteria for determining the validity of the reference standard should be pre-specified. This can include the choice of the reference standard(s) and its timing in relation to the index test. The validity of the reference standard can be determined through quality appraisal of the study (Whiting et al. 2003).

[e] Well-designed population-based case-control studies (e.g. screening studies where test accuracy is assessed on all cases, with a random sample of controls) do capture a population with a representative spectrum of disease and thus fulfil the requirements for a valid assembly of patients. However, in some cases the population assembled is not representative of the use of the test in practice. In diagnostic case-control studies a selected sample of patients already known to have the disease is compared with a separate group of normal/healthy people known to be free of the disease. In this situation patients with borderline or mild expressions of the disease, and conditions mimicking the disease, are excluded, which can lead to exaggeration of both sensitivity and specificity. This is called spectrum bias or spectrum effect because the spectrum of study participants will not be representative of patients seen in practice (Mulherin & Miller 2002).

[f] This also includes controlled before-and-after (pre-test/post-test) studies, as well as adjusted indirect comparisons (i.e. utilising A vs B and B vs C to determine A vs C, with statistical adjustment for B).

[g] Comparing single-arm studies, i.e. case series from two studies. This would also include unadjusted indirect comparisons (i.e. utilising A vs B and B vs C to determine A vs C, but where there is no statistical adjustment for B).

[h] Studies of diagnostic yield provide the yield of diagnosed patients, as determined by an index test, without confirmation of the accuracy of this diagnosis by a reference standard. These may be the only alternative when there is no reliable reference standard.

Note A: Assessment of comparative harms/safety should occur according to the hierarchy presented for each of the research questions, with the proviso that this assessment occurs within the context of the topic being assessed. Some harms (and other outcomes) are rare and cannot feasibly be captured within randomised controlled trials, in which case lower levels of evidence may be the only type of evidence that is practically achievable; both physical and psychological harms may need to be addressed by different study designs; harms from diagnostic testing include the likelihood of false positive and false negative results; harms from screening include the likelihood of false alarm and false reassurance results.

Note B: When a level of evidence is attributed in the text of a document, it should also be framed according to its corresponding research question, e.g. level II intervention evidence; level IV diagnostic evidence; level III-2 prognostic evidence.

Note C: Each individual study that is attributed a 'level of evidence' should be rigorously appraised using validated or commonly used checklists or appraisal tools to ensure that factors other than study design have not affected the validity of the results.

DIRECT EVIDENCE

B.3–5. RISK OF BIAS ASSESSMENT, CHARACTERISTICS OF THE EVIDENCE-BASE AND OUTCOME MEASURES

No studies met the inclusion criteria for safety or direct effectiveness regarding genetic CFTR testing. Due to the lack of direct evidence, only the results regarding safety of amniocentesis and CVS were narratively synthesised in section B.8.1, based on a separate search for systematic reviews on the safety of prenatal invasive tests. No statistical analysis was done.

B.6. RESULTS OF THE DIRECT EVIDENCE

Summary – What is the safety and effectiveness of prenatal CFTR mutation testing of couples carrying a fetus with a high clinical suspicion of CF, or testing of a fetus conceived by parents that are both CF carriers, compared with determining the diagnosis of the child after the birth?

No studies were identified on the safety or direct effectiveness of prenatal CFTR mutation testing. The key areas of concern regarding the safety of CFTR testing are thought to be in regards to deriving the samples to test.

No studies on the safety or direct effectiveness have been identified that were able to determine the patient-relevant outcomes for prenatal CFTR mutation testing of couples carrying a fetus with high clinical suspicion of CF, compared with determining the diagnosis after birth. Therefore, a linked analysis approach was conducted, and the results are shown below.

They key areas of concern regarding the safety of CFTR testing are thought to be in regards to deriving the samples to test. Therefore, a separate search was conducted to investigate the safety of amniocentesis and CVS, as shown in section B.8.1.

LINKED EVIDENCE – DIAGNOSTIC ACCURACY

B.3.I RISK OF BIAS ASSESSMENT

Summaries of the risk of bias for the studies that report diagnostic accuracy outcomes, as determined using the QUADAS-2 tool (Whiting et al. 2011), are shown in Table 21 to Table 30. The accuracy results (and summary of bias etc.) are divided into the following different populations:

- patients with classic CF symptoms
- men with CBAVD
- carriers of CFTR mutations
- fetuses with carrier parents
- fetuses with FEB.

Thirteen studies met the inclusion criteria to assess the accuracy of CFTR mutation testing in patients with classical CF symptoms. Four level III-2 studies (1 of good quality with a low risk of bias, 1 of intermediate quality with some risk and 2 of poor quality with a high risk of bias) compared a CFTR mutation test with DNA sequencing (Table 21). Four studies (1 level III-1 of good quality, and 3 level III-2 studies—1 of good and 2 of intermediate quality) compared DNA sequencing with a clinical diagnosis (Table 22); and 5 level III-2 studies (2 of good, 2 of intermediate and 1 of poor quality) compared a CFTR mutation test with clinical diagnosis (Table 23).

Table 21 Risk of bias and applicability judgements for diagnostic accuracy studies comparing CFTR mutation testing with DNA sequencing in CF patients

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Houdayer et al. (1998)	☹	?	?	?	☹	☺	☺
Ravnik-Glavac et al. (2002)	☹	?	☹	☹	☹	☺	☺
Ravnik-Glavac et al. (1994)	☹	?	☹	☹	☹	☺	☺
Tomaiuolo, Spina & Castaldo (2003)	☹	?	☺	☺	☹	☺	☺

Source: Whiting et al. (2011)
CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; DNA = deoxyribonucleic acid; Risk of bias: ☺ = low risk; ☹ = high risk; ? = unclear risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

Table 22 Risk of bias and applicability judgements for diagnostic accuracy studies comparing DNA sequencing with clinical diagnosis in CF patients

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Bickmann et al. (2009)	☺	☹	☺	☺	☺	☺	☺
Bonizzato et al. (1995) Gasparini et al. (1993)	☺	☹	☺	☹	☹	☺	☺
Kanavakis et al. (2003)	☺	☹	☺	☹	☹	☺	☺
Strom et al. (2003)	☺	☺	☺	☺	☹	☺	☺

Source: Whiting et al. (2011)
CF = cystic fibrosis; DNA = deoxyribonucleic acid; Risk of bias: ☺ = low risk; ☹ = high risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

Table 23 Risk of bias and applicability judgements for diagnostic accuracy studies comparing CFTR mutation testing with clinical diagnosis in CF patients

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Bonizzato et al. (1999)	☺	☹	☺	☺	☹	☺	☺
Frentescu & Budisan (2009)	?	☹	☺	☺	☹	☺	☺
Heim, Sugarman & Allitto (2001)	☺	☹	?	☹	☺	☺	☺
Lay-Son et al. (2011)	☺	☹	?	☺	☹	☺	☺
Wall, Cai & Chehab (1995)	?	?	?	☺	☺	☺	☺

Source: Whiting et al. (2011)
CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; Risk of bias: ☺ = low risk; ☹ = high risk; ? = unclear risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

The only studies that included patients with non-classical CF were conducted in those diagnosed with CBAVD. One level III-2 study of intermediate quality compared CFTR mutation testing with the evidentiary standard in this patient group (Table 24), 5 level III-2 studies (1 of good, 2 of intermediate and 1 of poor quality) compared DNA sequencing with clinical diagnosis (Table 26), and 4 level III-2 studies of good quality compared CFTR mutation testing with clinical diagnosis (Table 25).

Table 24 Risk of bias and applicability judgements for diagnostic accuracy studies comparing CFTR mutation testing with DNA sequencing in patients with CBAVD

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Giuliani et al. (2010)	☺	☹	☺	☹	☹	☺	☺

Source: Whiting et al. (2011)
CFTR = cystic fibrosis transmembrane conductance regulator; CBAVD = congenital bilateral absence of the vas deferens; DNA = deoxyribonucleic acid; Risk of bias: ☺ = low risk; ☹ = high risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

Table 25 Risk of bias and applicability judgements for diagnostic accuracy studies comparing DNA sequencing with clinical diagnosis in patients with CBAVD

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Bareil et al. (2007)	?	☹	☺	☹	☺	☺	☺
Bernardino, Lima & Zatz (2003)	?	☹	☺	☹	☹	☺	☺
Danziger et al. (2004)	☺	☹	☺	☺	☺	☺	☺
Gallati et al. (2009)	☺	☹	☺	☹	☺	☺	☺
Giuliani et al. (2010)	☺	☹	☺	☹	☹	☺	☺

Source: Whiting et al. (2011)
CBAVD = congenital bilateral absence of the vas deferens; DNA = deoxyribonucleic acid; Risk of bias: ☺ = low risk; ☹ = high risk; ? = unclear risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

Table 26 Risk of bias and applicability judgements for diagnostic accuracy studies comparing CFTR mutation testing with clinical diagnosis in patients with CBAVD

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Donat et al. (1997)	☺	☹	☺	☺	☺	☺	☺
Durieu et al. (1995)	☺	☹	☺	☺	☺	☺	☺
Giuliani et al. (2010)	☺	☹	☺	☹	☹	☺	☺
Wang et al. (2002)	☺	?	☺	☺	☺	☺	☺

Source: Whiting et al. (2011)
CBAVD = congenital bilateral absence of the vas deferens; CFTR = cystic fibrosis transmembrane conductance regulator; Risk of bias: ☺ = low risk; ☹ = high risk; ? = unclear risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

No studies were identified that reported on CFTR mutation testing in the partners of CF carriers. Similarly, no studies were identified that could inform on the accuracy of CFTR mutation testing in parents with a fetus suspected of CF. However, 1 level III-2 study of good quality reported on the diagnostic accuracy of CFTR mutation testing compared with DNA sequencing in CF carriers with a known CFTR mutation, and was included (Table 27).

Table 27 Risk of bias and applicability judgements for diagnostic accuracy studies comparing CFTR mutation testing with DNA sequencing in CFTR mutation carriers

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Tomaiuolo, Spina & Castaldo (2003)	☹	?	☺	☺	☹	☺	☺

Source: Whiting et al. (2011)
CFTR = cystic fibrosis transmembrane conductance regulator; DNA = deoxyribonucleic acid; Risk of bias: ☺ = low risk; ☹ = high risk; ? = unclear risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

To assess the diagnostic accuracy of CFTR mutation testing in fetuses suspected of having CF, 4 studies were included—all reported on CFTR mutation testing in fetuses where both parents were CF carriers (Table 28). However, only 2 studies were comparative (level III-2 of good quality) and reported the clinical outcomes after birth, but only for those with carrier or normal status. The other 2 studies were non-comparative (level IV studies, 1 of intermediate and 1 of poor quality).

One of the level III-2 studies also reported on the diagnostic accuracy of CFTR mutation testing of fetuses diagnosed with FEB. However, the clinical outcomes of these fetuses were not reported, making this a level IV study for this population. The study was still of good quality (Table 29).

Table 28 Risk of bias and applicability judgements for diagnostic accuracy studies comparing CFTR mutation testing with clinical diagnosis in fetuses with carrier parents

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Castaldo et al. (2000)	☺	☺	NA	☹	☹	☺	NA
Collazo et al. (2014)	☺	☺	?	☹	☹	☺	☺
Kanavakis et al. (2003)	☺	☺	?	☹	☹	☺	☺
Saker et al. (2006)	?	☺	NA	☹	☹	☺	NA

Source: Whiting et al. (2011)
CFTR = cystic fibrosis transmembrane conductance regulator; NA = not applicable (level IV study with no reference standard); risk of bias: ☺ = low risk; ☹ = high risk; ? = unclear risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

Table 29 Risk of bias and applicability judgements for diagnostic accuracy studies comparing CFTR mutation testing with clinical diagnosis in fetuses with FEB

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Kanavakis et al. 2003	☺	☺	NA	☹	☹	☺	NA

Source: Whiting et al. (2011)
CFTR = cystic fibrosis transmembrane conductance regulator; NA = not applicable (level IV study with no reference standard); FEB = fetal echogenic bowel; Risk of bias: ☺ = low risk; ☹ = high risk; ? = unclear risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

Five studies, 1 level III-2 of good quality and 4 non-comparative level IV studies of poor quality, reported on the failure rates of 7 different CFTR mutation tests in symptomatic patients (Table 30).

Table 30 Risk of bias and applicability judgements for studies reporting CFTR mutation testing failure rates

Study	Risk: Patient selection	Risk: Index test	Risk: Reference standard	Risk: Flow and timing	Applicability Patient selection	Applicability Index test	Applicability Reference standard
Axton & Brock (1995)	☹	☺	NA	☹	☹	☺	NA
Edelmann et al. (2004)	☹	☺	NA	☹	☹	☺	NA
Nagy et al. (2007)	☹	☺	NA	☹	☹	☺	NA
Strom et al. (2003)	☺	☺	☺	☺	☹	☺	☺
Strom et al. (2004)	☹	☺	NA	☹	☹	☺	NA

Source: Whiting et al. (2011)
NA = not applicable (level IV study with no reference standard); risk of bias: ☺ = low risk; ☹ = high risk
Note: Quality appraisal: good quality with low risk of bias = at least two ☺ and no more than one ☹; poor quality with high risk of bias = at least two ☹ and no more than one ☺; intermediate quality with some risk of bias = the remainder

B.4.I CHARACTERISTICS OF THE EVIDENCE-BASE

The CFTR mutation tests used in the included studies could be clearly separated into three groups. The first group consisted of panel-based tests that used PCR amplification of specific mutations with different detection methodologies to detect common CFTR mutation panels of between 12 and 100 mutations. The detection methodologies included amplification refractory mutation system (ARMS), reverse dot-blot hybridisation, allele-specific oligonucleotide hybridisation, oligonucleotide ligation assay (OLA), restriction fragment length polymorphism (RFLP) analysis and heteroduplex analysis. The second group were exon-scanning tests that used methodologies such as denaturing high-performance liquid chromatography (DHPLC), single-stranded conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE) to screen PCR amplification products, usually covering all 27 CFTR exons and the flanking regions. In most studies the PCR products that showed an abnormal pattern, indicating the presence of a mutation, were sequenced to confirm its presence. When a panel-based test was compared with an exon-based test plus DNA sequencing, the latter was considered to be an incomplete reference standard, as only those samples that had a

mutation were sequenced. The third group of tests were DNA sequencing-based assays such as pyrosequencing and an automated sequencing assay.

Summaries of the characteristics of the diagnostic accuracy studies are shown in Table 31 to Table 40. Further details about these characteristics are listed in Appendix D.

Table 31 Key features of the included evidence for diagnostic accuracy studies comparing CFTR mutation testing with DNA sequencing in CF patients

Study Country	Study design Risk of bias	Patient population	Index test (mutations detected)	Reference standard	Key outcome(s)
Tomaiuolo, Spina & Castaldo (2003) Italy	Level III-2 Low risk of bias	N=129 chromosomes from 85 DNA samples from CF subjects with known mutations	CF(12) ARMS kit OLA PCR kit (31) ASOH dot-blot (13) INNO-LiPA reverse dot-blot CF kit (30)	DGGE of the whole CFTR coding region followed by DNA sequencing confirmation	Sensitivity
Houdayer et al. (1998) France	Level III-2 Some risk of bias	N=40 DNA samples of known CFTR mutations	CF(12) ARMS kit	DNA typing and sequencing by two genetic testing laboratories	Sensitivity
Ravnik-Glavac et al. (1994) USA	Level III-2 High risk of bias	N=133 DNA samples of known CFTR mutations	SSCP (all exons)	DNA samples of known mutations obtained from previous study	Sensitivity
Ravnik-Glavac et al. (2002) USA	Level III-2 High risk of bias	N=73 DNA samples of known CFTR mutations	DHPLC (all exons)	DNA samples of known mutations obtained from previous study	Sensitivity

ARMS = amplification refractory mutation system; ASOH = allele-specific oligonucleotide hybridisation; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; DGGE = denaturing gradient gel electrophoresis; DHPLC = denaturing high-performance liquid chromatography; DNA = deoxyribonucleic acid; OLA = oligonucleotide ligation assay; PCR = polymerase chain reaction; SSCP = single-stranded conformation polymorphism

Table 32 Key features of the included evidence for diagnostic accuracy studies comparing DNA sequencing with clinical diagnosis in CF patients

Study Country	Study design Risk of bias	Patient population	Index test (mutations detected)	Reference standard	Key outcome(s)
Strom et al. (2003) USA	Level III-1 Low risk of bias	N=14 chromosomes from 7 confirmed CF patients	Automated DNA sequence analysis-based assay (991)	Confirmed CF patients; no diagnostic criteria were provided.	Sensitivity
Bickmann et al. (2009) Germany	Level III-2 Low risk of bias	N=184 chromosomes from 92 CF patients	Pyrosequencing panel assay (46) Pyrosequencing plus conventional DNA sequencing (all exons)	CF patients had typical symptoms and positive sweat test results.	Sensitivity
Kanavakis et al. (2003) Greece	Level III-2 Some risk of bias	N=874 chromosomes from 437 CF patients	DGGE plus DNA sequencing (all exons)	Diagnostic criteria involved positive sweat tests and typical clinical findings.	Sensitivity
Bonizzato et al. (1995) Gasparini	Level III-2 Some risk of bias	N=225 chromosomes from 133 CF patients	RFLP, RNA-SSCP or DGGE and DNA sequencing (99)	Diagnosis was confirmed by at least two positive sweat tests.	Sensitivity

Study Country	Study design Risk of bias	Patient population	Index test (mutations detected)	Reference standard	Key outcome(s)
et al. (1993) Italy					

CF = cystic fibrosis; DGGE = denaturing gradient gel electrophoresis; DNA = deoxyribonucleic acid; RFLP = restriction fragment length polymorphism; RNA = ribonucleic acid; SSCP = single-stranded conformation polymorphism

Table 33 Key features of the included evidence for diagnostic accuracy studies comparing CFTR mutation testing with clinical diagnosis in CF patients

Study Country	Study design Risk of bias	Population	Index test (mutations detected)	Reference standard	Key outcome(s)
Bonizzato et al. (1999) Italy	Level III-2 Low risk of bias	N=806 chromosomes from 403 CF patients	Reverse dot-blot hybridisation (15)	Diagnosis of CF was by sweat test.	Sensitivity
Lay-Son et al. (2011) Chile	Level III-2 Low risk of bias	N=578 chromosomes from 289 CF patients	OLA assay or INNO-LiPA CFTR19/ CFTR17+Tn Update reverse dot-blot hybridisation (32–36)	Clinical diagnostic criteria were not described.	Sensitivity
Wall, Cai & Chehab (1995) USA	Level III-2 Some risk of bias	N=246 chromosomes from 123 CF patients	Reverse dot-blot hybridisation (31)	CF was diagnosed by clinical criteria as well as abnormal sweat chloride levels.	Sensitivity
Frentescu & Budisan (2009) Romania	Level III-2 Some risk of bias	N=42 chromosomes from 21 patients with CF	Multiplex PCR, heteroduplex analysis and RFLP (18)	Diagnosis was based on clinical symptoms and sweat test values.	Sensitivity
Heim, Sugarman & Allitto (2001) USA	Level III-2 High risk of bias	N=5,840 chromosomes from 2,920 patients with CF	ASOH (93)	Clinical diagnostic criteria were not described.	Sensitivity

ASOH = allele-specific oligonucleotide hybridisation; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; OLA = oligonucleotide ligation assay; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism

Table 34 Key features of the included evidence for diagnostic accuracy studies comparing CFTR mutation testing with DNA sequencing in men with CBAVD

Study Country	Study design Risk of bias	Population	Index test (mutations detected)	Reference standard	Key outcome(s)
Giuliani et al. (2010) Italy	Level III-2 Some risk of bias	N=46 chromosomes from 23 CBAVD patients	INNO-LiPA CFTR19, 17 + Tn and Italian Regional Kits reverse dot-blot hybridisation (58)	DHPLC with DNA sequencing confirmation DHPLC with DNA sequencing confirmation plus MLPA	Sensitivity Specificity

CBAVD = congenital bilateral absence of the vas deferens; CFTR = cystic fibrosis transmembrane conductance regulator; DHPLC = denaturing high-performance liquid chromatography; DNA = deoxyribonucleic acid; MLPA + multiplex ligation-dependent probe amplification

Table 35 Key features of the included evidence for diagnostic accuracy studies comparing DNA sequencing with clinical diagnosis in men with CAVD/CBAVD

Study Country	Study design Risk of bias	Population	Index test	Reference standard	Key outcome(s)
Danziger et al. (2004) USA	Level III-2 Low risk of bias	N=16 male patients with CAVD (13 with CBAVD)	mTTGE and DNA sequencing confirmation	Diagnosis of CAVD was based on physical examination findings.	Sensitivity
Gallati et al. (2009) Switzerland	Level III-2 Some risk of bias	N= 25 azoospermic men diagnosed with CAVD	SSCP and DNA sequencing confirmation	Diagnostic criteria for CAVD were not described.	Sensitivity
Giuliani et al. (2010) Italy	Level III-2 Some risk of bias	N=23 CBAVD patients	Reverse dot-blot, DHPLC and DNA sequencing	Clinical diagnosis of CBAVD was based on azoospermia with the absence of palpable vas deferens.	Sensitivity
Bareil et al. (2007) France	Level III-2 High risk of bias	N=182 samples from men with a clinical diagnosis of CBAVD	DGGE or DHPLC and DNA sequencing confirmation	Clinical diagnosis of CBAVD was based on clinical examination with impalpable vas deferens.	Sensitivity
Bernardino, Lima & Zatz (2003) Brazil	Level III-2 High risk of bias	N=17 patients with CBAVD	SSCP and DNA sequencing confirmation	Diagnosis of CBAVD was based on scrotal examination, ultrasound and semen analysis.	Sensitivity

CAVD = congenital absence of the vas deferens; CBAVD = congenital bilateral absence of the vas deferens; DGGE = denaturing gradient gel electrophoresis; DHPLC = denaturing high-performance liquid chromatography; DNA = deoxyribonucleic acid; mTTGE = modified temporal temperature gradient electrophoresis; SSCP = single-stranded conformation polymorphism

Table 36 Key features of the included evidence for diagnostic accuracy studies comparing CFTR mutation testing with clinical diagnosis in men with CBAVD

Study Country	Study design Risk of bias	Population	Index test (mutations detected)	Reference standard	Key outcome(s)
Wang et al. (2002) USA	Level III-2 Low risk of bias	N=92 patients with CBAVD	Restriction enzyme analysis (26) Multiplex PCR plus mass spectrometry (100)	Diagnosis of CBAVD was made clinically by urologists.	Sensitivity
Donat et al. (1997) UK	Level III-2 Low risk of bias	N=30 patients with CBAVD	Multiplex PCR with restriction enzyme analysis (14)	Clinical diagnosis of CBAVD was made if azoospermia was confirmed and the vasa were impalpable.	Sensitivity
Durieu et al. (1995) France	Level III-2 Low risk of bias	N=14 CBAVD patients	PCR amplification with restriction enzyme or heteroduplex analysis (22)	Clinical diagnosis of CBAVD was made on azoospermia with non-palpable vas deferens.	Sensitivity
Giuliani et al. (2010) Italy	Level III-2 Some risk of bias	N=23 CBAVD patients	INNO-LiPA CFTR19, 17 + Tn and Italian Regional Kit reverse dot-blot hybridisation (58)	Clinical diagnosis of CBAVD was based on azoospermia and absence of palpable vas deferens.	Sensitivity

CBAVD = congenital bilateral absence of the vas deferens; PCR = polymerase chain reaction

Table 37 Key features of the included evidence for diagnostic accuracy studies comparing CFTR mutation testing with DNA sequencing in CFTR mutation carriers

Study Country	Study design Risk of bias	Population	Index test (mutations detected)	Reference standard	Key outcome(s)
Tomaiuolo, Spina & Castaldo (2003) Italy	Level III-2 Low risk of bias	N=129 chromosomes from 85 DNA samples from CF subjects with known mutations	CF(12) ARMS kit OLA PCR kit (31) ASOH dot-blot (13) INNO-LiPA reverse dot-blot CF kit (30)	DGGE of the whole CFTR coding region followed by DNA sequencing confirmation	Sensitivity

ARMS = amplification refractory mutation system; ASOH = allele-specific oligonucleotide hybridisation; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; DGGE = denaturing gradient gel electrophoresis; DNA = deoxyribonucleic acid; OLA = oligonucleotide ligation assay; PCR = polymerase chain reaction

Table 38 Key features of the included evidence for diagnostic accuracy studies comparing CFTR mutation testing with clinical diagnosis in fetuses with carrier parents

Study Country	Study design Risk of bias	Patient population	Index test	Reference standard	Key outcome(s)
Kanavakis et al. (2003) Greece	Level III-2 Low risk of bias	N=115 fetus samples from carrier parents	DGGE and PCR-mediated site-directed mutagenesis	Clinical diagnosis of child after birth	Sensitivity
Collazo et al. (2014) Cuba	Level III-2 Low risk of bias	N=72/108 fetus samples from couples with some risk of having a child affected by CF	ARMS and PCR-based restriction enzyme analysis		Sensitivity
Castaldo et al. (2000) Italy	Level IV Low risk of bias	N=33 fetus samples from 32 high-risk couples (1 dizygotic twin pregnancy)	ASOH and STR genotyping	No reference standard	Yield
Saker et al. (2006) France	Level IV Some risk of bias	N=12 fetus samples from carrier couples	PCR and STR genotyping	No reference standard	Yield

ARMS = amplification refractory mutation system; ASOH = allele-specific oligonucleotide hybridisation; CF = cystic fibrosis; DGGE = denaturing gradient gel electrophoresis; PCR = polymerase chain reaction; STR = short tandem repeats

Table 39 Key features of the included evidence for diagnostic accuracy studies comparing CFTR mutation testing with clinical diagnosis in fetuses with FEB

Study Country	Study design Risk of bias	Patient population	Index test	Reference standard	Key outcome(s)
Kanavakis et al. (2003) Greece	Level IV Low risk of bias	N=49 fetus samples for FEB detected by ultrasound	DGGE and DNA sequencing confirmation	No reference standard	Yield

DGGE = denaturing gradient gel electrophoresis; DNA = deoxyribonucleic acid; FEB = fetal echogenic bowel

Table 40 Key features of the included evidence for diagnostic accuracy studies that reported CFTR mutation test failure rates

Study Country	Study design Risk of bias	Patient population	CFTR mutation test	Key outcome(s)
Strom et al. (2003) USA	Level III-2 Low risk of bias	Carrier screening program: no. of patients screened not reported	CF testing for the ACMG panel using the Roche CF Gold Linear Array strips	Failure rate
Strom et al. (2004) USA	Level IV High risk of bias	N=1,092 patient samples previously tested with the CF Gold line probe assay chosen at random N=1,076 patient samples previously tested with the Applera CF OLA, Ver. 3.0, platform	The CF Portrait™ system multiplex PCR followed by automated hybridisation and detection Roche CF Gold line probe strips Applera CF OLA, Ver 3.0 assay	Failure rates
Edelmann et al. (2004) USA	Level IV High risk of bias	N=507 patients samples referred for CF screening and 12 proficiency samples	eMAP BeadChip assay multiplex PCR followed by ASOH	Failure rate
Axton & Brock (1995) UK	Level IV High risk of bias	N=193 mouthwash samples from CF patients	Restriction generation PCR	Failure rate
Nagy et al. (2007) Hungary	Level IV High risk of bias	N=116 DNA samples	qPCR and melting curve analysis Fluorescent PCR and DNA fragment analysis	Failure rate

ACMG = American College of Medical Genetics; ASOH = allele-specific oligonucleotide hybridisation; CF = cystic fibrosis; DNA = deoxyribonucleic acid; eMAP = elongation mediated multiplexed analysis of polymorphisms; OLA = oligonucleotide ligation assay; PCR = polymerase chain reaction; qPCR = quantitative real-time PCR

B.5.I OUTCOME MEASURES AND ANALYSIS

To assess the diagnostic accuracy of CFTR mutation testing, data was extracted into a classic 2×2 table (Table 41), in which the results of the index diagnostic test or the comparator were cross-classified against the results of the reference standard (Armitage, Berry & Matthews 2002; Deeks 2001), and Bayes' Theorem was applied:

Table 41 Diagnostic accuracy data extraction for CFTR mutation testing

		Reference standard	(DNA sequencing ± gene deletion analysis)	
		Disease +	*Disease* –	
Index test	*Test* +	true positive	false positive	Total test positive
(CFTR mutation testing)	*Test* –	false negative	true negative	Total test negative
		Total with CF	Total without CF	

PRIMARY MEASURES

Meta-analysis could not be conducted to determine the accuracy of CFTR mutation testing in patients with a high clinical suspicion of CF. The studies that compared CFTR mutation testing with DNA sequencing methods used DNA samples from patients with known CFTR mutations, and all were detected by DNA sequencing. Hence, there were no true negative results in these studies and only the sensitivity and false negative rate could be reported. Similar restrictions applied to the studies comparing CFTR mutation testing or DNA sequencing with clinical diagnosis. As all patients definitively diagnosed with CF are considered to have two CFTR mutations, there could be no true negative results. As a consequence, only the sensitivity and false negative rate could be reported.

Men with CBAVD are more likely to have CFTR mutations that are not identifiable than are patients with classic CF symptoms (see 'Context' in Section A). In fact, 25% of CBAVD patients have one and 28% have two CFTR alleles for which a mutation cannot be identified using panel-based CFTR mutation tests. Even after extensive DNA sequencing of all coding and flanking regions, a mutation could be detected on only one *CFTR* gene in 16% of the CBAVD patients, and no mutations could be identified in 13% (Claustres et al. 2000). One study compared the accuracy of CFTR mutation testing with a DNA sequencing method with or without a deletion/insertion detection method. In this study those CBAVD patients who had no detectable mutations after DNA sequencing could be considered true negatives, and both sensitivity and specificity outcomes could be reported for this study. However, as all included patients had been clinically diagnosed with CBAVD, there were no true negatives in the studies that used clinical diagnosis as the reference standard. Therefore, only sensitivity and false negative rates could be reported.

Test sensitivity was calculated as the proportion of people with CF (as determined by the reference standard) who had a positive test result using CFTR mutation testing:

Sensitivity (true positive rate) = number with true positive result / total with CF

Test specificity was calculated as the proportion of people without CF (as determined by the reference standard) who had a negative test result using CFTR mutation testing:

Specificity (true negative rate) = number with true negative result / total without CF

The 95%CI was calculated by exact binomial methods. Where possible the median sensitivity was calculated.

B.6.I. RESULTS OF THE TEST ACCURACY STUDIES

Summary – What is the diagnostic accuracy of CFTR mutation testing in patients with a high clinical suspicion of CF (population group 1)?

CFTR mutation testing in patients with classical CF symptoms

Thirteen studies (k=13) were included to determine the diagnostic accuracy of CFTR mutation testing in CF patients.

The median analytical sensitivity of CFTR mutation testing compared with DNA sequencing was 85% (range 71–97; k=4) when all mutations were included in the analysis; and 97% (range 90–100; k=4) when only those mutations that could be detected by each test were included. These results indicate that CFTR mutation tests are highly accurate compared with DNA sequencing at detecting those mutations that the tests were designed to recognise; only 3% of samples that should have been correctly identified were falsely negative.

The median clinical sensitivity of panel-based CFTR mutation tests compared with clinical diagnosis was 80% (range 52–91; k=5); and 91% (range 86–100; k=4) for DNA sequencing compared with clinical diagnosis. This difference is largely due to the reduced number of CFTR mutations that can be detected by the panel-based assays. Overall, 20% and 9% of all clinically diagnosed CF patients had one CFTR mutation that could not be detected using panel-based CFTR mutation tests and DNA sequencing, respectively.

CFTR mutation testing in men diagnosed with CBAVD

Eight studies were included to determine the diagnostic accuracy of CFTR mutation testing in men with CBAVD.

One study compared panel-based testing with exon-scanning testing plus DNA sequencing confirmation and MLPA deletion detection. The panel-based CFTR mutation test was able to detect the mutations included in the panel that were present among the 23 men with CBAVD (sensitivity = 100%). However, the analytical sensitivity of the panel-based test to detect all mutations was 94% (95%CI 81, 99) when compared with DNA sequencing; and 89% (95%CI 75, 97) when compared with DNA sequencing plus MPLA. The specificity of the assay compared with DNA sequencing with or without MPLC was 100% as there were no false positive results.

The median clinical sensitivity of exon-scanning testing plus DNA sequencing confirmation was higher than for panel-based testing when compared with clinical diagnosis (64% [range 47–88] k=5, versus 52% [range 45–72] k=4 for *CFTR* genes; and 75% [range 59–100] k=5, versus 70% [range 64–100] k=4 for patients having at least one CFTR mutation). These values are much lower than in CF patients due to the large proportion of men (median 25%, range 0–41) and chromosomes (median 36%; range 12–53) for which a CFTR mutation could not be identified.

What is the diagnostic accuracy of CFTR mutation testing in parents of a fetus suspected of CF (population group 2)?

One study that met the inclusion criteria reported on the diagnostic accuracy of four different panel-based CFTR mutation tests compared with exon-scanning CFTR mutation testing plus DNA sequencing confirmation in known CFTR mutation carriers, and was included to inform on the testing of parents with a fetus at risk of CF due to having a previous child clinically diagnosed with CF. The panel-based tests had a sensitivity of 100% compared with DNA sequencing for those mutations that could be detected; and 92% when all mutations were included in the analysis. These results suggest that the CFTR mutation carried by most parents presenting with a fetus suspected of having CF could be identified by panel-based testing; however, 8% of parents would require further testing.

What is the diagnostic accuracy of CFTR mutation testing in fetuses where both parents are CF carriers (population group 2a)?

Four studies reported on the accuracy of CFTR mutation testing in fetuses from carrier parents. Only 2 studies reported that there were no false negative results (i.e. all fetuses diagnosed as either carriers or normal were born without CF; sensitivity = 100%), and only 1 study reported on the fate of the fetuses diagnosed with CF (all fetuses were aborted). The results suggest that CFTR mutation testing compared with clinical diagnosis after birth in fetuses with carrier parents is likely to be highly sensitive, but the specificity cannot be determined as most fetuses from carrier parents diagnosed as having CF are aborted.

What is the diagnostic accuracy of CFTR mutation testing in fetuses diagnosed with an echogenic bowel (population group 2b)?

One study reported on the use of exon-scanning CFTR mutation testing plus DNA sequencing confirmation to diagnose CF in fetuses with an echogenic bowel; however, no clinical outcomes were reported for the fetuses. Thus, the accuracy of CFTR mutation testing compared with clinical diagnosis after birth in fetuses with an echogenic bowel could not be determined.

Test failure rates and the limitations of CFTR mutation testing

Five studies reported on the failure rates of seven panel-based CFTR mutation tests (median 4.5%, range 0.0001–9), suggesting that, in diagnostic laboratories using panel tests, about 4.5% of tests would need to be repeated.

In summary, a panel-based CFTR mutation test will not detect any mutation other than those included in the CFTR mutation panel. Both panel-based and exon-scanning tests, as well as DNA sequencing-based tests, cannot detect large deletion or insertion mutations, which occur in about 2% of CF patients worldwide. Methods such as MLPA are required to detect these rare deletion/insertion mutations. Even extensive DNA sequencing of all exons and flanking regions plus deletion/insertion analysis will not detect all mutations. Thus, in the case of a negative result, it is important for the diagnostic laboratory to explain the scope of the mutation testing that was undertaken and the likelihood of the patient being truly negative to the clinician requesting the test.

The accuracy of CFTR mutation testing was assessed in a number of different populations. A summary of the diagnostic accuracy data, divided by population group, is presented in Table 42.

Table 42 Summary of diagnostic accuracy data

Patient population	CFTR mutation testing compared with DNA sequencing (detectable mutations [a])	CFTR mutation testing compared with DNA sequencing (all mutations [b])	CFTR mutation testing compared with clinical diagnosis	DNA sequencing compared with clinical diagnosis
Alleles from patients diagnosed with CF	Median sensitivity = 97% (range 90–100); k=4 3% false negatives	Median sensitivity = 85% (range 71–97); k=4 18% false negatives	Median sensitivity = 80% (range 52–91); k=5 20% false negatives	Median sensitivity = 92% (range 90–100); k=4 8% false negatives
Alleles from men diagnosed with CBAVD	Sensitivity =100% [95%CI 89, 100]; k=1 No false negatives Specificity = 100% [95%CI 72, 100]; k=1 No false positives DNA sequencing plus MLPA Sensitivity =100% [95%CI 89, 100]; k=1 No false negatives Specificity = 100% [95%CI 66, 100]; k=1 No false positives	Sensitivity = 94% [95%CI 81, 99]; k=1 6% false negatives Specificity = 100% [95%CI 72, 100]; k=1 No false positives DNA sequencing plus MLPA Sensitivity = 89% [95%CI 75, 97]; k=1 11% false negatives Specificity = 100% [95%CI 66, 100]; k=1 No false positives	Median sensitivity = 52% (range 45–72); k=4 48% false negatives	Median sensitivity = 64% (range 47–88); k=5 36% false negatives
Men diagnosed with CBAVD	-	-	Median sensitivity = 70% (range 64–100); k=4 30% false negatives	Median sensitivity = 75% (range 59–100); k=5 25% false negatives
CFTR mutation carriers with known mutations	Median sensitivity = 100% (range 100–100); k=1; 4 CFTR mutation tests 2% false negatives	Median sensitivity = 92% (range 88–96); k=1; 4 CFTR mutation tests 8% false negatives	-	-
Fetuses with CFTR mutation carrier parents	-	-	Median sensitivity = 100%; k=2 Yield (k=4): 8–22% with CF 38–58% were carriers 24–33% were normal	-
Fetuses with FEB	-	-	Yield (k=1): 0% with CF 6% were carriers	-

Patient population	CFTR mutation testing compared with DNA sequencing (detectable mutations [a])	CFTR mutation testing compared with DNA sequencing (all mutations [b])	CFTR mutation testing compared with clinical diagnosis	DNA sequencing compared with clinical diagnosis
			94% were normal	

[a] Many CFTR mutation tests only detect a limited number of CFTR mutations, and only those that could be detected by the test were included in the analysis.
[b] All mutations detectable by DNA sequencing are included in the analysis.
CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CI = confidence interval; DNA = deoxyribonucleic acid; FEB = fetal echogenic bowel; k = number of studies; MLPA = multiple ligation-dependent probe amplification

CFTR MUTATION TESTING IN PATIENTS WITH CLASSICAL CF SYMPTOMS

Four studies compared the accuracy of CFTR mutation testing with DNA sequencing in CF patients. Three studies used DNA samples from patients who had had their genotype determined previously by DNA sequencing, and 1 study (Tomaiuolo, Spina & Castaldo 2003) used DNA sequencing to confirm the presence of mutations detected by DGGE exon scanning of the whole CFTR coding region. Two studies used four different panel-based CFTR mutation index tests, detecting between 12 and 31 specific mutations, and 2 studies used exon-scanning CFTR mutation index tests to detect the known mutations without DNA sequencing confirmation. When all mutations were included in the analysis, the median sensitivity (85%; range 71–97) reflected the inability of panel-based CFTR mutation tests to detect mutations not included in the CFTR mutation panel used in the test. When only those mutations that could be detected were included in the analysis, the median sensitivity increased to 97% (range 90–100; see Table 88 in Appendix D). These results indicate that CFTR mutation tests are highly accurate compared with DNA sequencing at detecting those mutations that the tests were designed to recognise; only 3% of samples that should have been correctly identified were falsely negative.

