NATIONAL PATHOLOGY ACCREDITATION ADVISORY COUNCIL

REQUIREMENTS FOR HUMAN MEDICAL GENOME TESTING UTILISING MASSIVELY PARALLEL SEQUENCING TECHNOLOGIES

(First Edition 2017)

NPAAC Tier 4 Document

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Contents

The National Pathology Accreditation Advisory Council (NPAAC) was established in 1979 to advise the Australian, State and Territory governments on matters relating to the accreditation of pathology laboratories. A key role of NPAAC is to develop and maintain pathology quality standards for accreditation. NPAAC also advises on pathology accreditation policy initiatives and initiates and promotes education programs about quality in the provision of pathology services.

Publications produced by NPAAC are issued as accreditation materials to provide guidance to medical pathology laboratories and accrediting agencies about minimum standards considered acceptable for good laboratory practice.

Failure to meet these minimum standards may pose a potential risk to public health and patient safety.

Scope

The *Requirements for Human Medical Genome Testing Utilising Massively Parallel Sequencing Technologies (First Edition 2017)* is a Tier 4 NPAAC document and must be read in conjunction with the Tier 2 document *Requirements for Medical Pathology Services.* The latter is the overarching document broadly outlining standards for good medical pathology practice where the primary consideration is patient welfare, and where the needs and expectations of patients, laboratory staff and referrers (both for pathology requests and inter-laboratory referrals) are safely and satisfactorily met in a timely manner.

Whilst there must be adherence to all the Requirements in the Tier 2 document, reference to specific standards in that document are provided for assistance under the headings in this document.

This document outlines the minimum best practice requirements for medical pathology laboratories undertaking the performance and implementation of human genetic testing utilising massively parallel sequencing (MPS) for all applications (ie. single gene, panel of genes, somatic testing, whole exome or whole genome or non-invasive prenatal screening) also known as next generation sequencing. It takes a risk based approach to defining standards for the implementation of these new technologies.

While MPS testing will have a marked impact in the area of microbial testing and is already being used, particularly in the area of microbial epidemiology, it is outside the scope of this document and will have other requirements. However, many of the practices will be similar.

Abbreviations

AS	Australian Standard
CFTR gene	Cystic Fibrosis Transmembrane Conductance Regulator gene
ctDNA	Cell-free (or circulating) tumour Deoxyribonucleic Acid
DNA	DeoxyriboNucleic Acid
dbSNP	Single Nucleotide Polymorphism Database
FASTA	Text-based nucleotide sequence, using single-letter codes
FASTQ	Text base nucleotide sequence with attached quality scores
FFPE	Formalin-Fixed Paraffin-Embedded (tissue)
HGNC	HUGO Gene Nomenclature Committee
HGVS	Human Genome Variation Society
HUGO	Human Genome Organisation
HGSA	Human Genetics Society of Australasia
ILAC	International Laboratory Accreditation Cooperation
ISO	International Organization for Standardization
IVD	In Vitro Diagnostic device
LRG	Locus Reference Genomic
MPS	Massively Parallel Sequencing
NATA	National Association of Testing Authorities, Australia
NHMRC	National Health and Medical Research Council
NIPS	Non-Invasive Prenatal Screening
NPAAC	National Pathology Accreditation Advisory Council
PCR	Polymerase Chain Reaction
QA	Quality Assurance
QC	Quality Control
qPCR	Quantitative PCR
RCPA	Royal College of Pathologists of Australasia
RM	Reference Materials
SNP	Single Nucleotide Polymorphism
SNV	Single Nucleotide Variant
TGA	Therapeutic Goods Administration
VCF	Variant Call Format
WGS	Whole Genome Sequencing
WES	Whole Exome Sequencing

Definitions

Term	Definition
Analytical validity	means the process and results of a test to determine that the presence or absence of the intended target has been assessed and shown to be reproducible and specific.
Bioinformatics pipeline	means the primary, secondary and tertiary analysis of MPS data that is performed computationally by individual computer algorithms. The pipeline is the specific combination and order of these algorithms used to analyse the data.
dbSNP	means the Single Nucleotide Polymorphism Database, a free public archive for genetic variation within and across different species developed and hosted by the National Center for Biotechnology Information (NCBI) in collaboration with the National Human Genome Research Institute (NHGRI).
Exome	means the collection of protein-coding genes in the genome and is made up of exons.
GC-content	means guanine cytosine-content of a gene or region of DNA.
Genome	means the complete set of DNA from an organism consisting of 3 billion nucleic acid base pairs in humans and found in every cell with a nucleus.
Genomic testing	means comprehensive analysis of large segments of the genome (e.g. whole genome sequencing, whole exome sequencing or large panel sequencing).
Incidental findings	means any finding that has clinical significance that was identified by the test but is not related to the request for testing. These may also be interchangeably referred to as secondary findings or unsolicited findings.
Indel	means a mutation class where there has been either an insertion or a deletion of nucleotides, or a combination of both, compared to the reference sequence.
Massively Parallel Sequencing	means the collection of technologies used to enable the sequencing of many, usually short fragments of DNA, at the same time to provide greatly increased sequencing coverage of either individual samples or multiple samples that can be distinguished by the use of introduced sample indexing. This may also be referred to as Next Generation Sequencing.

Term	Definition
Medical consultation framework	means the structure and process of patient consultation that is predetermined and laid out for staff to follow providing consistency of care.
Orthogonal testing	means the utilisation of different validation or confirmation techniques that are functional and statistically independent from the original testing.
Primary analysis	means the analysis of hardware generated data, machine statistics, production of sequence reads and quality scores.
Reference materials	means a set of materials that have predetermined and known properties for use in experimentation to provide control and comparison. In the context of MPS, they provide reference genomes for alignment of the small DNA fragments.
Requirements for Medical Pathology Services	means the overarching document broadly outlining standards for good medical pathology practice where the primary consideration is patient welfare, and where the needs and expectations of patients, laboratory staff and referrers (both for pathology requests and inter-laboratory referrals) are safely and satisfactorily met in a timely manner.
Re-sequencing	means sequencing a DNA region and comparing that region to a previously available reference sequence.
Sample indexing	means the embedding of markers directly onto sample molecules, which enables the identification of MPS samples. This technique is important in modern MPS systems where multiple samples are multiplexed into the same sequencing reaction.
Secondary analysis	means QA filtering of reads, assembly and alignment of reads, QA and variant calling on aligned reads.
Sequencing	means to determine the sequence of nucleotides.
Tertiary analysis	means QA/QC of variant calls, annotation and filtering of variants, assessment of pathogenicity and clinical significance, genome browser driven assessment, and other ‘sense making analyses’ such as population frequency and structure assessment, treatment/prognostic/classification associations or consequences in tumours.
Wet laboratory	means a laboratory that utilises chemicals or biological matter that are handled in liquid solutions or phases for analysis.

