Australian Commission on **Safety and Quality** in Health Care

NPAAC
National Pathology Accreditation Advisory Council

Requirements for Medical Testing for Human Genetic Variation

Fourth Edition

2025

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Acknowledgement

We acknowledge the Traditional Owners and Custodians of Country throughout Australia. We recognise their continuing connection to land, waters and community and acknowledge their ongoing contribution to the health system and community. We pay our respects to Elders past and present.

2012 First edition published; originally part of the Laboratory accreditation standards and guidelines for nucleic acid detection and analysis (2006)

2021 Second edition reprinted and reformatted to be read in conjunction with the Requirements for medical pathology services

2022 Third edition is a consolidation of Requirements for medical testing of human nucleic acids (2013) and Requirements for cytogenetic testing (2013) Content updated to best practice, redundant definitions not used throughout document deleted and restructured to align with the Commission's National Safety and Quality Health Service Standard

2025 Fourth edition includes amendments made to S1.01 regarding referring practitioners to include nurses and midwives as a response to new and specific MBS item numbers for a single genomic test (*Non-invasive prenatal testing of blood from an RhD negative pregnant patient)* and updates to the formatting and template to align with the National Pathology Standard.

Contents

Background

About the Australian Commission on Safety and Quality in Health Care

The Australian Commission on Safety and Quality in Health Care (the Commission) partners with the Australian Government, state and territory governments and the private sector to achieve a safe, high-quality, sustainable health system. It also works closely with patients, carers, clinicians, medical scientists, managers, healthcare organisations and policymakers.[1]

Key functions of the Commission include:

- developing national safety and quality standards
- developing clinical care standards to improve the implementation of evidence-based health care
- coordinating work in specific areas to improve outcomes for patients
- providing information, publications and resources about safety and quality.

The Commission works in four priority areas:

- High-quality care in an evolving environment
- Strong outcome-focused clinical governance
- Empowered patients, carers and communities
- An improvement driven workforce culture.

About the National Pathology Accreditation Scheme

The National Pathology Accreditation Scheme (NPAS) is an accreditation scheme that requires pathology practices to meet relevant standards for their pathology services to be eligible for Medicare benefits. The *Health Insurance (Accredited Pathology Laboratory-Approval) Principles 2017* (the Approval Principles) underpin NPAS. The Approval Principles set the categories of accredited pathology laboratories, specify the standards to be met and the kind of pathology services provided.

The Approval Principles ensure that pathology practices providing Medicare eligible pathology services met and maintain compliance with the standards. The Approval Principles objectives include:

- supporting the diagnosis and treatment of illness by linking Medicare benefits to pathology services that provide reliable results
- reducing the risk of misdiagnosis from pathology services that provide unreliable results
- maintaining public confidence in pathology services.

The Commission administers the NPAS behalf of the Australian Government Department of Health, Disability and Ageing (the Department). The Department manages the policy and regulatory framework for pathology practice accreditation that are approved to provide Medicare eligible pathology services.

About the National Pathology Accreditation Advisory Council

The National Pathology Accreditation Advisory Council (NPAAC) was established in 1979 to consider and make recommendations to the Australian, state and territory governments on matters related to the accreditation of pathology practices and the introduction and maintenance of uniform standards of practice in pathology practices throughout Australia. NPAAC is responsible for formulating standards which pathology practices are assessed.

The Approval Principles give effect to NPAAC endorsed standards by listing the standards and accreditation materials pathology practices seeking approval to provide Medicare eligible pathology services must meet. The pathology practice's conformity with the standards is assessed by the accrediting agencies defined in the Approved Principles.

Introduction

The *Requirements for Medical Testing for Human Genetic Variation* set the expected level of practice for laboratories performing testing for human genetic variation, where testing occurs following a request by or on behalf of a medical practitioner, nurse, midwife or paramedical.

The *Requirements for Medical Testing for Human Genetic Variation* is a tier 4 document that sets out the requirements for medical tests for heritable and non-heritable genetic/genomic changes associated with human disease**.**

These tests involve the extraction, detection, characterisation and quantification of nucleic acids, morphological assessment of chromosomes and *in situ* assessment of specific genetic changes in human tissue or cultured cells.

This standard applies in laboratory disciplines including (but not limited to) molecular, cytogenetic, and anatomical pathology laboratories. This standard also applies to testing using massively parallel sequencing, also known as next generation sequencing. Specific requirements relating to this work can be found in the *Requirements for human medical genome testing utilising massively parallel sequencing.*

Human genetic variation includes:

- nucleic acid (DNA/RNA) sequence variants
- copy number variants, including aneuploidy and ploidy changes
- structural variants such as small indels and gross chromosome rearrangements, for example translocation
- modification of nucleic acids such as methylation

Application of the standards

Tests for paternity, kinship, and identity and forensic analysis of specimen for use by law enforcement authorities are not in scope for this standard.