The 4 studies comparing DNA sequencing with clinical diagnosis in CF patients used different DNA sequencing methods designed to detect different numbers of CFTR mutations (see Table 89 in Appendix D). Strom et al. (2003) used an automated DNA sequence analysis assay that could detect 991 different CFTR mutations, whereas Bickmann et al. (2009) used both a pyrosequencing panel assay designed to detect only 46 common mutations (although the assay could easily be adapted to detect most mutations) and conventional DNA sequencing. Kanavakis et al. (2003) used a DGGE exon-scanning CFTR mutation test covering all 27 exons and neighbouring intronic regions of the *CFTR* gene, plus DNA sequencing of all samples, which showed a shift in mobility and did not present a pattern of a known mutation. The 4th study used DNA sequencing to search for 99 common CFTR mutations (Bonizzato et al. 1995; Gasparini et al. 1993). The median sensitivity of DNA sequencing compared with clinical diagnosis was 92% (range 90–100), indicating that 8% of patients diagnosed with CF had an unidentified CFTR mutation after DNA sequence analysis (Table 42). This false negative rate is predictable, as it is estimated that 2% of CF patients have large deletion/insertion mutations that cannot be detected by DNA sequencing, and up to 5% have an as-yet-unidentifiable CFTR mutation (Castellani et al. 2008).

Five studies compared the accuracy of panel-based CFTR mutation testing with clinical diagnosis of CF (see Table 90 in Appendix D). The studies used tests that detected between 15 and 93 CFTR mutations. The median sensitivity of panel-based tests compared with clinical diagnosis was 80% (range 52–91; see Table 42). This was lower than for DNA sequencing compared with clinical diagnosis (92%; range 90–100) and is likely due to the reduced number of CFTR mutations that could be detected by the panel-based assays. Thus, 20% of all clinically diagnosed CF patients had at least one CFTR mutation that could not be detected using panel-based tests.

In patients with CF symptoms the accuracy of the test would have little impact on health outcomes, given that it is clinical diagnosis that influences treatment decisions. However, those with the specific G551D mutation (which is common and would be tested for on a panel-based test) may be eligible for the drug ivacaftor, and other mutation-specific drugs might become available in the future. Other groups could benefit from mutation testing as it would allow family planning options for themselves, or allow cascade screening for family members to detect carriers, if they are planning on having children.

CFTR mutation testing in patients diagnosed with CBAVD

A study by Giuliani et al. (2010) compared the accuracy of a panel-based CFTR mutation test using a reverse dot-blot hybridisation detection system against a DHPLC exon-scanning CFTR mutation test plus DNA sequencing confirmation and MLPA for detection of large deletion/insertion mutations. The *CFTR* gene was sequenced for those exons where DHPLC detected a mutation but not in those patients where no mutations were detected; therefore, not all patients were tested with the evidentiary standard. The CFTR mutation test used two common mutation panels that detected 36 CFTR mutations plus IVS8-T5 polymorphism, and a third panel that detected a further 21 CFTR mutations commonly found in Italy; the test was able to detect all of the mutations included in the panels that were present among the 23 CBAVD patients (sensitivity = 100% for detectable mutations; see Table 42). However, there were two mutations detected by DHPLC plus DNA sequencing and two large deletions detected by MLPA that could not be detected by the reverse dot-blot assay (see Table 91 in Appendix D). Thus, the sensitivity of the reverse dot-blot assay compared with DNA sequencing was 94% (95%CI 81, 99), and 89% (95%CI 75, 97) when compared with the evidentiary standard (DNA sequencing plus MPLA). The specificity of the assay compared with DNA sequencing with or without MPLC was 100% as there were no false positive results (see Table 91 in Appendix D).

Five studies compared the accuracy of an exon-scanning CFTR mutation test plus DNA sequencing confirmation against clinical diagnosis of CBAVD (see Table 92 in Appendix D). The *CFTR* genes in those patients where a mutation was not detected were not sequenced, as DNA sequencing was only carried out to confirm the presence of at least one mutation. Four studies compared panel-based CFTR mutation tests designed to detect between 14 and 100 mutations with clinical diagnosis of CBAVD (see Table 93 in Appendix D). The median sensitivity of exon-scanning CFTR mutation

testing plus DNA sequencing confirmation (75% [range 59–100] in patients having at least one CFTR mutation; and 64% [range 47–88] per chromosome) was higher than for panel-based CFTR mutation testing (70% [range 64–100] in patients having at least one CFTR mutation; and 52% [range 45–72] per chromosome) when compared with clinical diagnosis (see Table 42). These values are much lower than in CF patients due to the large proportion of patients (median 25%; range 0–41) and chromosomes (median 36%; range 12–53) for which a CFTR mutation could not be identified and therefore received a false-negative result.

CFTR mutation testing in parents with a fetus suspected of CF

One study met the inclusion criteria and reported on the diagnostic accuracy of CFTR mutation testing compared with DNA sequencing of known CFTR mutation carriers. This provides the only evidence that can inform on the testing of parents with a fetus at risk of CF due to a previous child being clinically diagnosed with CF. The study by Tomaiuolo, Spina & Castaldo (2003) investigated the accuracy of four different panel-based CFTR mutation tests designed to detect between 12 and 31 CFTR mutations compared with DGGE exon-scanning CFTR mutation testing plus DNA sequencing confirmation in 25 CFTR-mutation carriers with known mutations. The panel-based tests had a sensitivity of 100% compared with DNA sequencing for those mutations that could be detected by the test. When the CFTR mutations not included in the panels were included in the analysis, the median sensitivity decreased to 92%; 4% (1/25) of patients had a false negative result in two of the tests and 12% (3/25) of patients in the other two. These results suggest that the CFTR mutation carried by most carrier parents could be identified by panel-based CFTR mutation testing. However, at least 8% of parents will require further testing if there is a strong suspicion of their carrier status.

CFTR mutation testing in fetuses where both parents are CF carriers

Four studies reported on the accuracy of CFTR mutation testing in fetuses from carrier parents (see Table 94 in Appendix D). However, only 2 studies reported on the rate of false negative results (indicating that there were no false negatives, i.e. all fetuses diagnosed as either carriers or normal were born without CF; sensitivity = 100%), and only 1 study reported the fate of the fetuses diagnosed with CF (all fetuses were aborted). In these 4 studies 8–22% of the fetuses were diagnosed with CF, 38–58% were diagnosed as CFTR mutation carriers and 24–33% were normal. These results appear to roughly fit within the expected parameters for the Mendelian inheritance patterns of a recessive trait. The results suggest that CFTR mutation testing in fetuses with carrier parents is likely to be highly sensitive compared with clinical diagnosis after birth, as no false negative results were recorded in 2 studies; however, the specificity cannot be determined, as most fetuses from carrier parents diagnosed as having CF are aborted (see Table 44 and Table 45 in section B.6.ii).

CFTR mutation testing in fetuses with FEB

One study reported on the use of DGGE exon-scanning CFTR mutation testing plus DNA sequencing confirmation to diagnose CF in fetuses with FEB (see Table 95 in Appendix D); however, no clinical

outcomes were reported for the fetuses, as there was no reference standard against which to determine the accuracy of the testing. Most of the fetuses (94%; 46/49) had no detectable CFTR mutations and the remaining 6% (3/49) were found to be carriers with one CFTR mutation. No fetus was diagnosed with CF; this was likely due to the small size of the study and the low rate of CF among fetuses diagnosed with FEB (2–13%, see Table 44 in section B.6.ii). The accuracy of CFTR mutation testing compared with clinical diagnosis after birth in fetuses with FEB could not be determined.

Test failure rates, mutation identification errors and the limitations of CFTR mutation testing

Five studies reported on the test failure rates of seven different panel-based CFTR mutation tests. The test failures were resolved by either repeating the test or sequencing the appropriate amplicon for all seven tests. The median failure rate was 4.5% (range 0.0001–9), suggesting that in diagnostic laboratories using panel-based CFTR mutation tests, about 4.5% of tests would need to be repeated.

There were very few false negative and no false positive results in the included studies that were due to mutation identification errors. The largest number of identification errors arose from 2 studies that used a panel-based CFTR mutation test with ARMS detection technology, which cannot distinguish between the heterozygous and homozygous state. In both studies 5% of the samples were homozygous and, therefore, the second mutation could not be determined using ARMS technology (Houdayer et al. 1998; Tomaiuolo, Spina & Castaldo 2003).

Ravnik-Glavač, Glavač & Dean (1994) found that the SSCP exon-scanning CFTR mutation test requires optimisation for best performance and that there was no optimal condition suitable for all exons. In their laboratory a 10% gel with 1.3% cross-linking in the presence of glycerol had a sensitivity of 100% for most exons and 80–98% for exons 4, 7, and 13. Under these conditions only 3% (4/133) of known mutations were missed. Ravnik-Glavač et al. (2002) found that the DHPLC exon-scanning CFTR mutation test was dependent on denaturation conditions, and that 10% (7/73) of CFTR mutations were not detected at the recommended denaturation temperature. However, when the melting temperature was optimised for each amplicon, they could detect all CFTR mutations.

Only 2 other included studies reported misidentification errors. One level IV study reported that a panel-based CFTR mutation test using an oligonucleotide ligation-based detection assay made 0.7% (7/1076) miscalls of the IVS8 5T/7T/9T polymorphism (Strom et al. 2004). Another level IV study by Nagy et al. (2007) reported that their panel-based CFTR mutation test using fluorescent PCR and fragment analysis did not recognise a one-base-pair difference and could not detect the F508Cdel mutation (caused by the deletion of a T).

Conclusion

In summary, a panel-based CFTR mutation test will not detect any mutation other than those included in the CFTR mutation panel used by the test. Both panel-based and exon-scanning CFTR mutation tests, as well as DNA sequencing-based tests, cannot detect large deletion or insertion

mutations, which occur in about 2% of CF patients worldwide[9]. Methods such as MLPA are required to detect these rare mutations. Even extensive DNA sequencing of all exons and flanking regions plus deletion/insertion analysis will not detect all mutations. Thus, in the case of a negative result, it is important for the diagnostic laboratory to explain the scope of the mutation testing that was undertaken and the likelihood of the patient being truly negative to the clinician requesting the test.

This was highlighted by the results of the external European QAP for CF in 2014[10], which reported that 27% (3/11) of Australian laboratories did not correctly report the results. Two laboratories did not interpret the results correctly and one laboratory made an error in the risk calculation. This could lead to future problems, especially for falsely negative CFTR mutation carriers, and emphasises the importance of proper pre- and post-test counselling to make patients aware that the tests are not 100% specific.

LINKED EVIDENCE – CHANGE IN MANAGEMENT

B.3–5.II RISK OF BIAS ASSESSMENT, CHARACTERISTICS OF THE EVIDENCE-BASE AND OUTCOME MEASURES

Nine studies were included on the impact of prenatal CFTR testing on TOP rates and CF birth rates. To assess the quality of the studies, the Institute of Health Economics (IHE) checklist for case series was used, as none of the studies had a valid comparator (Moga et al. 2012). As all studies were non-comparative, they were included as level IV evidence. Six of these studies included pregnant women where an FEB was detected, and 4 studies included pregnant women with a 1:4 risk of having a fetus with CF (1 study included both groups, FEB and high-risk). The quality/risk of bias scores of the included studies are shown in Table 43, in order of quality (highest to lowest). In case of multiple studies with the same quality score, the study with the bigger study population is listed first. The study of Scotet et al. (2003) included both groups (FEB and carrier parents), with separate results. See Appendix C for more detailed information of the individual studies included in the evidence-base.

[9] Available from <http://www.genet.sickkids.on.ca/StatisticsPage.html> (accessed 27 January 2015)

[10] CF network, Biomedical Quality Assurance Research Unit, Department of Public Health and Primary Care, Catholic University Leuven, Leuven, Belgium. Pers. comm. via email on 31 January 2015.

Table 43 Study quality and key features of the included evidence for change in management studies

Study	N (tested)	Indication (population)	Study quality
Ghose et al. (2000)	48	FEB	15/18 (high)
Muller et al. (2002)	641	FEB	14.5/18 (high)
Scotet et al. (2008)	268	1:4 risk of CF	14.5/18 (high)
de Becdelievre et al. (2011)	694	FEB	14/18 (high)
Ameratunga et al. (2012)	33	FEB	14/18 (high)
Tomaiuolo et al. (2013)	149	1:4 risk of CF	13/18 (medium)
Scotet et al. (2003)	173 (FEB), 148 (1:4 risk of CF)	FEB + 1:4 risk of CF (separate results)	13/18 (medium)
Slotnick & Abhamabad (1996)	53	FEB	12/18 (medium)
Castaldo et al. (2000)	33	1:4 risk of CF	12/18 (medium)

CF = cystic fibrosis; FEB = fetal echogenic bowel

Outcomes reported regarding change in management were the number of pregnancies terminated after a positive test result. From this outcome, the rate of CF births and 'CF births avoided' were calculated. No statistical analysis could be conducted due to the absence of a comparator.

B.6.II RESULTS OF THE CHANGE IN MANAGEMENT STUDIES

Summary – Does prenatal CFTR mutation testing (common mutation analysis) affect the clinical management of a pregnancy where the fetus is suspected of having CF, compared with determining the diagnosis of the child after the birth?

Nine studies were included to assess the impact of prenatal CFTR testing on change in management. All studies were level IV evidence. Six studies included evidence on TOP in pregnancies where an FEB was detected: the TOP rate in this group was 65% (50/77), but was in the range 0–100% in the different studies (median 69.4%). In the group with a 1:4 risk that tested positive during prenatal testing, 155/163 couples chose TOP (95% from 4 studies; 92.3–100%). This shows that there is a change in management after a positive CFTR test result, assuming that no TOP would occur in the absence of genetic testing.

As none of the studies reported a comparison with pregnancy management after no prenatal testing, all 9 studies were non-comparative (i.e. level IV evidence). All case series were medium to high quality, based on the IHE checklist. Although 9 studies were included, it is possible that the populations in the studies by Scotet et al. (2003; 2008) are partly overlapping, as well as in the studies by Castaldo et al. (2000) and Tomaiuolo et al. (2013). Only 1 Australian study was detected (Ameratunga et al. 2012), which included 66 cases of FEB. However, only 33 cases underwent parental CFTR mutation testing and only one couple was identified as both being carriers. Their fetus was tested and had two CFTR mutations. The pregnancy was continued and a child with CF was born, which is likely to have been the same outcome had no mutation testing occurred. The included studies that provided information on the percentage of pregnancies terminated after a positive CF result from prenatal CFTR testing are shown in Table 44 and Table 45. The population with a 1:4 risk that tested positive during prenatal testing chose to terminate the pregnancy 92.3–100% of the time (155/163; 95%, median: 96.2%). This was slightly lower where an FEB was detected, which reported

TOP in 50/77 (65%) cases prenatally diagnosed to have CF. However, this percentage was in the range 0–100% (median 69.4%), depending on the study. Heterogeneity between studies would be expected, due to religious and cultural variations regarding the acceptability of TOP. However, given that the studies all came from Europe, UK, USA and Australia, there is no simple explanation for the differences, other than chance, given the very small samples. The percentage of children born with CF in these high-risk populations was relatively low due to the high percentage of TOPs. In conclusion, there is a change in management (i.e. TOP) after a positive test result from prenatal testing in the majority of cases, whereas it is assumed that no TOP would occur in the absence of prenatal genetic testing.

Table 44 TOP and CF birth rate in pregnancies where an echogenic bowel is detected

Study	N tested / N total population (%)	N TOP / N CF +ve diagnosed through PND (%)	N CF +ves diagnosed after birth	N children born with CF (%)
de Becdelievre et al. (2011)	694/694 (100)	15/30 (50)	0 (although status remains unknown for 3 children)	15/694 (2.2)
Muller et al. (2002)	641/641 (100)	16/18 (88.9)	2	4/641 (0.6)
Scotet et al. (2003)	173/173 (100)	18/22 (81.8)	0	4/173 (2.3)
Slotnick & Abuhamad (1996)	53/143 (37.6)	0/5 (0)	2	7/143 (4.9)
Ghose et al. (2000)	48/60 (80)	1/1 (100)	2	2/60 (3.3)
Ameratunga et al. (2012)	1/33 (3.0) (of 33 FEB pregnancies, only 1 carrier couple)	0/1 (0)	1	1/33 (3.0)

CF = cystic fibrosis; FEB = fetal echogenic bowel; PND = prenatal diagnosis; TOP = termination of pregnancy

Table 45 TOP and CF birth rate in pregnancies with a 1:4 risk of CF

Study	N tested / N total population (%)	N TOP / N CF +ve diagnosed through PND (%)	N CF +ves diagnosed after birth	N children born with CF (%)
Scotet et al. (2008) [a]	268/268 (100)	70/74 (94.6)	0	4/268 (1.5)
Scotet et al. (2003) [a]	148/148 (100)	36/39 (92.3)	1	3/148 (2.0) + 1 CF +ve fetus lost
Tomaiuolo et al. (2013) [b]	149/181 (82.3)	42/43 (97.7)	0	1/181 (0.6)
Castaldo et al. (2000) [b]	33/33 (100)	7/7 (100)	0	0/33 (0)

[a] Populations of these studies are possibly overlapping.
[b] Populations of these studies are possibly overlapping.
CF = cystic fibrosis; PND = prenatal diagnosis; TOP = termination of pregnancy

LINKED EVIDENCE – IMPACT OF CHANGE IN MANAGEMENT

B.3–5.III RISK OF BIAS ASSESSMENT, CHARACTERISTICS OF THE EVIDENCE-BASE AND OUTCOME MEASURES

No studies on impact of change in management met the inclusion criteria regarding prenatal genetic CFTR testing.

In the absence of more relevant evidence, a narrative synthesis of the evidence regarding the adverse events of TOP and psychological consequences after TOP due to fetal abnormalities (compared with psychological impact of no TOP and caring for a child with CF) was undertaken and is shown in section B.8.1.

B.6.III RESULTS OF THE TREATMENT STUDIES

Summary – If there are alterations in clinical management (e.g. termination of pregnancy) and treatment options available to parents of a fetus suspected of CF, does this have an impact on the health outcomes of the parents?

No studies were identified on parental psychological health after TOP due to a CF-affected fetus, compared with no TOP and raising a child with CF.

No studies were identified on parental psychological health after TOP due to a CF-affected fetus, compared with no TOP and raising a child with CF.

B.7. EXTENDED ASSESSMENT OF COMPARATIVE HARMS

No unpublished data on the harms of CFTR mutation testing or post-market surveillance of CFTR mutation testing have been identified.

B.8. INTERPRETATION OF THE CLINICAL EVIDENCE

B.8.1. BROADER CLINICAL CONSIDERATIONS

Safety of amniocentesis and CVS

No studies on the safety of prenatal CFTR testing were identified. Therefore, a separate search was conducted to identify risk factors associated with amniocentesis and CVS, which are currently commonly used to retrieve fetal DNA for prenatal genetic CFTR testing. Amniocentesis would usually be done when pregnant women are diagnosed with FEB on ultrasound, in the second trimester and usually at around 15–17 weeks. CVS can be done at lower gestational age (usually around 10–12 weeks), so this would be suitable for women who already know they are carriers and choose to be tested earlier (Moreira, Muggli & Halliday 2007).

Safety of amniocentesis

A 2003 Cochrane systematic review (Alfirevic, Mujezinovic & Sundberg 2003; search updated in 2008) reported that an RCT by Tabor et al. (1986) provides the best estimate of an excess pregnancy loss in low-risk women caused by amniocentesis. This study, conducted in 4,606 women, showed an increase of 1% in total pregnancy loss (3.2% in the intervention group and 2.2% in the control group). This difference did not reach statistical significance; however, there was a statistically significant increase in miscarriages of 0.8% (2.1% in the intervention group and 1.3% in the control group), which gives a relative risk (RR) of 1.60 (95%CI 1.02, 2.52).

The occurrence of complications in the first 6 weeks after amniocentesis (or in the comparator group—the ultrasound) was higher in the intervention group (272 women, 12.1%) compared with the control group (131 women, 5.8%, p<0.001). The RR of early complications after amniocentesis was 2.2 (95%CI 1.8, 2.7). Abdominal pain and amniotic fluid leakage were more prevalent in the study group (184 (8.1%) and 39 (1.7%), respectively) compared with the control group (67 (3.0%) and 10 (0.4%), respectively), with p<0.001. There was no difference in the risk of vaginal bleeding between the two groups (Table 46).

Table 46 Complications and fetal loss after amniocentesis compared with a control group

	Intervention group (n=2,302)	Control group (n=2,304)	p-value	Relative risk (95%CI)
Occurrence of complications (<6 weeks after amniocentesis)	272 (12.1%)	131 (5.8%)	<0.001	2.2 (1.8, 2.7)
Abdominal pain	184 (8.1%)	67 (3.0%)	<0.001	
Amniotic fluid leakage	39 (1.7%)	10 (0.4%)	<0.001	
Vaginal bleeding	55 (2.4%)	58 (2.6%)	NS	
Miscarriage after 16th week	1.7%	0.7%	<0.01	2.3 (1.3, 4.0)
Total fetal loss (miscarriages plus stillbirths)	58 (2.5%)	42 (1.8%)	<0.05	

Source: Tabor et al. (1986)
NS = not significant

During the newborn evaluation, more congenital malformations were found in the control group (113; 4.98%) than in the study group (78; 3.48%), (p<0.05), most likely due to a lower rate of TOP in the control group.

However, there are limitations. This study was conducted nearly 30 years ago in a low-risk population. It is unclear if it is applicable to the current study population of women at risk of having a baby with CF. Nowadays it is ethically and practically impossible to conduct an RCT with the sample size required to detect a risk reduction this small. Ultrasound machines have improved since the 1980s and the number of amniocenteses performed has increased. This is why many clinicians today do not believe that the miscarriage rate related to amniocentesis is as high as 1.0% (Tabor, Vestergaard & Lidegaard 2009). This more-recent registry-based non-comparative cohort aimed to

investigate the miscarriage rate after amniocentesis and CVS in an unselected group of women. It included all singleton pregnancies in Denmark in which amniocentesis or CVS had been performed between 1 January 1996 and 31 December 2006, leading to the inclusion of data from 32,852 women undergoing an amniocentesis. The post-procedural miscarriage rate following amniocentesis decreased from 1.5% to 1.2% (p=0.25) during the 10-year study period, with an overall rate of 1.4% (95%CI 1.3, 1.5). In comparison, in 633,308 women who did not undergo an invasive procedure, the miscarriage rate after 15 weeks gestation was 0.9% (5,692/633,308) (Tabor, Vestergaard & Lidegaard 2009). The total loss rate (miscarriages and intra-uterine deaths) in the amniocentesis cohort was 2.0% (95%CI 1.8, 2.1) from 651 pregnancies.

Two other systematic reviews were identified on the safety of amniocentesis, including more-recent studies from the past 15–20 years. A systematic review of studies (Mujezinovic & Alfirevic 2007) published after 1995 reported similar (pooled) rates to the cohort study (Tabor, Vestergaard & Lidegaard 2009): 1.28% in the amniocentesis group and 0.64% in the control group, but with wide variation between studies. The 5 included controlled studies reported a pooled RR for total pregnancy loss of 1.25 (95%CI 1.02, 2.49) and an RR of 1.46 (95%CI 0.86, 2.49) when fetal losses before 24 weeks and 28 weeks were combined (Mujezinovic & Alfirevic 2007). However, relatively small studies were included in the analysis, rendering the findings less generalisable. The second systematic review was carried out recently (search date 31 January 2014 for studies published after the year 2000) estimating the risk of miscarriage before 24 weeks' gestation in women undergoing amniocentesis and CVS, with a minimum of 1,000 procedures per study (Akolekar et al. 2015). The background risk of miscarriage in the absence of invasive testing in appropriate controlled studies and the procedure-related risk of miscarriage following amniocentesis were also determined here. Fourteen studies were included, comprising 6 observational retrospective cohort studies without a control group, 4 retrospective cohort studies with a control group and 4 case-control studies. In total, there were 1,107 fetal losses in 124,001 women undergoing amniocentesis, with a weighted pooled miscarriage rate of 0.70% (95%CI 0.50, 0.92) and a background miscarriage rate of 0.70% (95%CI 0.53, 0.90; 6,634 losses in 771,963 women). No significant difference was detected in the rate of miscarriage: 0.81% (95%CI 0.58, 1.08) in the amniocentesis group and 0.67% (95%CI 0.46, 0.91; p=0.14) in the background risk group, as estimated from 7 studies with control groups. In this systematic review, based on the period 2000 – 1/2014, the estimate of a loss attributable to amniocentesis was as low as 0.1% (Akolekar et al. 2015).

Safety of chorionic villus sampling (CVS)

The most recent systematic review on the safety of amniocentesis and CVS (search date 31 January 2014 for studies published after 2000) included 7 studies with a minimum of 1,000 procedures per study in the CVS part of the review (Akolekar et al. 2015); 3 observational retrospective cohort studies without control group, 2 cohort studies with a control group (1 matched and 1 unmatched), 1 database study with control group and 1 prospective observational study with unselected women undergoing routine screening as a control group. A total of 53,890 women underwent CVS and there

were 1,186 fetal losses prior to 24 weeks' gestation, corresponding to a pooled loss rate of 2.36% (95%CI 1.68, 3.16). The background loss rate was 2.26% (95%CI 0.81, 4.41), based on 25,597 losses out of 670,696 women who did not undergo an invasive procedure. The background risk for CVS is higher than for amniocentesis, which is performed at an older gestational age, when the risk of spontaneous miscarriage is lower. From 3 included studies the weighted pooled procedure-related risk of miscarriage following CVS was estimated using an incidence-rate difference meta-analysis. A total of 207 out of 8,899 women undergoing CVS miscarried, compared with 534 out of 37,388 who did not have an invasive procedure. The pooled procedure-related risk of miscarriage before 24 weeks' gestation (following CVS) was 0.22% (95%CI –0.71 –1.16%, p=0.64), meaning that the estimate of a loss attributable to CVS was around 0.2%.

A second systematic review of studies published after 1995 included reports of CVS carried out transabdominally at between 10 and 14 weeks' gestation with a minimum of 100 procedures per study (Mujezinovic & Alfirevic 2007). No controlled studies comparing pregnancy loss after transabdominal CVS with appropriate controlled groups were identified. No statistical heterogeneity was found in the results for pregnancy loss within 14 days (k=4), within 30 days (k=4) and before 24 weeks (k=8). The pooled pregnancy loss rates (fixed effects) for before 14, and after 30, days following CVS and before 24 weeks of pregnancy were 0.7% (95%CI 0.3, 1.4), 1.3% (95%CI 0.5, 2.3) and 1.3% (95%CI 1.0, 1.7), respectively, with a total of 44 losses out of 3,402 pregnancies. This is lower than in the study by Akolekar et al. (2015), which estimated a pooled loss rate of 2.36%. However, the number of procedures per study in the systematic review by Mujezinovic et al. (2007) was significantly lower, with reported loss rates in pregnancies of less than 24 weeks' gestation varying from 1.1% to 3.1% in the included studies. The data for multiple insertions during CVS are even more heterogeneous, with loss rates ranging from 1.4% to 26.6%. It is likely that operator skill and experience played an important role here.

A Cochrane review (Alfirevic, Mujezinovic & Sundberg 2003) did not provide a direct comparison between CVS and a control group. However, they did compare the safety of transabdominal CVS versus transcervical CVS. The report stated that total pregnancy loss and spontaneous miscarriages were higher after transcervical CVS compared with transabdominal CVS. However, this was mostly due to the excess loss in the transcervical arm of one of the trials (Smidt-Jensen et al. 1992). This RCT was conducted between 1985 and 1990 and reported a total pregnancy loss after transcervical CVS of 12.4% compared with 7.4% after transabdominal CVS. Corresponding spontaneous pregnancy loss figures were 8.2% and 3%. Results from other studies (4 other trials) included in the systematic review were almost identical in both transcervical and transabdominal CVS. There was significant heterogeneity between included trials, with I^2 = 72.3%, and with a random effects model there were no significant differences in pregnancy loss and miscarriage between the two methods. All studies in this analysis were conducted over 23 years ago and it is unclear if these results are still applicable to current clinical practice.

Safety and psychological impact of TOP

As no studies were identified on parental psychological health after TOP due to a CF-affected fetus compared with no TOP and raising a child with CF, an additional broader (non-systematic) search was conducted in PubMed to assess parental psychological outcomes after the diagnosis of fetal anomalies (non-comparative). One systematic review was identified in this search and the relevant articles included in the systematic review. Articles included from the search and pearling of relevant studies are narratively discussed below and in Appendix E (Wool 2011), to show the consequences of change in management (i.e. TOP) in affected pregnancies. In addition to psychological effects, a search was done to identify systematic reviews on adverse events associated with TOP. The results are described below.

Adverse events and physical impact of TOP

There are a number of different methods for terminating a pregnancy: pharmaceutical, with a variety of pharmacological agents (mifepristone or methotrexate in combination with misopristol or gemeprost, or a prostaglandin analogue alone), and surgical (vacuum aspiration, dilatation and curettage, dilatation and evacuation, induction of labour, hysterotomy or hysterectomy). The method selected often depends on the gestational age of the fetus, availability, and doctor or patient preference.

<u>First-trimester TOP</u>

A Cochrane review compared medical and surgical methods of TOP (Say et al. 2005). Morbidity due to surgical termination (with a sufficiently skilled practitioner) depended on gestational age, the method of termination, age and parity. The complications due to surgical termination are infection, cervical laceration, incomplete evacuation, uterine perforation, haemorrhage and complications due to anaesthesia. Side effects from pharmaceutical TOPs are bleeding (moderate to heavy), pain, nausea, vomiting and diarrhoea. The review found that pharmaceutical termination is an effective alternative to surgical termination for first-trimester TOP.

A second Cochrane review (Kulier et al. 2011) compared pharmaceutical methods for first-trimester TOP. The most widely researched drugs for TOP are prostaglandins, mifepristone and methotrexate alone, mifepristone with prostaglandins and methotrexate with prostaglandins. More-frequent side effects such as nausea and diarrhoea were seen when misoprostol was administered orally compared with the vaginal route. Combined regimens were in most cases more effective than the administration of a single agent. The authors concluded that there are safe and effective pharmaceutical TOP methods available.

A third Cochrane review (Kulier et al. 2001) investigated surgical methods for first-trimester TOP. No reports of maternal deaths or cases of uterine perforation were identified. When vacuum aspiration was compared with dilatation and curettage, no statistically significant differences were found for adverse events such as excessive blood loss, blood transfusion, febrile morbidity, incomplete or

repeat uterine evacuation procedure, re-hospitalisation, post-operative abdominal pain or therapeutic antibiotic use.

Second-trimester TOP

Second-trimester TOP would be done mostly for women carrying a fetus with an echogenic bowel detected on ultrasound and subsequently found to have two CFTR mutations. Couples who are known carriers have the opportunity to test (and therefore undergo TOP) at an earlier gestation. Surgical (dilatation and evacuation) and pharmaceutical methods for second-trimester TOP were examined in another Cochrane review (Lohr, Hayes & Gemzell-Danielsson 2008). Only 2 trials were included in this review. The incidence of combined minor (e.g. haemorrhage not requiring transfusion, requirement for additional curettage) and major (e.g. haemorrhage requiring blood transfusion, complication requiring unintended major surgery) complications was lower with the surgical method than the pharmaceutical method (intra-amniotic prostaglandin $F_{2\alpha}$), with an odds ratio (OR) of 0.12 (95%CI 0.03, 0.46). However, the risk of major complications was not statistically different between the two groups. Fewer adverse events were reported with the surgical method compared with mifepristone combined with misoprostol (OR 0.06; 95%CI 0.01, 0.76).

One of the Cochrane reviews (Wildschut et al. 2011) compared different pharmaceutical methods for second-trimester TOP at 12–24 weeks. Included in the review were 38 RCTs, mostly limited by small numbers and lack of blinding, that compared 20 different drug regimens. Many of the studies reported the need for surgical evacuation due to retained products of the placenta and heavy vaginal bleeding. Other side effects reported were mild, self-limiting diarrhoea (common among women who received misoprostol), pain, nausea and vomiting. Side effects from vaginal misoprostol were usually mild and self-limiting. The risk of uterine rupture during pharmaceutical TOP with misoprostol was very low (0.3%), even for women with a uterine scar (Kapp et al. 2013).

Psychological impact of TOP after diagnosis of a fetal abnormality

Post-traumatic stress

Eight studies were identified reporting on post-traumatic stress after TOP following prenatal diagnosis of a fetal abnormality in pregnancy (Davies et al. 2005; Kersting et al. 2005, 2009; Korenromp, Christiaens et al. 2005; Korenromp, Page-Christiaens et al. 2005; Korenromp et al. 2007, 2009; Salvesen et al. 1997). In these studies post-traumatic stress was measured using the 15-item Impact of Event Scale (IES) or the 22-item Impact of Event Scale Revised (IES-R), where a higher score represented more stress. Post-traumatic stress was prevalent in the population undergoing TOP, with 1 study reporting up to 67% women affected at 6 weeks after TOP (n=30) (Davies et al. 2005). This decreased to 41% at 1 year after TOP. One of the studies, by Korenromp et al. (2009), reported a post-traumatic stress rate of 45.8% at 4 months after TOP compared with only 20% at 16 months after TOP. A rate of 17.3% was reported in a different study (Korenromp, Christiaens et al. 2005) for women 2–7 years after TOP. In the study by Salvesen et al. (1997) (n=24) severe intrusive distress as

measured through the IES was seen in 48% of women immediately after TOP, which decreased to 19% at 1 year after TOP. A severe avoidance response (measured through IES) was reported in 15% in the acute phase and 0% at 1 year after TOP. These results are confirmed by studies that also show a (slight) decrease in mean IES-R scores: from 44.03 (SD 19.17) at 14 days after TOP to 41.78 (SD 24.46) at 2–7 years after TOP in the first study (Kersting et al. 2005), and from 45.0 (SD 17.45) at 14 days after TOP to 30.9 (SD 21.35) at 14 months after TOP in the second study (Kersting et al. 2009). In comparison, the mean IES score after delivery of a healthy child is 7.97 (SD 8.04), and a score lower than 18 was seen as normal.

Women were showing more stress on IES-R following TOP compared with their partners, with men scoring 12.8 (SD 16.6) on average at 2–7 years after TOP and women having a mean score of 18.1 (SD 18.0) around this time (Korenromp, Page-Christiaens et al. 2005). A second study (Korenromp et al. 2007) measured post-traumatic stress scores in men and women at 4 months after TOP and reported scores of 16.9 (SD 12.6) and 25.1 (SD 15.2) for men and women, respectively.

Grief

Grief was prevalent in women who underwent TOP due to a fetal abnormality. Grief was measured as a score of >90 on the Perinatal Grief Scale / Inventory of Traumatic Grief. At 6 weeks Davies et al. (2005; n=22) reported that 47% of women scored above the cut-off value (90), with 27% still experiencing an abnormal level of grief at 12 months after TOP. One study (n=86) observed that grief dominated in 36% of women at 6 weeks after TOP, compared with 13% after 6 months (Geerinck-Vercammen & Kanhai 2003). Iles & Gath (1993; n=61) reported physical grief in 78% of women at 4–6 weeks after TOP, decreasing to 31% after 13 months. Decreasing grief over time was also shown in studies by Korenromp, Christiaens et al. (2005) and Korenromp et al. (2009): at 4 months after TOP 8.8% (mean score was 58.8, SD 19.6) of 147 women scored above the threshold for grief, in comparison with only 2.1% (mean score was 50.1, SD 16.5) at 16 months (Korenromp et al. 2009). After 2–7 years 2.6% of women scored above the threshold in a population of 196 women (5/196) in a second study (Korenromp, Christiaens et al. 2005). Patients who underwent TOP before 14 weeks' gestation had significantly lower scores for grief (mean 40.0, SD 10.8, n=44) than those who underwent TOP after 14 weeks' gestation (mean 46.9, SD 17.4, n=150, p=0.014).

Women showed more grief than men, with mean scores of 59.0 (SD 20.4) in women and 47.8 (SD 16.6) in men at 4 months after TOP (Korenromp et al. 2007), and 44.1 (SD 16.2) for women and 38.6 (SD 11.4) for men at 2–7 years after TOP (Korenromp, Page-Christiaens et al. 2005).

Depression and anxiety

Depression (and in some cases anxiety) after TOP was measured using the Beck Depression Inventory (BDI, scale 0 to 63, where a score >9 is positive for depression), the Symptom checklist-90 (90-item questionnaire with a depression score of >41 for women and >33 for men, and an anxiety score of >26 for women and >21 for men), and the Edinburgh Postnatal Depression Scale (EPDS,

scale 0 to 30, cut-off level of 12). No decrease in depression rates over time was detected in a study by Davies et al. (2005, n=22), with 30% and 32% of women scoring above the BDI threshold for depression at 6 weeks and 12 months after TOP, respectively. Kersting et al. (2009, n=36) did show a decrease over time in BDI scores, with a mean score of 12.3 (SD 7.54) in women at 2 weeks after TOP compared with a mean of 7.6 (SD 6.45) at 14 months after TOP. Similar results were shown in Korenromp et al. (2009, n=147), in which women had an average score of 8.2 (SD 5.7) on the EPDS and 27.9% above the cut-off score at 4 months, compared with a mean score of 5.3 (SD 4.4) and 13.1% above the cut-off at 16 months.

Men showed slightly less anxiety and depression in the long term (2–7 years after TOP, n=151) compared with women (Korenromp, Page-Christiaens et al. 2005). Men scored an average of 12.1 (SD 4.5) and 20.8 (SD 7.5) for anxiety and depression, respectively, whereas women had an average score of 14.0 (SD 6.0) for anxiety and 26.0 (SD 11.0) for depression.