Introduction

The *Requirements for Human Medical Genome Testing Utilising Massively Parallel Sequencing Technologies (First Edition 2017)* is a Tier 4 NPAAC document and together with the *Requirements for Medical Pathology Services*, sets out the minimum requirements for best practice in the development and performance of genomic or massively parallel sequencing (MPS).

MPS testing is used within a broad spectrum of human genetic testing and includes testing of: a few genes; a panel of genes associated with a specific phenotype; somatic mutations in the cancer setting; the whole exome and whole genome. Non-invasive prenatal screening (NIPS) performed using MPS technologies are also covered by these standards.

The *Requirements for Human Medical Genome Testing Utilising Massively Parallel Sequencing* is a companion document to the *Requirements for Medical Testing of Human Nucleic Acids.*

Any MPS test that is not approved by the TGA as a commercial test is considered to be an in-house IVD device and therefore subject to regulation in accordance with the TGA's IVD regulatory framework.

The unclear boundary between validated medical testing and medical research is recognised across all pathology testing. In a rapidly evolving field like medical genomics the boundary is constantly moving and a risk based approach has been taken to the setting of standards. When undertaking testing where clinical validity has not been established, the testing constitutes potential discovery and proper attention to ethical and legal obligations applicable to medical research must be observed.

These Requirements are intended to serve as minimum standards in the accreditation process and have been developed with reference to current Australian legislation and other standards from the International Organization for Standardization (ISO) including:

AS ISO 15189 *Medical laboratories – Requirements for quality and competence*.

These Requirements should be read within the national pathology accreditation framework including the current versions of the following NPAAC documents:

Tier 2 Document

- *Requirements for Medical Pathology Services*

All Tier 3 Documents

Tier 4 Documents

- *Requirements for Medical Testing of Human Nucleic Acids*

In addition to these standards, laboratories must comply with relevant state and territory legislation (including any reporting requirements).

In each section of this document, points deemed important for practice are identified as either 'Standards' or 'Commentaries'.

- A standard is the minimum requirement for a procedure, method, staffing resource or facility that is required before a laboratory can attain accreditation – standards are printed in bold type and prefaced with an 'S' (e.g. **S2.2**). The use of the word '**must**' in each standard within this document indicates a mandatory requirement for pathology practice.

- A Commentary is provided to give clarification to the standards as well as to provide examples and guidance on interpretation. Commentaries are prefaced with a 'C' (e.g. C1.2) and are placed where they add the most value. Commentaries may be normative or informative depending on both the content and the context of whether they are associated with a standard or not. Note that when comments are expanding on a standard or referring to other legislation, they assume the same status and importance as the standards to which they are attached. Where a Commentary contains the word '**must**' then that commentary is considered to be **normative**.

Please note that any Appendices attached to this document may be either **normative** or **informative** and should be considered to be an integral part of this document.

All NPAAC documents can be accessed at -
www.health.gov.au/internet/main/publishing.nsf/Content/health-npaac-publication.htm

While this document is for use in the accreditation process, comments from users would be appreciated and can be directed to:

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1. Ethical Issues and Clinical Governance

(Refer to Standard 1 and Standard 2 in *Requirements for Medical Pathology Services*)

Medical testing by genomic methods shares many ethical, legal and social issues with other forms of clinical investigation. Existing issues of informed consent, incidental findings, the right not to know, family studies and re-contacting are potentially magnified due to the volume of information that these tests yield and the potential wider implications of the findings.

Comprehensive genomic analyses (e.g. whole genome sequencing or exome sequencing) can generate information pertinent to the management of diseases other than the targeted clinical condition being investigated. Genomic testing can, therefore, be viewed as comprising both a diagnostic and a screening function. The scale of this overlap in test function is unprecedented. The implications of this complex testing scenario for the individual patient (and their family) will require clear explanation in order to obtain informed consent for testing.

The interpretation of MPS data carries the risk of both over-interpretation and under-interpretation. It is possible to falsely attribute pathogenicity to variants when there is insufficient evidence or even evidence indicating the variant is benign. Equally important, it is possible that the laboratory may miss vital evidence confirming the pathogenicity of a variant and an important variant may be missed. Each of these scenarios has serious consequences and can lead to irreversible actions thereby requiring formal, documented systems to minimise over- or under-interpretation of genomic data.

This chapter on ethical and legal implications of genomic testing precedes the chapters detailing the analytical, interpretive, reporting and resource requirements for such testing because of the broader issues.

S1.1 Medical testing on the human genome must be in accordance with relevant ethical guidelines, legal requirements and current pathology accreditation standards.

C1.1 As with all medical testing used in clinical management, medical genomic testing **must** have undergone an evaluation of the evidence for its safety, analytical and clinical validity, and clinical utility prior to implementation in a clinical setting.

S1.2 There must be an explicit medical consultation framework within which the clinician and the laboratory operate whenever genomic testing is undertaken.

C1.2(i) While all pathology test requests imply a consultation between the referring clinician and the laboratory professional supervising the test, this **must** be a requirement for Level 2 referrals (refer to ***Appendix A***) for genomic testing.

C1.2(ii) The laboratory **must** ensure that the indications and benefits of genomic testing are agreed between the laboratory and referring clinician and consent of the patient for the testing is both informed and unambiguous.

S1.3 The laboratory must ensure that written informed patient consent has been obtained prior to genomic testing.

C1.3(i) The laboratory **must** comply with the NPAAC *Requirements for Medical Testing of Human Nucleic Acids* for Level 2 tests (refer to ***Appendix A***) and ensure that for tests which have the potential to lead to complex clinical issues, an indication that consent has been obtained should be documented.

C1.3(ii) Consent for Level 1 DNA tests as defined by the NPAAC *Requirements for Medical Testing of Human Nucleic Acids* (refer to ***Appendix A***) can be implied by the request for testing and does not require additional evidence or documentation of informed patient consent.

S1.4 Patients must provide additional informed consent if their genomic data derived from clinical testing is to be analysed in a research setting.

C1.4(i) Collaborations between pathology laboratories providing diagnostic testing and researchers are subject to the provisions of privacy legislation in the various jurisdictions and to the NHMRC *National Statement on Ethical Conduct of Human Researc*h.[1]

C1.4(ii) Collaborations between institutions are subject to the framework outlined in the *Australian Code for the Responsible Conduct of Research.*[2]

C1.4(iii) Patients will have provided informed consent for the use of their samples or data for research. This consent **must** be distinct from the consent process for clinical testing. Consent may be:

(a) specific to a project under consideration

(b) extended where consent is given for the use of data or tissue in future research projects that are an extension of or closely related to the original project or in the same general area of research

(c) unspecified where consent is given for the use of data or tissue in any future research.