Anger and guilt, general psychological malfunctioning and psychiatric diagnoses

The study by Iles at al. (1993) reported that anger and guilt were experienced by 41/71 (58%) and 34/71 (48%) of women at 4–6 weeks after TOP, respectively. These rates decreased to 19/61 (31%) and 19/61 (31%), respectively, at 13 months after TOP. Korenromp et al. (2005) reported that only 8% and 10% of women reported feelings of regret and doubt after TOP, respectively. Other outcomes that were mentioned were emotional distress (53% after 6 weeks and 43% after 12 months, n=22) (Davies et al. 2005), psychiatric diagnoses (25% after 14 days and 16.7% after 14 months, n=36) (Kersting et al. 2009), and general psychological malfunctioning (12.2% of women scoring above the threshold after 4 months and 4.8% after 16 months) (Korenromp et al. 2009). This was more prevalent in women compared with their partners when measured at 4 months after TOP, with women scoring 145.6 (SD 53.1) on the Symptom Checklist-90 and men scoring an average of 121.5 (SD 36.6).

Psychological impact of caring for a child with CF (no TOP)

Recently the results of the International Depression Epidemiological Study on the prevalence of depression and anxiety in patients with CF and parent caregivers were published. This study was conducted at 154 CF centres in nine countries (Belgium, Germany, Italy, Spain, Sweden, The Netherlands, Turkey, UK and USA), and included 3,127 mothers and 975 fathers of young children with CF. These results were used as the comparator to be able to make an indirect rough comparison between the (psychological) consequences of caring for a child with CF and the impact of TOP (Quittner et al. 2014).

Depression and anxiety

In the study by Quittner et al. (2014) depression and anxiety were measured using the Hospital Anxiety and Depression Scale (HADS), with patients categorised using established cut-off scores

(scale 0–21: mild = 8–10, moderate = 11–15, severe ≥16). Depression was also measured using the Center for Epidemiologic Studies – Depression scale (CES-D; scale 0–60). Higher scores in the CES-D indicate more depressive symptoms, with a score ≥16 indicating depression. The results of the depression and anxiety questionnaires are shown in Table 47. Both depression and anxiety were prevalent among mothers of children with CF (mean children's age was 8.90 ± 5.08 years), with 20–34% of mothers scoring above the threshold for depression, depending on which questionnaire was used, and 48% for anxiety. Fathers scored lower in both depression (18–25%) and anxiety (36%), with significant differences in the CES-D and the HADS-anxiety. The study also reported that mothers reporting anxiety were 15.5 times more likely to score above the threshold on depression; 31% reported elevated scores on both. In fathers there was also a correlation between depression and anxiety: fathers with anxiety were 9.2 times more likely to report elevated depression (21% reported both).

Table 47 Depression and anxiety in parents caring for a child with CF

Positive scores (%)	Mothers (n=3,127)	Fathers (n=975)	OR (mothers compared with fathers)	p-value (mothers compared with fathers)
HADS—depression	618 (20%)	173 (18%)	1.15	0.142
CES-D	1,057 (34%)	240 (25%)	1.58	<0.001
Either HADS or CES-D	1,165 (37%)	305 (31%)	1.34	<0.001
HADS—anxiety	1,496 (48%)	343 (36%)	1.70	<0.001

Source: Quittner et al. (2014)
CES-D = Center for Epidemiologic Studies – Depression Scale, HADS = Hospital Anxiety and Depression Scale; OR = odds ratio

Depression and anxiety in mothers were associated with their children undergoing recent intravenous antibiotics, receiving psychotherapy and the child being a younger age. In fathers the only variable associated with depression was their child recently undergoing intravenous antibiotics.

B.8.2. CONCLUSIONS ON CLINICAL EFFECTIVENESS AND SAFETY

It is important to classify the diagnostic accuracy of CFTR mutation testing and the associated change in management (the option of TOP) in relation to no genetic testing. The diagnostic accuracy was assessed for all included indications, but the further implications of testing were only considered for prenatal CFTR testing. The reasons for not assessing the implications of the other tests are outlined in Table 9.

No studies meeting the PICO criteria regarding direct effectiveness or safety of CFTR mutation testing were identified. However, DNA retrieval for a genetic test (for NBS, symptomatic patients or carrier testing) is usually done through a routine blood test and is generally considered safe. More-invasive testing is involved with prenatal CFTR testing (CVS and amniocentesis), the safety of which is investigated in section B.8.1. The absolute risk of pregnancy loss increased by 1% in women who underwent an amniocentesis, compared with non-invasive imaging (3.2% versus 2.2%), as reported in the only large RCT on amniocentesis performed (in 1986). Since then, procedures have improved

and the most recent systematic review showed a loss attributable to amniocentesis of only 0.1% (95% CI –0.04, 0.26) (Akolekar et al. 2015). For CVS the systematic review reported a loss estimate of around 0.2% (95%CI –0.71, 1.16) attributable to the procedure. No RCTs examining the risks of pregnancy loss in women undergoing CVS compared with no invasive testing have been conducted. Akolekar et al. (2015) reported that the findings of the most recent systematic review demonstrate that the risk of miscarriage before 24 weeks' gestation in women undergoing CVS or amniocentesis was not significantly different from that of those not undergoing an invasive procedure. Other less severe (rare) complications of amniocentesis are abdominal pain, amniotic fluid leakage and vaginal bleeding.

For assessment of the body of evidence relating to test accuracy, an evidence rating from A (excellent) to D (poor) was assigned to each of the components in the body of evidence matrix provided in Table 48, adapted from the NHMRC FORM grading system (Hillier et al. 2011). This was also done for change in management (provided in Table 49), as the GRADE profile could not be used as stated in the research protocol due to the absence of a proper comparator.

The evidence-base for accuracy was satisfactory, with mostly level III-2 studies (i.e. those comparing a CFTR mutation panel against whole gene sequencing or clinical diagnosis, although many studies did not provide information on those patients/samples considered disease negative). Sensitivity was fairly consistent, with some variation between population groups. The accuracy results indicated that in the group of patients with classical CF symptoms, CFTR mutation tests are highly accurate when compared with DNA sequencing at detecting the mutations the tests were designed to diagnose. Overall, 20% of clinically diagnosed CF patients had one or two CFTR mutations that could not be detected with a panel-based CFTR mutation test. In the group of men diagnosed with CBAVD, CFTR testing was also highly accurate compared with DNA sequencing. However, when compared with a clinical diagnosis, the sensitivity was much lower due to the high proportion of men with CBAVD for whom a CFTR mutation could not be identified.

In the case of prenatal CFTR testing, the panel-based CFTR test identified all mutations that it was designed to detect. However, as not all mutations are included in the common mutation test, a small number of cases (around 8%) would require DNA sequencing. Because it is not able to detect large deletion or insertion mutations, around 2% of mutations would be missed even with DNA sequencing, and MPLA would have to be done to detect these rare mutations. The test failure rate is around 4.5%, which means that these tests would have to be repeated. It is very important that in the case of a negative test result, an explanation is given on the scope of the testing and the likelihood of the patient or fetus being truly negative.

Even though no comparative studies on the impact of CFTR testing on subsequent management were identified, a change in management has been detected after prenatal CFTR testing. Mothers with fetuses that tested positive for CFTR mutations terminated the pregnancy in around 95% of cases when both parents were carriers and in 65% of cases when an FEB was detected. However, the

TOP rate varied widely in the FEB group, in the range 0–100%. Even though this wide range could not be explained, the studies in which the TOP rate was 0% had a very low number of test positives (1 and 5). The difference in TOP rate between population groups could be due to the possibility of testing earlier when both parents are carriers (compared with testing in the second trimester after an FEB is detected on ultrasound), and thus terminating the pregnancy at a lower gestational age. Making the assumption that in the absence of prenatal testing the couple would choose not to abort the fetus, it is concluded that testing for CFTR mutations does result in an increase in the rate of TOP when two mutations are identified.

An additional non-systematic search was done to identify psychological outcomes after the prenatal diagnosis of fetal anomalies, either after TOP or after the birth of a child with CF, to investigate the impact of change in management that results from testing (i.e. increase in the rate of TOP, decrease in the rate of children with CF being born). Post-traumatic stress, grief, anger, guilt and depression were prevalent in the population undergoing TOP but this did decrease over time (months to years). Around 30% of women suffered from depression in the first months after TOP. Abnormal scores for grief occurred in around half the women in the weeks after TOP but this decreased strongly to around 2% after more than 16 months following TOP. Women who underwent TOP before 14 weeks' gestation scored lower for grief than women who terminated after 14 weeks' gestation. Furthermore, women showed more grief than men after TOP. Men also showed slightly less anxiety and depression in the long term compared with women.

Depression and anxiety were also prevalent among mothers of children with CF, with 20–34% and 48% of mothers scoring above the threshold (CES-D and HADS questionnaires) for depression and anxiety, respectively. In fathers depression and anxiety was less prevalent, with 18–25% and 36% experiencing these symptoms, respectively. Depression in mothers was associated with the child being a younger age and the severity of disease.

The short-term depression and anxiety rates were similar in women who underwent TOP to women who had a child with CF. Less is known about the long-term effects (years to decades) and other potential health impacts. However, Korenromp et al. (2005) reported that only 8% and 10% of women reported feelings of regret and doubt after their decision to undergo TOP, respectively, meaning that the availability of informed choice and the option to terminate is seen as a positive development.

On the basis of the evidence profile, it is suggested that, relative to no genetic testing (and diagnosis after birth), prenatal genetic CFTR testing and associated interventions have superior effectiveness at reducing the rate of people being born with CF. Prenatal genetic CFTR testing has slightly inferior safety, due to the risk of miscarriage and other adverse events from sampling procedures and physical adverse events due to TOP. Both terminating a pregnancy and having a child with CF are associated with poor psychological outcomes, at least in the short term, but no direct comparison could be made.

Table 48 Body of evidence assessment matrix for diagnostic accuracy results

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence base [a]			One or two level III studies with a low risk of bias, or level I or II studies with a moderate risk of bias	
Consistency [b]		Most studies consistent and inconsistency may be explained		
Generalisability			Population(s) studied in body of evidence differ to target population for guideline but it is clinically sensible to apply this evidence to target population [c]	
Applicability		Applicable to Australian healthcare context with few caveats		

[a] Level of evidence determined from the NHMRC evidence hierarchy – Table 20
[b] If there is only 1 study, rank this component as 'not applicable'.
[c] For example, results in adults that are clinically sensible to apply to children OR psychosocial outcomes for one cancer that may be applicable to patients with another cancer
SR = systematic review, several = more than two studies

Table 49 Body of evidence assessment matrix for change in management results

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence base [a]				Level IV studies, or level I to III studies/SRs with a high risk of bias
Consistency [b]		Most studies consistent and inconsistency may be explained		
Generalisability		Population(s) studied in the body of evidence are similar to the target population for the guideline		
Applicability		Applicable to Australian healthcare context with few caveats		

[a] Level of evidence determined from the NHMRC evidence hierarchy – Table 20
[b] If there is only 1 study, rank this component as 'not applicable'.
SR = systematic review, several = more than two studies

SECTION C TRANSLATION ISSUES

C.1. TRANSLATION ISSUES ADDRESSED

The evidence presented in Section B concluded that, in terms of comparative efficacy, prenatal CFTR testing is effective at reducing the number of babies born with CF compared with diagnosis after birth. However, it has an inferior safety profile compared with NBS, due to the slight increase in miscarriage risks associated with sampling procedures and the physical adverse events associated with termination of pregnancy (TOP). Subsequently, a cost-effectiveness analysis comparing the incremental costs associated with prenatal CFTR testing with those associated with no prenatal testing (i.e. diagnosis after birth) is presented in detail in Section D.

Additional information beyond what has been presented in Section B is required to translate the systematic review of the clinical evidence to the base-case economic evaluations presented in Section D. Specifically, the following questions needing to be addressed are presented as headings, and the methods used to conduct these analyses, and the results, are described below.

C.1.1. WHAT IS THE ANALYTICAL VALIDITY OF CFTR MUTATION TESTING IN THE PRENATAL POPULATION?

Three types of genetic tests are considered in the economic model: (i) common mutation test, (ii) known mutation test (single mutation test or common mutation test) and (iii) whole gene test. The common mutation test includes a panel of the most prevalent CFTR mutations in the population and is used to perform mutation testing in the parents of a fetus suspected of CF. If both parents carry the most common mutation, F508del, a single mutation test would be performed on a sample from the fetus. If the parents are found to carry other mutations on the common mutation test, the fetus would also be tested using the common mutation test. The whole gene screen may be performed in fetuses based on the results of the parental testing (discussed in sections D.2 and D.3).

The analytical validity of these tests in the modelled populations is assessed in section B.6.i. Common mutation tests were found to be 100% sensitive to the detectable mutations in parent carriers. The mutation testing in fetuses from carrier parents was also reported to be 100% sensitive. However, as most fetuses from carrier parents diagnosed as having CF are aborted, the specificity of these tests has not been determined (see Table 94 and Table 95). Additionally, the accuracy of CFTR mutation testing in fetuses with FEB cannot be determined due to the lack of evidence. No evidence around test accuracy was identified for whole gene screen or single mutation analysis in fetuses.

Therefore, analytical validity of 100% is assumed in the present evaluations for all these tests. This is consistent with a systematic review of 14 economic studies focusing on preconception or prenatal

CF screening that found that most economic studies have assumed 100% diagnostic accuracy for fetal diagnosis and a specificity of 100% for CF carrier detection (Radhakrishnan et al. 2008).

C.1.2. WHAT IS THE CLINICAL SENSITIVITY (DETECTION RATE) OF THE CFTR MUTATION TEST?

Common mutation panel test

The clinical assessment (see section B.6.i) found only 1 study performed in Italy that provided evidence related to the accuracy of four different panel-based CFTR mutation tests designed to detect between 12 and 31 CFTR mutations compared with DNA sequencing in carrier parents. These tests identified 88–96% of mutations in the carrier parents with a median sensitivity of 92%. It is uncertain how applicable these results are to the Australian ethnic composition and the proposed listing.

The clinical sensitivity or detection rate of a mutation panel depends on the number of most-prevalent CFTR mutations selected for the panel testing. The prevalence of CFTR mutations varies widely across different ethnic groups. The proposed MBS item for common mutation testing described in this report suggests that the minimum number of mutations tested be 10; however, guidelines from the American College of Obstetricians and Gynaecologists (ACOG), American College of Medical Genetics (ACMG) and Human Genetics Society of Australia (HGSA) recommended that all CFTR mutations with allele frequency ≥0.1% are included in the population screening panels. The ACOG/ACMG panel currently includes 23 mutations that will identify around 88% of carriers, whereas HGSA recommends a panel of 17 mutations (after excluding unclear phenotypes[11]) with a test sensitivity of around 83.5% in Australia (Mishra, Greaves & Massie 2005). In accordance with these guidelines, laboratories currently listed on the RCPA 'Catalogue of genetic tests and laboratories' website offer panel-based common mutation testing for 11–44 of the most common mutations, and some laboratories in the private sector even test up to 90 mutations (see Table 102, Appendix I, for more details).

The base-case analysis, as per the proposed listing recommended by PASC[12], assumes only 10 common mutations. Therefore, an estimate of clinical sensitivity corresponding with panel-based mutation test detecting the 10 most-prevalent mutations in the Australian population was sought in the literature. In the economic studies estimating cost-effectiveness of CF carrier screening in Australia, Maxwell et al. (2010) and Norman et al. (2012) considered that the 10-mutation panel would have a minimum sensitivity of 80% in Australia. Given a lack of alternative data, this estimate is used in the base-case evaluation of a 10 common mutations panel.

[11] <http://www.hgsa.org.au/documents/item/1282>; accessed on 1 April 2015
[12] Protocol Advisory Sub-Committee

The clinical sensitivity associated with the common mutation panel will impact the estimated cost-effectiveness; for a given test cost, a higher sensitivity test will be more cost-effective. The sensitivity of the test may be improved by increasing the number of detectable mutations based on the population frequency in the common testing panel.

Based on the additional evidence available, the proposed listing of a 10 common mutations panel does not appear to reflect current clinical practice. Therefore, economic analysis is performed for a number of scenarios varying the number of mutations and the clinical sensitivity associated with common mutation testing. These scenarios are summarised in Table 50.

Table 50 Scenarios of alternative numbers of mutations included on 'common mutations' panel and analysed in the economic models

Number of common mutations included in the panel	Clinical sensitivity	Source for clinical sensitivity estimates	Model usage
10 (PASC-recommended minimum)	80.0%	Maxwell et al. (2010) and Norman et al. (2012)	Base-case scenario
17 (HGSA-recommended)	83.5%	HGSA report, Mishra et al. (2005)	Additional scenario 1
23 (ACOG-recommended)	88.0%	ACOG report	Additional scenario 2
32 (clinical evidence; see section B.6)	92.0%	Section B.6.i	Additional scenario 3

Sources: Maxwell et al. (2010); Norman et al. (2012); Mishra, Greaves & Massie (2005); <http://www.hgsa.org.au/documents/item/1282>, accessed on 1 April 2015; <http://www.acog.org/Resources-And-Publications/Committee-Opinions/Committee-on-Genetics/Update-on-Carrier-Screening-for-Cystic-Fibrosis>, retrieved on 1 April 2015.
ACOG = American College of Obstetricians and Gynaecologists; HGSA = Human Genetics Society of Australasia; PASC = Protocol Advisory Sub-Committee

Of note, there are more than 1,900 known mutations of the *CFTR* gene and if a test panel of 32 mutations has a sensitivity of 92%, the unidentified mutations will contribute to the remaining 8%. Therefore, after a certain point, increasing the number of mutations to be tested does not significantly increase the clinical sensitivity of the test due to the very low frequency of occurrence of the additional mutations included in the extended panel.

Whole gene sequencing

As discussed in section A.2.3 and section B.6.2.1, DNA sequencing-based tests cannot detect large deletion or insertion mutations, which occur in about 2% of CF patients worldwide. Methods such as MLPA or quantitative fluorescent multiplex PCR are required to detect these rare deletion/insertion mutations.

During the clinical assessment (see section B.6.2.1) no studies were identified reporting the clinical sensitivity of the whole gene test in fetuses suspected of CF. Since these mutations are very rare, whole gene sequencing is assumed to be 100% sensitive in the model.

C.1.3. WHAT IS THE INCIDENCE OF CF IN FETUSES SHOWING ECHOGENIC BOWEL?

The incidence of CF in the FEB population in Australia was not investigated during the clinical assessment. Therefore, a literature search was conducted to estimate the incidence of CF in fetuses showing an echogenic bowel in the Australian context.

Two studies were identified that analysed CFTR mutations within fetuses with an echogenic bowel in the Australian population (Ameratunga et al. 2012; Nicholls et al. 2003). In the study by Nicholls et al., 35 cases referred to the National Referral Laboratory at the Women's and Children's Hospital, Adelaide, for prenatal diagnosis of CF following detection of isolated FEB on second-trimester ultrasound between 1992 and 2002 were studied. Two of these 35 fetuses (6%) were diagnosed with CF. Ameratunga et al. performed a retrospective analysis of ultrasound scans performed between 2004 and 2009 at the Royal Women's Hospital, Melbourne, to assess the association between FEB diagnosed during the second trimester and adverse perinatal outcomes. Of the 66 cases identified, 33 pregnancies (53%) were tested for CF and 1 baby was confirmed to have CF, providing an incidence of 3%.

Both these studies (Ameratunga et al. 2012; Nicholls et al. 2003) have small sample sizes, vary in their study period and duration, and provide different estimates of the incidence of CF in FEB. Further investigation was conducted to verify the incidence of CF estimated in fetuses with FEB in other populations. A summary of the available data is shown in Table 98, Appendix G. Considerable uncertainty around the estimated risk of CF in these fetuses with FEB is observed in these studies, with estimates of incidence varying between 2% and 13%. The estimates of incidence of CF (3% and 6%) observed in both Australian studies mentioned above are within this observed range and are thus considered reasonable to be used in economic models. An average of these two values (4.5%) is assumed in the base-case economic evaluations.

The cost-effectiveness of prenatal CFTR mutation testing in fetuses with an echogenic bowel is likely to be sensitive to the incidence of CF, with higher values resulting in higher cost-effectiveness in this population. Therefore, the impact of varying the incidence of CF (from 2% to 13%) on the cost-effectiveness of prenatal CFTR testing in model 2 is assessed in the sensitivity analysis.

C.1.4. WHAT IS THE RISK OF MISCARRIAGE ASSOCIATED WITH INVASIVE TESTING?

Prenatal CFTR testing involves invasive testing, amniocentesis and CVS, and is associated with a risk of miscarriage additional to the background risk of miscarriage, which varies with the gestational age. The safety of these procedures is assessed in section B.8.1. The evidence from the literature was inconsistent, with older studies presenting a higher risk of miscarriage associated with invasive testing compared with the control group (i.e. no invasive testing). However, a recent literature review by Akolekar et al. (2015) concluded that the risk of miscarriage before 24 weeks' gestation in women undergoing CVS or amniocentesis is not significantly different from that of those not undergoing an invasive procedure. This study reported a miscarriage rate of 0.81% (95%CI 0.58,

1.08) in the amniocentesis group and 0.67% (95%CI 0.46, 0.91) in the control group. A pooled loss rate of 2.18% (95%CI 1.61, 2.82) was reported in the CVS group compared with the background loss rate of 1.79% (95%CI 0.61, 3.58) in women who did not undergo an invasive procedure. The weighted pooled procedure-related risks of miscarriage for amniocentesis and CVS were 0.11% (95%CI –0.04, 0.26) and 0.22% (95%CI –0.71, 1.16), respectively. The estimates from this study are used in the base-case evaluations as they provide the most recent evidence for risks of miscarriage in those undergoing invasive testing.

The choice of invasive test performed on the fetus is dependent on gestational age, with CVS being offered at around 10–14 weeks and amniocentesis performed usually between 15 and 24 weeks (Moreira, Muggli & Halliday 2007). Parents who have had a previous child born with CF or who are known carriers can have early prenatal diagnosis and thus have the choice of CVS or amniocentesis performed for the invasive testing. Therefore, the miscarriage rates are determined by weighting the proportions of CVS and amniocentesis performed in this population.

According to a report on prenatal diagnostic testing (Moreira, Muggli & Halliday 2007), the proportions of CVS and amniocentesis performed in Victoria at under 25 weeks are around 34% and 64%, respectively. The report also provides proportions of these tests over the past 10 years, which are similar to these proportions. A weighted risk of miscarriage (background: 1.05% and procedure-related: 0.15%) using these proportions is assumed in the economic analysis for fetuses with both parents known to be CF carriers (Table 51).

In fetuses presenting with FEB the perinatal mortality (including miscarriages and stillbirths) is reported to be higher than in the general fetal population—5.5% in a review of 11 studies (Carcopino et al. 2007). However, no data were found relating to the additional risk of miscarriage attributable to invasive testing in this population. Therefore, the risk of miscarriage as associated with the general infant population is assumed in this model. As the FEB is diagnosed on the second-trimester ultrasound, amniocentesis would probably be used. Therefore, the background miscarriage rate for this population is 0.67% and the additional procedure-related miscarriage rate is 0.11%.

Table 51 Estimated risk of miscarriage of fetuses that have invasive testing

Procedure	Background risk of miscarriage (95%CI)	Procedure-related risk of miscarriage (95%CI)
Chorionic villus sampling (CVS)	1.79% (0.61, 3.58)	0.22% (–0.71, 1.16)
Amniocentesis	0.67% (0.46, 0.91)	0.11% (–0.04, 0.26)
Parents known CF carriers (34% CVS and 66% amniocentesis)		
Weighted risk of miscarriage (base-case)	1.05% (0.66*0.67%+0.34*2.26%)	0.15% (0.66*0.81%+0.34*2.36%)
Weighted risk of miscarriage (values used in sensitivity analysis)	Not varied	(0%, 0.57%) [a]
Fetus has FEB (0% CVS and 100%		

Procedure	Background risk of miscarriage (95%CI)	Procedure-related risk of miscarriage (95%CI)
amniocentesis)		
Risk of miscarriage (base-case)	0.67%	0.11%
Risk of miscarriage (values used in sensitivity analysis)	Not varied	(0%, 0.26%) [a]

[a] Invasive testing being protective is not plausible; therefore, the lower limit chosen in 95%CI is 0%.
CF = cystic fibrosis; CI = confidence interval; FEB = fetal echogenic bowel

A sensitivity analysis is performed using the 95%CIs for risk of miscarriage in the intervention groups, as reported from the study by Akolekar et al. (2015). Table 51 summarises the estimated risk of miscarriage used in the economic evaluation and the sensitivity analysis for these procedures.

C.1.5. WHAT IS THE UPTAKE RATE OF TERMINATION OF PREGNANCY?

The clinical evidence showed that the parents with fetuses that tested positive for CFTR mutations terminated the pregnancy in around 95% of cases when both parents were CF carriers and in 65% of cases when an FEB was detected (see section B.6.ii). However, the TOP rate varied widely in the FEB group, with a range of 0%–100%, whereas the termination rate was in the range 92.3%–100% in study groups with carrier parents.

In the base-case evaluations a 95% pregnancy termination rate is assumed in the population where both parents are CF carriers, and 65% for the population where the fetus has an FEB. The TOP rate will affect the economic results. Therefore, the effect of varied uptake rates of TOP within the identified range (Table 52) has been analysed in the sensitivity analysis. Recent studies in the Australian community have reported much higher TOP acceptance rates than elsewhere when the fetus is diagnosed with anomalies (Ioannou et al. 2014; Massie, J et al. 2007). However, some CF-affected pregnancies with FEB may be diagnosed too late for termination. Therefore, a broader range of termination uptake rates (30%–100%), as reported by Radhakrishnan et al. (2008) in a review of economic evaluations of CF screening is tested in the sensitivity analysis for this population. For the population of fetuses with both parents known to be CF carriers, termination uptake rates are varied from 80% (an arbitrary value chosen to evaluate the impact of a lower termination uptake rate) to 100%.

Table 52 Estimated uptake rate of TOP in parents of fetuses diagnosed with CF

Contents	Fetus with both parents known CF carriers (%)	Fetus has FEB (%)
Termination of pregnancy (TOP) uptake rate	95	65
Range identified in section B.6.ii	(92.3–100)	(0–100)
Values used in sensitivity analysis	(80–100)	(30–100)

CF = cystic fibrosis; FEB = fetal echogenic bowel

C.2. SUMMARY OF RESULTS OF PRE-MODELLING STUDIES AND THEIR APPLICATION IN THE ECONOMIC EVALUATION

Table 53 provides a summary of the findings of each pre-modelling study and its implications to the economic evaluation presented in Section D.

Table 53 Summary of results of pre-modelling studies and their implications in the economic evaluation

Section	Pre-modelling study	Results and applications in Section D
Section C.1.1	Diagnostic accuracy of CFTR mutation tests	Diagnostic accuracy of all CFTR mutation tests is assumed to be 100%
Section C.1.2	Clinical sensitivity (detection rate) of CFTR mutation tests	Sensitivity of panel-based test is assumed as follows: Number of mutations (clinical sensitivity) 10 (80%) 17 (83.5%) 23 (88%) 32 (92%) Whole gene test is assumed to be 100% sensitive
Section C.1.3	Incidence of CF in fetuses showing FEB	Incidence of FEB is assumed to be 4.5%; value range of 2%–13% is tested in sensitivity analysis
Section C.1.4	Risk of miscarriage attributable to invasive testing	Parents known carriers: 0.15% Fetus has FEB: 0.11%
Section C.1.5	TOP uptake rate	Parents known carriers: (80%–100%) Fetus has FEB: (30%–100%)

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; FEB = fetal echogenic bowel; TOP = termination of pregnancy

SECTION D ECONOMIC EVALUATION

Economic analyses are presented only for prenatal testing (populations 3a and 3b), as per section A.8.

D.1. OVERVIEW

The clinical evaluation suggested that, relative to no genetic testing, prenatal genetic CFTR testing and associated interventions have slightly inferior safety and superior effectiveness based on the evidence profile given in section B.8. It was therefore decided that a cost-effectiveness analysis would be undertaken for the economic evaluation.

Most CF cases are currently diagnosed through NBS in Australia. Therefore, absence of prenatal testing and CF diagnosis after birth through NBS was considered current clinical practice and, thus, chosen as a comparator. Since the risks and subsequent decisions vary across populations 2a and 2b, separate models were constructed for each. Model 1 evaluates the economic implications of introducing prenatal testing for a population of fetuses with parents known to be CFTR carriers (population 2a), and model 2 comprises a population of fetuses who have FEB detected on the second-trimester ultrasound (population 2b). The economic analysis takes the form of decision tree analyses, incorporating the incidence of CF, test sensitivity and parental choices for diagnostic tests and terminations. The time horizon for the model is pregnancy to birth (including NBS). The resource implications of providing prenatal testing take into account the costs of diagnostic tests, termination, miscarriage, birth and NBS. Outcomes were measured as number of CF cases diagnosed prenatally, number of procedure-related fetal losses, number of pre-informed CF births and number of CF births averted. Cost-effectiveness is presented as cost per CF case diagnosed prenatally, cost per pre-informed CF birth and cost per CF birth averted.

Four scenarios were considered in the economic analysis, based on the number of mutations included and the clinical sensitivity of a common mutation test. Various sensitivity analyses were also undertaken. Under the baseline assumptions (10-mutation panel with 80% test sensitivity and cost of $135), the incremental costs per CF birth averted are $1,898 and $23,254 for model 1 and model 2, respectively. Although the mutation panels (additional scenarios) with higher sensitivity result in higher effectiveness, this is offset by the higher costs of the mutation test. The incremental costs per CF birth averted were observed to be driven largely by the cost of the diagnostic tests (common mutation test and whole gene sequencing). In model 1 the incremental cost per CF birth averted is most sensitive to decreases in the uptake rate of terminations of CF-affected pregnancies, whereas in model 2 the incremental cost per CF birth averted is most sensitive to the incidence of CF in this population and the uptake rate of terminations of CF-affected pregnancies.

D.2. POPULATIONS AND SETTINGS

The economic analysis compares the costs associated with prenatal CF diagnosis in pregnancies where fetuses are assessed at high risk of having CF (populations 2a and 2b in Table 9) with no prenatal genetic testing, as these populations have not been assessed elsewhere. This includes prenatal diagnosis of couples who have a previous child with CF or a CFTR-related disorder, or who are found to be carriers of a CFTR mutation (population with 1:4 risk) and where the fetus is found to have an 'echogenic gut' on ultrasound during the second trimester.

Echogenic gut identified on mid-trimester ultrasound is a marker for poor fetal outcomes such as aneuploidy, congenital infection, intra-uterine growth restriction, amniotic haemorrhage and CF. This means that amniocentesis is often indicated not just for CFTR mutation testing. The pathway followed is generally dictated by risk assessment based on various factors such as maternal age, results of biochemical serum screening, presence of other fetal structural anomalies, family history, ethnicity and parental preferences (Scotet et al. 2010). Therefore, only a part of this population—those who are being assessed as being at higher risk of CF and indicative of invasive testing—are assumed to undertake prenatal CFTR diagnostic testing.

Prenatal testing for CF consists of a search for the most common CFTR mutations in parental DNA samples. Their mutations are sought in CVSs or amniotic fluid cells, depending on the term of pregnancy. The fetus is considered affected with CF when it carries mutations from both parents, who then have an option of TOP. It is important that fetal testing is done within the valid timeframe to allow for TOP if CF is diagnosed.

The following CFTR mutation analyses would be available for prenatal CFTR testing in each population:

- common mutation test: a minimum of 10 mutations tested (in parents)
- known mutation test: single mutation test for F508del or common mutation test (in fetus)
- whole gene test (in fetus).

In the parents of a fetus where no familial mutations are known, a common mutation analysis would be done. If a mutation is found or known in both parents, known mutation analysis in the fetus would be performed (single mutation testing if both parents are carriers of F508del and panel-based test in others), to target the previously identified parents' known mutations. In cases where no common mutations have been identified in parents with a previous CF birth, a whole gene screen may be warranted in the fetus as the parents would be definite carriers of unidentified rare mutations. In fetuses with FEB the residual risk of CF when no common mutations are identified in the parents would be low. However, the risk of CF would be higher in the presence of FEB when only one parent is identified as a carrier (Hodge et al. 1999; Ogino et al. 2004). Therefore, a whole gene screen may be suggested in this case (see section D.3).

All parents undergoing prenatal genetic testing for CF should undergo genetic counselling; therefore, prenatal genetic CFTR testing should be restricted to specialist medical services that provide accredited genetic counselling. Obstetricians specialising in prenatal diagnosis or clinical geneticists should provide the service.

In the absence of prenatal genetic testing (comparator), the CF diagnosis is made during NBS, which includes testing for elevated levels of IRT, DNA testing for common mutations, and a sweat test as described in section A.2.2.

D.3. STRUCTURE AND RATIONALE OF THE ECONOMIC EVALUATION

Literature review

A literature search was conducted to identify published economic evaluations of prenatal diagnostic testing for CFTR mutations in fetuses at high risk of CF due to parents with a previous child with CF (or CFTR-related disorders) and for fetuses showing echogenic bowel on the second-trimester ultrasound. The search queries and results obtained are listed in Table 99 and Table 100, Appendix G.

No studies were identified that evaluated the cost-effectiveness of prenatal diagnostic testing for CFTR mutations in the targeted high-risk population (i.e. a fetus with a 1:4 risk of CF or showing FEB on second-trimester ultrasound) compared with the current situation of no prenatal testing.

A broader search strategy identified a number of economic studies assessing the cost-effectiveness of different prenatal screening strategies for CF in the general pregnant population. Two of the identified studies were conducted in the Australian setting (Maxwell et al. 2010; Norman et al. 2012). Maxwell et al. compared the costs, outcomes and cost savings of three models of prenatal CF carrier screening (stepwise versus simultaneous screening of both parents) compared with no screening. Norman et al. estimated the cost and outcomes from national carrier screening for CF (stepwise screening of parents), including both initial and subsequent pregnancies. Both studies reported outcomes as costs per CF birth averted. These studies are summarised in Table 101, Appendix H.

An approach similar to the above studies is used in the models presented here. The parents are screened first for the common CFTR mutations, followed by invasive testing in the fetus. Parents have the choice to terminate the pregnancy if the fetus tests positive for CF. However, the target populations in the present models are restricted to the high-risk population and the clinical pathways are based on the clinical management algorithm presented in section A.5 (see Figure 3). Outcomes are reported as costs per CF diagnosed and costs per CF birth averted, similar to the above studies, but the time horizon of the models is limited from pregnancy to birth (including NBS) and the lifetime costs associated with CF management are not considered in the models presented. A discussion of the lifetime costs of treating someone with CF is provided in section D.4.

Structure of the economic evaluation

Two economic models are presented for the populations discussed in section D.2.

- **Model 1 (population 2a):** Fetus at risk of CF due to parents being CF carriers (known CF carriers or have a previous child clinically diagnosed with CF)
- **Model 2 (population 2b):** Fetus at risk of CF due to diagnosis of echogenic bowel on the second-trimester ultrasound.

In both models the comparator is no prenatal CFTR mutation testing followed by NBS or clinical diagnosis after birth. The models take the form of cost-effectiveness analyses, estimating the costs per CF case diagnosed prenatally, costs per pre-informed CF birth and costs per CF birth averted. A time horizon of pregnancy to birth (including NBS) was chosen. Where applicable, costs derived from older sources are updated to 2014 dollars using the consumer price index inflation calculator[13]. No discounting is applied to costs or outcomes since the modelled period is less than 1 year.

The prenatal testing of CFTR mutations in a fetus enables an early diagnosis of CF and provides the parents with a choice regarding whether to terminate the pregnancy if the fetus tests positive for CF. If they decide to proceed with the affected pregnancy, they would be better prepared for managing CF once the child is born.

A summary of the key characteristics of the economic evaluation is provided in Table 54.

Table 54 Summary of the economic evaluation

Perspective	Australian healthcare
Comparator	No prenatal diagnosis for cystic fibrosis (CF)
Type of economic evaluation	Cost-effectiveness
Sources of evidence	Systematic review
Time horizon	Pregnancy to NBS
Outcomes	Costs per prenatal CF case detected, costs per CF birth averted, costs per pre-informed CF birth
Costs	Australian dollars, 2014 prices
Methods used to generate results	Decision tree analysis
Discount rate	Not applicable
Software packages used	TreeAge Pro Software 2015, MS Excel 2010

Model 1 (population 2a)

In model 1 parents are known to be carriers either due to a previous CF birth or previous diagnostic testing. In the prenatal CFTR mutation testing arm, both parents undergo genetic counselling followed by common CFTR mutation testing. If both parents are identified as carriers for common

[13] <http://www.rba.gov.au/calculator/annualDecimal.html>

mutations, a CVS/amniocentesis followed by a known mutation analysis is performed for fetal CF diagnosis. If only one parent is identified as a common mutation carrier or no common mutations are identified in either parent, it is highly likely that one or both parents is/are a carrier of a rare mutation; therefore, a whole gene screen for CFTR mutation would be performed for fetal diagnosis. Miscarriages are known risks of these procedures and are accounted for in the models. If a fetal diagnosis of CF is made, parents would have the option of terminating the pregnancy. Pregnancies that are continued would follow the same path as in the no-testing arm.

In the no-testing arm (comparator), no prenatal CFTR testing is performed and the pregnancy is continued to full term with natural outcomes (i.e. miscarriage, stillbirth and live birth). The CF diagnosis is made after the child's birth through NBS. As the modelled neonates are at higher risk of CF due to family history, genetic counselling and common CFTR mutation testing are offered irrespective of the results for IRT levels (Wilcken et al. 1995). Sweat tests are offered when one mutation is identified in the neonate.

Model 2 (population 2b)

The presence of FEB increases the probability that the fetus is affected with one of a range of conditions such as CF aneuploidy, growth restrictions, congenital infection and amniotic haemorrhage. For this reason the European and American societies recommend consideration of invasive diagnostic testing for karyotype, viral infection titres and CF screening following the identification of FEB at 19–20 weeks' gestation. However, to simplify the model structure these outcomes are not considered in the model. This is a conservative approach and will underestimate the cost-effectiveness of prenatal CFTR testing because invasive diagnostic testing would nevertheless be performed for other medical conditions in FEB fetuses, and the additional costs incurred would be those of testing mutations in the parents and the fetus's blood sample.

Model 2 consists of parents with fetuses showing echogenic bowel and assessed at high risk of CF based on their risk profile. No data for the CFTR carrier frequencies among the parents of fetuses with FEB were found in the literature. Therefore, a prevalence-based approach (based on Bayesian conditional probabilities) is used in modelling the prenatal testing arm, where the expected values are estimated in two categories based on the actual outcome—fetuses with CF and fetuses with no CF. The remainder of the structure is similar to model 1. Parents undergo genetic counselling followed by common CFTR mutation testing. If both parents are identified as carriers for common mutations, amniocentesis followed by known mutation analysis is performed in the fetus. The probability of CF is higher when FEB is present and one parent is identified as a carrier, compared with the risk of CF in the general population (Hodge et al. 1999; Ogino et al. 2004). Thus, if only one parent is identified as a carrier of a common mutation, they would have the choice of invasive testing and a whole gene screen in the fetus due to the risk of unidentified rare mutations. No fetal testing would be done in cases where no mutations are identified in the parents. However, there may be a residual risk of CF in the fetus due to unidentified rare mutations. The residual risk is

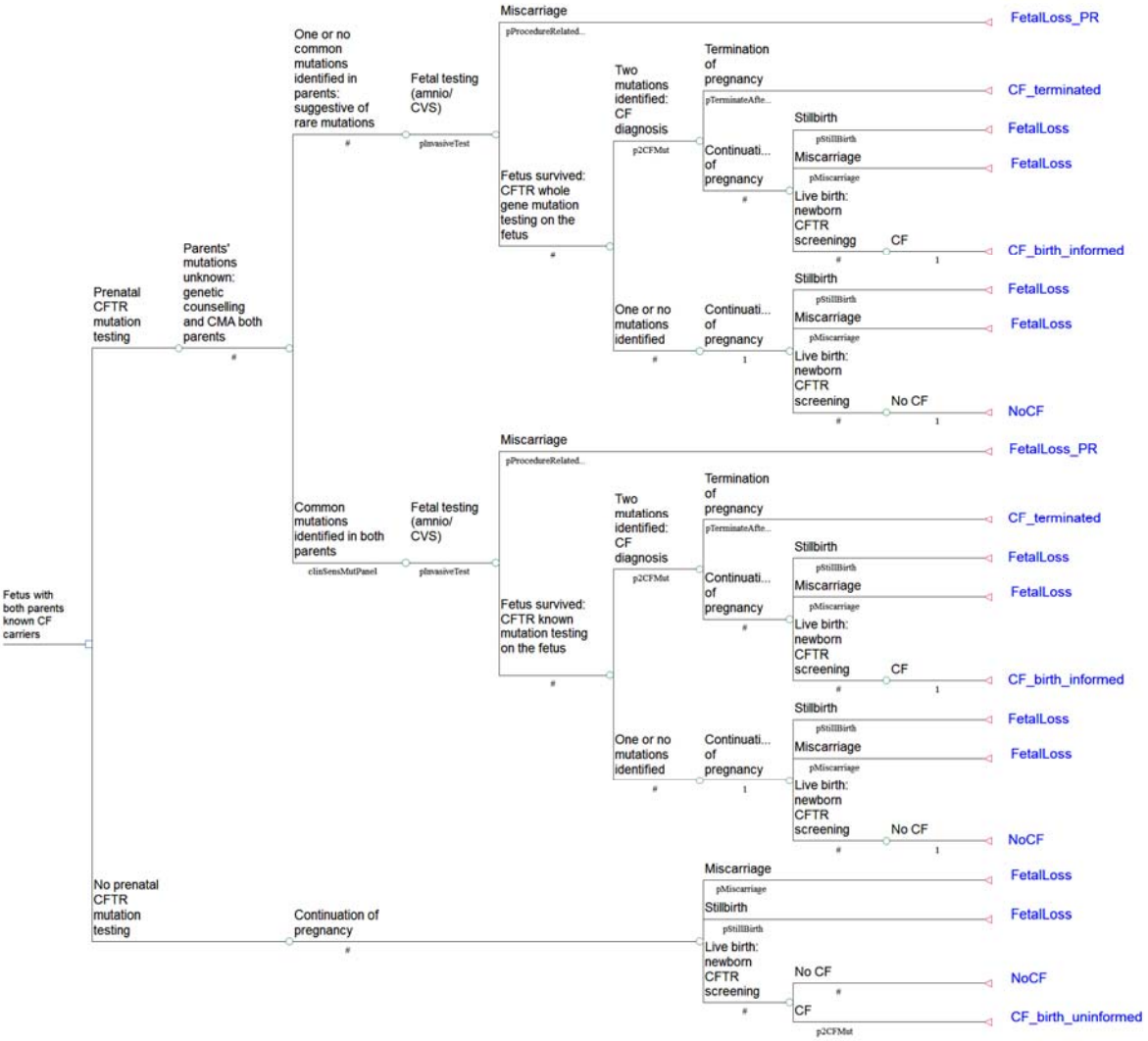
dependent on the sensitivity of the common mutation panel selected for the testing (see discussion under section C.1.2). As in model 1, parents would have the option of terminating the pregnancy if the fetus tests positive for CF, and pregnancies that are continued would follow the same path as in the no-testing arm.

In the comparator arm modelled pathways are similar to the comparator arm in model 1. The diagnosis of CF is made through NBS (IRT / DNA testing / sweat test) after the child's birth. Unlike model 1, only a small proportion of infants would undergo DNA and sweat testing additional to IRT assay. This is discussed in detail in section D.4.

Model assumptions

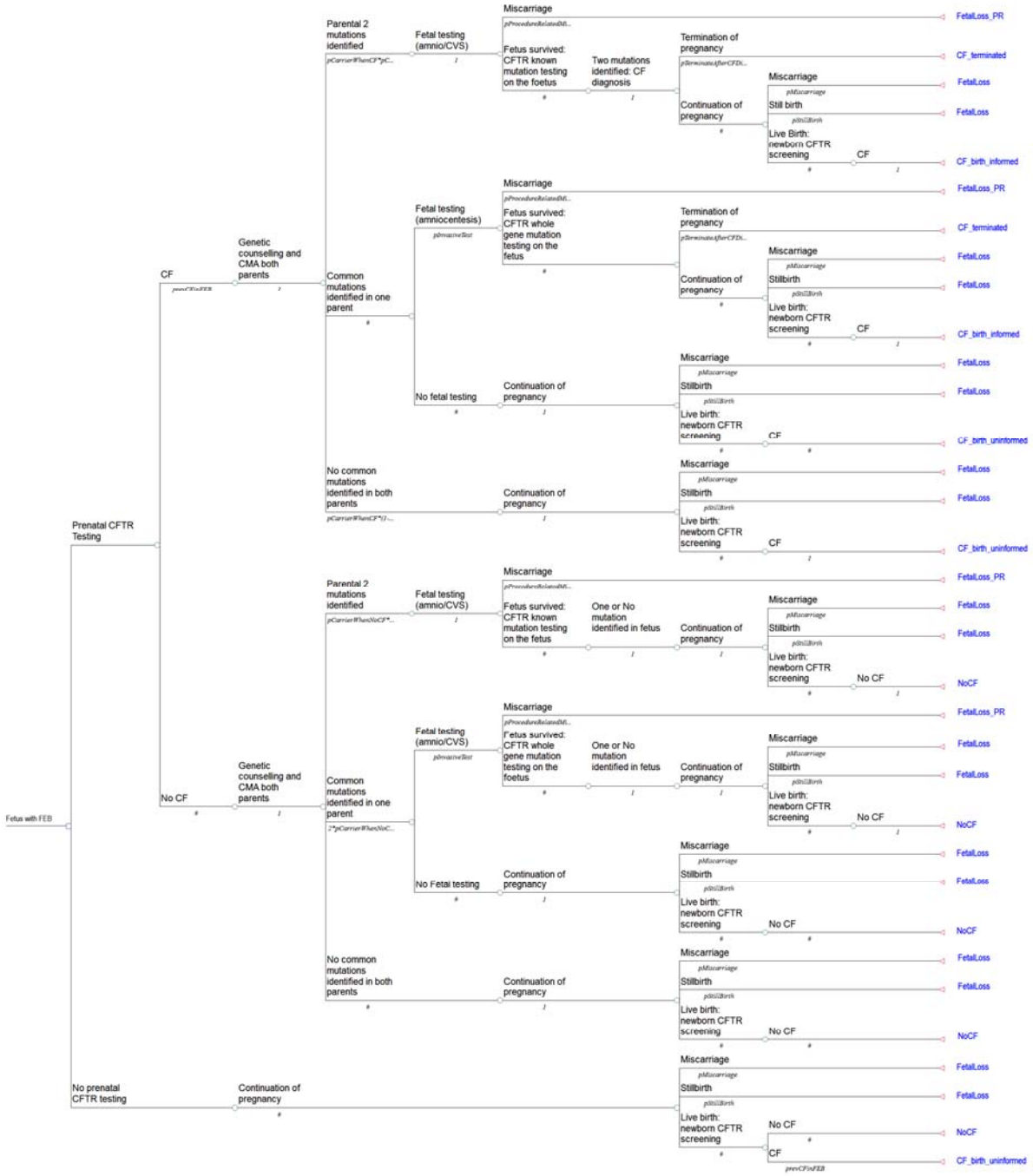
- Prenatal diagnostic CFTR testing is done within the valid timeframe to allow for TOP if CF is diagnosed.
- The parents accepting the test are prepared to take the risk of invasive testing.
- Consistent with the limited available evidence-base, mutation tests for this population group are assumed to be 100% sensitive and it is assumed that the tests used are appropriate for the mutations being identified. In the absence of any evidence, it is assumed that the tests are also 100% specific. The analytical validity of the diagnostic tests is, therefore, considered to be 100%; that is, the mutation tests are assumed to accurately detect the presence or absence of specific mutations. The impact of varying the test accuracy was not assessed in the sensitivity analyses (see section C.1.1 for further discussion).
- Parenting partnerships are considered to be stable and transparent (i.e. parents with a previous CF child between them are both assumed to be carriers).
- *De novo* mutations are very rare and thus not included in the model.
- To simplify the model structure, it is assumed that whole gene testing will detect all CFTR mutations. However, as discussed in section B.6.i, the residual risk for unidentified mutations is still 2% as large deletions/duplications involving the *CFTR* gene are not detectable by any sequencing assay.
- It is assumed that NBS will identify all infants with CF; however, in clinical practice it is found to be around 95% sensitive and nearly 5% of cases are missed and diagnosed later (Mishra, Greaves & Massie 2005). This will underestimate the cost-effectiveness of prenatal testing.

The structure of the economic evaluations is shown in Figure 6 and Figure 7.



amnio = amniocentesis; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CMA = common mutation analysis; CVS = chorionic villus sampling; FetalLoss_PR = procedure-related fetal loss;

Figure 6 Decision analytic structure of the economic evaluation for fetus at 1:4 risk of CF due to parents being carriers



amnio = amniocentesis; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CMA = common mutation analysis; CVS = chorionic villus sampling; FEB = fetal echogenic bowel; FetalLoss_PR = procedure-related fetal loss

Figure 7 Decision analytic structure of the economic evaluation for fetus at high risk of CF due to echogenic bowel diagnosed on second-trimester ultrasound

Model outcomes

The decision trees presented in Figure 6 and Figure 7 culminate in six different categories according to the chosen outcome measure. These are referred to as 'outcome states' and summarised in Table 55.

Table 55 Summary of decision tree final outcome states in the economic evaluation

Outcome state	Inference
Fetal loss	Loss of fetus due to natural miscarriage or stillbirth
Procedure-related fetal loss	Loss of fetus due to procedure-related miscarriage
No CF	Birth of a baby not affected with CF
CF-terminated	CF-affected pregnancy detected during prenatal testing and terminated
CF-birth-informed	Baby born with CF (pregnancy continued after CF diagnosis)
CF-birth-uninformed	Baby born with CF (CF-affected fetus undetected during prenatal testing and CF-affected birth in the comparator arm)

CF = cystic fibrosis

D.4. INPUTS TO THE ECONOMIC EVALUATION

D.4.1. TEST PARAMETERS

Analytical validity of CFTR *mutation testing*

As discussed in section C.1.1, the analytical validity of all CFTR mutation tests is assumed to be 100%.

Clinical sensitivity of CFTR *mutation testing*

A clinical sensitivity of 80% for a 10-mutation panel is assumed for CFTR common mutation testing in the base-case evaluations. Economic analyses relevant to current practice are presented for alternative scenarios, comprising panels with higher clinical sensitivity (see section C.1.2 and Table 50 for further details).

Probability of identifying CFTR *mutations in both parents*

In model 1 both parents are assumed to be definite carriers because of having a previous child with CF. However, the parental carrier status determination will depend on the clinical sensitivity of the panel selected for the common mutation testing. Therefore, common mutation analysis will identify around 64% of couples with two mutations and 36% with one or no mutations in the base-case evaluations. The detailed derivation of these probabilities is provided in Table 103, Appendix J.

In Australia 1 in 25 people are carriers of CFTR mutations. However, the carrier rate in model 2 is assumed to be greater than the carrier rate in the general population, due to the presence of FEB. The chances of identifying mutations in parents will depend on the clinical sensitivity of the common mutation panel and the prevalence of CF in FEB. Parents of fetuses diagnosed with CF are definite carriers, and the chances of identifying two mutations, one mutation and no mutations are 64%, 32% and 4%, respectively, using common mutation testing. For parents of fetuses with no CF the carrier rate as prevalent in the general population (4%) is assumed, and the identification of CFTR mutations is derived based on that. The model arms with one or no mutations identified in these parents will also include those who are not CFTR carriers (see Table 103, Appendix J, for detailed derivations).

Risk of CF in fetus

In cases where both parents are known carriers of CFTR mutations, there is a 1 in 4 chance (25%) that the child will have CF, compared with approximately 0.04% in the general population.

An incidence of 2% of CF cases is assumed in the FEB population in the base-case analysis. The effect of a higher incidence of CF (2%–13%) on the cost-effectiveness of CFTR prenatal testing in this population is investigated in the sensitivity analysis (see section C.1.3 for further details).

Risk of miscarriage

The background and procedure-related risks of miscarriage, as estimated in Table 51, section C.1.4, are used in the base-case analysis and sensitivity analysis. In summary, a background risk of 1.05% and a procedure-related risk of 0.15% are assumed for model 1, and a background risk of 0.67% and a procedure-related risk of 0.11% are assumed for model 2. In sensitivity analysis 95%CI ranges of background and procedure-related risks are tested.

Termination acceptance rate

Different termination acceptance rates are used for the modelled populations, as discussed in section C.1.5. In model 1 (fetus with parents known CF carriers) the termination uptake rate of 95% is assumed in the base-case analysis, whereas an uptake rate of 65% is assumed in model 2 (fetus with FEB).

The uptake of TOP can affect the outcome costs per CF birth averted; therefore, the effect of uptake rates between 80% and 100% for model 1 and between 30% and 100% in model 2 are assessed in the sensitivity analysis (Table 52).

Risk of stillbirth

In Australia stillbirth is classified as an infant who dies after 20 weeks' gestation or when they weigh more than 400 grams; babies lost before 20 weeks are termed miscarried. The risk of stillbirth is estimated to be 0.006 (6 per 1,000) from ABS data (2012)[14] and is assumed to be constant between model arms in both models 1 and 2.

Test parameters used in the economic models are summarised in Table 56.

Table 56 Summary of estimated values for test parameters used in the economic models

Parameter	Model 1: Both parents known CF carriers		Model 2: Fetus has FEB	
	Base-case	*Sensitivity analysis*	*Base-case*	*Sensitivity analysis*
Clinical sensitivity common mutation test	80%	80%–92%	80%	80%–92%
Risk of CF	25%	Not varied	4.5%	2%–13%
Background risk of miscarriage	1.05%	(0.51%, 1.8%)	0.67%	(0.46%, 0.91%)
Procedure-related risk of miscarriage	0.15%	(0%, 0.57%)	0.11%	(0%, 0.26%)

[14] <http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/3303.0main+features100062012>, accessed on 12 January 2015

Parameter	Model 1: Both parents known CF carriers		Model 2: Fetus has FEB	
Risk of stillbirth	0.006	Not varied	0.006	Not varied
TOP acceptance rate	95%	80%–100%	65%	30%–100%

CF = cystic fibrosis; FEB = fetal echogenic bowel; TOP = termination of pregnancy

D.4.2. HEALTHCARE RESOURCES

Cost of genetic testing

The applicant did not provide a proposed item fee for each of the proposed tests. At present the cost of different CFTR mutation tests and the number of mutations tested varies across Australia (see Appendix I). The cost of common mutation testing is identified as between $168 and $300 per test, for testing of between 11 and 44 CFTR mutations. Single mutation testing varies between $50 to $160, and whole gene sequencing costs around $1,000. In accordance with proposed MBS listing (see Table 8, section A.3), the cost of a panel-based test corresponding to detecting 10 mutations was searched. Maxwell et al. (2010) suggested a figure of $116.77 per test for prenatal screening based on a 10-mutation panel, including costs for sample collection, DNA extraction, labour and consumables. This estimate is considered reasonable and is assumed in the base-case evaluations, converted to 2014 dollars (i.e. $135).

Among other scenarios, only test costs corresponding to 32 mutations were found, ranging between $200 and $290 (see Table 102). The technology and cost of DNA diagnostic testing for a CF mutation are changing rapidly. These costs will likely decline and the number of mutations screened will quickly increase. Therefore, the cost of a 32-mutation panel test is assumed to be $200. For panels including 17 mutations and 23 mutations, costs of $150 and $170, respectively, are assumed. Some of the laboratories in Australia test higher number of mutations for a lower price; for example, Healthscope Pathology in Victoria charges $150 for testing 44 CFTR mutations[15]. However, the corresponding data for clinical sensitivity was not available; therefore, these scenarios were not included in the analysis. Table 57 summarises the costs estimated for common mutation testing for all scenarios. The diagnostic tests used in the analysis are assumed to include costs for sample collection, DNA extraction, labour, consumables and additional costs associated with test failure, and these costs are not distinctly included in the model.

[15] <http://www.healthscopepathology.com.au/index.php/general-pathology/self-sampling/cystic-fibrosis-carrier-screening/>, accessed on 13 March 2015

Table 57 Costs of common mutation testing used for the scenarios analysed in the economic models

Number of common mutations included in the panel	Cost	Source	Model usage
10	$135	Maxwell et al. (2010)	Base-case analysis
17	$150	Assumption [a]	Additional scenario 1
23	$170	Assumption [b]	Additional scenario 2
32	$200	Table 102, Appendix I	Additional scenario 3

[a] Based on lowest identified published price of 'common mutation test' available—in actual fact this price is quoted for 38- and 44-mutation panels (see Table 101, Appendix I).
[b] Arbitrary value chosen within the range of costs of 17 mutation panel and 32 mutations panel

A cost of $80 is assumed for single mutation testing (Table 102). According to Australian data (Figure 1), approximately 85% of CF patients have at least one copy of the F508del mutation, with 52% having two copies. Therefore, it is assumed that single mutation testing is performed in 65% (52%–80%) of the fetuses of parents identified as carriers, and the remaining 35% will have common mutation testing to identify the other known mutations. Therefore, a weighted cost of common mutation and single mutation testing ($99) is used for known mutation analysis for diagnosis of CF in fetuses in both models. The cost of whole gene sequencing is assumed to be $1,000 (see Table 102) in the base-case evaluations. The applicant advised that this cost may be valid for gene sequencing done using conventional Sanger sequencing. However, the recent next-generation sequencing (NGS) based on throughput is promising as a more cost-effective option for whole gene sequencing, potentially halving this cost[16]. Subsequently, the impact of lowering the cost of whole gene testing to $500 is assessed in the sensitivity analysis. The estimated costs for different genetic tests used in the evaluation are summarised in Table 58.

Table 58 Estimated cost of CFTR mutation testing used in base-case economic evaluations

Test type	Cost
Common mutation test (parents)	$135
Single mutation test [a] (fetus)	$80
Known mutation test (fetus)	$99
Whole gene sequencing (fetus)	$1,000

[a] A single mutation test is performed to identify one or two F508del mutations.
CFTR = cystic fibrosis transmembrane conductance regulator

Cost associated with medical procedures

The costs of CVS, amniocentesis and a sweat test were estimated using MBS item numbers 16603 ($121.85), 16600 ($63.50) and 66686 ($50.65), respectively.

[16] Pers. comm. from applicant, received 12 January 2015

In model, 1 where the fetus has known parent carriers, the proportions of invasive tests performed by amniocentesis and CVS are estimated to be 66% and 34%, respectively (see section C.1.4). Therefore, a weighted cost of $83.34 (0.66*$63.50 + 0.34*$121.85) is used in model 1. However, fetuses in model 2 will undergo amniocentesis only (as CVS is unavailable at the gestational age when FEB is identified), and so the cost of amniocentesis alone ($63.50) is used in model 2 for invasive testing.

The costs of termination, delivery and miscarriage were estimated using Australian Diagnosis-Related Group (DRG) data from 2011 to 2012. These data provide an estimate of the total cost of managing a specific type of patient including a wide range of different types of cost. In the present model DRGO05Z, DRGO60C and DRGO63Z were used, thus estimating the total cost of a termination to be $2,257 ($2,412 in 2014 dollars); a single, uncomplicated vaginal delivery to be $3,588 ($3,834 in 2014 dollars); and a termination without procedures to be $1,775 ($1,897 in 2014 dollars).

Cost of medical practitioner (clinical geneticist)

The cost of genetic counselling by a clinical geneticist is estimated to be $263.90 (for the initial consult) and $132.10 (for subsequent consults) from MBS item number 132, which covers specialist professional attendance of at least 45 minutes' duration for an initial assessment of a patient, and MBS item number 133 for specialist professional attendance of at least 20 minutes' duration subsequent to the first attendance, in a single course of treatment for a review of a patient.

Cost of newborn CF diagnosis

As discussed in section A.2.2, NBS for CFTR is a three-tier process with IRT immunoassay offered to all neonates, CFTR mutation analysis offered to infants with elevated IRT and at a high risk of having CF (i.e. with a family history or other relevant medical conditions such as meconium ileus), followed by sweat tests and parental genetic counselling for those identified with one mutation. It is unclear how much of each of these levels of screening activity is being done currently, and the likely degree of substitution if prenatal testing were to be implemented. Therefore, a weighted cost of newborn CFTR diagnosis is derived based on the number of tests estimated to be performed for the infants in each group, conditional on the associated risk of CF.

In model 1, due to a family history of CF, all infants will undergo IRT and CFTR testing, and approximately 50% of the infants will undergo a sweat test (i.e. the carrier rate in the 1:4 risk population is 50%).

It is estimated that the majority (66%) of FEB pregnancies will be normal at birth (i.e. benign and transient FEB), and others will be potentially associated with medically significant outcomes such as CF, aneuploidy, growth restrictions and infections (Simon-Bouy et al. 2003). Also, in infants with

bowel obstructions the IRT level is quite often not elevated and genetic testing is therefore recommended due to the higher incidence of CF in this population[17]. Consequently, in the model 2 base-case analysis, 34% of infants with a history of FEB are considered to be at higher risk and will undergo IRT and CFTR testing followed by sweat tests in carriers. However, as discussed in section D.3, prenatal invasive diagnostic testing may be able to detect other congenital abnormalities, although this evaluation has focused on CFTR diagnostic testing alone. Nevertheless, to the extent that ultrasound screening may be capable of detecting other significant abnormalities, the overall benefits and estimates of cost-effectiveness associated with the process are underestimated.

The carrier rate in the FEB population is expected to be higher compared with that in the general population, as sourced from the literature. Scotet et al. (2010) reported a carrier rate of 6.6% in a review of cases of FEB diagnosed in pregnant women living in Brittany and referred for *CFTR* gene analysis over the period 1992–2007. In a study of a neonatal CFTR screening program in South Australia, Ranieri et al. (1994) found that approximately 7% of the neonates selected for mutation analysis, based on elevated IRT or other risk factors, were carriers for five of the common mutations included in the mutation panel. Therefore, in model 2 it is assumed that 7% of those tested for mutations will be offered sweat tests and parental genetic counselling.

The unit costs of IRT and CFTR genetic screening are difficult to estimate since these are performed as part of neonatal screening and are block funded. The CFTR genetic testing as part of NBS may be limited to either three mutations (F508del, G542X and G551D) or a broader mutation panel via a CFTR diagnostic lab. There are no common sets of mutations being tested across laboratories in Australia[18]. Victorian Clinical Genetics Services (VCGS), which operates Victoria's NBS program, was contacted during the assessment. VCGS indicated that a first-tier IRT immunoassay would cost around $5 per sample (including consumables, labour and overheads) and around $100 per sample for testing CFTR mutations[19].

According to HESP member advice, the CF screen is only one part of NBS, and all babies should undergo screening (including CF) to minimise the risk of inadvertently missing cases irrespective of prenatal testing results[20]. Therefore, NBS costs for neonates who have had prenatal CF testing would be similar to those without prenatal screening except that the cost of genetic counselling in these cases is assumed to be $132.10 (MBS item number 133 for subsequent professional consultations). A sensitivity analysis is performed excluding costs of CFTR testing from the NBS costs in prenatally tested infants, assuming that genetic testing is not required once the CF status is determined. Table 59 provides the estimated cost of newborn CF screening used in the base-case economic models.

[17] Pers. comm. with an HESP member, received 26 March 2015
[18] Pers. comm. with an HESP member, received 26 March 2015
[19] Pers. comm. with Victorian Clinical Genetics Services, received 27 March 2015
[20] Pers. comm. with an HESP member, received 26 March 2015

Only common mutation panel testing is included as part of an NBS program, and whole gene testing for infants with inconclusive results is not considered. These are included as part of the current proposed MBS item number 4 (see section A.3).

Table 59 Weighted cost of newborn screening for cystic fibrosis

Resource	Cost (A)	Model 1 – Both parents known CF carriers (B)	Model 2 – Fetus has FEB (C)
IRT immunoassay	$5.00	100%	100%
CFTR testing	$100.00	100%	34%
Sweat test	$50.65	50%	2.4% [b]
Genetic counselling [a]	$263.90	50%	2.4% [b]
Weighted NBS cost (for infant with no prenatal CFTR testing)		∑A×B = $262.28	∑A×C = $46.49
Weighted NBS cost (for infant with prenatal CFTR testing)		$196.38	$43.35

[a] Cost of genetic counselling for NBS in infant who had prenatal testing is assumed to be $132.10 (MBS item number 133).
[b] This is calculated as 7% of the 34% of infants tested for CFTR mutations.
CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; FEB = fetal echogenic bowel; IRT = immunoreactive trypsin; IRT = immunoreactive trypsin; NBS = newborn screening

Prenatal diagnostic testing provides parents with information on fetal status and may allow them to consider the options of either terminating or continuing the affected pregnancy. Identifying a fetus at risk presents a change in routine management of pregnancy. The primary outcomes of interest in this assessment are the cost per prenatal CF detected and the cost per CF birth averted. The incremental cost per prenatal CF detected and the incremental cost per pre-informed CF birth will provide the incremental cost for additional information (prenatal diagnosis), and the ICER per CF birth averted will provide the incremental cost for integrating change in pregnancy management (i.e. termination of affected pregnancy) as a result of testing. Accordingly, the cost per CF diagnosis takes into account the costs of prenatal testing (parental mutation testing and fetal diagnostic testing), termination, miscarriage, stillbirth, live birth and NBS. Table 60 summarises the costs of medical tests and services used in models 1 and 2.

Table 60 Cost of health resources used in the economic evaluations

Resource	Model 1 – Both parents known CF carriers	Model 2 – Fetus has FEB	Source
Invasive test (weighted cost)	$83.34	$63.50	MBS item numbers 16600 and 16603
Sweat test	$50.65	$50.65	MBS Item number 66686
Genetic counselling (initial)	$263.90	$263.90	MBS Item number 132
Genetic counselling (subsequent session)	$132.10	$132.10	MBS Item number 133
Termination of pregnancy (in 2014 dollars)	$2,412.00	$2,412.00	DRG O05Z
Miscarriage (in 2014 dollars)	$1,897.00	$1,897.00	DRG O63Z

Resource	Model 1 – Both parents known CF carriers	Model 2 – Fetus has FEB	Source
Single, uncomplicated vaginal delivery (in 2014 dollars)	$3,834.00	$3,834.00	DRGO60C
NBS (for infant with no prenatal CFTR testing)	$262.28	$127.02	Table 59

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; FEB = fetal echogenic bowel; DRG = Diagnosis-Related Group; MBS = Medicare Benefits Schedule; NBS = newborn screening

Costs excluded

The cost of other health resources, such as antenatal care and management by general physicians / obstetricians, routine blood tests and ultrasounds, apply similarly across both intervention and comparator arms of the models, and therefore are not included in the analysis.

Of note, some economic studies include the lifetime extra cost of care for infants born with CF, which are then used to offset the costs of prenatal diagnosis to show plausible savings associated with prenatal CF screening. As CF is an irreversible and progressive disease, the healthcare costs associated with managing CF are very high and rise with disease progression. In a recent study Van Gool et al. (2013) analysed costs of care of CF by age and health states based on data from the Australian Cystic Fibrosis Data Registry (ACFDR), and reported the mean annual cost for managing patients with CF to be $26,279, with a lifetime healthcare cost of $516,413 (3.5% discount rate)[21]. The average costs for patients with severe disease were reportedly three times higher than those for patients with mild disease.

In a simple cost analysis, including such costs will greatly favour intervention. However, where cost-effectiveness analysis is required, a time horizon including lifetime costs necessitates (to eliminate bias) a requirement for estimates of lifetime outcomes such as quality-adjusted life years—that is, lifetime utility estimates associated with CF and utility effects associated with TOP—and potentially but not necessarily subsequent pregnancies and children that are dependent on the decision to terminate or not. Given that such estimates are not able to be reliably estimated, including only the cost of care for a CF child would provide a distorted picture of the cost-effectiveness of prenatal CFTR testing. Therefore, the economic analysis undertaken for this assessment considers the time horizon of pregnancy to NBS but does not attempt to make comparisons regarding lifetime utilities and costs of care in CF or non-CF patients.

[21] Costs were reported in 2009 USD and are converted to 2014 AUD using the Campbell and Cochrane Economics Methods Group (CCEMG) – 'Evidence for policy and practice information and co-ordination (EPPI)-Centre cost converter. <http://eppi.ioe.ac.uk/costconversion/default.aspx>, accessed on 8 April 2015.

D.5. RESULTS OF THE ECONOMIC EVALUATION

The model provides several possible outcomes, including fetal loss (i.e. miscarriage and stillbirth), unplanned fetal loss (i.e. procedure-related miscarriage), elective termination of a CF-affected fetus, birth of a healthy baby with no CF, and birth of a CF-affected baby (Table 55). The cost-effectiveness measures analysed relate to the costs associated with the clinically important outcomes. These are the cost per case of CF diagnosed prenatally, the cost per pre-informed CF birth and the cost per CF birth averted. The results of the economic evaluation are presented for four scenarios based on the number of mutations in the common mutation panel test. The different panel sizes vary with respect to clinical sensitivity and the cost associated with common mutation testing, as discussed in section C.1.2 and section D.4. The scenarios are summarised below:

- Base-case scenario: common mutation panel test identifying 10 mutations with 80% sensitivity at a cost of $135.
- Additional scenario 1: common mutation panel test identifying 17 mutations with 83.5% sensitivity at a cost of $150.
- Additional scenario 2: common mutation panel test identifying 23 mutations with 88% sensitivity at a cost of $170.
- Additional scenario 3: common mutation panel test identifying 32 mutations with 92% sensitivity at a cost of $200.

The following is a summary of the results of the economic evaluation:

- disaggregated by decision tree probabilities, results per outcome states for base-case scenario only (additional scenarios are presented in Appendix J)
- for the base-case scenario: the incremental cost-effectiveness for prenatal CF detected, pre-informed CF birth and CF birth averted (additional scenarios in Appendix J)
- for each of the three additional scenarios, incremental cost-effectiveness
- numerous sensitivity analyses (see section D.6).

D.5.2. OUTCOME PROBABILITIES AND INCREMENTAL EFFECTS

The results of the decision tree analysis are presented in Figure 10 (model 1) and Figure 11 (model 2), Appendix J. The probability at each decision tree terminal of the intervention (prenatal testing) arm is derived from a composite of the incidence of CF in the tested population, clinical sensitivity of the common mutation panel, carrier rate in the parents, risk of fetal loss and uptake rate of termination of affected pregnancy. The comparator arm presents the natural outcomes of pregnancy in the absence of prenatal CFTR testing followed by diagnosis of CF through NBS.

The outcomes and incremental effects (per 100 pregnancies), as derived from the decision tree analysis, are presented in Table 61 and Table 62. All outcomes may be considered clinically relevant.

Table 61 Outcome and incremental effects (per 100 pregnancies) for base-case scenario in model 1 (Both parents known CF carriers)

Outcome	Prenatal testing	No prenatal testing	Incremental effectiveness	Nature of effect
Prenatal CF diagnosed	24.94	0	24.94	Benefit
CF births total:	1.23	24.59	–23.36	
informed	1.23	0	1.23	Benefit
uninformed	0.0	24.59	–24.59	
CF birth averted	23.72	0	23.72	Benefit
Fetal loss	1.26	1.65	–0.40	
Procedure-related fetal loss	0.15	0	0.15	Harm
No CF	73.65	73.76	–0.11	-

CF = cystic fibrosis

Table 62 Outcome and incremental effects (per 100 pregnancies) for base-case scenario in model 2 (Fetus with FEB)

Outcome	Prenatal testing	No prenatal testing	Incremental effectiveness	Nature of effect
Prenatal CF diagnosed	3.58	0	3.58	Benefit
CF births total:	2.13	4.44	–2.31	
informed	1.24	0	1.24	Benefit
uninformed	0.89	4.44	–3.55	
CF birth averted	2.34	0	2.34	Benefit
Fetal loss	1.24	1.27	–0.03	
Procedure related fetal loss	0.007	0	0.007	Harm
No CF	94.28	94.29	0.00	-

CF = cystic fibrosis; FEB = fetal echogenic bowel

Since fetuses are diagnosed through NBS in the comparator arm, the prenatal CF diagnosis is zero in this strategy. Similarly, the procedure-related fetal loss is assumed to be zero in this strategy since there is no invasive testing performed on the fetuses for CFTR mutation testing. There are fewer CF-affected births in the intervention arm compared with the comparator since prenatal testing is associated with a change in pregnancy management (generally termination of affected fetuses), resulting in fewer CF births. The number of babies born without CF would be similar across the two strategies in both models.

The incremental effectiveness is derived by subtracting outcomes of the no prenatal testing strategy from outcomes of the prenatal testing strategy. Overall, prenatal testing is associated with benefits due to the increased rate of prenatal CF diagnoses and the lower number of babies born with CF, and is driven by a change in management (i.e. terminations of CF-affected fetuses) and the lower number of uninformed CF births, although there is a small risk of harm due to procedure-related fetal loss (i.e. miscarriages due to invasive testing).

D.5.2. INCREMENTAL COSTS

The incremental cost and cost-effectiveness of prenatal CFTR testing compared with no prenatal testing for both modelled populations are presented in Table 63. Prenatal CFTR testing is associated with incremental costs ($450 for model 1 and $544 for model 2) largely driven by the cost of CFTR mutation testing in parents and fetuses, and the costs associated with elective terminations, offset by the costs incurred during childbirth and NBS. The incremental costs are calculated per individual pregnancy in Table 63 below. These are multiplied by 100 to estimate the incremental costs for a cohort of 100 pregnancies as in Table 64.

Table 63 Incremental costs, base-case scenario

Cost	Prenatal testing	No prenatal testing	Increment
Model 1 (Parents are known CF carriers)			
Cost per pregnancy	$4,521.66	$4,071.61	$450.05
Model 2 (Fetus has FEB)			
Cost per pregnancy	$4,410.45	$3,866.92	$543.54

CF = cystic fibrosis; FEB = fetal echogenic bowel

D.5.3. INCREMENTAL COST-EFFECTIVENESS

The incremental cost-effectiveness estimates are calculated as below:

- Cost per outcome of interest

$$ICER = \frac{cost\ of\ prenatal\ testing - cost\ of\ current\ practice}{number\ of\ outcomes\ (of\ interest)\ with\ testing\ available - number\ of\ outcomes\ (o)}$$

Table 64 Incremental cost-effectiveness ratios, base-case scenario

Clinically relevant outcomes	Incremental outcomes (per 100 pregnancies)	ICER ($/outcome)
Model 1 (Parents are known CF carriers)	*Incremental cost: $45,005*	
Diagnosis of CF in utero	24.94	$1,804 / prenatal CF detected
CF birth averted	23.72	$1,898 / CF birth averted
Pre-informed CF birth	1.23	$36,649 / pre-informed CF birth
Model 2 (Fetus has FEB)	*Incremental cost: $54,354*	
Diagnosis of CF in utero	3.58	$15,182 / prenatal CF detected
CF birth averted	2.34	$23,254 / CF birth averted
Pre-informed CF birth	1.24	$43,727 / pre-informed CF birth

CF = cystic fibrosis; FEB = fetal echogenic bowel; ICER = incremental cost-effectiveness ratio

Consideration of the identified costs in the context of the estimated lifetime costs of CF-related healthcare (estimated at ~$516,413/CF-affected person (discounted); see section D.4 under *Excluded costs*) indicates that genetic testing results in a significant saving in healthcare costs. However, from an economic perspective this should be interpreted with caution since it is a cost

analysis only and does not take into account the value associated with clinical outcomes; that is, life, CF-affected or otherwise.

Furthermore, the calculated ICERs above consider only one relevant outcome at a time and do not simultaneously incorporate the multiple outcomes of clinical relevance. This makes the overall value difficult to interpret for decision-making purposes. Therefore, an alternative presentation of the economic model that incorporates all outcomes is presented, and calculation of the collective outcomes resulting from a given expenditure associated with the proposed testing is presented.

Summary of model 1: Both parents are known CF carriers

For every additional net spend of $50,000 associated with the listing—that is, $60,991 will be spent directly on testing but a saving of $10,991 will be made on other associated costs (e.g. birth of CF-affected child, diagnosis of CF after birth and genetic counselling)—the following outcomes will be obtained:

Benefits

- 111.1 pregnancies will be tested, and from this
- 27.7 in-utero diagnoses of CF will be made, enabling:
    - 26.35 CF births to be averted, and
    - 1.36 CF births to occur with pre-informed parents.

Harms

- 0.17 additional unplanned fetal losses (i.e. 1/654 pregnancies tested) will occur.

Summary model 2: Fetus with FEB

For every additional $50,000 spent associated with the listing—that is, $28,489 will be spent directly on genetic testing and $21,511 will be the associated costs—the following outcomes will be obtained:

Benefits

- 92 pregnancies will be tested
- 3.29 in-utero diagnoses of CF will be made, enabling:
    - 2.15 CF births to be averted, and
    - 1.14 CF births to occur with pre-informed parents.

Harms

- 0.01 additional unplanned fetal losses (i.e. 1/9,200 pregnancies tested) will occur, and
- 0.82 births with CF undetected despite in-utero testing (i.e. 1/112 pregnancies tested) will occur.

The overall and incremental costs and key outcomes (i.e. CF births diagnosed, averted) for the test and comparator in the model, for the additional scenarios with varying common mutation panels, are calculated and presented in Appendix J. A summary of the incremental costs per CF births averted is shown in Table 65.