C1.4(iv) The informed consent process should clearly state the protocol with respect to re-contacting the patient about findings, including incidental findings, identified during subsequent research projects.

C1.4(v) All requests from researchers for the release of de-identified data from samples submitted for diagnostic testing and of associated laboratory data to biobanks or databases will require approval by the Human Research Ethics Committee with responsibility for oversight of activities of the pathology service.[1, 2]

C1.4(vi) Provided a suitable ethical framework is in place, the diagnostic laboratory can provide samples and data to researchers, but should retain sufficient sample for the minimum retention period and the laboratory records in accordance with the pathology accreditation retention requirements.[*]

S1.5 Where all or part of the genomic assay is outsourced the referring laboratory must take responsibility for evaluating the competence of the referral laboratory selected.†

C1.5(i) Where non-accredited referral laboratories are selected the referring laboratory **must** evaluate the laboratory against relevant local and/or international standards initially and periodically thereafter.

C1.5(ii) Records of these evaluations **must** be maintained.

C1.5(iii) A competent referral laboratory is generally considered to be a laboratory accredited by a signatory to the ILAC mutual recognition arrangements for the referred activity**.**

C1.5(iv) Where appropriate, the selection of the referral laboratory should be made in consultation with the clinicians using the service.

S1.6 The laboratory must have a policy on the reporting of incidental findings which must be made available on request to patients and clinicians.

C1.6(i) In terms of data security and privacy, patient autonomy relates to a patient's wish of learning, or not, of incidental findings that may arise in the course of testing and the general scope of testing to which the patient has consented.

C1.6(ii) Data management strategies should consider the masking of information that is outside the scope of testing for a given patient sample. This may involve masking of loci other than those targeted for analysis for a given patient. Masking may be performed at any stage during the bioinformatics analysis pipeline, but **must** be performed prior to providing annotated variant calls for review.

S1.7 The reporting laboratory must have a written policy regarding the review of variants and this policy must be available to clinicians.

C1.7 Laboratories should have a formal process for evaluating new evidence, re-interpreting previous individual patient results, re-contacting clinicians and contributing to patient reviews, where required. However, a bi-directional flow from the clinic through continued review of patient files can also contribute to timely review.

[*] *Requirements for the Retention of Laboratory Records and Diagnostic Material*
[†] *Requirements for Medical Testing of Human Nucleic Acids*

S1.8 Laboratories must have a policy for the submission of variants to relevant clinical databases.

C1.8(i) Laboratories are encouraged to submit genotypic data from genomic testing to facilitate consistency of interpretation across laboratories.

C1.8(ii) Genomic testing would benefit from the availability of clinically vetted, regularly updated databases of annotated variants that ideally would include population frequencies and referenced clinical relevance for each variant. Thus, there is a need for consolidation of the various genotype-phenotype databases into a commonly available and perhaps centralised clinical-grade resource that is publicly accessible.

C1.8(iii) The availability of phenotypic information is essential for the investigation of genotype-phenotype relationships both at the individual patient level and at the global level. Integrating the phenotype information into these clinical-grade resource databases can greatly assist with interpretation of variants identified from genomic analyses.

C1.8(iv) Laboratories are encouraged to contribute phenotypic information in consideration of the issues posed by privacy concerns, data complexity and lack of uniform methods for collection of phenotypic data.

S1.9 The laboratory must ensure that nucleic acid samples and laboratory records, including data generated by MPS, are retained in accordance with the current pathology accreditation requirements.[‡]

C1.9 As a minimum retaining an aliquot of the DNA and the corresponding VCF file (or equivalent) should be considered.

[‡] *Requirements for the Retention of Laboratory Records and Diagnostic Material*

2. Personnel

(Refer to Standard 4 in *Requirements for Medical Pathology Services*)

S2.1 Laboratories providing MPS-based assays must be under the direction, control and full-time supervision of a supervising Pathologist or Senior Scientist who has relevant qualifications, competency and experience in genetic testing.[§]

S2.2 Laboratories implementing MPS-based assays must have suitably qualified and competent staff for the testing provided.

C2.2(i) Staff involved in the reporting of MPS results **must** have, as a minimum, a demonstrated understanding of the bioinformatics analysis steps and resources used for annotation.

C2.2(ii) Staff **must** demonstrate an understanding of the limitations of informatics interpretations and provide adequate review of automatically generated interpretations in the clinical context of testing.

[§] *Requirements for the Supervision of Pathology Laboratories*

3. Laboratory Scope of Testing

(Refer to Standard 5 and Standard 6 in *Requirements for Medical Pathology Services*)

Applications for MPS include the investigation of patients with a Mendelian phenotype or family history which strongly implicates a genetic aetiology. This may range from MPS testing for a single gene such as the CFTR gene through to testing for a large panel of genes known to be causative for a specific phenotype such as sudden cardiac arrest.

MPS testing may play a role in the investigation of families with a Mendelian phenotype where the specific genetic aetiology is not established i.e. genome-wide diagnostic testing. It may also play a role in the investigation of multiple affected individuals from different families or of single individuals with very rare genetic disorders, where randomized clinical trials to assess clinical utility and other measures of efficacy of genomic testing are not possible. These referrals are based on clinical judgment. This approach is analogous to investigations such as cytogenetic analysis, microarray or tissue biopsy where the target pathology is unknown.

Non-Invasive Prenatal Screening (NIPS), often referred to as Non-Invasive Prenatal Testing, is a screening test that employs either targeted or whole genome approaches for the detection of circulating cell-free fetal DNA in maternal plasma to identify fetal copy number variation. Currently the two major methodologies used for NIPS are MPS and DNA microarray. This testing can be performed at earlier gestation, and with greater sensitivity and specificity, than already existing maternal screening methodologies. For these reasons it has resulted in a rapid rate of uptake and there are now strong/emerging recommendations to consider NIPS for obstetric patient care. In the application of MPS to NIPS, the strength of this approach is the ability to generate tens of millions of sequence reads across either targeted regions of specific chromosomes or the entire genome. These sequences can then be aligned and uniquely mapped (tagged) to the human genome reference sequence and hence their specific chromosome. Following mapping, the tags are counted to determine chromosome ploidy. The capacity to count millions of tags results in very high sensitivity to detect aneuploidy in a given sample. If aneuploidy is present, there is either an increase (trisomy) or decrease (monosomy) in the relative number of tags on the affected chromosome compared to the euploid chromosomes.

The use of MPS for testing of somatic mutations in the setting of cancer will be a major application of the technology. MPS testing of tumour DNA has the capacity to identify variants that are drivers of tumourigenesis and to assess the tumour for potentially actionable drug targets.