Table 65 Incremental cost-effectiveness ratios for additional scenarios with increased numbers of mutations included in the common mutation testing panel

Strategy	17-mutation panel	23-mutation panel	32 mutation panel
Model 1 (Parents are known CF carriers)			
Incremental cost per prenatal CF diagnosed	$1,816	$1,840	$1,977
Incremental cost per CF birth averted	$1,910	$1,935	$2,079
Model 2 (Fetus has FEB)			
Incremental cost per prenatal CF diagnosed	$15,331	$15,537	$16,304
Incremental cost per CF birth averted	$23,480	$23,794	$24,972

CF = cystic fibrosis; FEB = fetal echogenic bowel

Prenatal testing is most cost-effective in the base-case scenario (i.e. testing for 10 CFTR mutations with 80% sensitivity and a cost of $135) in both models. In this scenario the cost of the common mutation test is the lowest. In model 1 the higher clinical sensitivity results in higher effectiveness as a larger number of mutations will be detected using the common mutation test, reducing the use of costlier whole gene sequencing. In model 2 the higher clinical sensitivity will reduce the use of whole gene testing as well as the number of undiagnosed cases of CF. While scenarios with higher test sensitivity result in an increased incremental effectiveness, this is also associated with an increased cost of the extended mutation panel.

D.6. SENSITIVITY ANALYSIS

Sensitivity analysis considers variations on key assumptions including clinical sensitivity of the common mutation test, cost of whole gene sequencing, incidence of CF in fetuses with FEB, uptake of pregnancy termination and uptake of invasive testing in FEB (where only one parent is identified as a carrier), and fetal loss due to procedure-related miscarriages for the base-case (10-mutation panel) scenario using 95%CIs or plausible upper and lower limits. The analyses are presented in a tornado analysis for both model 1(Figure 9), and key results (reported ICERs are for incremental cost per CF birth averted) are summarised in Table 66.

In model 1 the tornado analysis indicates that the ICER with respect to $/birth averted is most sensitive to the uptake rate of terminations of CF-affected pregnancies (i.e. ICER exceeding $2,500/CF birth averted, where termination rate decreases to 80% for CF-affected pregnancies) followed by the cost of NBS in infants tested prenatally, the test sensitivity of the common mutation panel and the cost of whole gene sequencing.

In model 2 the ICER is most sensitive to changes in the incidence of CF in the tested population (i.e. ICER exceeding $53,000/CF birth averted when the incidence of CF within those with FEB is 2%) and

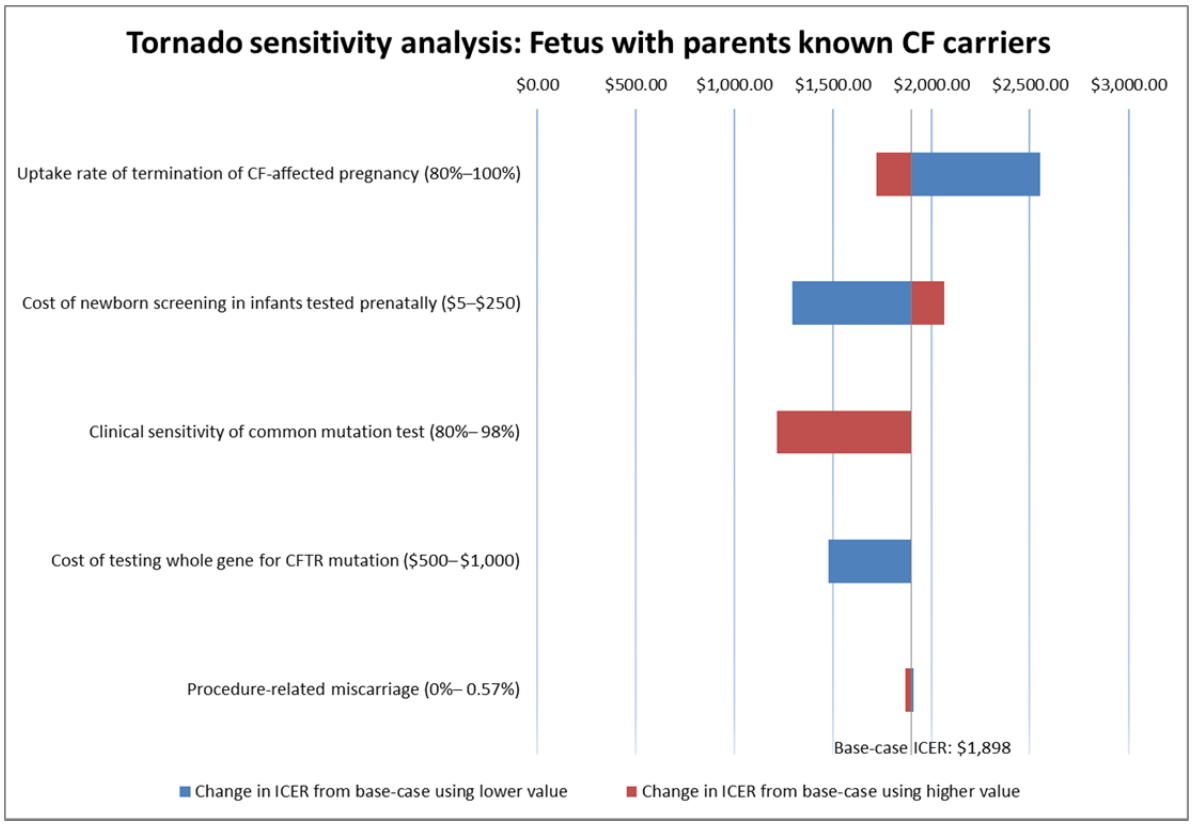
termination of pregnancy uptake rates. The uptake rates of invasive testing when one parent is identified as a carrier and the clinical sensitivity of the common mutation test have a lower impact on the cost-effectiveness results in this model.

Termination rate in CF-affected pregnancies

The termination uptake rates of CF-affected pregnancies in the tested populations could vary considerably depending on the personal choices of parents and the timely diagnosis of affected pregnancies. As previously discussed in section C.1.5, parents with a previous child diagnosed with CF are expected to have higher uptake rates of terminations of affected pregnancies compared with parents of fetuses with FEB, as some CF-affected pregnancies with FEB may be diagnosed too late for termination. Therefore, the termination uptake rates were varied in the range 80%–100% for model 1 and 30%–100% for model 2 in the sensitivity analyses. The higher rate of terminations will result in fewer CF births and thus higher cost-effectiveness of the intervention, in contrast to a higher number of CF births and lower cost-effectiveness (i.e. ICER exceeding $52,077/CF birth averted for 30% terminations in FEB) with a lower rate of terminations.

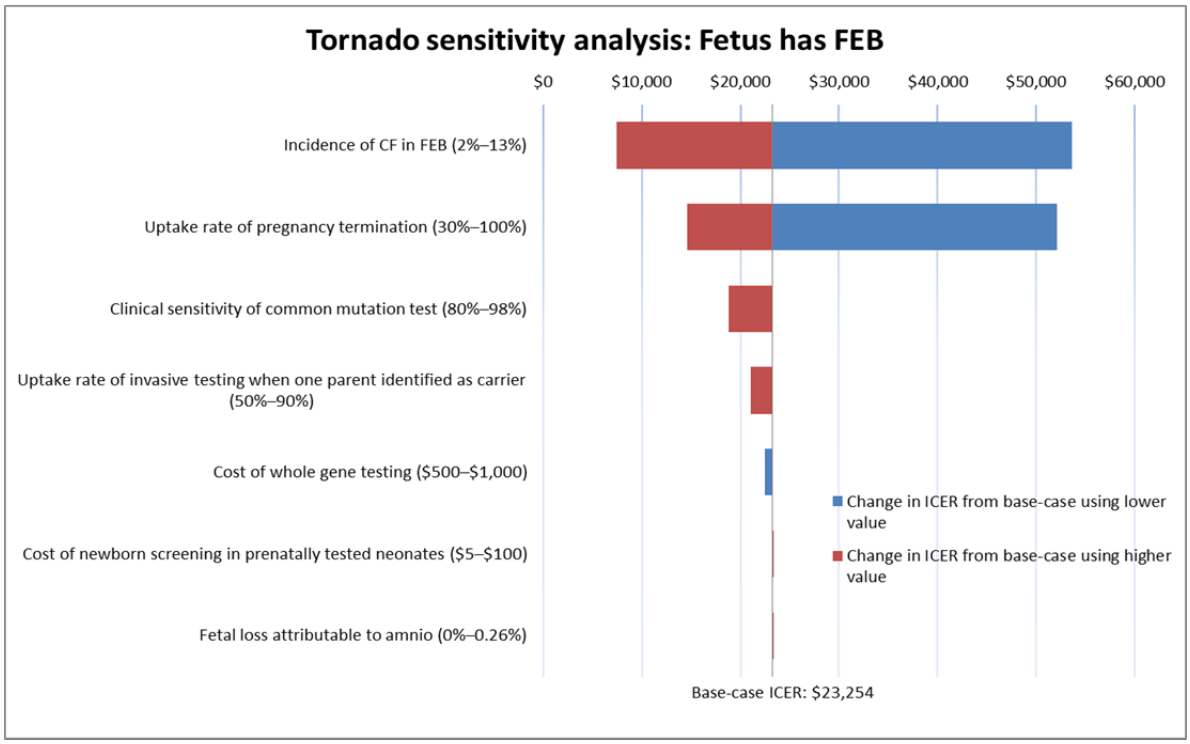
Incidence of CF in the FEB population

There is considerable uncertainty in the estimated incidence of CF in the FEB population. The literature review identified that the incidence of CF varied in the range 2%–13%, whereas the two Australian studies reported incidence rates of 3% and 6%. Therefore, a range of 2%–13% was applied in the tornado sensitivity analysis. Increasing the incidence was observed to increase the cost-effectiveness of prenatal testing; and, conversely, decreasing the incidence decreased the cost-effectiveness of prenatal testing, increasing the incremental cost per CF birth averted to $53,565 in the FEB population (model 2).



CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; ICER = incremental cost-effectiveness ratio

Figure 8 Tornado sensitivity analysis, model 1 (Both parents known CF carriers)



CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; FEB = fetal echogenic bowel; ICER = incremental cost-effectiveness ratio

Figure 9 Tornado sensitivity analysis, model 2 (Fetus has FEB)

Duplication of prenatal genetic testing costs in postnatal screening

In the economic models NBS is not substituted by prenatal testing in the intervention arm, and is assumed to be performed for all infants as per the standard NBS protocol, irrespective of prenatal testing[22]. This increases the costs per diagnosis in the intervention arm as the cost of *CFTR* testing is counted twice. A range of $5 (the cost of IRT immunoassay only)–$250 (costs including IRT, mutation testing in infants, sweat test in carriers and genetic counselling) was applied for the cost of NBS in infants who had prenatal CFTR testing in model 1, and the range in model 2 was $5–$100. In model 1 (where parents are known carriers) the ICER ($1,898/CF birth averted) is sensitive to this parameter, and resulting ICERs ranged from $1,294 to $2,067 per CF birth averted). In model 2 (where fetus has FEB) these costs do not significantly change the cost-effectiveness, possibly due to the low incidence of CF and CF carriers.

The cost of whole gene sequencing

In model 1 (where parents are known carriers) when the test sensitivity is 80% (base-case scenario), 64% of couples will be identified as homozygous for common mutations and offered known mutation testing in the fetus; whereas the remaining 36% will be either heterozygous for common and rare mutations, or homozygous for rare mutations, and thus will be offered whole gene sequencing for fetal testing. Subsequently, halving the cost of whole gene sequencing to $500 reduces the cost per diagnosis, decreasing the ICER ($1,477/CF birth averted for whole gene test cost is $500). This change is not observed in model 2 (where the fetus has FEB), where the frequency of CF mutations is much lower.

Invasive testing uptake rate

Uptake rates of invasive testing have a low impact on the ICER in model 2 (fetuses with FEB) in the base-case scenario due to low incidence of CF (3%), but would probably have a higher impact if the incidence of CF was higher in this population. Procedure-related miscarriages seem to have no impact on the ICERs in both models, likely due to the very low rate of this outcome.

The key results of the sensitivity analyses are summarised in Table 66.

Table 66 Key drivers of the economic models

Description	Values assessed in sensitivity analysis	ICER using lower value $/*CF birth averted*	ICER using higher value $/*CF birth averted*	Impact
Model 1 – Parents are known CF carriers				*(Base-case ICER $1,898/CF birth averted)*

[22] Pers. comm. with an HESP member, received 26 March 2015

Description	Values assessed in sensitivity analysis	ICER using lower value $/*CF birth averted*	ICER using higher value $/*CF birth averted*	Impact
Uptake of termination of affected pregnancy	Varied in the range 80%–100%	$2,553	$1,723	High; lower termination uptake rates favour comparator
Cost of NBS in infants tested prenatally	Varied in the range $5–$250	$1,294	$2,067	High; retesting neonates for CFTR mutations during NBS favours comparator
Clinical sensitivity of common mutation test	Varied in the range 80%–98%	$1,898	$1,215	High; higher clinical sensitivity favours intervention
Cost of whole gene sequencing	Varied in the range $500–$1,000	$1,477	$1,898	Moderate; lowering the cost favours intervention
Model 2 – Fetus has FEB				*(Base-case ICER $23,254/CF birth averted)*
Incidence of CF in FEB population	Varied in the range 2%–13%	$53,565	$7,398	High; higher incidence of CF in FEB population favours intervention
Uptake of termination of affected pregnancy	Varied in the range 30%–100%	$52,077	$14,607	High; lower termination uptake rates favour comparator
Clinical sensitivity of common mutation test	Varied in the range 80%–92%	$23,254	$18,800	Low; higher clinical sensitivity favours intervention
Uptake of invasive testing when one parent is identified as a carrier	Varied in the range 50%–90%	$23,254	$20,993	Low; higher values favour intervention

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; FEB = fetal echogenic bowel; ICER = incremental cost-effectiveness ratio; NBS = newborn screening

SECTION E FINANCIAL IMPLICATIONS

A market-based approach is used to estimate the financial implications of listing CFTR mutation testing on the MBS. This approach is based on extrapolations from data collected in the RCPA Genetic Testing Survey conducted in 2011. This survey recorded the number of CFTR tests by broad reason: diagnostic, predictive, screening or prenatal diagnostic. Data could not be distinguished by indication, and as the proposed indications for MBS listing do not include all current indications for testing (i.e. NBS, or testing in newborns with one mutation identified through NBS, are not proposed), the proportion of current tests eligible for MBS listing were sought from the literature and expert opinion.

In the populations eligible for MBS funding, CFTR mutation testing is currently provided for by the states/territories or is privately funded. With listing on the MBS, it is expected that the cost of testing will shift from the states/territories or patients to the MBS (with no cost offsets to the MBS anticipated).

E.1. JUSTIFICATION OF THE SELECTION OF SOURCES OF DATA

The sources of data used in estimating the financial implications of CFTR mutation testing to the MBS are presented in Table 67.

Table 67 Sources of data and justification for use in the financial implication analysis

Data	Source
Number of CFTR mutation tests	RCPA Genetic Testing Survey 2011 raw data (provided by the applicant). The number of tests observed is projected using the genetic testing growth rate (see below) to estimate the number of CFTR mutation tests in 2015–19. These data do not distinguish by indication or the type of test used.
Genetic testing growth rate	RCPA Genetic Testing Survey 2011 raw data (provided by the applicant). 15%, based on an estimate of the current growth of genetic testing; this is used to project the number of tests in 2015–19. The growth rate in genetic testing overall may overestimate the growth rate in CFTR mutation testing, as higher growth may be observed in newly identified genetic targets. Sensitivity analysis is tested around this variable.
Number of births in Australia, 2011	Australian Bureau of Statistics, births statistics, 2013 (Australian Bureau of Statistics 2014). Used (with the proportion of newborns screened with elevated IRT—see below) to estimate the number of tests associated with NBS, to determine the proportion of screening tests ineligible for MBS funding as they are due to NBS.

Data	Source
Proportion of newborns screened with elevated IRT	Massie et al. (2012), who reported the number of neonates born in Victoria during 2007–08 with elevated IRT (above the 99th percentile threshold) as 1.45% Ranieri et al. (1994) and Wilcken et al. (1995), who reported slightly lower figures (1.13% and 1.04%, respectively) based on neonates born in South Australia during 1989–93 and those born in NSW during 1994–95. These figures are used in sensitivity analysis.
Proportion of newborns with elevated IRT that have one mutation identified and recorded a positive sweat test	Ranieri et al. (1994), who reported the number of neonates born in South Australia during 1989–93 with elevated IRT, one CFTR mutation identified and a positive sweat test as 0.60%. This estimate is used to determine the proportion of diagnostic tests that are ineligible for MBS funding as they result from NBS.
Number of births in Victoria, 2006–13	Australian Bureau of Statistics, births statistics, 2013 (Australian Bureau of Statistics 2014). Used (with the number of general screening tests—see below) to estimate the number of general screening tests per pregnancy, to determine the proportion of screening tests ineligible for MBS funding as they are due to general population (i.e. no family history) screening.
Number of general screening tests conducted in Victoria, 2006–13	Archibald et al. (2014) reported n=10,489 individuals with no family history screened prior to or in the early stages of pregnancy in Victoria during 2006–13. These estimates observed in Victoria are assumed to apply nationwide; however, it is unknown if such services are provided in all states and territories, and so this approach may overestimate the proportion of screening tests in individuals with no family history of CF. This is tested in sensitivity analysis.
Proportion of predictive tests eligible for MBS funding	Expert (HESP member) opinion is used to estimate the proportion of predictive tests projected that would be eligible for MBS funding, as alternative data sources could not be identified. Given the uncertainties associated with using such data, sensitivity analysis is performed around this variable.
Test used in each indication	Clinical management algorithms, from section A.5
Clinical sensitivity of common mutation panel	From section C.1.2 10-mutation panel: 80% As per section D, scenario analyses are presented that assume the cost to be as per a 32-mutation panel and the clinical sensitivity of the test to be 92%.
CFTR mutation test cost	From section D.4 Single mutation analysis: $80 Common mutation panel: $135 Whole gene screen: $1,000 Alternative scenario analyses are presented using alternative test fees.
Funding sources for current CFTR mutation testing	RCPA Genetic Testing Survey 2011 raw data (provided by the applicant). To estimate the net cost implication of MBS funding of CFTR mutation testing to the states/territories and the patient. As survey data were not provided by indication, it is uncertain how these data apply to the specific indications proposed for MBS funding, as indications that are not proposed for MBS funding may be more likely to receive state/territory-based funding, and, conversely, those not proposed for MBS funding may be more likely to be currently funded by the patient.

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; HESP = Health Expert Standing Panel; IRT = immunoreactive trypsinogen; MBS = Medicare Benefits Schedule; NBS = newborn screening; RCPA = Royal College of Pathologists of Australasia

E.2. USE AND COSTS OF CFTR MUTATION TESTING

As CFTR mutation testing is current practice in all states and territories, a market-based approach has been used to estimate the financial implications of its introduction on the MBS. This approach uses the number of observed CFTR mutation tests reported in Australia in the RCPA Genetic Testing Survey 2011. These, by broad indication for testing, are presented in Table 68.

Table 68 Number of CFTR tests observed in the RCPA Genetic Testing Survey 2011

Broad indication	Definition of broad indication	Number of tests
Diagnostic	Testing of an affected patient (of any age) to determine the genetic basis for their disease	3,110
Predictive	Testing of an unaffected person (of any age, not including prenatal) who is at increased risk of carrying the mutation on the basis of family history	1,266
Screening	Testing of an unaffected person who is not recognised as being at increased risk of carrying a heritable mutation	10,194
Prenatal diagnostic	Diagnostic testing on a fetus	792

CFTR = cystic fibrosis transmembrane conductance regulator

The RCPA estimates that the current growth of genetic testing is 15% per year (see Table 67). This has been used to estimate the total number of CFTR mutation tests for the period 2015–19. The total numbers of tests projected during 2012–19 are presented in Table 113, Appendix K, and the projected estimates used in the financial analysis (2015–19) are provided in Table 69.

Table 69 Projected number of CFTR mutation tests, 2015–19

	2015	2016	2017	2018	2019
Diagnostic	5,439	6,255	7,194	8,273	9,514
Predictive	2,214	2,546	2,928	3,368	3,873
Prenatal diagnostic	1,385	1,593	1,832	2,107	2,423
Screening	17,829	20,504	23,579	27,116	31,184
Total	26,868	30,898	35,533	40,863	46,993

CFTR = cystic fibrosis transmembrane conductance regulator

This approach assumes that:

- The estimated growth rate of genetic testing is accurate and constant over the projected period.
- The distribution of the indications for testing is constant over the projected period.
- The growth rate observed in genetic testing broadly is applicable to the growth rate in CFTR mutation testing. Given that general growth in genetic testing may be driven by the identification of new targets, this assumption may overestimate growth in CFTR mutation testing in Australia. Sensitivity analyses are presented assuming lower rates of growth (10% and 5%), as per conservative estimates provided by the RCPA.

These data represent the total number of CFTR mutation tests across all indications. However, proposed MBS funding does not include all indications (e.g. newborn or general population screening are not eligible). As RCPA survey data are not disaggregated by indication, an estimate of the proportion of tests conducted for ineligible indications in Australia was sought from the literature and expert opinion.

As all prenatal indications of CFTR mutation testing are proposed to be eligible for funding, all projected prenatal tests observed in the RCPA survey data are assumed to be eligible for MBS funding.

Indications ineligible for MBS funding under the screening include NBS and general population screening (i.e. no family history of CF). In NBS genetic testing occurs in newborns with an elevated IRT. The proportion of newborns requiring genetic testing is based on that observed in a Victorian study (Massie, JH et al. 2012), which reported 2,019 cases of elevated IRT in 139,695 newborns screened (i.e. 1.45%). Marginally lower figures were observed in older South Australian data (Ranieri et al. 1994), where 1,004 mutation tests were performed from 88,752 newborns screened (i.e. 1.13%); and were estimated during HESP-member feedback (1%)[23], and so a lower value is tested in the sensitivity analysis.

This proportion of newborns requiring genetic testing is then applied to the total number of births in Australia for 2011 (the year of the survey) (Australian Bureau of Statistics 2014) to estimate the number of tests in 2011, and therefore the proportion of tests that would have been ineligible for proposed MBS funding. The proportion of tests that are then due to NBS is estimated in Table 70.

Table 70 Estimated proportion of screening tests, newborn screening

	Parameter	Value	Source
A	Number of screening tests, 2011	10,194	Table 68
B	Number of births in Australia, 2011	297,073	ABS (2014)
C	Proportion of births requiring genetic CFTR testing	1.45%	Massie et al. (2012)
D	Number of births requiring genetic CFTR testing	4,294	B × C
E	Proportion of tests that are due to NBS	42.1%	D/A

ABS = Australian Bureau of Statistics; CFTR = cystic fibrosis transmembrane conductance regulator; NBS = newborn screening

Data from Victoria are used to estimate the proportion of screening tests that occur in the general population. In Victoria carrier screening is available to individuals and couples (as a fee-for-service program) prior to or in the early stages of pregnancy (Archibald et al. 2014; Massie, J et al. 2009). Between 2006 and 2013, 10,489 individuals with no family history of CF were screened by the carrier screening program (Archibald et al. 2014). The total number of births observed in Victoria during this

[23] Pers. comm., received 17 March 2015

same period (n = 561,691) (Australian Bureau of Statistics 2014) is used to estimate the number of CFTR tests conducted per pregnancy (10,489/561,691 = 0.0187).

This estimate is then applied to the total number of births in Australia for 2011 (Australian Bureau of Statistics 2014) to estimate the number of tests in 2011, and therefore the proportion of tests that would have been ineligible for proposed MBS funding. The proportion of tests that are then due to general population carrier screening is estimated in Table 71.

Table 71 Estimated proportion of carrier screening tests, general population

	Parameter	Value	Source
A	Number of screening tests in Australia, 2011	10,194	Table 68
B	Number of carrier screening tests in Victoria, 2006–13	10,489	Archibald et al. (2014)
C	Number of births in Victoria, 2006–13	561,691	ABS (2014)
D	Number of screening tests per pregnancy	0.0187	B/C
E	Number of births in Australia, 2011	297,073	ABS (2014)
F	Number of carrier screening tests in Australia, 2011	5,548	D × E
G	Proportion of tests that are general population carrier screening	54.4%	F/A

ABS = Australian Bureau of Statistics

The total proportion of tests ineligible for MBS funding is then the sum of the NBS (Table 70, Row E) and general population screenings (Table 71, Row G), which equals 97%. This approach assumes that the uptake of carrier screening from Victoria is applicable to the whole of Australia. Sensitivity analyses are conducted around this estimate, as the extent of carrier screening programs and uptake of carrier screening in other states and territories is unknown.

Indications ineligible for MBS funding that would be considered diagnostic testing include newborns with one mutation identified in NBS who record a positive sweat test (and so require diagnostic testing to identify the other mutation). To estimate this population, data from the South Australian NBS program are used (Ranieri et al. 1994). This study reported that, of the newborns indicated for genetic testing (n = 1,004), six (0.6%) recorded a positive sweat test after one CFTR mutation was identified (Ranieri et al. 1994). This proportion is applied to the estimated number of births in 2011 that required genetic CFTR testing (Table 70, Row D) to estimate the number of diagnostic tests conducted for this indication.

Table 72 Estimated proportion of diagnostic tests, newborns with one mutation and positive sweat test

	Parameter	Value	Source
A	Number of diagnostic tests, 2011	3,110	Table 68
B	Number of births requiring genetic CFTR testing	4,294	Table 70, Row D
C	Proportion with one mutation and positive sweat test	0.60%	Ranieri et al. (1994)
D	Number with one mutation and positive sweat test	26	B × C
E	Proportion of diagnostic tests that are ineligible	0.83%	D/A

CFTR = cystic fibrosis transmembrane conductance regulator

No data could be identified to estimate the proportion of predictive tests that would be eligible for proposed MBS funding. HESP-member opinion was sought and it was estimated that the proportion could represent 50% of tests[24]. This is tested in sensitivity analysis, taking into consideration leakage of testing into relatives (e.g. siblings) of a person with CF.

Table 73 presents a summary of the eligible and ineligible indications, by broad indication, and the estimated proportion of tests eligible for MBS funding based on the literature and, where applicable, HESP-member opinion. Given the uncertainties associated with the estimates, sensitivity analyses are presented using the estimates as indicated.

Table 73 Proportion of CFTR mutation tests eligible for MBS funding

Broad indication	Proposed listing	Not proposed	Proportion of tests eligible
Diagnostic	- People suspected of classic CF (population 1b, Table 9) - People suspected of non-classic CF (including men with CBAVD) (populations 1c & 1d, Table 9)	- Newborns with one mutation identified in NBS and a positive sweat test (and so require diagnostic test to identify other mutation) (population 1, Table 10)	99%
Predictive	- Pregnant couples who have a previous child with CF; or in the current pregnancy who have a fetus with an echogenic gut (populations 2a & 2b, Table 9)	- Cascade testing of other family members	50% [a] Sensitivity analysis: 100%
Prenatal diagnostic	- Fetuses at high risk of having CF (populations 2a & 2b, Table 9)	- None	100%
Screening	- Partners of a person with CF or partners of a known CF carrier (population 3, Table 9)	- NBS - Screening in the general population	3% Sensitivity analysis: 25% [a]

[a] HESP member pers. comm., 16 March 2015
CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule; NBS = newborn screening

These estimates are applied to the total number of tests, as presented in Table 69, and the numbers of tests eligible for MBS funding are presented in Table 74. This approach assumes no leakage, that the proportion of tests eligible remains constant over time and that there is no growth in the market anticipated with MBS listing of CFTR mutation testing. As there is some indication that patients are currently funding a large proportion of CFTR mutation tests privately (see Table 8, Section A.3), some growth in the market may occur.

[24] Pers. comm., received 16 March 2015

Table 74 Estimated numbers of CFTR mutation tests eligible for MBS funding

	2015	2016	2017	2018	2019
Diagnostic	5,395	6,204	7,134	8,204	9,435
Predictive	1,107	1,273	1,464	1,684	1,936
Prenatal diagnostic	1,385	1,593	1,832	2,107	2,423
Screening	617	710	816	939	1,080
Total	8,504	9,780	11,247	12,934	14,874

Note: Estimated by multiplying the number of tests listed in Table 69 by the proportion of tests eligible in Table 73.
CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule

There are three CFTR mutation tests proposed for listing and each of these is associated with a different test cost. The type of test used, by broad indication, is assumed to be based on the proposed clinical management algorithms (see Figure 2, Figure 3 and Figure 4).

All patients with diagnostic indications will receive a common mutation panel test. Those in whom rare mutation(s) are suspected will also receive a whole gene screen test. If the clinical sensitivity of a common mutation panel test is 80% (assuming a 10-mutation panel, see section C.1.2), it is assumed that 20% of diagnostic patients will receive both tests. Therefore, estimating the proportion of diagnostic tests that are either a common mutation panel or a whole gene screen uses all tests (100% + 20%) as the denominator—the proportion of common mutation panels is 100%/120% (i.e. 83%) and the proportion of whole gene screens is 20%/120% (i.e. 17%).

Fetuses with a high risk of CF are eligible for known mutation analysis if both parents are identified with common mutations, or a whole gene screen if rare mutation(s) are suspected. The type of known mutation analysis depends on what mutations the parents have: single mutation analyses are performed to identify F508del mutations, while common mutation panels are performed to identify other known common mutations. As the common mutation panel will identify 80% of mutations, it is assumed that 80% of prenatal tests are known mutation analyses. The proportions of common mutation panels and single mutation analyses are those used to weight known mutation testing in the economic model (i.e. 28% and 52%, respectively). The remaining 20% of prenatal diagnostic tests are assumed to be whole gene screens.

According to clinical management pathways, common mutation panels are proposed only for use in pregnant couples who have a previous child with CF, or those in a current pregnancy who have a fetus with an echogenic gut; and partners of a person with CF or who is a known CF carrier. All predictive and screening tests are therefore assumed to be common mutation panels.

These uses are summarised in Table 75.

Table 75 Proportion of tests, by indications eligible and test type

	Diagnostic	Predictive	Prenatal diagnostic	Screening
Single mutation analysis [a]	-	-	52%	-

	Diagnostic	Predictive	Prenatal diagnostic	Screening
Common mutation panel	83%	100%	28%	100%
Whole gene screen	17%	-	20%	-
Total	100%	100%	100%	100%

[a] Single mutation analyses are performed to identify one or two F508del mutations.

These estimates are applied to the number of tests eligible for MBS funding, as presented in Table 74, and the number of tests by test type are presented in Table 76.

Table 76 Projected number of tests, by test type, 2015–19

	2015	2016	2017	2018	2019
Single mutation analysis [a]	720	828	953	1,096	1,260
Common mutation panel	6,608	7,599	8,739	10,049	11,557
Whole gene screen	1,176	1,353	1,555	1,789	2,057
Total	8,504	9,780	11,247	12,934	14,874

Note: Estimated by multiplying the number of tests listed in Table 74 by the proportion of tests by type in Table 75.
[a] Single mutation analyses are performed to identify one or two F508del mutations.

The costs of testing are assumed as per section D.4:

- single mutation analysis: $80
- common mutation panel: $135
- whole gene screen: $1,000.

Therefore, the cost implications of CFTR mutation testing are as presented in Table 77.

Table 77 Estimated total cost implications of CFTR mutation testing, by test type, 2015–19

	2015	2016	2017	2018	2019
Single mutation analysis [a]	$57,625	$66,269	$76,209	$87,640	$100,786
Common mutation panel	$892,040	$1,025,846	$1,179,723	$1,356,681	$1,560,183
Whole gene screen	$1,176,131	$1,352,551	$1,555,434	$1,788,749	$2,057,061
Total	$2,125,796	$2,444,665	$2,811,365	$3,233,070	$3,718,030

[a] Single mutation analyses are performed to identify one or two F508del mutations.
CFTR = cystic fibrosis transmembrane conductance regulator

The number of tests and financial implications have been disaggregated by proposed indications and are presented in Appendix K.

E.3. CHANGES IN USE AND COST OF OTHER MEDICAL SERVICES

In the populations eligible for MBS funding CFTR mutation testing is currently provided for by the states/territories or is privately funded. No changes in use or costs of other medical services attributable to the MBS are anticipated with the proposed listing.

E.4. FINANCIAL IMPLICATIONS TO THE MBS

The financial implications to the MBS resulting from the proposed listing of CFTR mutation testing are summarised in Table 78. It is assumed that all tests are conducted in an outpatient setting (and so a rebate of 85% applies).

Table 78 Estimated total costs to the MBS associated with CFTR mutation testing, 2015–19

	2015	2016	2017	2018	2019
Total services	8,504	9,780	11,247	12,934	14,874
Total cost	$2,125,796	$2,444,665	$2,811,365	$3,233,070	$3,718,030
MBS rebate (85%)	$1,806,926	$2,077,965	$2,389,660	$2,748,109	$3,160,326
Patient contributions	$318,869	$366,700	$421,705	$484,960	$557,705

CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule

E.5. FINANCIAL IMPLICATIONS FOR GOVERNMENT HEALTH BUDGETS

In the populations eligible for MBS funding CFTR mutation testing is currently provided for by the states/territories or is privately funded. Under the proposed listing, the costs of CFTR mutation testing will shift from the states/territories and patients to the MBS.

Current funding sources for CFTR mutation testing are presented in section A.3, based on data from the RCPA Genetic Testing Survey 2011. These figures have been used to estimate the proportion of costs that will shift from the states/territories and those that will shift from patients. Of tests where the funding source was provided, 46% of CFTR mutation tests were funded by the states/territories and 54% by patients. As data in the survey were not provided by indication, how these data apply to the specific indications proposed for MBS funding is uncertain, as indications that are not proposed for MBS funding may be more likely to receive state/territory-based funding, and, conversely, those not proposed for MBS funding may be more likely to be currently funded by the patient. This approach may overestimate the net cost savings incurred by patients and understate those incurred by the states/territories, thus overestimating the net implication to government health budgets.

Estimated savings to state/territory budgets, by test type, are presented in Table 79.

Table 79 Estimated savings to state/territory budgets for CFTR mutation testing, by test type

	2015	2016	2017	2018	2019
Single mutation analysis [a]	$26,287	$30,230	$34,764	$39,979	$45,976
Common mutation panel	$406,922	$467,961	$538,155	$618,878	$711,710
Whole gene screen	$536,517	$616,994	$709,543	$815,975	$938,371
Total	$969,726	$1,115,184	$1,282,462	$1,474,831	$1,696,056

[a] Single mutation analyses are performed to identify one or two F508del mutations.
CFTR = cystic fibrosis transmembrane conductance regulator

The total net cost implication to the government health budget is presented in Table 80.

Table 80 Estimated net cost implications of CFTR mutation testing to government health budget, 2015–19

	2015	2016	2017	2018	2019
Cost to MBS [a]	$1,806,926	$2,077,965	$2,389,660	$2,748,109	$3,160,326
Savings to states/territories	$969,726	$1,115,184	$1,282,462	$1,474,831	$1,696,056
Total cost to government	$837,201	$962,781	$1,107,198	$1,273,278	$1,464,270

[a] From Table 78
CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule

As the proposed CFTR mutation testing listing does not attempt to change existing clinical practice, the net cost effect should be zero. The net cost implication to government health budgets reflects the expected savings in out-of-pocket costs for patients (as presented in Table 81).

Table 81 Estimated net savings to patients associated with MBS funding of CFTR mutation testing, 2015–19

	2015	2016	2017	2018	2019
MBS CFTR *funding*					
Patient contribution [a]	$318,869	$366,700	$421,705	$484,960	$557,705
Current CFTR *funding*					
Total cost of current testing	$2,125,796	$2,444,665	$2,811,365	$3,233,070	$3,718,030
Cost borne by states/territories	$969,726	$1,115,184	$1,282,462	$1,474,831	$1,696,056
Cost borne by patients	$1,156,070	$1,329,481	$1,528,903	$1,758,238	$2,021,974
Net savings to patients	$837,201	$962,781	$1,107,198	$1,273,278	$1,464,270

[a] From Table 78
CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule

If there is growth in the market, the net overall cost effect of CFTR mutation testing may be positive. However, as it is likely that the indications eligible for MBS funding are more likely to be funded by the states/territories, this effect may be small.

E.6. IDENTIFICATION, ESTIMATION AND REDUCTION OF UNCERTAINTY

Uncertainties flagged around estimates used in the financial analysis were tested in sensitivity analyses (Table 82).

The analyses were most sensitive to changes that increased the number of common mutation screening tests, increasing the implication to the MBS by 25%. Reducing the growth rate, improving the clinical sensitivity of the common mutation panel and reducing the cost of the whole gene screen resulted in substantial reduced costs to the MBS (25%–50%).

While changes in the current funding arrangements between the states/territories and patients does not affect the financial implications to the MBS, if the states/territories are funding 85% or more of tests eligible under proposed MBS funding, net savings to government will be observed, with patients bearing the cost of the change, assuming that tests are not bulk-billed.

Table 82 Financial implications of sensitivity analyses

	2015	2016	2017	2018	2019
Base-case					
Cost to MBS	$1,806,926	$2,077,965	$2,389,660	$2,748,109	$3,160,326
Savings to states/territories	$969,726	$1,115,184	$1,282,462	$1,474,831	$1,696,056
Net savings to government health budgets	$837,201	$962,781	$1,107,198	$1,273,278	$1,464,270
Growth rate 10% (base-case: 15%)					
Cost to MBS	$1,512,585	$1,663,844	$1,830,228	$2,013,251	$2,214,576
Savings to states/territories	$811,761	$892,937	$982,231	$1,080,454	$1,188,499
Net savings to government health budgets	$700,824	$770,907	$847,997	$932,797	$1,026,077
Growth rate 5% (base-case: 15%)					
Cost to MBS	$1,255,759	$1,318,547	$1,384,474	$1,453,698	$1,526,383
Savings to states/territories	$673,930	$707,626	$743,008	$780,158	$819,166
Net savings to government health budgets	$581,829	$610,921	$641,467	$673,540	$707,217
Genetic testing in newborns 1.13% (base-case: 1.45%)					
Cost to MBS	$1,996,483	$2,295,955	$2,640,348	$3,036,401	$3,491,861
Savings to states/territories	$1,071,455	$1,232,173	$1,416,999	$1,629,549	$1,873,981
Net savings to government health budgets	$925,028	$1,063,782	$1,223,349	$1,406,851	$1,617,879
Proportion of eligible screening tests 50% (base-case: 25%)					
Cost to MBS	$2,247,574	$2,584,710	$2,972,417	$3,418,279	$3,931,021
Savings to states/territories	$1,206,208	$1,387,140	$1,595,211	$1,834,492	$2,109,666
Net savings to government health budgets	$1,041,365	$1,197,570	$1,377,206	$1,583,787	$1,821,355
Proportion of eligible predictive tests 100% (base-case: 50%)					
Cost to MBS	$1,679,884	$1,931,867	$2,221,647	$2,554,894	$2,938,128
Savings to states/territories	$901,546	$1,036,778	$1,192,294	$1,371,138	$1,576,809
Net savings to government health budgets	$778,339	$895,089	$1,029,353	$1,183,756	$1,361,319
Clinical sensitivity 92% (base-case: 80%) [a]					
Cost to MBS	$1,317,456	$1,515,075	$1,742,336	$2,003,686	$2,304,239
Savings to states/territories	$707,041	$813,097	$935,062	$1,075,321	$1,236,619
Net savings to government health budgets	$610,415	$701,978	$807,274	$928,365	$1,067,620

	2015	2016	2017	2018	2019
Base-case					
Cost to MBS	$1,806,926	$2,077,965	$2,389,660	$2,748,109	$3,160,326
Savings to states/territories	$969,726	$1,115,184	$1,282,462	$1,474,831	$1,696,056
Net savings to government health budgets	$837,201	$962,781	$1,107,198	$1,273,278	$1,464,270
Proportion of tests funded by state 75% (base-case: 46%)					
Cost to MBS	$1,806,926	$2,077,965	$2,389,660	$2,748,109	$3,160,326
Savings to states/territories	$1,594,347	$1,833,499	$2,108,524	$2,424,802	$2,788,523
Net savings to government health budgets	$212,580	$244,467	$281,137	$323,307	$371,803
Proportion of tests funded by state 100% (base-case: 46%)					
Cost to MBS	$1,806,926	$2,077,965	$2,389,660	$2,748,109	$3,160,326
Savings to states/territories	$2,125,796	$2,444,665	$2,811,365	$3,233,070	$3,718,030
Net savings to government health budgets	–$318,869	–$366,700	–$421,705	–$484,960	–$557,705

[a] no change in test cost
MBS = Medicare Benefits Schedule

Scenario analyses conducted using alternative MBS fees are presented in Table 83.

Table 83 Financial implications of CFTR test cost scenario analyses

	2015	2016	2017	2018	2019
Base-case					
Cost to MBS	$1,806,926	$2,077,965	$2,389,660	$2,748,109	$3,160,326
Savings to states/territories	$969,726	$1,115,184	$1,282,462	$1,474,831	$1,696,056
Net savings to government health budgets	$837,201	$962,781	$1,107,198	$1,273,278	$1,464,270
Cost of known mutation analysis $50 (base-case: $80)					
Cost to MBS	$1,788,559	$2,056,842	$2,365,369	$2,720,174	$3,128,200
Savings to states/territories	$959,868	$1,103,848	$1,269,426	$1,459,839	$1,678,815
Net savings to government health budgets	$828,690	$952,994	$1,095,943	$1,260,335	$1,449,385
Cost of whole gene screen $500 (base-case: $1,000)					
Cost to MBS	$1,307,071	$1,503,131	$1,728,601	$1,987,891	$2,286,075
Savings to states/territories	$701,467	$806,687	$927,691	$1,066,844	$1,226,871
Net savings to government health budgets	$605,603	$696,444	$800,910	$921,047	$1,059,204

CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule

A scenario analysis assuming 92% clinical sensitivity and a $200 cost of common mutation panel testing is presented in Table 84.

Table 84 Financial implications of common mutation panel scenario analyses

	2015	2016	2017	2018	2019
Base-case					
Cost to MBS	$1,806,926	$2,077,965	$2,389,660	$2,748,109	$3,160,326
Savings to states/territories	$969,726	$1,115,184	$1,282,462	$1,474,831	$1,696,056
Net savings to government health budgets	$837,201	$962,781	$1,107,198	$1,273,278	$1,464,270
Common mutation panel, clinical sensitivity (92%) and cost ($200) (base-case: 80% and $135)					
Cost to MBS	$1,719,313	$1,977,210	$2,273,791	$2,614,860	$3,007,089
Savings to states/territories	$922,706	$1,061,112	$1,220,279	$1,403,320	$1,613,818
Net savings to government health budgets	$796,607	$916,098	$1,053,513	$1,211,540	$1,393,271

MBS = Medicare Benefits Schedule

SECTION F OTHER RELEVANT CONSIDERATIONS

F.1. ETHICAL CONSIDERATIONS CONCERNING PRENATAL GENETIC TESTING

F.1.1. INTRODUCTION

Prenatal testing for CFTR mutations is typically used to enable reproductive choices or family planning to proceed on the basis of the health of the fetus, with the opportunity being given to terminate the pregnancy when the fetus is affected (Munthe 2015). In this way the goal of prenatal testing is to help couples make an informed choice, namely about what they feel would be best for themselves and their family (Aksoy 2001). In the scenarios where parents are carriers or where an echogenic gut has been detected in the fetus, prenatal testing is considered standard care.

Genetic tests pose their own specific ethical considerations, which are discussed below. The aim of the assessment report is to review and synthesise the available evidence to inform a public funding decision. In the case of ethical considerations, evidence synthesis equates to reviewing the relevant literature and assessing the balance of arguments. The synthesis is mainly descriptive but it is also normative insofar as it seeks to identify ethical ideals for framing policy on how medical professionals should conduct themselves.

F.1.2. METHODS OF EVIDENCE SYNTHESIS

A literature search was performed for papers that linked ethical theory to genetic testing. Articles identified as potentially relevant were selected (Giarelli 2001; Hildt 2002; Kinder 1998; Wilcken 2011; Winslow, Kodner & Dietz 2005). These papers constituted the main body of evidence. Where possible, these papers were supported by additional articles that presented (i) material from an Australian perspective and (ii) issues relating specifically to prenatal mutation testing. Some of these additional articles were identified in the 'ethics' literature search, while others were identified in a separate search on ethics in prenatal testing for CF. Additional key texts in medical ethics (Beauchamp & Childress 2001; Rogers & Braunack-Mayer 2004) and web resources (ALRC 2003; Andersen et al. 2005) were also sourced.

F.1.3. ETHICAL FRAMEWORK

The philosophical approach adopted by this assessment is the 'four-principles approach', also called 'principlism' since it is predominant within the field of biomedical ethics (Beauchamp & Childress 2001; Munson 2000; Rogers & Braunack-Mayer 2004). The articles included in the assessment all used the four-principles approach.

F.1.3.1. The 'four-principles approach'

Principlism comprises respect for autonomy, non-maleficence, beneficence and justice, which are used to analyse and assess the ethical considerations relating to the provision of health care, including genetic testing (Andersen et al. 2005; Beauchamp & Childress 2001; Winslow, Kodner & Dietz 2005). Each of the principles is briefly described below.

Respect for autonomy

Autonomy refers to self-rule. The principle of respect for autonomy emphasises the importance of personal freedom and choice in personal as well as political life. It is deeply rooted in the Western moral and political tradition. It includes different aspects; for example, independence, privacy, self-realisation and voluntariness (i.e. freedom from coercion and manipulation) (Hildt 2002). Autonomy requires two things: agency—the capacity for intentional action; and liberty—the absence of constraints imposed by others that prevent or impede one's intentional choices and actions (Beauchamp & Childress 2001; Hildt 2002; Winslow, Kodner & Dietz 2005). The principle of respect for autonomy underpins the widely acknowledged ethical requirement that, as a rule with limited exceptions, medical treatment should proceed only with the 'informed consent' of the patient.

In the case of prenatal genetic testing, the principle of respect for autonomy commends that the pregnant patient or couple should be able to make their own informed decisions regarding whether testing and TOP occur. However, the availability of testing may itself negatively impact on the people involved, since the pregnant patient or couple can thus be assigned the major share of responsibility for health outcomes (Schmitz 2013). External social influences play a significant role here, since the availability of testing and TOP may result in a reduction in social support for people having 'avoidable' disabilities caused by genetic mutations. The feeling may emerge that parents of an affected child are responsible for the disease suffered by the child and have to face the consequences (e.g. without the support of public funds). The importance of the principle of respect for autonomy, together with the tendency of society to moralise in terms of individual responsibility, could result in genetic testing being increasingly promoted and used (Hildt 2002), with blame being cast on those who choose not to utilise available technologies.

Non-maleficence

Non-maleficence refers to not inflicting harm or injury to others, and is associated with the dictum *Primum non nocere*: 'Above all, (first) do no harm'. The principle also finds expression in the modern Hippocratic oath: 'I will use treatment to help the sick according to my ability and judgement, *but I will never use it to injure or wrong them*'. In clinical practice the principle of non-maleficence is often combined with, and sometimes balanced against, the principle of beneficence, a version of which is expressed in the first half of the above Hippocratic oath (Beauchamp & Childress 2001; Giarelli 2001). For instance, even the best diagnostic tests and treatments can carry certain risks of harm (e.g. amniocentesis), and it is practically impossible for medical professionals to act without ever causing harm—causing some harm might be worthwhile in the light of greater potential benefits.

Hence, the avoidance of *unwarranted* or *unnecessary* harm, even if that harm is unintentional, is paramount to the non-maleficent conduct of health professionals.

Beneficence

The principle of beneficence asserts that it is not enough to respect the autonomy of patients and to avoid causing them harm; clinicians and providers of health services should also act in ways that actively promote the welfare or best interests of patients (Kinder 1998). Just as there are standards of due care that define appropriate conduct in the protection of patients from harm, so too are there standards of beneficence. For example, an obvious expectation in medical care is the physician's duty to improve the health of patients by providing appropriate treatment.

Justice

Justice refers to giving people what they are owed, for example honesty, courtesy and a fair share in the available resources. In the present context the principle of justice finds expression in the belief that everyone deserves equal access to advances in medicine. Different theories of justice focus on conditions of entitlement; fair and equal treatment; and how the distribution of social goods such as health care ought to occur on the basis of morally relevant factors such as a person's degree of need, capacity to benefit and/or particular rights. Distributive justice concerns how resources are distributed, to whom and for what reasons. For instance, difficult choices are sometimes made between greatly benefiting the few (e.g. those with rare diseases) and benefiting to a lesser degree the many (Giarelli 2001; Winslow, Kodner & Dietz 2005); and are also made concerning the degree to which benefits going to the worst off may be more important to secure.

F.1.4. ETHICAL ISSUES SPECIFIC TO PRENATAL TESTING

Provisional questions such as those proposed by Hoffmann (2005) may help identify where documentation is needed. They elicit reflection on the possible implications of a technology, and on other dimensions such as the social construction of the technology; interactions between various actors and institutions; conflicting interests between stakeholders; and historical, economic, social and cultural considerations (Andersen et al. 2005). The main ethical issues associated with prenatal genetic testing and their relevant ethical principles are shown in Table 85 and discussed below.

Table 85 Main ethical issues for prenatal genetic testing and their most relevant principles

Issue	Most relevant principle(s)
Informed choice and counselling	Respect for autonomy, non-maleficence, beneficence
Disability rights critique	Justice
Access to testing and TOP	Justice
Privacy and confidentiality	Respect for autonomy, non-maleficence, beneficence
Weighing risks and benefits	Non-maleficence, beneficence

TOP = termination of pregnancy

F.1.4.1. Aim of prenatal testing

The aim of prenatal screening and testing differs from that of most other forms of genetic screening and testing. Whereas most genetic testing provides an individual with options for treatment and for the possible prevention of disease, prenatal testing serves as a basis for reproductive decision making and family planning (Aksoy 2001; de Jong & de Wert 2015; de Jong et al. 2011). In other words, prenatal testing cannot serve to prevent the development of disease in a particular individual. It can only inform parents of the presence of disease in the fetus, while any subsequent TOP prevents the birth of an individual with a disease. However, it has also been argued that the aim of prenatal testing is to serve societal goals, and there have been controversial calls for prenatal testing to be used to reduce the number of otherwise costly people in society (Munthe 2015). The Department of Health and Social Security in the UK, for instance, released a governmental document in 1977 that states: '… because caring for the handicapped can impose great burdens on our society the prevention of handicaps … in addition to its other benefits may save money' (Aksoy 2001). If the aim of prenatal testing was to reduce the birth prevalence of CF, this would be morally problematic because it would favour the use of TOP as a means of reducing the number of people with specific disorders or other medical needs. This has led to the general view that prenatal screening for fetal abnormalities should be regarded as serving the aim of providing pregnant couples with reproductive options, to then be freely chosen (respect for autonomy).

F.1.4.2. Informed choice and counselling

Counselling before and after prenatal testing is crucial. If pre-test information is incomplete or unbalanced, if support is minimal or absent, or if counselling is directive, the aim of 'providing options for meaningful reproductive choice' for couples is not met. Without adequate information and counselling, the aim of prenatal testing would, in effect, change to protecting society against the birth of children with (costly) disorders by encouraging selective terminations of pregnancy (de Jong & de Wert 2015). Already, the fact that the offer of testing comes from authoritative institutions or people (physicians) means that there is an initial pressure on women and couples to consider prenatal testing, and the more suggestive the offer and less clear the opportunity to freely decline it, the greater the pressure (Munthe 2015). The legality and availability of TOP in the case of an abnormal test may alone contribute to pressure and bias toward testing.

The three most common reasons that women cite as reasons for changing their mind about prenatal testing are (Aksoy 2001):

- the level of risk they have for the condition in question (in this case, CF)
- the miscarriage risk of the test (amniocentesis or CVS)
- the method that would be offered if they opted for TOP following an abnormal test result.

These considerations are relevant during counselling, especially since counselling professionals and women seem to place different degrees of importance on different aspects of testing. Women value

the safety of the test most highly, whereas health professionals foremost value test accuracy (de Jong, Maya & Van Lith 2015).

F.1.4.3. Disability rights critique

The 'disability rights critique' disagrees with selective termination as a means to avoid the birth of children with a certain disorder or disability—it is mostly understood as a claim that prenatal testing sends a discriminatory message about people with the condition tested for (de Jong & de Wert 2015). Prenatal testing sends to society the message that it would have been better if those living with the targeted condition had not been born (de Jong et al. 2011). Hildt (2002) stated, 'It is well known that one of the main arguments against PND is that a widespread use may lead to further discrimination of disabled people'. There is a concern that prenatal testing will lead to a degradation of society's willingness to accept and care for children deemed 'abnormal', while at the same time enlarging the category of unacceptable 'abnormality' and narrowing the range of acceptable 'normality'. Furthermore, it has been argued that widespread acceptance of selective TOP would diminish the importance of, and the motivation toward, developing cures for genetic disorders (Aksoy 2001). However, if informed decisions, rather than termination rates, are taken as a measure of success in prenatal testing, the disability rights critique is arguably less convincing (de Jong et al. 2011).

F.1.4.4. Access to testing and TOP

In Australia access to medical services is generally adequate and equitable; however, access problems among the rural population are well known. Genetics services are no exception (Wilcken 2011).

Currently, most genetic tests are expensive and not listed on the MBS. In most cases, but not all, diagnostic CFTR testing is paid for by the states/territories. When people are referred by a private facility, they are billed directly and must cover the entire cost themselves. Pre-implantation genetic diagnosis (PGD) is not included in this assessment, since this service is assessed elsewhere (see MSAC 1165), and at the time of writing no subsidies are offered for PGD. PGD is often preferred by families to avoid the risk of having to abort an affected fetus, although it is prohibitively expensive for most couples (Wilcken 2011). In the event that MBS funding for PGD is not recommended, the number of people who opt for prenatal testing might increase, leaving PGD for those individuals who can afford to pay for it privately. This is a relevant consideration in the decision making that is to follow both the present assessment (MSAC 1216) and assessment of MSAC 1165.

F.1.4.5. Privacy and confidentiality

The principle of respect for autonomy commends access to voluntary genetic testing, including proper counselling and information on its risks and benefits. It also affirms the individual's right to privacy. In the case of prenatal testing, an individual may choose to reveal information about their carrier status to their partner and medical personnel, but this information must usually be kept

confidential by medical personnel. Genetic information is special insofar as genetic abnormalities in one individual could also have implications for the health of genetically related family members. Therefore, if the individual chooses to keep his/her carrier status private, this limits the ability of other family members to make informed reproductive choices. Because CFTR mutations are a matter for both individuals and families, ethical dilemmas can occur when a clinician is torn between maintaining the confidentiality of test results and informing family members of their own risk of having a child with CF. Judging which specific clinical situations warrant a breach of confidentiality remains one of the most difficult ethical issues raised by genetic testing. In the case of CF-carriers, genetic counsellors may seek to persuade patients to disclose their carrier status to siblings, parents and/or children instead of providing non-directive counselling, or ask patients to allow the counsellors to disclose this information (Beauchamp & Childress 2001). The exceptions that permit or even mandate disclosure are circumstances in which it is necessary to lessen or prevent a serious threat to the life, health or safety (whether or not the threat is imminent) of an individual who is a genetic relative of the person to whom the genetic information relates (Suthers, McCusker & Wake 2011). The Institute of Medicine's Committee on Assessing Genetic Risks, together with other institutions, has proposed that the following criteria must be met to justify a breach of confidentiality: (1) all attempts to elicit voluntary disclosure must be exhausted, (2) the seriousness of the harm posed by the mutations must be certain, and (3) the availability of preventative or therapeutic interventions must be clear (Winslow, Kodner & Dietz 2005). It is important to consider that, in the case of carriers, there is only a risk for family members if they are going to have children.

It is possible that, in some cases, the prenatal test may indicate non-paternity (Hill, J 2004). Disclosure of non-paternity is a separate ethical issue. If a spontaneous mutation is a possible cause of this genetic discrepancy, it is possible and ethical for the physician to advise the family about the fetus's status with regard to mutations, and to indicate that presumably this was a rare case of spontaneous mutation (Campbell 2004). Unless a physician has solid evidence of non-paternity, they have no right to share these suspicions with the father, since doing so could have devastating consequences. However, if there is evidence of non-paternity, sharing the medical (genetic) fetal information with the couple might lead to the conclusion of non-paternity, since withholding information about paternity might only be possible by misinforming them about the test results, which would not be justified (Hill, J 2004).

F.1.4.6. Fetal perspective

For the fetus, the difference is not between being born with CF and being born without CF; rather, it is between a worldly existence or none at all. The difference between existing and not existing is beyond comparison here (Aksoy 2001).

F.1.4.7. Weighing risks and benefits

The principles of non-maleficence and beneficence could be taken to entail that the risks of harm should be outweighed (perhaps substantially) by the probable benefits before a genetic test is

accepted into general practice. Thus, relevant factors include the predictive value of the test, the benefits and harms provided by interventions associated with the test (i.e. amniocentesis and CVS) and with a positive test result (i.e. TOP), and the availability and acceptability of those interventions (Burke & Press 2006). It is important to weigh all the benefits and risks of genetic testing before undergoing prenatal testing. For example, what would a positive test result mean to the prospective parents? What are the harms of the test? What is the accuracy of the test? What are the consequences in case of a false-negative or false-positive result? What would be the psychological consequences when choosing TOP? What would be the psychological impact of caring for a child with CF? In general, do the benefits of testing outweigh the risks?

F.1.5. SUMMARY

Important ethical considerations regarding prenatal genetic testing include the following:

- Prenatal testing for fetal abnormalities should be regarded as serving the aim of providing pregnant couples with reproductive options, not as serving the aim of reducing the number of people deemed costly to society.
- Non-directive counselling is crucial for ensuring proper informed consent and for minimising the risks of harm, both psychological and physical.
- There is a concern that prenatal testing may lead to a degradation of society's willingness to accept and care for children with CF, while at the same time enlarging the category of unacceptable abnormality and narrowing the range of acceptable normality. This is why the success of testing is ideally gauged in terms of the number of informed decisions produced, rather than the number of CF births prevented.
- Testing and counselling should be available, and not financially burdensome, to all women at increased risk of having a child with CF.
- Test results should be kept confidential by medical personnel; however, the couple should be counselled on sharing information with family members who may benefit from it.

F.1.6. CONCLUSIONS

The above ethical considerations suggest that prenatal genetic testing should only be offered on the MBS in conjunction with non-directive pre- and post-test genetic counselling from accredited counsellors.

F.2. NON-INVASIVE PRENATAL DIAGNOSIS (NIPD)

Prenatal care for couples at risk of single-gene disorders such as CF has the potential to change through the implementation of earlier prenatal testing using non-invasive prenatal diagnosis (NIPD) (Schmitz 2013). NIPD is based on cell-free fetal DNA in maternal plasma and enables prenatal diagnosis using a maternal blood test. This means that the risk of miscarriage and other risks of

injury associated with amniocentesis or CVS would be avoided (Hill, M et al. 2014). Another advantage of NIPD is that there is enough cell-free fetal DNA in maternal plasma for testing between 7 and 9 weeks' gestation, meaning that the test can be performed earlier than invasive tests. This would allow more time to psychologically and practically prepare for a CF child, a less invasive method of TOP, and the potential for less psychological attachment to pregnancy due to less delay in testing. It is anticipated that non-invasive prenatal tests for CF will be available in the near future (Hill, M et al. 2014). NIPD is predominantly seen as a positive development; however, there are some concerns about the following ethical issues.

NIPD must be accurate. It has to be assessed whether NIPD will be as accurate as invasive prenatal testing regarding the detection of CFTR mutations.

Difficulties in ensuring informed consent. The decreased risk of miscarriage that NIPD brings also increases the risk of people being more easily lured into prenatal testing—which could resemble a 'harmless routine blood test'—without serious reflection on the long-term consequences, including possible TOP (Munthe 2015). There is an increased risk of the test's 'routinisation' and this may lead to an erosion of informed decision making (de Jong, Maya & Van Lith 2015). Evidence is available showing that NIPD may be considered by parents as routine rather than optional (Hill, M et al. 2014; Schmitz 2013; Skirton, Goldsmith & Chitty 2014), emphasising the importance of counselling and differentiating prenatal genetic testing from more-routine blood tests.

Increased pressure to undergo testing. The decreased risks of NIPD may not only lead to difficulties in ensuring informed consent, but may increase pressure on couples to undertake prenatal testing. Society, family and health professionals could all suggest that testing is the routine thing to do. Couples should not have to justify these choices to society or family.

Widening the scope. With any increasing use and routinisation of NIPD, one should expect that more gene disorders will be able to be tested for (i.e. beyond just CF). In turn, this widening of the scope of prenatal testing could lead to further difficulties in ensuring informed consent and to the trivialisation of selective terminations on the part of society and medical personnel (de Jong & de Wert 2015; Schmitz 2013).

F.3. LEGAL IMPLICATIONS

TOP is state-regulated and subject to criminal law in almost all states and territories, except the Australian Capital Territory. In Victoria, South Australia, Western Australia, Tasmania and the Northern Territory, legislation is in place to provide a statutory explanation of when a termination is legal, with respect to personal circumstances and timing. In New South Wales and Queensland the common law recognises exceptions to the Crimes Act and Criminal Code that enable lawful TOP in a large number of women who meet particular criteria. However, whether TOP is lawful when CF is prenatally diagnosed could differ between states, as the life expectancy of CF patients continues to

increase. This should be considered in the decision-making process regarding prenatal diagnosis of CF, since its usefulness is questionable in circumstances where lawful TOP would not be available.

APPENDIX A CLINICAL EXPERTS AND ASSESSMENT GROUP

HEALTH EXPERT STANDING PANEL (HESP)

Member	Expertise or affiliation
Dr Felicity Collins	Clinical Geneticist, Children's Hospital, Westmead
Dr Carolyn Shalhoub	Senior Genetic Counsellor, Sydney Children's Hospital
Dr Michael Gattas	Clinical Geneticist, Brisbane Genetics, Wesley Medical Centre
Dr John Massie	Paediatric Respiratory Medicine / CF physician, The Royal Children's Hospital Melbourne

ASSESSMENT GROUP

Adelaide Health Technology Assessment (AHTA), University of Adelaide, Adelaide, South Australia

Name	Position
Ms Sharon Kessels	Research Officer
Dr Judy Morona	Senior Research Officer
Dr Ruchi Mittal	Senior Research Officer
Ms Arlene Vogan	Health Economist
Ms Camille Schubert	Senior Health Economist
Ms Skye Newton	Team Leader
Assoc. Prof. Tracy Merlin	Managing Director

NOTED CONFLICTS OF INTEREST

There were no conflicts of interest.

APPENDIX B SEARCH STRATEGIES

BIBLIOGRAPHIC DATABASES

Electronic database	Time period
Cochrane Library – including, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, the Cochrane Central Register of Controlled Trials (CENTRAL), the Health Technology Assessment Database, the NHS Economic Evaluation Database	1/1989 – 10/2014
Current Contents	1/1989 – 10/2014
Embase	1/1989 – 10/2014
PubMed	1/1989 – 10/2014
Web of Science – Science Citation Index Expanded	1/1989 – 10/2014
Cinahl	1/1989 – 10/2014
Econlit	1/1989 – 10/2014
Scopus	1/1989 – 10/2014

ADDITIONAL SOURCES OF LITERATURE (INCLUDING WEBSITES)

HTA WEBSITES

INTERNATIONAL	
International Network of Agencies for Health Technology Assessment	http://www.inahta.org/
AUSTRALIA	
Australian Safety and Efficacy Register of New Interventional Procedures – Surgical (ASERNIP-S)	http://www.surgeons.org/for-health-professionals/audits-and-surgical-research/asernip-s/
Centre for Clinical Effectiveness, Monash University	http://www.monashhealth.org/page/Health_Professionals/CCE/
Centre for Health Economics, Monash University	http://www.buseco.monash.edu.au/centres/che/
AUSTRIA	
Institute of Technology Assessment / HTA unit	http://www.oeaw.ac.at/ita
CANADA	
Institut national d'excellence en santé et en services sociaux (INESSS)	http://www.inesss.qc.ca/en/publications/publications/
Alberta Heritage Foundation for Medical Research (AHFMR)	http://www.ahfmr.ab.ca/
Alberta Institute of Health Economics	http://www.ihe.ca/
The Canadian Agency for Drugs And Technologies in Health (CADTH)	http://www.cadth.ca/index.php/en/
The Canadian Association for Health Services and Policy Research (CAHSPR)	https://www.cahspr.ca/en/about/vision
Centre for Health Economics and Policy Analysis (CHEPA), McMaster University	http://www.chepa.org/
Centre for Health Services and Policy Research (CHSPR), University of British Columbia	http://www.chspr.ubc.ca/

INTERNATIONAL	
Institute for Clinical and Evaluative Studies (ICES)	http://www.ices.on.ca/
Saskatchewan Health Quality Council (Canada)	http://www.hqc.sk.ca/
DENMARK	
Danish National Institute Of Public Health	http://www.si-folkesundhed.dk/?lang=en
FINLAND	
Finnish National Institute for Health and Welfare	http://www.thl.fi/en/web/thlfi-en/
FRANCE	
L'Agence Nationale d'Accréditation et d'Evaluation en Santé (ANAES)	http://www.anaes.fr/
GERMANY	
German Institute for Medical Documentation and Information (DIMDI) / HTA	http://www.dimdi.de/static/en/
Institute for Quality and Efficiency in Health Care (IQWiG)	http://www.iqwig.de
THE NETHERLANDS	
Health Council of the Netherlands (Gezondheidsraad)	http://www.gezondheidsraad.nl/en/
NEW ZEALAND	
New Zealand Health Technology Assessment (NZHTA)	http://www.otago.ac.nz/christchurch/research/nzhta/
NORWAY	
Norwegian Knowledge Centre for the Health Services	http://www.kunnskapssenteret.no
SPAIN	
Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud "Carlos III"I / Health Technology Assessment Agency (AETS)	http://www.isciii.es/
Andalusian Agency for Health Technology Assessment (Spain)	http://www.juntadeandalucia.es/
Catalan Agency for Health Technology Assessment (CAHTA)	http://www.gencat.cat
SWEDEN	
Center for Medical Health Technology Assessment	http://www.cmt.liu.se/?l=en&sc=true
Swedish Council on Technology Assessment in Health Care (SBU)	http://www.sbu.se/en/
SWITZERLAND	
Swiss Network on Health Technology Assessment (SNHTA)	http://www.snhta.ch/
UNITED KINGDOM	
National Health Service Health Technology Assessment (UK) / National Coordinating Centre for Health Technology Assessment (NCCHTA)	http://www.hta.ac.uk/
NHS Quality Improvement Scotland	http://www.nhshealthquality.org/
National Institute for Clinical Excellence (NICE)	http://www.nice.org.uk/
The European Information Network on New and Changing Health Technologies	http://www.euroscan.bham.ac.uk/
University of York NHS Centre for Reviews and Dissemination (NHS CRD)	http://www.york.ac.uk/inst/crd/
UNITED STATES	
Agency for Healthcare Research and Quality (AHRQ)	http://www.ahrq.gov/clinic/techix.html

INTERNATIONAL	
Harvard School of Public Health	http://www.hsph.harvard.edu/
Institute for Clinical and Economic Review (ICER)	http://www.icer-review.org/
Institute for Clinical Systems Improvement (ICSI)	http://www.icsi.org
Minnesota Department of Health (US)	http://www.health.state.mn.us/
National Information Centre of Health Services Research and Health Care Technology (US)	http://www.nlm.nih.gov/nichsr/nichsr.html
Oregon Health Resources Commission (US)	http://www.oregon.gov/oha/OHPR/HRC/Pages/index.aspx
Office of Health Technology Assessment Archive (US)	http://ota.fas.org/
U.S. Blue Cross / Blue Shield Association Technology Evaluation Center (Tec)	http://www.bcbs.com/blueresources/tec/
Veteran's Affairs Research and Development Technology Assessment Program (US)	http://www.research.va.gov/default.cfm

SPECIALTY WEBSITES

Cystic Fibrosis Australia	http://www.cysticfibrosis.org.au/
Cystic Fibrosis Foundation (US)	www.cff.org/
Cure4CF Foundation (US)	http://www.cure4cf.org/
Australian Heart/Lung Transplants Association	http://www.ahlta.com.au/
Lung Foundation Australia	http://lungfoundation.com.au/

ADDITIONAL SOURCES OF LITERATURE

Source	Location
Internet	
NHMRC—National Health and Medical Research Council (Australia)	http://www.nhmrc.gov.au/
US Department of Health and Human Services (reports and publications)	http://www.hhs.gov/
New York Academy of Medicine Grey Literature Report	http://www.greylit.org/
Trip database	http://www.tripdatabase.com
Current Controlled Trials metaRegister	http://controlled-trials.com/
National Library of Medicine Health Services/Technology Assessment Text	http://text.nlm.nih.gov/
UK National Research Register	http://www.nihr.ac.uk/Pages/NRRArchive.aspx
Google Scholar	http://scholar.google.com/
Australian and New Zealand Clinical Trials Registry	www.anzctr.org.au
Pearling	
All included articles had their reference lists searched for additional relevant source material	

APPENDIX C STUDIES INCLUDED IN THE SYSTEMATIC REVIEW

Table 86 Study profiles of included studies on diagnostic accuracy

Study Country	Study design Quality	Study population	Inclusion criteria / Exclusion criteria	Index test(s)	Reference standard
Axton & Brock (1995) UK	Level IV: A study of diagnostic yield (no reference standard) Quality: poor with a high risk of bias	N=193 mouthwash samples from CF patients	None reported	Restriction generation PCR. This technique uses a mismatch primer to introduce a base substitution designed to create or destroy a restriction site when a mutation is present.	No reference standard
Bareil et al. (2007) France	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: poor with a high risk of bias	N=182 samples from men with a clinical diagnosis of CBAVD	Inclusion criteria: Clinical diagnosis of CBAVD Exclusion criteria: Patients with renal abnormalities	A complete scan of the 27 coding/flanking sequences of the *CFTR* gene was performed by either DGGE or DHPLC. In addition, two intronic mutations, 18111.6kbAG in IVS11 and 384910kbCT in IVS19, and variations at locus IVS8-Tn were screened by specific PCR restriction tests. Samples with only 1 or no CFTR disease-causing mutations were further investigated for large rearrangements by a semi-quantitative fluorescent PCR assay. Samples showing abnormal profiles were directly sequenced with BigDye Terminator sequencing.	Clinical diagnosis of CBAVD was based on clinical examination with impalpable vas deferens, transrectal ultrasonography, semen analysis and sperm count in accordance with World Health Organization guidelines.
Bernardino, Lima & Zatz (2003) Brazil	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: poor with a high risk of bias	N=17 patients with CBAVD	Inclusion criteria: 20 unrelated men (ages 26–49 years) with obstructive azoospermia; 17 were diagnosed with CBAVD; none had been diagnosed with CF	Mutation detection studies were carried out on all 27 exons and exon–intron boundaries of the *CFTR* gene by SSCP and heteroduplex analysis. *CFTR* gene variants of the T tract length of intron 8 were also investigated. Abnormally migrating fragments were subsequently sequenced to confirm the	Diagnosis of CBAVD was based on scrotal examination, ultrasound and semen analysis.

Study Country	Study design Quality	Study population	Inclusion criteria / Exclusion criteria	Index test(s)	Reference standard
				presence of mutations.	
Bickmann et al. (2009) Germany	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: good with a low risk of bias	N=92 CF patients	Not reported	Pyrosequencing: single-stranded DNA was prepared and used for pyrosequencing, and the quantitative signal was detected with the PSQ 96MA instrument.	CF patients had typical symptoms and positive sweat test results.
Bonizzato et al. (1995) Gasparini et al. (1993) Italy	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: intermediate with some risk of bias	N=225 chromosomes from 133 CF patients N=24 chromosomes (12 patients) from whom DNA was not available N=17 chromosomes shared by 8 siblings and 2 cousins were excluded from analysis	Inclusion criteria: All CF patients born between 10 January 1973 and 12 December 1981 in two northern Italian regions (Veneto and Trentino-AltoAdige) Exclusion criteria: For mutation frequency analysis, 8 brothers or sisters and 1 common chromosome shared by cousins were discarded	RFLP, RNA-SSCP and DGGE analysis of the *CFTR* gene The presence of a given mutation in electrophoretically altered fragments was always determined by DNA sequencing.	Diagnosis was confirmed by at least two positive sweat tests.
Bonizzato et al. (1999) Italy	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: good with a low risk of bias	N=806 chromosomes from 403 CF patients	Not reported	Reverse dot-blot hybridisation assay designed to detect the 15 most common CFTR mutations in Italy	Diagnosis of CF was by sweat test.

Study Country	Study design Quality	Study population	Inclusion criteria / Exclusion criteria	Index test(s)	Reference standard
Castaldo et al. (2000) Italy	Level IV: A study of diagnostic yield (no reference standard) Quality: good with a low risk of bias	N=32 fetal samples collected through CVS from high-risk couples (1 dizygotic twin pregnancy)	Inclusion criteria: Pregnant couples were identified during a CF-carrier screening study and offered prenatal screening; 32/33 couples gave informed consent for prenatal testing.	An ASOH dot-blot semi-automated procedure using a panel of 13 CF mutations was used on all the CF families. When no mutations were detected, STR genotyping of intragenic polymorphisms by PCR and gel electrophoresis was followed by extragenic polymorphisms by PCR and restriction enzyme analysis.	No reference standard
Collazo et al. (2014) Cuba	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: good with a low risk of bias	N=61 fetal samples collected through amniocentesis from couples with some risk of having children affected by CF	Inclusion criteria: Couples with some risk of having children affected by CF, referred from all parts of Cuba during 1988—2011	ARMS to detect F508del, G542X and R1162X PCR-based restriction enzyme analysis to detect R334W, R553X and 3120+1G>A	Clinical diagnosis at birth
Danziger et al. (2004) USA	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: good with a low risk of bias	N=16 male patients with CAVD (n=13 with CBAVD and n=1 CUAVD) or with idiopathic epididymal obstruction (n=2)	Prospectively selected infertile men attending the male infertility clinic over a 3-year period who were diagnosed with obstructive azoospermia Inclusion criteria: (i) new diagnosis with no prior genotyping (ii) members of an ethnic group with an assumed low detection rate by common mutation panel	mTTGE and DNA sequencing The Ambry CF Test includes a full mutation scan of all CFTR exons as well as relevant intronic regions by mTTGE analysis followed by DNA sequencing of suspect regions.	A detailed history and physical examination was performed on all patients by an experienced urologist. The diagnosis of CAVD or idiopathic obstruction was based on physical examination findings.

Study Country	Study design Quality	Study population	Inclusion criteria / Exclusion criteria	Index test(s)	Reference standard
Donat et al. (1997) UK	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: good with a low risk of bias	N=30 patients with CBAVD	Inclusion criteria: Patients with CBAVD presenting consecutively to the Edinburgh infertility clinic	All patients were tested for 14 *CFTR* gene mutations using a single-tube PCR multiplex system with restriction enzyme analysis.	Clinical diagnosis of CBAVD was made if the semen analysis confirmed azoospermia, the volume was low (<2 mL), the pH was low (<7), the vasa were impalpable and the testes were otherwise normal.
Durieu et al. (1995) France	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: good with a low risk of bias	N=14 CBAVD patients	Inclusion criteria: Patients were consecutively included between December 1992 and July 1993	PCR amplification with restriction enzyme or heteroduplex analysis	The clinical diagnosis of CBAVD was made on azoospermia with normal or slightly smaller sized testes, non-palpable vas deferens, and small volume and low pH ejaculate.
Edelmann et al. (2004) USA	Level IV: A study of diagnostic yield (no reference standard) Quality: poor with a high risk of bias	N=507 patients' samples referred for CF screening, 12 proficiency samples	Not reported	CF testing was initially performed by multiplex PCR followed by ASOH. Then samples were tested with eMAP BeadChip assay, which combines multiplex amplification of genomic DNA and multiplex detection of mutations and polymorphisms using ASOs with variable 3'-terminal sequences displayed on colour-encoded beads that are assembled into random arrays on semiconductor chips. Single-stranded PCR products are annealed to the bead-displayed ASOs and rendered visible by incorporation of a fluorescently labelled nucleotide analogue.	No reference standard

Study Country	Study design Quality	Study population	Inclusion criteria / Exclusion criteria	Index test(s)	Reference standard
Frentescu and Budisan (2009) Romania	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: intermediate with some risk of bias	N=42 chromosomes from 21 patients with a clinical suspicion of CF from 21 unrelated families	Inclusion criteria: Patients were recruited from paediatric hospitals in Bucharest, Cluj-Napoca and Constanta	Multiplex PCR, heteroduplex and RFLP analysis	Diagnosis was based on clinical symptoms and sweat test values.
Gallati et al. (2009) Switzerland	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: intermediate with some risk of bias	N=25 azoospermic men diagnosed with CAVD	Inclusion criteria: Men aged between 27 and 57 years who had consulted for primary couple infertility and were diagnosed with CAVD	Screening of the entire coding sequence of the *CFTR* gene including intron–exon boundaries and the promoter region was performed by SSCP and heteroduplex analysis. *CFTR* gene variants of the T tract length of intron 8 were also investigated. DNA sequencing of CFTR variants using an ABI 377 sequencing system	The diagnosis of primary infertility was based on physical examination, ultrasonography and semen analysis. All patients were investigated for testicular volumes, pathological findings and CAVD.
Giuliani et al. (2010) Italy	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: intermediate with some risk of bias	N=23 CBAVD patients	Inclusion criteria: The patients did not have a family history of CF, and were enrolled in an assisted reproduction technology program	Screening of the *CFTR* gene was performed by analysis of 57 mutations and the 5T allele by using a reverse dot-blot approach (INNO-LiPA CFTR19 and INNO-LiPA CFTR17 + Tn Update Kits). INNO-LiPA CFTR Italian Regional Kit	1. When no mutation was detected, MLPA to detect deletions and/or duplications and DHPLC with DNA sequencing were undertaken. 2. Clinical diagnosis of CBAVD was based on azoospermia with low seminal fluid volume. presence of globus major and absence of palpable vas deferens.