The application of MPS technologies to tumour assessment introduces a requirement for analysing DNA derived from samples other than blood thereby introducing increased variability due to the size and quality of tissue samples from resections, biopsies and even cytology specimens. Variable tissue processing from individual laboratories providing samples will further compound this problem. The intrinsic potential for heterogeneity of tumour samples adds a further level of complexity with clonal populations of tumour cells within a single sample. Analogous to NIPS, the monitoring of blood samples for circulating tumour DNA (ctDNA) is likely to be a routine application of MPS technologies in the future. However, at the present time larger studies are required to correlate the findings from such

testing with both prognostic value and the efficacy of targeted therapies based on the analysis of ctDNA. The use of MPS for such applications will require additional measures to ensure sufficient sensitivity and specificity of the specific MPS technologies applied.

There is debate about the use of genomic methods in preconception carrier screening for relevant mutations, prenatal screening and as a first tier approach for newborn screening. A recent report from the Foundation for Genomics and Population Health in the UK concludes that "Extensive interrogation of genomic data for preventive purpose is not recommended."[3]

S3.1 The laboratory must define the scope of testing in advance of the provision of any genomic pathology test with a view to ensuring it is able to provide a quality pathology service.

C3.1(i) Targeting only genes relevant to the disease being investigated will assist with minimising incidental findings.

C3.1(ii) Where the phenotype is non-specific or not recognised as a particular syndrome, wider capture of data with targeted data interrogation that is performed in a tiered manner is useful.

C3.1(iii) 'Genome-wide' diagnostic testing should be considered if it is clear that testing with a narrower scope (using filters) will yield insufficient results.

4. Wet Laboratory Procedures

(Refer to Standard 5 and Standard 6 in *Requirements for Medical Pathology Services*)

Assays involving nucleic acid amplification require measures to control contamination. General requirements for contamination control in assays involving amplification of nucleic acids are outlined in the NPAAC *Requirements for the Medical Testing of Human Nucleic Acids.*

The use of indexing is a fundamental component of MPS. Indexes allow multiple patients to be sequenced in one run. Individual FASTQ files can be assigned to specific patients on the basis of the indices. The use and rotation of indexes have an important role in the monitoring of contamination.

S4.1 In addition to general requirements for contamination control, the risk of contamination specific to MPS wet laboratory procedures must be mitigated.

C4.1(i) If library preparation involves nucleic acid amplification, amplified nucleic acid should be isolated from non-amplified nucleic acid to reduce the risk of contamination.

C4.1(ii) Sample indexing is used to identify unique reads in pooled libraries and can be used to detect contamination.

S4.2 Sample indexing must be performed at the earliest possible stage of library preparation to mitigate the possibility of cross-contamination.

C4.2(i) Workflows that call for multiple manipulations, additions and incubation of samples prior to index ligation or amplification increase the risk of undetectable sample-to-sample cross-contamination.

C4.2(ii) Indexing should be re-used on the longest cycle to enable detection of carry-over contamination. Consecutive runs of the same sequencing instrument using the same barcode indexing should be avoided. Frequent reuse of the same set of barcode indexes will compromise the laboratory's ability to detect cross and carry-over contamination.

C4.2(iii) Laboratories may consider including ‘identity’ SNPs within MPS assays. These SNPs can be interrogated with a second method to confirm patient identity if no unique variants are identified within the genes analysed. In addition, these SNPs can be used to monitor and detect any contamination. Where members of the same pedigree have been analysed, bioinformatics analyses to confirm family relatedness may also prove useful to highlight any errors in specimen identification and processing or contamination.

Wet Workflow Validation

Consideration should be given to biases inherent in sequencing technologies. Particular attention should be given to ensuring that any systematic weaknesses or errors of the sequencing system do not limit the diagnostic specificity of the assay, or that if such flaws exist orthogonal testing is employed to detect variants in regions of bias. Examples include high GC-content or repetitive regions.

S4.3 The genomic platform used must meet the specifications required for the diagnostic purpose and be operated in accordance with the manufacturer's specifications.

S4.4 Testing methods must be validated and documented in respect to operational performance of the wet laboratory workflow used in molecular diagnosis. [**]

S4.5 Laboratories must regularly monitor the performance of the wet laboratory workflow used in molecular diagnosis.

C4.5 Inclusion of known DNA control/standard samples at regular intervals allows ongoing monitoring of assay performance.

Sample Preparation

Failure to exclude samples of poor quality or with insufficient quantity of amplifiable DNA can significantly affect the sensitivity and specificity of genomic diagnosis and lead to the possibility of inaccurate results. This is of particular significance where the sample type may be associated with limited amounts of DNA, for example FFPE tissue or cell-free circulating DNA.

In the case of measuring cell-free circulating DNA for the purpose of NIPS, the laboratory should have a process to ensure that adequate amounts of fetal DNA (i.e. in accordance with the sensitivity limit determined for the assay) are present in the sample prior to data analysis and interpretation of results.

S4.6 The laboratory must determine adequate sample acceptability criteria and QC measures before proceeding with key steps of a diagnostic application.

C4.6(i) The laboratory **must** have a procedure outlining how to manage specimens outside the acceptability criteria.

C4.6(ii) Consideration should be given to including appropriate controls in the analysis. For example sequencing trios (proband and both parents) to confirm a de novo change, or paired tumour and normal samples to exclude a somatic variant being classified as a germline variant.

C4.6(iii) When analysing limited amounts of, or damaged, nucleic acids, QC measures should be included to ensure sufficient amplifiable nucleic acids for a reliable result.

[**] *Requirements for the Development and Use of In House In Vitro Diagnostic Devices*

C4.6(iv) Assessment of adequate tissue integrity, type, volume and cellularity prior to nucleic acid extraction for tumour tissue is usually estimated using microscopic examination by a competent person. A sufficient purity or proportion of targeted cells can then be achieved through macro-dissection.

Library Preparation

S4.7 The laboratory must demonstrate its ability to track samples during the multistep process of library preparation.

C4.7 Laboratories should consider using a Laboratory Information Management System capable of tracking a multistep workflow with multiple samples and with QC.

S4.8 The laboratory must determine the optimal conditions for library preparation.

C4.8(i) The laboratory **must** have a procedure for the continual monitoring of QC measures.

C4.8(ii) Documented metrics of performance of the library preparation should be generated on all clinical samples and used to QC library preparation steps. For example, the effect of input mass of nucleic acids, fragmentation conditions, PCR cycles etc. should be assessed.

C4.8(iii) QC metrics in the form of bioanalyser traces, spectrophotometric readings or real-time PCR results should be produced and routinely collected and compared to those of an optimal validated run.

C4.8(iv) An accurate estimation of nucleic acids library quantity is essential for optimal clonal amplification.

S4.9 The laboratory must have a quality assessment procedure to assess the adequacy of clonal amplification that is used for template generation.

C4.9 Quality assessment of the clonal amplification procedure is essential to ensure an adequate representation of DNA samples in the template. This is critical for equal representation if multiple barcoded samples have been pooled during library preparation.