Study Country	Study design Quality	Study population	Inclusion criteria / Exclusion criteria	Index test(s)	Reference standard
Heim, Sugarman & Allitto (2001) USA	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: poor with a high risk of bias	N=5,840 chromosomes from 2,920 individuals with a clinical diagnosis of CF: 4.3% Hispanic 3.5% African American 0.7% Native American 0.3% Asian	<u>Inclusion criteria:</u> Patients were referred from all 50 US states, with 28.3% from the northeastern, 24.8% from the southeastern, 16.6% from the western, and 30.3% from the central, states.	Samples were analysed using one of two mutation panels comprising 70 or 86 mutations, using a pooled ASOH strategy. The 86-mutation panel included 63 mutations in common with the 70-mutation panel and an additional 23 mutations, and excluded 7 mutations in the 70-mutation panel	Clinical diagnosis; no details given
Houdayer et al. (1998) France	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: intermediate with some risk of bias	N=40 DNA samples of known CFTR mutations from CF patients or carriers	Not reported	The CF(12) ARMS kit was used as recommended by the manufacturer (Zeneca Diagnostics). CF(12) ARMS uses multiplexed ARMS technology, which allows the simultaneous identification of the more prevalent CFTR mutations in 1 working day.	DNA samples from CF patients or carriers were typed by two genetic testing laboratories by analysis of the 27 exons and the intron–exon boundaries of the *CFTR* gene.
Kanavakis et al. (2003) Greece	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: intermediate with some risk of bias	N=874 chromosomes from 437 CF patients	<u>Inclusion criteria:</u> Patients of Greek origin who attended the Cystic Fibrosis Unit of St Sophia's Children's Hospital	The 27 exons and neighbouring intronic regions of the *CFTR* gene were assessed by DGGE. All DNA samples showing a shift in mobility and not presenting a pattern of a known mutation were sequenced.	Diagnostic criteria involved positive sweat tests and typical clinical findings of pulmonary and gastrointestinal disease.
	Level III-2: Quality: good with a low risk of bias	N=115 prenatal diagnoses, the majority of families having at least 1 affected child	Not reported	A combination of DGGE and PCR-mediated site-directed mutagenesis was used for confirmation of the mutation(s) for each diagnosis, which included re-testing of sample DNA from parents and healthy and/or affected siblings.	Clinical diagnosis of child after birth
	Level IV: A study of diagnostic yield (no reference standard) Quality: good with a low risk of bias	N=49 samples collected through CVS or amniocentesis from fetuses due to FEB detected during routine ultrasound	Not reported	The 27 exons and neighbouring intronic regions of the *CFTR* gene were assessed by DGGE. All DNA samples showing a shift in mobility and not presenting a pattern of a known mutation were sequenced.	Clinical outcomes not reported

Study Country	Study design Quality	Study population	Inclusion criteria / Exclusion criteria	Index test(s)	Reference standard
Lay-Son et al. (2011) Chile	Level III-2: A comparison against independent, blinded reference standard among non-consecutive patients Quality: good with a low risk of bias	N=578 chromosomes from 289 patients with CF	Inclusion criteria: Patients from the CF National Program of the Ministry of Health of Chile recruited between March 2004 and March 2010	CFTR mutations were determined by two methods throughout the study period: OLA technology using 'Cystic Fibrosis v3.0' (32 mutations) was used from 2004 to 2008, and 'INNO-LiPA CFTR19/CFTR17+Tn Update'(36 mutations) was used since 2008.	Clinical diagnosis; details not described
Nagy et al. (2007) Hungary	Level IV: A study of diagnostic yield (no reference standard) Quality: poor with a high risk of bias	N=116 DNA samples: 84 blood samples 18 chorionic villus samples (at 12th gestational week) 14 amniotic fluids (at 18th gestational week)	Not reported	Samples were analysed for the presence of F508del. qPCR and melting curve analysis Fluorescent PCR and DNA fragment analysis	No reference standard
Ravnik-Glavac et al. (2002) USA	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: poor with a high risk of bias	N=73 DNA samples of known CFTR mutations obtained from CF patients: 53 single-base-pair substitutions 16 small deletions 4 small insertions	Inclusion criteria: CF patients who had heterozygous CFTR mutations Exclusion criteria: CF patients who had homozygous CFTR mutations	DHPLC of PCR products using Stanford MELT software followed by empirical determination of optimal melting temperatures for unresolved mutations	DNA samples of known mutations were obtained from previous study or kindly provided by the authors who first reported them.
Ravnik-Glavac et al. (1994) USA	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: poor with a high risk of bias	N=133 DNA samples of known CFTR mutations	Not reported	SSCP analysis of PCR products	DNA samples of known mutations were obtained from previous study or kindly provided by the authors who first reported them.
Saker et al. (2006) France	Level IV: A study of diagnostic yield (no reference standard)	N=12 fetus samples from carrier couples (non-invasive sampling) 10 couples were both carrying the F508del CFTR	Not reported	Samples were analysed for the presence of F508del by PCR. STR genotyping was by PCR amplification and DNA sequencing.	No reference standard

Study Country	Study design Quality	Study population	Inclusion criteria / Exclusion criteria	Index test(s)	Reference standard
	Quality: intermediate with some risk of bias	mutation			
Strom et al. (2003) USA	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: good with low risk of bias	N=7 confirmed CF patients with positive sweat tests 4 had 1 CF mutation identified by extended panel screening 3 had no mutations identified by either ACMG or extended panel screening	Inclusion criteria: Confirmed CF patients whose samples were submitted by physicians on a research basis for sequencing	DNA sequencing reactions were performed with an ABI Prism Big Dye™ Terminator v3.0 cycle sequencing reaction kit according to the manufacturer's protocol. ACMG panel (using the Roche CF Gold Linear Array strips) or extended panel screening	Confirmed CF patients; no diagnostic criteria provided
Strom et al. (2004) USA	Level IV: A study of diagnostic yield (no reference standard) Quality: poor with a high risk of bias	N=1,092 patient samples previously tested with the CF Gold line probe assay chosen at random N=1,076 patient samples previously tested with the Applera CF OLA, Ver. 3.0, platform	Although information such as patient ethnicity and pertinent family history was requested for each patient, in practice this information was rarely provided. Thus, it was not possible to distinguish between samples submitted for mutation detection in a patient with CF or for carrier detection for infertility evaluations.	The CF Portrait™ system includes a one-tube multiplex PCR followed by a completely automated process of hybridisation and detection in a 96-well microtiter plate containing an assay chip (with an 8x8 array of capture probes and controls to detect all requisite alleles) in the bottom of each well. Roche CF Gold line probe strips Applera CF OLA, Ver 3.0	No reference standard

Study Country	Study design Quality	Study population	Inclusion criteria / Exclusion criteria	Index test(s)	Reference standard
Tomaiuolo, Spina & Castaldo (2003) Italy	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: good with a low risk of bias	N=105 DNA samples from CF subjects bearing different genotypes N=80 from CF patients: 49 with 2 known mutations (15 homozygous, 34 heterozygous) 31 with 1 known and 1 unknown heterozygous mutation N=25 from CF carriers with known mutation	Not reported	1. CF(12) ARMS kit, based on ARMS technology, requiring 2 multiplex PCRs for 6 mutations each 2. The OLA PCR kit, based on a single multiplex PCR, screens for 31 CFTR mutations 3. INNO-LiPA CF, based on the reverse hybridisation principle, screens for 12 CFTR mutations (1 kit) and 17 CFTR mutations plus IVS8 polyT (second kit) 4. ASOH dot-blot assay was used according to manufacturer's instructions to detect the 13 most common mutations in southern Italy.	DGGE of the whole CFTR coding region was followed by sequence identification of each mutation.
Wall, Cai & Chehab (1995) USA	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: intermediate with some risk of bias	N=123 CF patients: 102 (83%) Caucasian 20 (16%) Hispanic 1 (1%) Native American	Not reported	PCR and reverse dot-blot hybridisation	CF was established by clinical criteria as well as abnormal sweat chloride levels.
Wang et al. (2002) USA	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: good with a low risk of bias	N=92 patients with CBAVD	Inclusion criteria: Patients were included consecutively.	Restriction enzyme analysis of either a CF25 mutation panel, or multiplex PCR followed by MALDI-TOF mass spectrometry of a CF100 mutation panel	Diagnosis of CBAVD was made clinically by urologists and most patients had renal ultrasound studies.

ACMG = American College of Medical Genetics; ARMS = amplification refractory mutation system; ASO = allele-specific oligonucleotide; ASOH = allele-specific oligonucleotide hybridisation; CAVD = congenital absence of the vas deferens; CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CUAVD = congenital unilateral absence of the vas deferens; CVS = chorionic villus sampling; DGGE = denaturing gradient gel electrophoresis; DHPLC = denaturing high performance liquid chromatography; DNA = deoxyribonucleic acid; eMAP = elongation-mediated multiplexed analysis of polymorphisms; FEB = fetal echogenic bowel; MALDI-TOF = matrix-assisted laser desorption ionisation – time of flight; MLPA = multiple ligation-dependent probe amplification; mTTGE = modified temporal temperature gradient electrophoresis; OLA = oligonucleotide ligation assay; PCR = polymerase chain reaction; qPCR = quantitative real-time PCR; RFLP = restriction fragment length polymorphism; RNA = ribonucleic acid; SSCP = single-stranded conformation polymorphism; STR = short tandem repeats

Table 87 Study profiles of included studies on change in management

Study setting	Study design / Quality appraisal	Study population and PNDs	Inclusion criteria	Intervention	Outcomes
Ameratunga et al. (2012) Melbourne, Victoria, Australia	Retrospective case series Level: IV Quality: 14/18	33/63 cases of FEB underwent parental carrier testing, 11/63 were referred from other hospitals or GP practices.	Inclusion: Pregnant women found to have FEB on mid-trimester ultrasound between 1 March 2004 and 1 March 2009	Parental carrier status was performed in 33 pregnancies: (32 fetuses underwent karyotyping, and maternal serology for toxoplasmosis was performed in 44 cases, and for cytomegalovirus in 49 cases)	1 carrier couple, no PND (no TOP), newborn was CF +ve
Castaldo et al. (2000) Southern Italy	Prospective case series Level: IV Quality: 12/18	33 high-risk (1:4) pregnant couples; 31 had ≥1 child with CF, 2 were identified as carriers; 1 declined prenatal diagnosis	Inclusion: Couples where the woman was pregnant and the risk of the fetus having CF was 1:4	In 22/32 cases both the mutations were known and direct analysis of the mutations was done. In 7 cases (6 with two unknown mutations and 1 with one unknown mutation) diagnosis was made by analysis of intragenic polymorphisms.	• CF +ve: 7 fetuses, 7 TOP • Carriers: 18 fetuses • CF –ve: 6 fetuses • Unknown: 1 fetus (familial unknown rare mutation)
de Becdelievre et al. (2011) France	Retrospective case series Level: IV Quality: 14/18	465 couples (group 1) directly referred to the researchers' laboratory because of fetal digestive anomalies at routine ultrasound investigation An additional 229 couples (group 2) referred for further investigations after screening for frequent mutations by other laboratories	Inclusion: Pregnant couples where fetal digestive anomalies were found on routine ultrasound investigation (echogenic bowel, intestinal loop dilatation, intra-abdominal calcifications, meconium peritonitis and non-visualisation of the gallbladder)	In 679/694 cases CFTR mutation analysis was first performed in the parents. The fetus was studied when a mutation was identified in at least one parent and/or when a fetal sample was available. First, common mutation analysis was performed according to patients' ethnic/geographical origins. If a mutation was identified, a complete scan of the CFTR coding regions of the partner was done. If both parents were carriers, prenatal diagnosis was performed.	Group 1: • CF +ve: 10/465 fetuses • Carriers: 13/465 fetuses Group 2: • CF +ve: 20/229 fetuses 15/30 CF +ve fetuses were born. 3 cases remained unresolved after complete molecular analysis (one TOP)

Study setting	Study design / Quality appraisal	Study population and PNDs	Inclusion criteria	Intervention	Outcomes
Ghose et al. (2000) Leeds, UK	Prospective case series Level: IV Quality: 15/18	48/60 couples with FEB consented to CF screening. 54 singleton pregnancies and 6 were twins or triplets. Maternal age range: 16–41 years	Inclusion: Women with a detected FEB in second-trimester ultrasound between February 1996 and December 1997	Parental blood DNA testing to determine the carrier status (first only dF508, G511D and R553X; after January 1997 the 12 most common mutations were screened) for CF when they consented to be screened	CF +ve diagnosed through PND: 1/48 (1 TOP) CF +ve diagnosed after birth (out of people with no PND): 2/12 (no TOP)
Muller et al. (2002) France	Prospective case series Level: IV Quality: 14.5/18	641 cases: FEB: 580 cases (481 had isolated FEB, 99 had FEB in combination with another anomaly) Intestinal loop dilatation: 37 (34 isolated) Intra-abdominal calcification (isolated): 15 Meconium peritonitis (isolated): 5 Absence of gallbladder (isolated): 4	Inclusion: Pregnant women with abnormal ultrasound signs of fetal bowel anomalies (FEB, intestinal loop dilatation, intra-abdominal calcifications, absence of gallbladder) that were referred to 21 French molecular genetics laboratories	CFTR mutation testing was done on fetal cells or parental blood cells, or on both. First step was a common mutation screen representing 70%–90% of CF chromosomes. In addition, depending on ethnic background, particular mutations were screened, giving a detection rate of ≥90%. If only one mutation was detected (in 1 parent and/or fetus), whole gene sequencing was conducted.	Outcome of pregnancies was known for 627/641 cases: • CF +ve: 20/627, 16 TOP, 2 continued pregnancy, 2 had 2nd mutation detected after birth (19 had FEB, 15 isolated) • CFTR-RD: 1 (no TOP) • Carriers: 18 (no TOP, no CF, FEB in 15, 12 isolated)
Scotet et al (2003) Brittany, France	Retrospective case series Level: IV Quality: 13/18	337 PNDs: 148 PNDs for couples related to a CF child (1:4 risk) 189 PNDs following the diagnosis of FEB during pregnancy	Inclusion: All women using PND between 1 January 1992 and 31 December 2001 (data from the two genetics laboratories in the region); these are women with a 1 in 4 chance of having a baby with CF or where an FEB was detected on ultrasound.	In the case of a 1:4 risk (parents are known to be carriers), the fetus was tested for the parental mutations through CVS (at 10 weeks) or amniocentesis (at 16–17 weeks). In cases of FEB, a molecular analysis of the *CFTR* gene was undertaken.	1:4 risk couples (148 PNDs, 39 CF +ve, 36 TOP, 1 fetal loss, 2 births) Related to CF child clinically diagnosed (72 PNDs) • CF +ve: 15/72 (13 TOP) • Related to screened CF child (76 PNDs) • CF +ve: 24/76 (23 TOP) Family testing (26 PNDs): • CF +ve: 6/26 (5 TOP) CFTR testing after FEB detection (173 PNDs, 22 CF +ve, 18 TOP) Initial molecular analysis (173 PNDs)

Study setting	Study design / Quality appraisal	Study population and PNDs	Inclusion criteria	Intervention	Outcomes
					• CF +ve: 17/173 (13 TOP) 1:4 risk identified through FEB in previous pregnancy (16 PNDs) • CF +ve: 5/16 (5 TOP)
Scotet et al (2008) Brittany, France	Retrospective case series Level: IV Quality: 14.5/18	290 PNDs: 268 PNDs in 165 couples with 1:4 risk (2 twin pregnancies) 22 PNDs after detection of FEB	<u>Inclusion:</u> Couples with a 1:4 risk of CF (due to previous child or cascade screening) or with FEB diagnosed on ultrasound during 1989–2006	Common mutation analysis in the fetus (30 most common mutations) and, if necessary, whole gene screen or a search for large rearrangements in the gene	<u>1:4 risk couples (268 PNDs, 74 CF +ve, 70 TOP):</u> Previous CF child (195 PNDs): • CF +ve: 55 (51 TOP, 3 births, 1 intra-uterine death) • CF –ve: 43 • Carriers: 96 Cascade screening (49 PNDs): • CF +ve: 13 (13 TOP) • CF +ve but diagnosed after birth: 1 (no TOP) • CF –ve: 9 • Carriers: 27 Positive parental CF testing after FEB (22 PNDs): • CF +ve: 6 (6 TOP) <u>CFTR testing directly after FEB (22 PNDs)</u> • CF +ve: 22 (18 TOP)
Slotnick & Abuhamad (1996) Virginia, USA	Prospective case series? Level: IV Quality: 12/18	143 couples with FEB were counselled; 58 parental CF tests and 53 amniocenteses were conducted: Grade 1 FEB: 40 Grade 2 FEB: 81 Grade 3 FEB: 24	<u>Inclusion:</u> Pregnant women (and their partners) identified as showing FEB at 16–20 weeks' gestation. The intervention was confined to white couples of European extraction (little was known about gene frequencies in other populations).	Parental carrier testing of CF: standard mutation analysis of 12 (and later 17) mutations and/or deletion analysis. When both parents were carriers, CF testing in the fetus was offered.	CF +ve diagnosed through PND: 5 (no TOP) CF +ve but diagnosed after birth: 2 (no PND)

Study setting	Study design / Quality appraisal	Study population and PNDs	Inclusion criteria	Intervention	Outcomes
Tomaiuolo et al. (2013) Southern Italy	Retrospective case series Level: IV Quality: 13/18	149 couples who underwent pre-test counselling for CF underwent 181 prenatal diagnoses. 11 couples did not have PND due to miscarriage, disagreeing with PND, too difficult to make a decision about termination, and mild mutations in 1 of the parents (risk of a child with a CFTR-related disorder) <u>Indications:</u> Affected child: 148 Cascade screening: 28 Consanguinity: 1 Preconceptional screen: 2 Echogenic bowel: 1 Screening for IVF: 1	<u>Inclusion:</u> Couples referred for molecular diagnostics for CF because of being at high risk of giving birth to a child affected by CF between January 1993 and December 2012	Fetal DNA was only tested for parental mutations, as the CFTR genotype of all couples was known.	1/181: PND not possible due to insufficient DNA in sample: • CF –ve: 38 • Carriers: 98 • CF +ve: 42, 41 TOP • Twins (1 CF +ve, 1 carrier): 1 (selective TOP) • Twins (1 carrier, 1 CF –ve): 1

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CFTR-RD = CFTR related disorder CVS = chorionic villus sampling; DNA = deoxyribonucleic acid; FEB = fetal echogenic bowel; GP = general practitioner; PND = prenatal diagnosis; IVF = in-vitro fertilisation; TOP = termination of pregnancy

APPENDIX D DIAGNOSTIC ACCURACY (SECTION B.6.I)

Table 88 Diagnostic accuracy of CFTR mutation testing compared with DNA sequencing in CF patients

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	True-positive results	False-positive results	False-negative results	True-negative results	Cannot be detected by index test	Accuracy for detectable CFTR mutations (all CFTR mutations)
Tomaiuolo, Spina & Castaldo (2003) Italy	Level III-2 Low risk of bias	N=129 chromosomes from 85 DNA samples from CF subjects with known mutations	CF(12) ARMS kit	12	92	0	6 (5%)	0	31 (24%)	Sensitivity = 94% (71%)
			OLA PCR kit	31	106	0	0	0	23 (18%)	Sensitivity = 100% (82%)
			ASOH dot-blot assay	13	123	0	0	0	6 (5%)	Sensitivity = 100% (96%)
			INNO-LiPA CF kit (reverse dot-blot)	29 + poly-T	105	0	0	0	24 (18%)	Sensitivity = 100% (81%)
Houdayer et al. (1998) France	Level III-2 Some risk of bias	N=40 DNA samples of known CFTR mutations	CF(12) ARMS kit	12	34	0	2 (5%)	0	4 (10%)	Sensitivity = 94% (85%)
Ravnik-Glavac et al. (1994) USA	Level III-2 High risk of bias	N=133 DNA samples of known CFTR mutations	SSCP All mutations	All 27 exons and their exon–intron boundaries	129	0	4 (3%)	0	0	Sensitivity = 97%
			Exon 10 mut		6	0	0	0	0	Sensitivity = 100%
			Exon 11 mut		8	0	0	0	0	Sensitivity = 100%
			Exon 4 mut		14	0	2	0	0	Sensitivity = 88%
Ravnik-Glavac et al. (2002) USA	Level III-2 High risk of bias	N=73 DNA samples of known CFTR mutations	DHPLC All mutations	10 exons with flanking introns	66	0	7 (10%)	0	0	Sensitivity = 90%
			Exon 10 mut		4	0	1	0	0	Sensitivity = 80%
			Exon 11 mut		8	0	0	0	0	Sensitivity = 100%
			Exon 4 mut		14	0	0	0	0	Sensitivity = 100%
			DHPLC at optimal Tm		73	0	0	0	0	Sensitivity = 100%

ARMS = amplification refractory mutation system; ASOH = allele-specific oligonucleotide hybridisation; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; DHPLC = denaturing high performance liquid chromatography; DNA = deoxyribonucleic acid; OLA = oligonucleotide ligation assay; PCR = polymerase chain reaction; SSCP = single-stranded conformation polymorphism

Table 89 Diagnostic accuracy of DNA sequencing compared with clinical diagnosis in CF patients

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	True-positive results	False-positive results	False-negative results	True-negative results	Accuracy
Strom et al. (2003) USA	Level III-1 Low risk of bias	N=14 chromosomes from 7 confirmed CF patients	Automated DNA sequence analysis-based assay	991	13	0	1 (7%)	0	Sensitivity = 93%
Bickmann et al. (2009) Germany	Level III-2 Low risk of bias	N=184 chromosomes from 92 CF patients	Pyrosequencing panel assay Pyrosequencing plus conventional DNA sequencing	46 All 27 CFTR exons including splice sites	158 183	0 0	26 (14%) 1 (1%)	0 0	Sensitivity = 86% Sensitivity = 99.5%
Kanavakis et al. (2003) Greece	Level III-2 Some risk of bias	N=874 chromosomes from 437 CF patients	DGGE plus DNA sequencing	All 27 exons and their exon–intron boundaries	794	0	80 (9%)	0	Sensitivity = 91%
Bonizzato et al. (1995) Gasparini et al. (1993) Italy	Level III-2 Some risk of bias	N=225 chromosomes from 133 CF patients	RFLP, RNA-SSCP or DGGE and DNA sequencing	99	203	0	22 (10%)	0	Sensitivity = 90%

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; DGGE = denaturing gradient gel electrophoresis; DNA = deoxyribonucleic acid; RFLP = restriction fragment length polymorphism; RNA = ribonucleic acid; SSCP = single-stranded conformation polymorphism

Table 90 Diagnostic accuracy of CFTR mutation testing compared with clinical diagnosis in CF patients

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	True-positive results	False-positive results	False-negative results	True-negative results	Accuracy
Bonizzato et al. (1999) Italy	Level III-2 Low risk of bias	N=806 chromosomes from 403 CF patients	Reverse dot-blot hybridisation	15	661	0	145 (18%)	0	Sensitivity = 82%
Lay-Son et al. (2011) Chile	Level III-2 Low risk of bias	N=578 chromosomes from 289 patients with CF	OLA assay or INNO-LiPA CFTR19/CFTR17+Tn Update reverse dot-blot hybridisation	32 or 36	338	0	240 (42%)	0	Sensitivity = 59%

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	True-positive results	False-positive results	False-negative results	True-negative results	Accuracy
Wall, Cai & Chehab (1995) USA	Level III-2 Some risk of bias	N=246 chromosomes from 123 CF patients	Reverse dot-blot hybridisation	31	216	0	30 (12%)	0	Sensitivity = 88%
Frentescu & Budisan (2009) Romania	Level III-2 Some risk of bias	N=42 chromosomes from 21 patients with CF	Multiplex PCR, heteroduplex analysis and RFLP	18	22	0	20 (48%)	0	Sensitivity = 52%
Heim, Sugarman & Allitto (2001) USA	Level III-2 High risk of bias	N=5,840 chromosomes from 2,920 individuals with CF	ASOH	93	4,664	0	1,176 (20%)	0	Sensitivity = 80%

ASOH = allele-specific oligonucleotide hybridisation; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; OLA = oligonucleotide ligation assay; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism

Table 91 Diagnostic accuracy of CFTR mutation testing compared with DNA sequencing in patients with CBVAD

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	True-positive results	False-positive results	False-negative results	True-negative results	Cannot be detected by index test	Accuracy for detectable CFTR mutations (all CFTR mutations)
Giuliani et al. (2010) Italy	Level III-2 Some risk of bias	N=46 chromosomes from 23 CBAVD patients	INNO-LiPA CFTR19, 17 + Tn and Italian Regional Kit (reverse dot-blot hybridisation)	57 + poly-T	33 + MPLA 33	0 + MPLA 0	2 (4%) + MPLA 4 (9%)	11 + MPLA 9	2 (4%) + MPLA 4 (9%)	Sensitivity = 100% (89–100) (94% [81–99]) Specificity = 100% (72–100) Sensitivity = 100% (89–100) (89% [75–97]) Specificity = 100% (66–100)

CBAVD = congenital bilateral absence of the vas deferens; CFTR = cystic fibrosis transmembrane conductance regulator; DNA = deoxyribonucleic acid; MPLA = multiplex ligation-dependent probe amplification

Table 92 Diagnostic accuracy of DNA sequencing compared with clinical diagnosis in patients with CBVAD

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	True-positive results	False-positive results	False-negative results	True-negative results	Accuracy	Additional information
Danziger et al. (2004) USA	Level III-2 Low risk of bias	N=16 male patients with CAVD (13 with CBAVD)	mTTGE and DNA sequencing: N=16 patients N=32 chromosomes	Full mutation scan of the *CFTR* gene	 12 15	 0 0	 4 (25%) 17 (53%)	 0 0	 Sensitivity = 75% Sensitivity = 47%	All mutations were heterozygous. 3 variants of unknown significance were identified.
Gallati et al. (2009) Switzerland	Level III-2 Some risk of bias	N= 25 azoospermic men diagnosed with CAVD	SSCP and DNA sequencing: N= 25 patients N=50 chromosomes	All 27 exons and their exon–intron boundaries	 17 32	 0 0	 8 (32%) 18 (36%)	 0 0	 Sensitivity = 68% Sensitivity = 64%	Two mutations were identified in 15 (60%) patients and 1 mutation in 2 (8%) patients.
Giuliani et al. (2010) Italy	Level III-2 Some risk of bias	N=23 CBAVD patients	Reverse dot-blot, DHPLC and DNA sequencing: N= 23 patients N=46 chromosomes	*CFTR* coding region	 23 35	 0 0	 0 (0%) 11 (24%)	 0 0	 Sensitivity = 100% Sensitivity = 76%	Two mutations were identified in 12 (52%) patients and 1 mutation in 11 (48%) patients.
Bareil et al. (2007) France	Level III-2 High risk of bias	N=182 samples from men with a clinical diagnosis of CBAVD	DGGE or DHPLC and DNA sequencing N= 182 patients N=364 chromosomes	All 27 exons and their exon–intron boundaries	 168 320	 0 0	 14 (8%) 44 (12%)	 0 0	 Sensitivity = 92% Sensitivity = 88%	Two mutations were identified in 152 (84%) patients and 1 mutation in 16 (9%) patients.
Bernardino, Lima & Zatz (2003) Brazil	Level III-2 High risk of bias	N=17 patients with CBAVD	SSCP and DNA sequencing: N= 17 patients N=34 chromosomes	All 27 exons and their exon–intron boundaries	 10 19	 0 0	 7 (41%) 15 (44%)	 0 0	 Sensitivity = 59% Sensitivity = 56%	Two mutations were identified in 9 (53%) patients and 1 mutation in 1 (6%) patient.

CAVD = congenital absence of the vas deferens; CBAVD = congenital bilateral absence of the vas deferens; CFTR = cystic fibrosis transmembrane conductance regulator; DGGE = denaturing gradient gel electrophoresis; DHPLC = denaturing high-performance liquid chromatography; DNA = deoxyribonucleic acid; mTTGE = modified temporal temperature gradient electrophoresis; SSCP = single-stranded conformation polymorphism

Table 93 Diagnostic accuracy of CFTR mutation testing compared with clinical diagnosis in patients with CBVAD

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	True-positive results	False-positive results	False-negative results	True-negative results	Accuracy	Additional information
Wang et al. (2002) USA	Level III-2 Low risk of bias	N=92 patients with CBAVD	Restriction enzyme analysis: N= 92 patients N=184 chromosomes	25 + poly-T	 59 85	 0 0	 33 (36%) 99 (54%)	 0 0	 Sensitivity = 64% Sensitivity = 46%	Two mutations were identified in 26 (28%) patients and 1 mutation in 33 (36%) patients.
			Multiplex PCR plus mass spectrometry: N= 92 patients N=184 chromosomes	100	 62 95	 0 0	 30 (33%) 89 (48%)	 0 0	 Sensitivity = 67% Sensitivity = 52%	Two mutations were identified in 33 (36%) patients and 1 mutation in 29 (32%) patients.
Donat et al. (1997) UK	Level III-2 Low risk of bias	N=30 patients with CBAVD	Multiplex PCR with restriction enzyme analysis: N= 30 patients N=60 chromosomes	14	 21 27	 0 0	 9 (30%) 33 (55%)	 0 0	 Sensitivity = 70% Sensitivity = 45%	Two mutations were identified in 6 (20%) patients and 1 mutation in 15 (50%) patients.
Durieu et al. (1995) France	Level III-2 Low risk of bias	N=14 CBAVD patients	PCR amplification with restriction enzyme or heteroduplex analysis: N= 14 patients N=28 chromosomes	22	 10 16	 0 0	 4 (29%) 12 (43%)	 0 0	 Sensitivity = 71% Sensitivity = 57%	Two mutations were identified in 3 (21%) patients and 1 mutation in 7 (50%) patients.
Giuliani et al. (2010) Italy	Level III-2 Some risk of bias	N=23 CBAVD patients	INNO-LiPA CFTR19, 17 + Tn and Italian Regional Kits (reverse dot-blot hybridisation) N=23 patients N=46 chromosomes	57 + poly-T	 23 33	 0	 0 13	 	 Sensitivity = 100% Sensitivity = 72%	Two mutations were identified in 10 (43%) patients and 1 mutation in 13 (57%) patients.

CBAVD = congenital bilateral absence of the vas deferens; CFTR = cystic fibrosis transmembrane conductance regulator; PCR = polymerase chain reaction

Table 94 Diagnostic accuracy of CFTR mutation testing compared with DNA sequencing in CFTR mutation carriers

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	True-positive results	False-positive results	False-negative results	True-negative results	Cannot be detected by index test	Accuracy for detectable CFTR mutations (all CFTR mutations)
Tomaiuolo, Spina & Castaldo (2003) Italy	Level III-2 Low risk of bias	N=25 DNA samples from CF carriers bearing a known mutation	CF(12) ARMS kit	12	22	0	0	0	3 (12%)	Sensitivity = 100% (88%)
			OLA PCR kit	31	24	0	0	0	1 (4%)	Sensitivity = 100% (96%)
			ASOH dot-blot assay	13	24	0	0	0	1 (4%)	Sensitivity = 100% (96%)
			INNO-LiPA CF kit (reverse dot-blot)	29 + poly-T	22	0	0	0	3 (12%)	Sensitivity = 100% (88%)

ARMS = amplification refractory mutation system; ASOH = allele-specific oligonucleotide hybridisation; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; DNA = deoxyribonucleic acid; OLA = oligonucleotide ligation assay; PCR = polymerase chain reaction

Table 95 Diagnostic accuracy of CFTR mutation testing compared with clinical diagnosis in fetuses

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	Fetus homozygous (has CF)	Fetus heterozygous (carrier)	Fetus has no CFTR mutations	Outcomes	Reference standard gaps
Kanavakis et al. (2003) Greece	Level III-2 Low risk of bias	N=115 fetus samples from carrier parents	DGGE and PCR–mediated site-directed mutagenesis	Specific mutation detection	22 (19%)	59 (51%)	34 (30%)	No false negatives Sensitivity = 100%	Fate of fetuses diagnosed with CF unknown
		N=49 fetus samples for FEB detected by ultrasound	DGGE and DNA sequencing	All 27 exons and their exon–intron boundaries	0 (0%)	3 (6%)	46 (94%)	None were diagnosed with CF.	No clinical outcomes reported
Collazo et al. (2014) Cuba	Level III-2 Low risk of bias	N=72/108 fetus samples from couples with some risk of having a child affected by CF	ARMS and PCR-based restriction enzyme analysis	6	16 (22%)	27 (38%)	20 (28%)	Conclusive diagnosis was possible in 72/108 cases: 9 (12%) were either carrier or normal No false negatives Sensitivity = 100%	Fate of fetuses diagnosed with CF unknown
Castaldo et al. (2000)	Level IV Low risk of	N=33 fetus samples from 32 high-risk couples	ASOH and STR genotyping	13 mutations and STRs	7 (21%)	18 (55%)	8 (24%)	All 7 CF fetuses were aborted.	No clinical outcomes for carrier and

Study Country	Evidence level and risk of bias	Population	Tests	Number of mutations detected	Fetus homozygous (has CF)	Fetus heterozygous (carrier)	Fetus has no CFTR mutations	Outcomes	Reference standard gaps
Italy	bias	(1 dizygotic twin pregnancy)							normal fetuses reported
Saker et al. (2006) France	Level IV Some risk of bias	N=12 fetus samples from carrier couples	PCR and STR genotyping	F508del and STRs	1 (8%)	7 (58%)	4 (33%)	Unknown	No clinical outcomes reported

ARMS = amplification refractory mutation system; ASOH = allele-specific oligonucleotide hybridisation; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; DGGE = denaturing gradient gel electrophoresis; DNA = deoxyribonucleic acid; FEB = fetal echogenic bowel; PCR = polymerase chain reaction; STR = short tandem repeats

Table 96 CFTR mutation test limitations, mutation identification errors and failure rates

Study Country	Evidence level and risk of bias	Population	Test(s)	Test limitations and resolutions	Mutation identification errors	Failure rate
Tomaiuolo, Spina & Castaldo (2003) Italy	Level III-2 Low risk of bias	N=129 chromosomes from 85 DNA samples from CF subjects with known mutations	CF(12) ARMS kit	ARMS technology cannot distinguish between heterozygous and homozygous point mutations.	5 samples with homozygous point mutations were correctly identified but the CF(12) ARMS assay could not distinguish between the heterozygous and homozygous state.	Not reported
Strom et al. (2003) USA	Level III-2 Low risk of bias	N=7 confirmed CF patients with positive sweat tests 4 patients had 1 CF mutation and 3 had no mutations identified by either the ACMG or extended panel screening.	DNA sequencing reactions were performed with an ABI Prism Big Dye™ Terminator v3.0 cycle sequencing reaction kit according to the manufacturer's protocol.	This sequencing assay detects 991 of the 1,004 (98.7%) described mutations as of August 2002. All but 2 of these mutations involve large deletions of CFTR and would not be detectable by any sequencing assay.	None reported	Not reported

Study Country	Evidence level and risk of bias	Population	Test(s)	Test limitations and resolutions	Mutation identification errors	Failure rate
		Carrier screening program: number of patients screened not reported	CF testing for the ACMG panel using the Roche CF Gold Linear Array strips	Irregularities such as unusually weak lines or missing lines can be present on the strip assay, and these ambiguities can be resolved by sequencing the appropriate amplicon.	None reported	There were 9 instances of ambiguous results (approximately 1 per 10,000 assays)
Giuliani et al. (2010) Italy	Level III-2 Some risk of bias	N=23 CBAVD patients	MLPA to detect deletions and/or duplications of the *CFTR* gene DHPLC with abnormal results were analysed by direct DNA sequencing to detect point mutations of the *CFTR* gene.	Large deletions involving the *CFTR* gene are not detectable by any sequencing assay.	MLPA detected 2 deletion mutations in 2 CBAVD patients that could not be detected with DHPLC plus sequencing.	Not reported
Houdayer et al. (1998) France	Level III-2 Some risk of bias	N=40 DNA samples of known CFTR mutations	CF(12) ARMS kit	ARMS technology cannot distinguish between heterozygous and homozygous point mutations.	2/40 patients with homozygous mutations were not identified as homozygous.	Not reported
Ravnik-Glavac et al. (1994) USA	Level III-2 High risk of bias	N=133 DNA samples of known CFTR mutations	SSCP analysis of PCR products	The mobility shift of single strands is dependent on electrophoretic conditions such as temperature, ionic strength, composition of the gel, addition of glycerol. The sensitivity of SSCP varied greatly with the conditions and between exons.	There was no optimal condition suitable for all exons. However, using a 10% gel with 1.3% cross-linking in the presence of glycerol had a sensitivity of 100% for most exons and 80–98% for exons 4, 7 and 13.	Not reported
Ravnik-Glavac et al. (2002) USA	Level III-2 High risk of bias	N=73 DNA samples of known CFTR mutations	DHPLC using Stanford MELT software	An increase of the denaturation temperature by 1–2 degrees above that recommended by the MELT program improved the resolution of mutation detection to 100%.	7/73 (9.6%) mutations not detected at the recommended denaturation temperature	Not reported

Study Country	Evidence level and risk of bias	Population	Test(s)	Test limitations and resolutions	Mutation identification errors	Failure rate
Strom et al. (2004) USA	Level IV High risk of bias	N=1,092 patient samples previously tested with the CF Gold line probe assay chosen at random N=1,076 patient samples previously tested with the Applera CF OLA, Ver. 3.0, platform	The CF Portrait™ system multiplex PCR followed by a completely automated process of hybridisation and detection	Compound mutations within the same codon or on nearby codons can affect the performance of other probes detecting nearby sequence regions. For example, expected visual spot patterns were missing one or more wild-type probe spots with a compound heterozygote (I506V/F508del) and a homozygous mutation F508del/F508del in exon 10.	There was no discordance when compared with the Roche CF Gold line probe strips.	9% The authors reported that this was higher than expected and was most likely a product of DNA quality and not assay sensitivity. The DNA samples were not fresh, and the chips were observed to have gross failure consistent with PCR failure.
		N=1,092 patient samples	Roche CF Gold line probe strips		No errors reported	5%
		N=1,076 patient samples	Applera CF OLA, Ver 3.0 assay		7/1,076 (0.7%) miscalls of the IVS-8 5T/7T/9T polymorphism	5%
Edelmann et al. (2004) USA	Level IV High risk of bias	N=507 patients' samples referred for CF screening and 12 proficiency samples	eMAP BeadChip assay Multiplex PCR followed by ASOH	eMAP assay failure was scored as the inability to produce a result for at least one mutation on the BeadChip. The PCR failure rate primarily reflects the quality of the DNA samples.	There was no discordance between the ASOH method and the eMAP BeadChip assay.	9/519 (1.7%) The PCR failure rate for eMAP BeadChip assay = 3.5%. The failure rate for multiplex PCR and ASOH = 6%.
Axton & Brock (1995) UK	Level IV High risk of bias	N=193 mouthwash samples from CF patients	Restriction generation PCR	5/8 samples that failed amplified partially yielding products in some exons and required only partial retesting.	None reported	8/193 (4%)
Nagy et al. (2007) Hungary	Level IV High risk of bias	N=116 DNA samples	qPCR and melting curve analysis Fluorescent PCR and DNA fragment analysis	The F508Cdel mutation is caused by the deletion of a T and it seems that the fluorescent-PCR system does not recognise this one-base-pair difference.	Two F508Cdel samples were detected correctly by qPCR, but were reported as normal by fluorescent-PCR.	Not reported