Data Generation and Storage

S4.10 The laboratory must empirically establish the depth of coverage necessary for accurate detection of variants and determine the false positive and false negative rates.

C4.10 The laboratory **must** have a policy regarding adequacy of coverage for the detection of variants in the intended application e.g. constitutional testing, somatic cancer.

S4.11 If testing requires a deviation from the laboratory's validated procedures, this must be recorded and an appropriate comment documented on the patient's test report.

5. IT Infrastructure

Genomic technologies introduce complex analytical methods that require substantial IT infrastructure and bioinformatics. This section provides guidance on IT infrastructure issues that should be addressed by laboratories performing MPS. This section must be read in conjunction with the NPAAC *Requirements for Information Communication.*

Given the vast potential of genomic methods to generate genome-wide data, laboratories should actively consider which data they will store and the retention times of that data. In some cases it may be that institutional IT departments, and their policies, may be able to accommodate data within centralised storage facilities. However, there may be many cases where this is not possible and the problems will need to be addressed locally.

Data Processing Infrastructure and Capacity

S5.1 The computing hardware and other IT infrastructure must be validated for the testing undertaken and maintained accordingly.

C5.1(i) The chosen computing hardware **must** be shown to be capable of performing the required analyses and/or capable of running the chosen software using training/control datasets (i.e. datasets with characteristics consistent with clinical samples to be analysed).

C5.1(ii) The laboratory **must** have policies in place to address the authentication and integrity of the data and to maintain confidentiality during data transfer.

S5.2 A privacy and security risk assessment must be undertaken prior to using cloud technology for MPS data analysis.

C5.2(i) The use of cloud based computing for MPS data **must** comply with relevant Australian State and Federal records and privacy requirements.

C5.2(ii) The laboratory **must** have a documented process for conducting privacy and security assessments for cloud based analysis of MPS data.

C5.2(iii) Controls **must** be in place to regularly monitor the security and privacy of cloud based applications for MPS data.

Data Management and Storage

S5.3 The laboratory must determine and justify which data are to be stored and the retention times.[††]

C5.3(i) Interpreted or annotated variant files, such as those after review of the initial calls, **must** be stored.

[††] *Requirements for the Retention of Laboratory Records and Diagnostic Material (Sixth Edition 2013)*

C5.3(ii) Where possible, the laboratory should determine the feasibility of very long term data retention. Some genomic data may need to be repeatedly accessed and analysed over a greater period than has been expected in non-genomic data retention policies (e.g. for whole genome or whole exome data).

6. Bioinformatics

(Refer to Standard 3 and Standard 5 in *Requirements for Medical Pathology Services*)

This chapter covers bioinformatics analysis applied to the range of MPS testing, i.e. for genes, panels of genes, exomes and whole genomes.

The Bioinformatics Pipeline

The term 'bioinformatics pipeline' refers to a number of computational tasks, generally applied sequentially, beginning with receipt of the output of an MPS sequencing instrument such as an image or FASTQ files and performing progressive analysis of the data. This process can be divided into three steps:

- primary analysis – conversion of instrument file to FASTQ or FASTA file
- secondary analysis – alignment and variant calling
- tertiary analysis – data annotation and interpretation.

Laboratories have a choice of bioinformatics pipelines. The requirements described in this section apply regardless of the source of the bioinformatics pipeline.

Validation and Verification of the Bioinformatics Pipeline Before Use

S6.1 The extent of the validation of the bioinformatics pipeline must be commensurate with the risk to the patient and the complexity of the testing.

S6.2 The analytical validity of the entire bioinformatics pipeline must be established.

C6.2(i) The ability to correctly detect and call sequence variants (secondary analyses) and to annotate and assess the pathogenicity of sequence variants (tertiary analyses) **must** be considered and be compatible with clinical validity.

C6.2(ii) Performance characteristics **must** be measured on an independent data set that has not been used for optimising the parameters of the pipeline.

C6.2(iii) The following performance characteristics should be considered[‡‡]:

(a) frequency of True Positive, True Negative, False Positive or False Negative results.

(b) bias - independent data sets for pipeline development and pipeline validation must be used to minimise bias

(c) accuracy and precision

(d) sensitivity

(e) specificity

[‡‡] *Requirements for the Development and Use of In House In Vitro Diagnostic Devices (Third Edition 2014)*

(f) reportable range

(g) reference range

(h) limit of detection

(i) reproducibility/ repeatability.

S6.3 Common sources of error which can compromise analytical validity of the pipeline must be identified and mitigated.

S6.4 Reference material must be used to validate the pipeline, where available.

C6.4(i) Validation of a bioinformatics pipeline requires its execution using input data where the correct status of the variant(s) is known (referred to as *Reference Materials*).

C6.4(ii) An example of bioinformatics is the consensus variant calls distributed by the Genome in a Bottle Consortium for NA12878.

S6.5 The laboratory must define the limitations of the informatics pipeline.

C6.5 Common limitations of bioinformatics pipelines include:

(a) the maximum size of indels detectable

(b) regions of poor mapping

(c) regions of poor sequence coverage

(d) repeat regions and homopolymer sequence regions that may affect variant calling

(e) specific limitations of individual specimens that can affect the capability of a given bioinformatics pipeline.

S6.6 Pipeline QC metrics must be established to verify ongoing acceptable performance.

C6.6(i) Critical quality metrics **must** be established for each category of MPS test.

C6.6(ii) QC metrics **must** be established which demonstrate that the pipeline is in control.

C6.6(iii) For the informatics pipeline, relevant critical quality metrics may include:

(a) the total number of reads passing quality filters

(b) the percentage of aligned reads

(c) the number of SNPs

(d) insertions and deletions (indels) called, Ti/ Tv ratio, and the percentage of variants in dbSNP.

S6.7 The laboratory must establish valid ranges and acceptability criteria for the pipeline QC metrics.

C6.7 Acceptability criteria **must** be documented for these metrics, along with actions to be taken if acceptability criteria are not met. Examples of such metrics include returning the expected number of variants from a data set of given size (e.g. 10,000 – 50,000 SNVs for WES), returning an expected percentage of variants contained in population databases such as dbSNP, and the expected Ti/Tv ratio.

S6.8 The laboratory must record and validate/ verify all changes to the informatics pipeline.

C6.8(i) Before each new version of the bioinformatic pipeline is used, QC metrics as described in S6.7 will be re-established for the new version and performance relative to previous versions documented. Any changes in performance **must** be explained.

C6.8(ii) A software revision control system must be established and each version start and end date recorded. Software changes **must** include a history that describes the reason for changes, the date the change was made, and the person who made the change.

C6.8(iii) The laboratory **must** document all components of the informatics pipeline, including software packages used, custom scripts and algorithms, reference sequences and database sets.

C6.8(iv) If information from public websites is used, the date of access **must** be recorded.

7. Quality Control (QC) and Quality Assurance (QA)

(Refer to Standard 2, Standard 3, Standard 4, Standard 5 and Standard 6 in *Requirements for Medical Pathology Services*)

S7.1 The laboratory must ensure appropriate quality metrics are in use that are suitable for MPS diagnostic tests.

C7.1(i) The limitations of genomic testing **must** be presented in the final report (Refer to Standard 8 – *Requesting and Reporting*).

C7.1(ii) Consideration should be given to cross platform confirmation. Sanger sequencing can be considered to reduce false positive and/ or negative rates, particularly in small indel variants.

C7.1(iii) QC of sequencing data may include:

(a) base call quality scores

(b) read depth

(c) uniformity of read coverage

(d) on target/ off target reads (for capture-based methods)

(e) percentage of PCR duplicates (for capture-based methods)

(f) allelic Read Percentage

(g) GC-content bias

(h) decline in signal intensity along a read.

S7.2 The laboratory must document and implement QC measures that evaluate the test process.

C7.2(i) Well-characterised DNA samples should be used as internal QC samples. Cell lines are renewable but may have some balanced or unbalanced chromosomal rearrangements. Blood samples from individuals who are <55 years are typically free from such rearrangements but are limited in supply. Rearrangements that are identified may reflect the age of the donor or be a consequence of the culture process.

C7.2(ii) Consideration should be given to obtaining certified reference materials to use as internal QC samples, where available.

C7.2(iii) The laboratory must determine and monitor the imprecision (repeatability and reproducibility) of the IVD; this can be done using internal QC samples.

S7.3 Laboratories performing diagnostic genomic testing must participate in an external genomic proficiency testing program.

C7.3(i) If such a program is unavailable, the laboratory **must** participate in an inter-laboratory sample exchange program to meet the requirements for external quality assessment.

C7.3(ii) Laboratories using MPS testing for specific phenotypes **must** enrol in the disease specific modules where an external QA program is available, such as hereditary breast cancer (*BRCA1* & *BRCA2*), as these cover aspects such as disease specific reporting comments and other disease relevant factors which may not always be covered in external genomic QA programs.

S7.4 The laboratory must monitor the quality metrics and acceptability criteria established during pipeline validation.

C7.4(i) Quality metrics **must** be recorded for each test performed and compared with acceptability criteria defined during pipeline validation.

C7.4(ii) If acceptability criteria are not met, the laboratory's actions **must** be documented.

S7.5 Deviations of quality metrics from defined acceptability criteria must be explained and documented.

C7.5(i) If acceptability criteria are not met, the data **must** be rejected or reference to the limitations and caveats of the data **must** be made in the report.

C7.5(ii) Deviation from acceptability criteria requires investigation and troubleshooting. It may require full or partial repetition of the process. For example, a deviation in the percentage of SNPs in the dbSNP observed may indicate a problem with variant calling for that sample.

8. Requesting and Reporting

(Refer to Standard 3, Standard 4 and Standard 6 in *Requirements for Medical Pathology Services*)

Laboratories providing genomic tests must have clearly defined, evidence based protocols for accepting genomic test requests, classifying the clinical significance of detected genetic variants and for addressing incidental findings.

This section must be read in conjunction with the NPAAC *Requirements for Information Communication.*

S8.1 Laboratories must have clearly defined protocols for the acceptance of genomic test requests.

C8.1(i) Laboratories must have explicit requirements regarding the level and quality of information in order to facilitate effective clinical handover.

C8.1(ii) Requesting of genomic tests can benefit from a multidisciplinary team approach to inform correct test choice.

S8.2 MPS test results must be reported clearly, consistently and unambiguously, using established nomenclature guidelines such as those available from the Human Genome Variation Society[4] and relevant standardised reporting formats.

C8.2 It should be recognised that the understanding of genomic information will vary amongst requesters.

S8.3 The MPS report must include the clinical context in which testing has been requested.

C8.3 Clinical information is essential to inform the correct choice of tests and interpretation of reports.

Variant Reporting

S8.4 The laboratory must consistently classify genomic variants according to their clinical significance.

C8.4(i) The laboratory **must** have evidence-based guidelines for the classification, interpretation and clinical significance of variants.

S8.5 Laboratories must establish and maintain an internal database of genomic findings to facilitate consistent classification of variants.

C8.5(i) A record of all variants relevant to the request and their classification **must** be maintained by the testing laboratory and should be readily accessible for review and may be disclosed upon request to a clinician.

C8.5(ii) Reporting laboratories **must** maintain an in-house database of variants that is curated to a standard acceptable for clinical use and that is capable of

submitting variant data to a clinical standard external database where available.

C8.5(iii) This database could serve the purpose of identifying common genomic variants specific to a patient population and/or recurrent false-positive calls associated with a particular genomic platform.

C8.5(iv) The curation of an internal laboratory and platform-specific list of common benign variants can assist with the interpretation process. This could aid with the process of systematic review of variant interpretations.

S8.6 The report must state the laboratory's policy regarding the classification of variants which are reported, and highlight the possible existence of variants which may not appear on the report.

C8.6(i) Reporting laboratories **must** minimize the potential for genomic test reports to misrepresent the clinical significance of genomic findings.

C8.6(ii) There is likely to be a substantial number of variants of 'unknown significance' for which there is currently no relevant evidence to assist interpretation.

Incidental Findings

Genomic analysis will inevitably detect clinically significant variants which are unrelated to the clinical features that prompted testing. The laboratory's policy related to the handling of incidental findings should inform the counselling process and be documented as part of the informed consent process prior to MPS testing. This information should include a clear explanation of the current limits of testing and interpretation, and how privacy and confidentiality are maintained.

Examples of incidental findings detected during genomic testing include:

- consanguinity and incest, where this was not known to ordering clinicians and /or individuals and their families
- carrier status for recessive disorders unrelated to the clinical indication for genomic testing
- variants involving highly penetrant genes associated with dominant, adult-onset conditions.

S8.7 Laboratories performing genomic testing must have clear policies in place for the disclosure of incidental findings.

C8.7(i) Laboratories **must** limit the reporting of incidental findings to variants that are unequivocally classified as pathogenic or likely pathogenic.

C8.7(ii) The variant reporting policy **must** consider the strength of evidence supporting the association between the variant and clinical outcome of interest.

C8.7(iii) Clinicians and patients should be informed of these policies and the types of incidental findings that will be reported, if any.

C8.7(iv) Clinicians may give patients the option of not receiving certain results. While these policies should be in place, exceptional circumstances may arise which need to be handled judiciously on a case-by case basis through laboratory-clinician consultation.