ACMG = American College of Medical Genetics; ARMS = amplification refractory mutation system; ASOH = allele-specific oligonucleotide hybridisation; CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; DHPLC = denaturing high performance liquid chromatography; DNA = deoxyribonucleic acid; eMAP = elongation mediated multiplexed analysis of polymorphisms; MLPA = multiple ligation-dependent probe amplification; OLA = oligonucleotide ligation assay; PCR = polymerase chain reaction; qPCR = quantitative real-time PCR; SSCP = single-stranded conformation polymorphism

APPENDIX E IMPACT OF CHANGE IN MANAGEMENT (SECTION B.8.1)

Table 97 Psychological impact of TOP due to fetal abnormalities

Study	Study type	Population	Results
Davies et al. (2005)	Cohort study (non-comparative)	30 women: 14 with first-trimester TOP, 16 with second-trimester TOP. Follow-up at 6 months was n=26 and follow-up at 12 months was n=22.	Post-traumatic stress disorder (score >18 on Impact of Event Scale): 6 weeks after TOP: 67%, mean 27 (SD: 14.6) 6 months after TOP: 50%, mean 23 (SD: 18.7) 12 months after TOP: 41%, mean 21 (SD: 18.9) Emotional distress (score >4 on the general health questionnaire) 6 weeks after TOP: 53% 6 months after TOP: 46% 12 months after TOP: 43% Grief (score >90 on Perinatal Grief Scale) 6 weeks after TOP: 47% 6 months after TOP: 31% 12 months after TOP: 27% Depression (score >9 on Beck Depression Inventory) 6 weeks after TOP: 30% 6 months after TOP: 36% 12 months after TOP: 32%
Geerinck-Vercammen & Kanhai (2003)	Prospective semi-structured interviews (before and after TOP)	89 couples who underwent TOP for fetal abnormality; 86 participated in at least one interview.	Grief at 6 weeks after TOP: Dominating: 27 (36%) women, 5 (8%) men Regular moments: 33 (43%) women, 21 (33%) men Few moments: 16 (31%) women, 5 (11%) men Grief at 6 months after TOP: Dominating: 8 (13%) women, 1 (2%) men Regular moments: 12 (20%) women, 5 (11%) men Few moments: 35 (58%) women, 25 (53%) men
Iles et al. (1993)	Prospective semi-structured interviews (4–6 weeks, 6 months and 13 months after TOP)	71 women who underwent mid-trimester TOP; 61 women were interviewed all three times	Physical grief (choking, sighing, blurred vision etc.): 4–6 weeks after TOP: 55/71 (78%) 6 months after TOP: 29/65 (45%) 13 months after TOP: 19/61 (31%) Numbness: 4–6 weeks after TOP: 45/71 (63%) 6 months after TOP: – 13 months after TOP: – Anger: 4–6 weeks after TOP: 41/71 (58%) 6 months after TOP: 27/65 (42%) 13 months after TOP: 19/61 (31%) Guilt: 4–6 weeks after TOP: 34/71 (48%) 6 months after TOP: 8/65 (12%) 13 months after TOP: 19/61 (31%)

Study	Study type	Population	Results
Kersting et al. (2005)	Cross-sectional study	83 women who underwent TOP 2–7 years ago and 60 women 14 days after TOP	Post-traumatic stress disorder (Impact of Event Scale–Revised score) 14 days after TOP (n=60): mean 44.03 (SD 19.17) 2–7 years after TOP (n=83): mean 41.78 (SD 24.46)
Kersting et al. (2009)	Prospective longitudinal study	62 women who underwent TOP; interviews and questionnaires were completed by 36 (58%)	Psychiatric diagnoses according to Structured Clinical Interview for DSM-IV: 14 days after TOP: 25% 6 months after TOP: 25% 14 months after TOP: 16.7% Post-traumatic stress disorder (Impact of Event Scale–Revised score >19) n=36 14 days after TOP: mean 45.0 (SD 17.54) 6 months after TOP: mean 35.3 (SD 21.53) 14 months after TOP: mean 30.9 (21.35) Depression (Beck Depression Inventory score) n=36 14 days after TOP: mean 12.3 (SD 7.54) 6 months after TOP: mean 7.7 (SD 8.11) 14 months after TOP: mean 7.6 (SD 6.45)
Korenromp et al. (2005) [a]	Cross-sectional retrospective study	254 women who underwent TOP 2–7 years ago (mean 4.1 years, SD 1.3); only 196 women completed the set of questionnaires (77%)	Grief (Inventory of Traumatic Grief score >90) 2.6% (n=5) Post-traumatic stress disorder (Impact of Event Scale–Revised score >39) 17.3% (n=33), unrelated to elapsed time since TOP Patients who underwent TOP before 14 weeks of gestation had significantly lower scores for grief (mean 40.0; SD 10.8, n=44) and post-traumatic stress (mean 14.1; SD 14.5, n=44) then women who underwent TOP after 14 weeks of gestation (mean 46.9; SD 17.4; n=150, p=0.014 and mean 21.5; SD 20.3; n=148, p=0.026, respectively).
Korenromp et al. (2005) [b]	Cross-sectional retrospective study	151 couples who underwent TOP 2–7 years ago (same women as Korenromp et al. (2005) [a])	Grief (Inventory of Traumatic Grief), mean score Men: 38.6 (SD 11.4) Women: 44.1 (SD 16.2) Post-traumatic stress disorder (Impact of Event Scale–Revised), mean score Men: 12.8 (SD 16.6) Women: 18.1 (SD 18.0) Anxiety (Symptom Checklist-90 anxiety score (women >26 and men >21), mean score Men: 12.1 (SD 4.5) Women: 14.0 (SD 6.0) Depression (Symptom Checklist-90 depression score (women >41 and men >33), mean score Men: 20.8 (SD 7.5) Women: 26.0 (SD 11.0)
Korenromp et al. (2007)	Prospective cohort study	217 women at 4 months after TOP and their partners (all men, n=169)	Grief (Inventory of Complicated Grief, cut-off level = 90), mean score Women: 59.0 (SD 20.4) Men: 47.8 (SD 16.6) Post-traumatic stress disorder (Impact of Event Scale,

Study	Study type	Population	Results
			cut-off level = 26), mean score Women: 25.1 (SD 15.2) Men: 16.9 (SD 12.6) General psychological malfunctioning (Symptom Checklist-90, cut-off level = 204 for women and 170 for men), mean score Women: 145.6 (SD 53.1) Men: 121.5 (SD 36.6) Post-partum depression (Edinburgh Postnatal Depression Scale, cut-off level = 12), mean score Women: 8.4 (SD 5.6) Men: 5.5 (SD 5.2)
Korenromp et al. (2009)	Prospective cohort study	300 women were initially included (at 4 months after TOP); 217 of them were also included in Korenromp et al. (2007) and 147 of them completed the study (i.e. filled in questionnaires at 4 months, 8 months and 16 months)	Grief (Inventory of Complicated Grief, cut-off level = 90), mean score (SD), % above cut-off level 4 months after TOP: 58.8 (19.6) 8.8% 8 months after TOP: 54.0 (18.2) 4.8% 16 months after TOP: 50.1 (16.5) 2.1% Post-traumatic stress disorder (Impact of Event Scale, cut-off level = 26), mean score (SD), % above cut-off 4 months after TOP: 25.2 (12.2) 45.8% 8 months after TOP: 21.4 (15.1) 36.7% 16 months after TOP: 15.5 (12.4) 20.5% Psychological malfunctioning (Symptom Checklist-90, cut-off level = 204), mean score (SD), % above cut-off 4 months after TOP: 144 (50) 12.2% 8 months after TOP: 128 (39) 7.5% 16 months after TOP:121 (33) 4.8% Depression (Edinburgh Postnatal Depression Scale, cut-off level =12), mean score (SD), % above cut-off 4 months after TOP: 8.2 (5.7) 27.9% 8 months after TOP: 6.9 (4.9) 19.7% 16 months after TOP: 5.3 (4.4) 13.1%
Salvesen et al. (1997)	Prospective cohort study	24 women who underwent TOP before 24 weeks of pregnancy	Intrusive distress: Impact of Event Scale (0–8 low, 9–19 medium, >20 severe) Acute phase: low 4%, medium 48%, severe 48% 7 weeks after TOP: low 26%, medium 48%, severe 26% 5 months after TOP: low 13%, medium 54%, severe 33% 1 year after TOP: 44%, medium 37%, severe 19% Avoidance response: Impact of Event Scale (0–8 low, 9–19 medium, >20 severe) Acute phase: low 74%, medium 11%, high 15% 7 weeks after TOP: low 80%, medium 20%, high 0% 5 months after TOP: low 87%, medium 13%, high 0% 1 year after TOP: low 80%, medium 20%, high 0%
Zeanah et al. (1993)	Retrospective case series	23 women at 2 months after TOP	Grief (Perinatal Grief Scale, score 1–5, higher score means more-intense symptom), mean (SD) 3.29 (0.83) Difficulty coping (Perinatal Grief Scale, score 1–5, higher score means more-intense symptom), mean (SD)

Study	Study type	Population	Results
			2.39 (0.97) Despair (Perinatal Grief Scale, score 1–5, higher score means more intense symptom), mean (SD) 1.98 (0.58) Depression (Beck Depression Inventory, 1–10 = none to minimal depression, 11–18 = mild to moderate, 19–29 = severe), mean (SD) 10.8 (7.5)

SD = standard deviation; TOP = termination of pregnancy

APPENDIX F EXCLUDED STUDIES

INCORRECT POPULATION/SAMPLE

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APPENDIX G LITERATURE SEARCH FOR ECONOMIC STUDIES

LITERATURE SEARCH FOR INCIDENCE OF CF IN FETUSES WITH ECHOGENIC BOWEL

Table 98 Incidence of CF in fetuses with echogenic bowel reported in previous studies

Study	Setting (period)	N fetuses with FEB tested	N CF +ve	Incidence of CF [a] (%)
Goetzinger et al. (2011)	USA (1990–2008)	260	6	2.3
Carcopino et al. (2007)	Review of the literature (11 studies)	1,682	40	2.4
Buiter et al. (2013)	Netherlands (2009–10)	37	1	2.7
Ameratunga et al. (2012)	Australia (2004–09)	33	1	3.0
Muller et al. (2002)	France (1997–98)	641	20	3.1
Ghose et al. (2000)	Leeds (1996 –97)	60	2	3.3
Dugueperoux et al. (2012)	France (2002 –09)	229	9	3.9
de Becdelievre et al. (2011)	France (1992–2009)	694	30	4.3
Mailath-Pokorny et al. (2012)	Austria (1998–2011)	66	3	4.5
Slotnick & Abuhamad (1996)	USA (16 months)	145	7	4.8
Nicholls et al. (2003)	Australia (1992–2002)	35	2	5.7
Scotet et al. (2010)	France (1992–2007)	289	23	8.0
Scotet et al. (2003)	France (1992–2001)	173	22	12.7

[a] Incidence of CF is calculated as N CF +ve / N tested.
CF = cystic fibrosis; FEB = fetal echogenic bowel

LITERATURE SEARCH FOR PREVIOUSLY PUBLISHED ECONOMIC EVALUATIONS OF PRENATAL CF DIAGNOSIS

Table 99 Results of literature search for economic evaluations for prenatal CFTR mutation testing

Search	Query	Results
Pubmed	(Search date: 17 November 2014)	
#11	Search (((((((cystic fibrosis conductance transmembrane regulator) OR CFTR)) OR ((((cystic fibrosis OR cystic fibrosis [MeSH])))) AND ((gene OR gene* OR carrier* OR prenatal OR antenatal OR fetus* OR fetus* OR fetal OR fetal)))))) AND ((((screen* OR test* OR diagnos*))) AND ((("Cystic Fibrosis Transmembrane Conductance Regulator/diagnostic use"[Mesh]) OR ("Cystic Fibrosis/prevention and control"[Mesh])) OR ("Cystic Fibrosis/diagnosis"[Mesh] AND "Cystic Fibrosis/genetics"[Mesh])))) AND (((((cost OR economic OR cost-effectiveness)) AND (evaluation OR analysis))) OR ((economics[MeSH Subheading]) OR ('decision analyses'[MeSH Terms] OR 'decision analysis'[MeSH Terms] OR cost-effectiveness[MeSH Terms] OR 'cost analysis"[MeSH Terms]))))	93
Embase	(12 November 2014)	
#8	CFTR OR 'cystic fibrosis transmembrane conductance' OR 'cystic fibrosis' AND ('gene' OR gene* OR carrier* OR prenatal OR antenatal OR fetal OR fetus OR fetal) AND (screen* OR test* OR diagnos*) AND (economic OR cost AND (analysis OR evaluation)	388

Search	Query	Results
	OR 'cost comparison' OR 'cost effectiveness')	

LITERATURE SEARCH FOR PREVIOUSLY PUBLISHED ECONOMIC EVALUATIONS OF FETUSUS SHOWING ECHOGENIC BOWEL ON THE SECOND-TRIMESTER ULTRASOUND

Table 100 Results of literature search for economic evaluations for prenatal CFTR mutation testing in fetusus showing echogenic bowel

Search	Query	Results
Pubmed	(Search date: 9 December 2014)	
#3	Search ((((((echogenic[Title/Abstract] OR hyperechogenic[Title/Abstract])) AND (bowel[Title/Abstract] OR gut[Title/Abstract])) AND (prenatal[Title/Abstract] OR antinatal[Title/Abstract] OR fetal[Title/Abstract] OR fetal[Title/Abstract] OR pregnancy[Title/Abstract] OR pregnant[Title/Abstract] OR fetus[Title/Abstract] OR fetus[Title/Abstract])) AND cystic fibrosis[Title/Abstract])) AND (((economic[Title/Abstract] OR cost-effectiveness[Title/Abstract] OR cost[Title/Abstract] OR decision[Title/Abstract])) AND (analysis[Title/Abstract] OR evaluation[Title/Abstract]))	0

APPENDIX H ECONOMIC STUDIES CONDUCTED IN AUSTRALIAN SETTING

Table 101 Economic evaluations identified that investigate cost-effectiveness of prenatal screening in Australia

Study	Setting	Results
Maxwell et al. (2010)	Australian healthcare perspective. Decision tree analysis to compare simultaneous screening and stepwise screening strategies for couples	Outcome measured as cost per CF birth averted.
Norman et al. (2012)	Australian healthcare system. Decision tree modelling to evaluate the cost-effectiveness of national carrier screening for CF, including both initial and subsequent pregnancies (for couples identified as carriers in initial pregnancies). Couples with subsequent pregnancies have reproductive choices such as pre-implantation diagnosis, abstaining from reproduction and planning for prenatal diagnosis.	Outcome was measured as cost per CF birth averted.

CF = cystic fibrosis

APPENDIX I COST OF CFTR GENETIC TESTING IN AUSTRALIA

Table 102 Costs associated with CFTR mutation testing

Test description	Cost	Reference Lab
Common mutation testing		
10 mutations	$135 (Australian dollars, 2014 prices)	Maxwell et al. (2010)
Microvillar enzyme analysis for prenatal diagnosis (11 mutations)	$168	National Referral Laboratory (Women's and Children's Hospital, SA)
CF (maximum of 13 mutations)	$300	Women's and Children Hospital, SA
29 mutations	$250	PaLMS-RNSH, NSW
32 mutations	$250	Gippsland Pathology, VIC
32 mutations	$200	Department of Molecular Genetics, NSW
32 mutations	$290	Molecular Genetics Laboratory, NSW
44 mutations	$150	Healthscope Pathology, VIC
38 mutations	$150	Victorian Clinical Genetics Services, VIC
30 mutations	$270	The Queensland Fertility Group, QLD
90 mutations	$350	Genea, NSW
145 mutations	$500	Expert opinion [a]
Single mutation testing		
F508del	$50	PaLMS-RNSH, NSW
delta F508	$50	Hunter genetics, NSW
Phe508del	$80	Department of Molecular Genetics, NSW
CF carrier screening – Poly-T	$84	PaLMS-RNSH, NSW
Single mutation (CVS)	$90	PaLMS-RNSH, NSW
F508del	$80	Sydney South West Pathology Service, NSW
CF known (one) mutation analysis	$160	SA Pathology, SA
Whole gene testing		
CF gene scanning and rare mutation detection (Sanger sequencing)	$1,000	PaLMS-RNSH, NSW

Sources: Massie, J, Ioannou & Delatycki (2014); Maxwell et al. (2010); RCPA website accessed on 8 January 2015 (data last updated on December 2009);
<http://genetictesting.rcpa.edu.au/component/search/?searchword=cystic+fibrosis&ordering=&searchphrase=all&areas[0]=all>;
<http://www.healthscopepathology.com.au/index.php/advanced-pathology/344/cystic-fibrosis-carrier-screening/>, accessed on 13 March 2015.

[a] Pers. comm. with RCPA member, received on 2 March 2015

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CVS = chorionic villus sampling; PaLMS-RNSH = Pacific Laboratory Medicine Services – Royal North Shore Hospital, NSW

APPENDIX J ADDITIONAL INFORMATION RELATING TO THE ECONOMIC EVALUATION

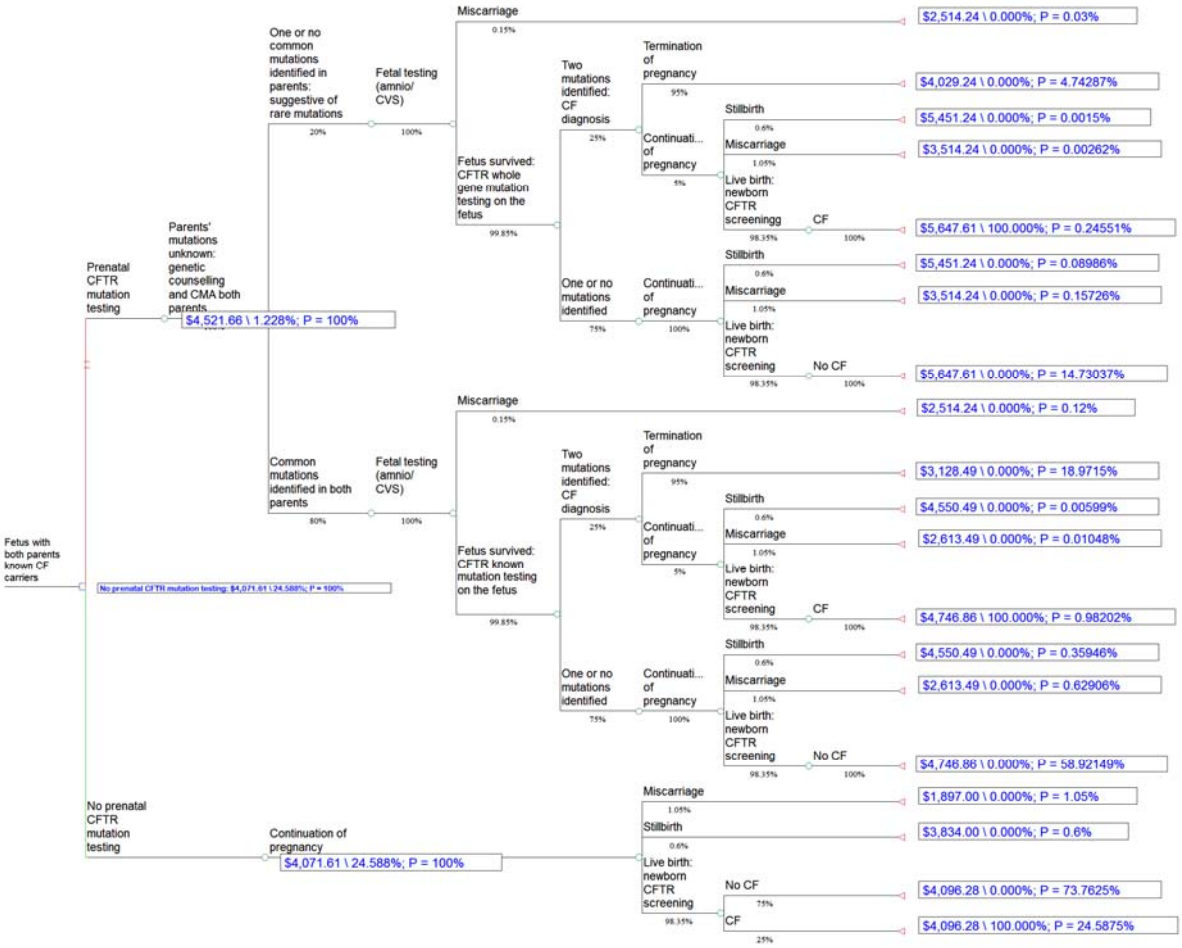
DERIVATION OF MODEL PROBABILITIES

Table 103 Model probabilities for identifying CFTR mutations in both parents in the base-case analysis

Model arm	Two mutations identified	One mutation identified	No mutation identified
Model 1			
Both parents known CF carriers	clin_sens * clin_sens	2 * clin_sens * (1-clin_sens)	(1-clin_sens) * (1-clin_sens)
(carrier rate 100%)	64%	32%	4%
Model 2			
Parents of fetus with CF	clin_sens * clin_sens	2 * clin_sens * (1-clin_sens)	(1-clin_sens) * (1-clin_sens)
(carrier rate 100%)	64%	32%	4%
Parents of fetus without CF	cr * clin_sens * cr * clin_sens	(2 * cr * clin_sens * cr * (1 -clin_sens)) + (2 * cr * clin_sens * (1 – cr))	(cr* (1-clin_sens) * cr * (1-clin_sens)) + (2 * cr* (1-clin_sens) * (1 – cr)) + ((1 – cr) * (1 – cr))
(carrier rate 4%)	0.10%	6.2%	93.7%

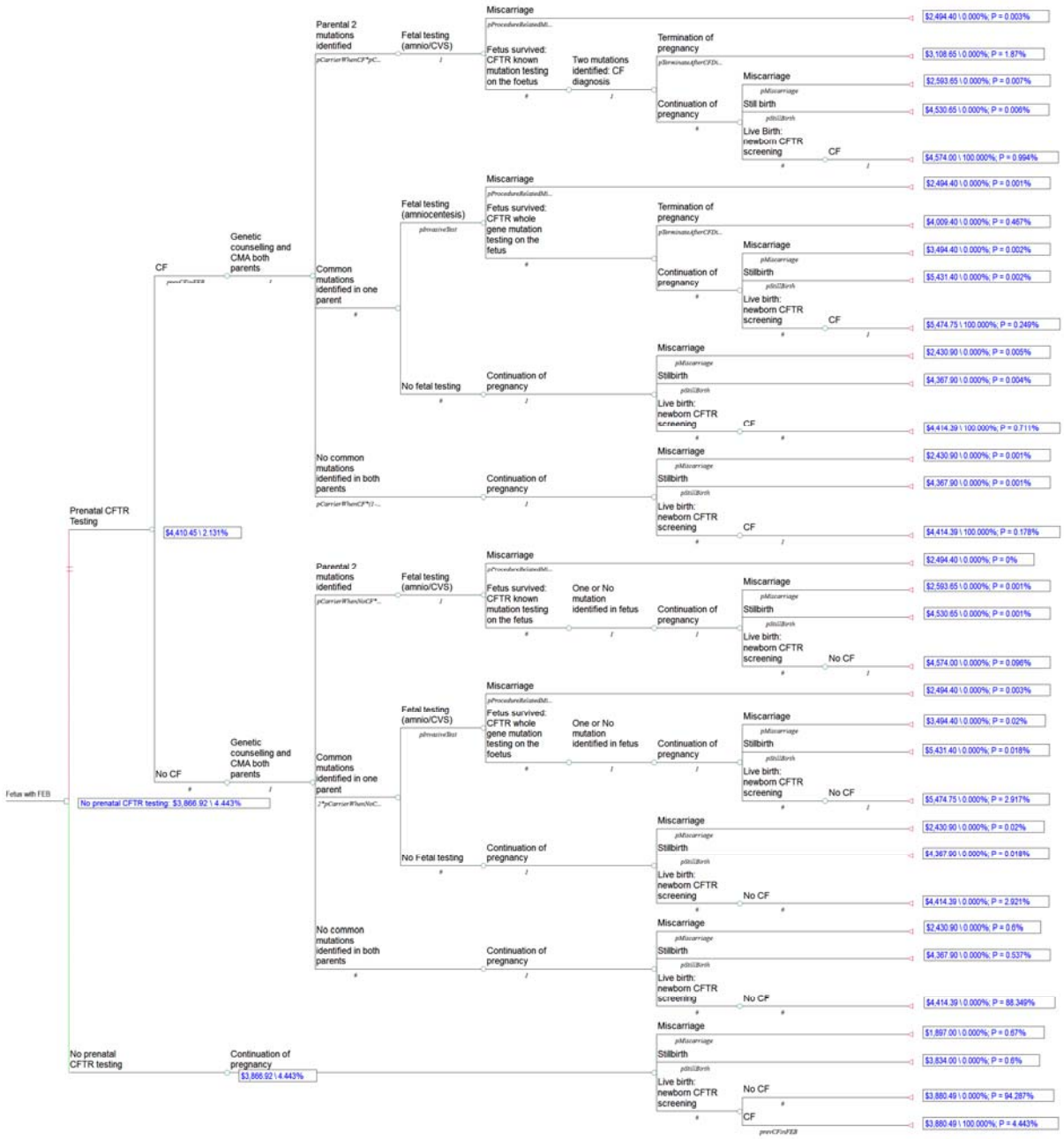
CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; clin_sens = clinical sensitivity (80%); cr = carrier rate

RESULTS OF THE ECONOMIC EVALUATION



amnio = amniocentesis; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CMA = common mutation analysis; CVS = chorionic villus sampling

Figure 10 Results of the economic evaluation (total CF births), model 1 (Both parents known CF carriers)



amnio = amniocentesis; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CMA = common mutation analysis; CVS = chorionic villus sampling; FEB = fetal echogenic bowel

Figure 11 Results of the economic evaluation (total CF births), model 2 (Fetus has FEB)

ADDITIONAL SCENARIOS

ADDITIONAL SCENARIO 1: 17 MUTATIONS PANEL TEST

Table 104 Outcome and incremental effects (per 100 pregnancies) for additional scenario 1

Outcome	Prenatal testing	No prenatal testing	Incremental effectiveness	Nature of effect
Model 1 (Parents are known CF carriers)				
Prenatal CF diagnosed	24.94	0	24.94	Benefit
CF births total:	1.23	24.59	–23.36	
informed	1.23	0	1.23	Benefit
uninformed	0	24.59	–24.59	
CF births averted	23.72	0	23.72	Benefit
Fetal loss	1.26	1.65	–0.39	
Procedure-related fetal loss	0.15	0	0.15	Harm
No CF	73.65	73.76	–0.11	-
Model 2 (Fetus has FEB)				
Prenatal CF diagnosed	3.74	0	3.74	Benefit
CF births total:	2.03	4.44	–2.41	
informed	1.30	0	1.30	Benefit
uninformed	0.73	4.44	–3.71	
CF births averted	2.44	0	2.44	Benefit
Fetal loss	1.24	1.27	–0.03	
Procedure-related fetal loss	0.007	0	0.007	Harm
No CF	94.28	94.29	0.00	-

FEB = fetal echogenic bowel; CF = cystic fibrosis

Table 105 Incremental costs, additional scenario 1

Cost	Prenatal testing	No prenatal testing	Increment
Model 1 *(Parents are known CF carriers)*			
Cost per pregnancy	$4,524.55	$4071.61	$452.94
Model 2 *(Fetus has FEB)*			
Cost per pregnancy	$4,439.82	$3,866.92	$572.90

FEB = fetal echogenic bowel; CF = cystic fibrosis

Table 106 ICER, additional scenario 1

Clinically relevant outcomes	Incremental outcomes (per 100 pregnancies)	ICER ($/outcome)
Modelled population 1 (Parents are known CF carriers)	*Incremental cost: $45,294*	
Diagnosis of CF in utero	24.94	$1,816 / prenatal CF detected

Clinically relevant outcomes	Incremental outcomes (per 100 pregnancies)	ICER ($/outcome)
CF births averted	23.72	$1,910 / CF birth averted
Pre-informed CF birth	1.23	$36,884 / pre-informed CF birth
Modelled population 2 (Fetus has FEB)	*Incremental cost: $57,290*	
Diagnosis of CF in utero	3.74	$15,331 / prenatal CF detected
CF births averted	2.44	$23,480 / CF birth averted
Pre-informed CF birth	1.30	$44,171 / pre-informed CF birth

CF = cystic fibrosis; FEB = fetal echogenic bowel; ICER = incremental cost-effectiveness ratios

ADDITIONAL SCENARIO 2: 23 MUTATIONS PANEL TEST

Table 107 Outcome and incremental effects (per 100 pregnancies) for additional scenario 2

Outcome	Prenatal testing	No prenatal testing	Incremental effectiveness	Nature of effect
Model 1 (Parents are known CF carriers)				
Prenatal CF diagnosed	24.94	0	24.94	Benefit
CF births total:	1.23	24.59	–23.36	
informed	1.23	0	1.23	Benefit
uninformed	0.0	24.59	–24.59	
CF births averted	23.72	0	23.72	Benefit
Fetal loss	1.26	1.65	–0.39	
Procedure-related fetal loss	0.15	0	0.15	Harm
No CF	73.65	73.76	–0.11	-
Model 2 (Fetus has FEB)				
Prenatal CF diagnosed	3.94	0	3.94	Benefit
CF births total:	1.90	4.44	–2.54	
informed	1.37	0	1.37	Benefit
uninformed	0.53	4.44	–3.91	
CF births averted	2.57	0	2.57	Benefit
Fetal loss	1.24	1.24	–0.03	
Procedure-related fetal loss	0.009	0	0.009	Harm
No CF	94.28	94.29	0.00	-

FEB = fetal echogenic bowel; CF = cystic fibrosis

Table 108 Incremental costs, additional scenario 2

Cost	Prenatal testing	No prenatal testing	Increment
Model 1 *(Parents are known CF carriers)*			
Cost per diagnosis	$4,530.47	$4071.61	$458.86

Cost	Prenatal testing	No prenatal testing	Increment
Model 2 *(Fetus has FEB)*			
Cost per diagnosis	$4,478.91	$3,866.92	$611.99

FEB = fetal echogenic bowel; CF = cystic fibrosis

Table 109 ICER, additional scenario 2

Clinically relevant outcomes	Incremental outcomes (per 100 pregnancies)	ICER ($/outcome)
Modelled population 1 (Parents are known CF carriers)	*Incremental cost: $45,886*	
Diagnosis of CF in utero	24.94	$1,840 / prenatal CF detected
CF births averted	23.72	$1,935 / CF birth averted
Pre-informed CF birth	1.23	$37,397 / pre-informed CF birth
Modelled population 2 (Fetus has FEB)	*Incremental cost: $61,199*	
Diagnosis of CF in utero	3.94	$15,537 / prenatal CF detected
CF births averted	2.57	$23,794 / CF birth averted
Pre-informed CF birth	1.37	$44,769 / pre-informed CF birth

CF = cystic fibrosis; FEB = fetal echogenic bowel; ICER = incremental cost-effectiveness ratios

ADDITIONAL SCENARIO 3: 32 MUTATIONS PANEL TEST

Table 110 Outcome and incremental effects (per 100 pregnancies) for additional scenario 3

Outcome	Prenatal testing	No prenatal testing	Incremental effectiveness	Nature of effect
Model 1 (Parents are known CF carriers)				
Prenatal CF diagnosed	24.94	0	24.94	Benefit
CF births total:	1.23	24.59	–23.36	
informed	1.23	0	1.23	Benefit
uninformed	0.0	24.59	–24.59	
CF births averted	23.71	0	23.71	Benefit
Fetal loss	1.26	1.65	–0.39	
Procedure-related fetal loss	0.15	0	0.15	Harm
No CF	73.65	73.76	–0.11	-
Model 2 (Fetus has FEB)				
Prenatal CF diagnosed	4.12	0	4.12	Benefit
CF births total:	1.78	4.44	–2.66	
informed	1.43	0	1.43	Benefit
uninformed	0.36	4.44	–4.09	
CF births averted	2.69	0	2.69	Benefit
Fetal loss	1.24	1.24	–0.03	

Outcome	Prenatal testing	No prenatal testing	Incremental effectiveness	Nature of effect
Procedure-related fetal loss	0.008	0	0.008	Harm
No CF	94.28	94.29	0.00	-

CF = cystic fibrosis; FEB = fetal echogenic bowel

Table 111 Incremental costs, additional scenario 3

Cost	Prenatal testing	No prenatal testing	Increment
Model 1 *(Parents are known CF carriers)*			
Cost per pregnancy	$4,564.63	$4071.61	$493.02
Model 2 *(Fetus has FEB)*			
Cost per pregnancy	$4,538.17	$3,866.92	$671.25

CF = cystic fibrosis; FEB = fetal echogenic bowel

Table 112 ICER, additional scenario 3

Clinically relevant outcomes	Incremental outcomes (per 100 pregnancies)	ICER ($/outcome)
Modelled population 1 (Parents are known CF carriers)	*Incremental cost: $49,302*	
Diagnosis of CF in utero	24.94	$1,977 / prenatal CF detected
CF births averted	23.71	$2,079 / CF birth averted
Pre-informed CF birth	1.23	$40,181 / pre-informed CF birth
Modelled population 2 (Fetus has FEB)	*Incremental cost: $67,125*	
Diagnosis of CF in utero	4.12	$16,304 / prenatal CF detected
CF births averted	2.69	$24,972 / CF birth averted
Pre-informed CF birth	1.43	$46,974 / pre-informed CF birth

CF = cystic fibrosis; FEB = fetal echogenic bowel; ICER = incremental cost-effectiveness ratios

APPENDIX K ADDITIONAL INFORMATION FOR THE FINANCIAL IMPLICATION ANALYSIS

Table 113 Projected numbers of CFTR mutation tests, 2012–19

	2011	2012	2013	2014	2015	2016	2017	2018	2019
Diagnostic	3,110	3,577	4,113	4,730	5,439	6,255	7,194	8,273	9,514
Predictive	1,266	1,456	1,674	1,925	2,214	2,546	2,928	3,368	3,873
Screening	10,194	11,723	13,482	15,504	17,829	20,504	23,579	27,116	31,184
Prenatal	792	911	1,047	1,205	1,385	1,593	1,832	2,107	2,423
Total	15,362	17,666	20,316	23,364	26,868	30,898	35,533	40,863	46,993

CFTR = cystic fibrosis transmembrane conductance regulator

The number of tests and financial implications have been disaggregated as best as possible by proposed indications and are presented below.

Populations 1b, 1c and 1d

- patients with symptoms of classic CF
- patients with chronic symptoms of non-classic Cf
- men with congenital absence of the vas deferens

Testing in these indications comes under the diagnostic indication. The numbers of tests and financial implications for these indications are presented in Table 114.

Table 114 Estimated numbers of CFTR mutation tests and cost implications for diagnostic indications eligible for MBS funding, populations 1b, 1c and 1d

	2015	2016	2017	2018	2019
Number of tests [a]	5,395	6,204	7,134	8,204	9,435
Number of single mutation analyses	0	0	0	0	0
Cost of known mutation analyses	$0	$0	$0	$0	$0
Number of common mutation panels	4,495	5,170	5,945	6,837	7,863
Cost of common mutation panels	$606,885	$697,918	$802,605	$922,996	$1,061,445
Number of whole gene screens	899	1,034	1,189	1,367	1,573
Cost of whole gene screens	$899,089	$1,033,952	$1,189,045	$1,367,402	$1,572,512
Total cost	$1,505,974	$1,731,870	$1,991,650	$2,290,398	$2,633,957

[a] From Table 74

CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule

Populations 2a and 2b

- prenatal diagnosis of fetuses of couples who have a previous child with CF or CFTR-related disorder, or of couples who are found to be carriers of a CFTR mutation (including testing of the parents)
- prenatal diagnosis of fetuses with an echogenic gut (including testing of the parents)

Testing in these indications comes under the predictive and prenatal indications. The numbers of tests and financial implications for these indications are presented in Table 115.

Table 115 Estimated numbers of CFTR mutation tests and cost implications for prenatal indications eligible for MBS funding, populations 2a and 2b

	2015	2016	2017	2018	2019
Number of tests [a]	2,492	2,866	3,296	3,791	4,359
Number of single mutation analyses	720	828	953	1,096	1,260
Cost of known mutation analyses	$57,625	$66,269	$76,209	$87,640	$100,786
Number of common mutation panels	1,495	1,719	1,977	2,274	2,615
Cost of common mutation panels	$201,822	$232,096	$266,910	$306,947	$352,989
Number of whole gene screens	277	319	366	421	485
Cost of whole gene screens	$277,043	$318,599	$366,389	$421,347	$484,549
Total cost	$536,490	$616,963	$709,508	$815,934	$938,324

[a] Sum of prenatal and predictive tests presented in Table 74
CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule

Population 3

- partners of someone who is known to have CF or be a carrier of a CFTR mutation

Testing in this indication comes under the screening indication. The numbers of tests and financial implications for this indication are presented in Table 116.

Table 116 Estimated numbers of CFTR mutation tests and cost implications for prenatal indications eligible for MBS funding, population 3

	2015	2016	2017	2018	2019
Number of tests [a]	617	710	816	939	1,080
Number of single mutation analyses	0	0	0	0	0
Cost of known mutation analyses	$0	$0	$0	$0	$0
Number of common mutation panels	617	710	816	939	1,080
Cost of common mutation panels	$83,332	$95,832	$110,207	$126,738	$145,749
Number of whole gene screens	0	0	0	0	0
Cost of whole gene screens	$0	$0	$0	$0	$0
Total cost	$83,332	$95,832	$110,207	$126,738	$145,749

[a] From Table 74
CFTR = cystic fibrosis transmembrane conductance regulator; MBS = Medicare Benefits Schedule

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