MPS Test Report

Producing a report that contains all the relevant information for accurate interpretation while avoiding information overload and possible distraction from the actual result required for patient care can be a challenge. One approach to solving this problem is to include a prominent one page patient summary in the report containing clinically significant variants[6] relevant to the request that is supplemented with further information relating to the actual test (limitations, bioinformatics pipeline description and metrics) and other identified variants, if appropriate to the request. Such multipage reports would need to comply with all relevant standards and guidelines for reporting and include page number, report date and patient demographics on each page to ensure unambiguous linking of the entire report.

A consistent approach to reporting genomic findings is important, particularly for families dispersed across state or national boundaries. Crucially, those responsible for reporting should appreciate that interpretive difference may influence medical management and patient choices. Even if a report is directed to the expert requesting clinician, it is important to note that reports may be included in medical records read by non-genomics experts involved in the patient's care. Hence, every effort should be made to ensure that the report is succinct, clear and interpretable by as wide a range of relevant clinicians as possible. It will also be used to inform the patient.

S8.8 The result section of the report must unambiguously state the clinically significant variant(s) detected by the assay, using established nomenclature guidelines.

C8.8(i) Non-standard variant nomenclature may be in common usage to describe variants. If this is the case, such nomenclature may be included in the report, but **must** supplement rather than replace standard nomenclature.

C8.8(ii) If the absence of a particular variant or set of variants is particularly pertinent for treatment decision(s), this may be further highlighted in the text of the report.

S8.9 The report must provide information regarding the regions targeted by the assay, and the variant type(s) that the assay can detect.

C8.9(i) The scope of the assay should be clearly delineated, to enable the requestor to determine its clinical utility for each patient.

C8.9(ii) Where pertinent, an estimate of the diagnostic yield of the assay for detecting established clinically significant variants may be provided.

C8.9(iii) For genomic testing, it is impractical to list each tested region in the report. In such cases, reference to a full test list available elsewhere (e.g. on a website) is acceptable. If reference is made to an external resource (e.g. the laboratory's website), the resource **must** be document controlled, and the report **must** make it clear which version of the resource has been used.

S8.10 When a sample is inadequate for MPS testing based on sample acceptance criteria and assay performance limits established during validation, the report must state that testing could not proceed.

C8.10(i) Acceptance/rejection criteria may include:

(a) the percentage of neoplastic nuclei in the sampled tumour

(b) the absolute number of sampled nuclei

(c) the amount and quality of extracted DNA

(d) various wet-laboratory and bioinformatics quality metrics indicating adequacy of assay performance (discussed in Sections 4 and 6)

(e) performance of assay internal control samples.

C8.10(ii) Some samples may be inadequate for MPS but may still be suitable for testing by other methods. The report should make this clear, and may suggest alternative testing where this is possible.

S8.11 Where relevant, laboratories must include recommendations for appropriate follow-up in reports.

C8.11 In situations where further genetic studies may be warranted (e.g. parental testing, segregation analysis, testing of other tissues and other family members) these recommendations should be included in the test report.

Reporting of Somatic Variants

The following additional considerations apply to the reporting of somatic variants.

S8.12 The laboratory must consistently classify somatic variants according to their clinical significance, in the context of a patient's tumour and the level of supporting evidence.

C8.12(i) The laboratory **must** have a documented process for classifying, interpreting and determining the clinical significance of somatic variants. This process should consider patient specific factors (e.g. tumour site and histology), and the quality of evidence supporting the clinical significance ('actionability') of the variant.

C8.12(ii) The clinical purpose of somatic variant testing often differs from germline variant testing. Clinically useful somatic variants may predict the response to a specific therapy, provide prognostic information, or subtype/classify neoplasms. The 5 level classification system used for germline variants (Pathogenic, Likely Pathogenic, Variant of Unknown Significance, Likely Benign, Benign) may not directly translate to somatic testing, and alternative schemas should be considered.

S8.13 If variants targeted by the assay are known to predict response to specific therapeutic agents, the relationship between the presence or absence of a variant and the likely efficacy of the agent must be indicated on the report.

C8.13(i) The established relationship between variant and therapy may be tumour-type specific; this **must** be indicated on the report. This information may be incorporated into the laboratory's variant classification system.

C8.13(ii) The limit of detection of the assay **must** be stated for targeted variants that predict response to specific therapeutic agents.

S8.14 In circumstances where the variant allele percentage is clinically significant, it must be included on the report.

C8.14 Variant allele percentage may be important in some clinical circumstances (e.g. monitoring of minimal residual disease, or if there is a clinical cut-point for using a targeted therapy). In these cases the variant allele percentage **must** be reported, along with its measurement uncertainty.

S8.15 The percentage of neoplastic cell nuclei in a malignant sample must be included in the report.

C8.15(i) The limit of detection of the assay for each targeted region may vary between assay runs, between samples and between targeted regions within samples. Targeted regions with suboptimal coverage or suboptimal performance of other quality metrics (which contribute to limit of detection) **must** be noted in the report.

C8.15(ii) The percentage of viable neoplastic cells in the tested material will inform the likelihood of a false negative result, in the context of the limit of

detection of the assay. If a sample has relatively low neoplastic cell content with respect to the limit of detection of the assay for clinically important variant(s), orthogonal testing or repeat sampling should be considered.

S8.16 The laboratory must have a policy regarding reporting (germline) findings of clinical significance.

C8.16(i) In the context of somatic mutation testing, the identification of pathogenic or likely pathogenic germline variants in familial cancer genes will have important implications for both the patient and their relatives. Such findings may be more likely in large panels or genome-wide testing than in small 'hotspot' panels.

C8.16(ii) As with germline testing, clinicians and patients should be informed of these policies, and the types of incidental findings that will be reported.

C8.16(iii) Testing of paired tumour and normal samples can assist with the classification of variants from tumour data as either germline or somatic.

C8.16(iv) Where a germline variant associated with a familial cancer syndrome is identified, referral to a clinic specialising in familial cancer should be recommended in the report.

Reporting of Non-invasive Prenatal Screening (NIPS)

The following additional considerations apply to the reporting of NIPS results.

S8.17 Variant(s) screened for by the assay must be clearly stated on the report.

C8.17(i) NIPS can potentially be used to screen for variants including aneuploidy, microdeletions, and single gene mutations. The variant(s) included in the screen should be stated in the report, in a manner clear to non-expert clinicians.

S8.18 NIPS reports must clearly indicate that NIPS is a screening test rather than a diagnostic test.

C8.18(i) Screen positive and screen negative results can be presented in various ways depending on the specific NIPS assay (e.g. as binary 'detected/not detected' or 'high risk'/ 'low risk' results, or as 'risk scores' based on modification of prior patient risk by the result of the assay).

C8.18(ii) Screen-positive results in particular **must** clearly state that NIPS is not a diagnostic test, and that irreversible decisions about pregnancy management should not be solely based on the result of NIPS.

S8.19 Relevant information regarding clinical performance of the test for detection of fetal variants must be provided to requesting clinicians.

C8.19(i) Performance information provided to clinicians should include the sensitivity and specificity of the test for detecting fetal variants in clinical samples.

C8.19(ii) Positive and negative predictive values ('patient specific risks') depend on both the clinical sensitivity/specificity of the assay, and the patient's prior risk. Note that an accurate estimate of the prior risk may be available to the requesting clinician, but not the laboratory. However, the laboratory should provide information for requesting clinicians regarding the distinction between sensitivity/specificity and individual, patient specific risk.

C8.19(iii) The report should address additional relevant test limitations including: variant(s) not screened or detected by the assay, biological factors contributing to the possible discordance between NIPS result and fetal genotype, and any limitations to patient eligibility for testing (e.g. multiple pregnancies and egg donor pregnancies in some forms of NIPS).

S8.20 The laboratory must have a policy regarding the measurement of fetal fraction.

C8.20(i) Where the policy is not to measure fetal fraction, the decision **must** be justified in terms of its potential impact on assay performance characteristics and QC.

C8.20(ii) The assay report **must** note whether fetal fraction is routinely measured as part of the assay. The measured fetal fraction may be provided on the report, as a low fetal fraction may have clinical utility in and of itself.[6] See also S8.10 regarding sample acceptance criteria.

S8.21 If the NIPS assay requires demographic information in addition to standard identifiers, this information must be displayed on the report.

C8.21(i) If the information forms part of the sample acceptance criteria and/or is used by the test algorithm, it **must** be displayed on the report.

C8.21(ii) Information on the report may include gestational age at sample collection, number of fetuses, whether the pregnancy is a result of in-vitro fertilisation etc. Displaying this information on the report enables the requestor to detect errors in the information provided on the initial request form.

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Appendix A Extension of Classification of Human Genetic Tests (Normative)

Levels of DNA testing

DNA testing is categorised into two levels of testing. These are outlined in Table 1.

Table 1 Levels of DNA testing

Type of DNA test for an inherited genetic disorder	Explanatory notes
Level 1 DNA test (standard)	Included here would be: a) DNA testing for diagnostic purposes (e.g. the patient has clinical indicators or a family history of an established inherited disorder, and DNA testing is being used to confirm the disorder) or any other DNA test that does not fall into Level 2. b) Population-based screening programs.
Level 2 DNA test (i.e. the test has the potential to lead to complex clinical issues)	DNA testing for which specialised knowledge is needed for the DNA test to be requested, and for which professional genetic counselling should precede and accompany the test. Predictive or pre-symptomatic DNA testing, for conditions for which there is no simple treatment would usually be included in this grouping. Specific written consent and counselling issues are associated with this grouping.

The distinction between Level 1 (standard DNA test) and Level 2 (DNA test with potential complex issues) would usually be made by the requesting clinician ordering the test, since that individual will be best placed to appreciate the short-term and long-term implications of the test for the patient and other family members.

Counselling and consent

Issues regarding counselling and consent for genetic testing have been considered in the National Health and Medical Research Council publication, *Medical Genetic Testing: Information for health professionals*[7]. Pathologists/Senior Scientists in charge of the laboratory and their senior staff should be familiar with the issues addressed in this publication so that meaningful discussion can take place between the laboratory and the requesting clinician in cases where appropriate test classification of a request remains unresolved.

Classification of human genetic tests

The decision schema outlined below has been designed to help classify human genetics tests and to provide guidance to the clinical scientist/pathologist in charge of the laboratory. While it is primarily a laboratory tool, it will be of value to health professionals involved in human genetic testing.

Irrespective of the classification of a test, the requesting clinician should ensure that the person or legal guardian provides consent for the investigation. The majority of requests for genetic testing (e.g. for diagnostic or medical screening purposes), will be Level 1. A test is classified as Level 2 (i.e. requiring professional genetic counselling and consent) only if it fulfils one or more of the criteria shown below. These criteria reflect the complexity of genetic or counselling issues commonly encountered. The criteria are not comprehensive and, in cases of doubt, it may be prudent for the requesting clinician to manage the test process as for a Level 2 genetic investigation.

Box 1 Schema for classifying human genetics tests

1. **Genetic test requests for somatic variants are classified as Level 1** (e.g. testing for the BCR/ABL fusion gene in chronic myeloid leukaemia [Level 1])
2. **Genetic test requests for heritable variants, including diagnostic testing and medical screening programs, are classified as Level 1 testing unless a request fulfils one or more of the following criteria:**
   - **2.1 Guidelines developed by the National Health and Medical Research Council or a national medical specialty college recommend pre-test genetic counselling and written consent** (e.g. testing for a familial BRCA1 mutation in a woman with breast cancer who is at high risk of having familial breast and ovarian cancer [Level 2])
   - **2.2 The specimen being tested is from a clinically affected child being tested for a disorder that typically presents in adulthood** (e.g. testing for the Huntington disease mutation in a child with a neurodegenerative disorder [Level 2])
   - **2.3 The specimen being tested is from an apparently unaffected child or fetus** (e.g. prenatal testing for a mutation already defined in the family [Level 2]; carrier testing for Duchenne muscular dystrophy during childhood [Level 2])
   - **2.4 The specimen for testing is from a clinically unaffected adult and the test is predictive of a disease for which interventions are of limited efficacy or carry substantial risks or costs** (e.g. pre-symptomatic testing for myotonic dystrophy [Level 2]).
   - **2.5 Widely targeted testing where there is a high probability of incidental findings** e.g. unfiltered exome or genome finding.

Appendix B Key Requirements of a Genomic Report (Informative)

The minimum suggested content for a report is described below. The following list is not a recommendation for the structure of the report.

Patient diagnosis context

- Clinical details on request
- Specimen
- Type (blood, tissue and site, fluid)
- Secondary specimen identifier (Block number, referring laboratory identifier)

Test description

- Test category
- Purpose of test (e.g to assist in the diagnosis of … or the exclusion of…)
- Genes tested list
- Methodology used including confirmation of variants by an orthogonal method if performed
- Limitations to test including any remaining uncertainty where it exists

Result summary

- Inheritance model used for sequencing data analysis if relevant
- Gene name using HGNC approved gene symbol
- Zygosity
- cDNA nomenclature utilising standardised nomenclature (HGVS recommended)
- Protein nomenclature utilising HGVS recommended nomenclature
- Genomic coordinates utilising HGVS recommended nomenclature based on an LRG where available and a RefSeqGene record if not available
- Reference sequences including genome build or reference sequence version
- Variant reporting policy for the reporting laboratory that complies with relevant guideline

Interpretive comment

- Variant classification as class 1 (benign) through to class 5 (pathogenic)
- Narrative comment indicating the relevance of the identified variants to the reason for the test request
- If applicable the need for follow up or confirmatory testing should be indicated on the report

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