All action items apply to all human genetic testing unless otherwise stated.

Human genetic testing remains a dynamic field that continues to evolve. Emerging technologies are likely to supersede existing technologies or allow for new diagnostic insights.

This standard is a Tier 4 document that covers emerging technologies. However, laboratories will need to continue to follow evidence based and internationally accepted best practise guidelines for technologies not covered in this standard. This standard has been developed with reference to current and proposed Australian regulations and should be implemented in conjunction with all relevant NPAAC documents.

These are laboratory standards that should be adhered to by all members of the workforce and applied specifically where they relate to their roles and responsibilities.

In addition to this standard, laboratories must comply with all relevant state and territory legislation, including any reporting requirements, related to medical testing of human genetics.

Structure of the standards

Each section identifies key points for practice as either Standards (S) or Commentaries (C):

- **Standard**: The minimum requirement for procedures, methods, staffing, or facilities. Standards required before a laboratory can attain accreditation are prefaced with an 'S' (e.g., S 2.02). The use of "must" indicates a mandatory requirement.
- **Commentary**: Provides clarification to the standards, examples, and guidance. Prefaced with a 'C' (e.g., C 1.2a), commentaries may be normative or informative. If a commentary uses "must," it is considered normative and holds the same weight as a Standard.

Appendices may also be normative or informative and are considered integral to the document.

Terminology

Analytical sensitivity

means the lowest amount of analyte in a sample which can be reliably detected (also referred to as the limit of detection).

Clinical sensitivity

means the proportion of true positives that are identified as positive by the assay.

Constitutional variant

means an alteration in the DNA, which is present within all cells, including germ cells, which can therefore be passed to subsequent generations

Copy number variation

means a DNA segment one kilobase or larger that is present at a variable copy number in comparison with a reference genome.

In control

means the assay continues to perform as it did when it was validated or verified.

Medical screening

means medical tests used for the early detection of diseases and health conditions before there are any signs or symptoms.

Positive controls

means particular samples included in an experiment that are treated the same as other samples, and which are known to include variant(s) of interest for the assay.

Prenatal

means before birth.

Predictive testing

can mean either:

a. presymptomatic testing used to detect germline genetic changes associated with conditions that appear after birth or later in life; or
b. somatic testing used to detect tissue specific genetic changes predictive of response to a specialised cancer therapy; or

c. pharmacogenetic testing used to detect germline genetic variation associated with variable responses to pharmaceutical agents.

Professional genetic counselling

refers to a communication process, which aims to help individuals, couples and families understand and adapt to the medical, psychological, familial and reproductive implications of the genetic contribution to specific health conditions by a certified health professional.

Sample

means a portion of the specimen assumed to contain the target analytes in the same proportion to that found in the original specimen. Also includes non-patient derived material such as controls, blanks, and reference materials.

Separate area

means a laboratory space that is functionally separated from other laboratory spaces by physical barriers, distance or strict laboratory practice, or by performance of the test within the working space of an instrument, as dictated by the methods and technology available in the laboratory.

Somatic variants

means an alteration in the DNA that occurs after conception and is not present within the germline.

Specimen

means any tissue or fluid from a patient that is submitted to the Medical Pathology Service for testing.

Specialised knowledge

means the knowledge of principles and techniques applicable to a particular discipline. It is obtained during the acquisition of professional/specialised qualifications and/or relevant experience.

Tissue type

means anatomical origin of a tissue specimen.

Unsolicited findings

means any findings identified by the test that are clinically significant but not related to the request for testing.

In this document, for the purposes of accreditation, unsolicited findings are used. Outside of this document, these may also be known as incidental, unanticipated, secondary or additional findings.

Validation

means the process of defining an analytical requirement and confirming that the method under consideration has performance capabilities consistent with that requirement.

Variant

means any alteration from a reference sequence. Variants may cause disease or be benign. In the case of “copy number” variants, such an alteration can often involve several genes, contiguously located.

Heteromorphic variant means a microscopically visible chromosomal region that is variable in size, morphology or staining between individuals.

Abbreviations

Acronym	Full text
cfDNA	Cell-free DNA
FISH	Fluorescence *in situ* hybridisation
ISCN	International System of Cytogenetic Nomenclature
ISH	*In situ* Hybridisation
ISO	International Organization for Standardization
NHMRC	National Health and Medical Research Council
NIPS	Non-invasive prenatal screening
NPAAC	National Pathology Accreditation Advisory Council
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment

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Standards

Pre-analytical

1. Ethical Responsibilities

An inherent risk in multi-gene and genome-wide assays, including microarrays, massively paralleling sequencing of gene-panels, exomes or genomes, lies in the potential to detect clinically relevant unsolicited findings unrelated to the reason for testing, and this risk increases with the complexity of the gene panel being used. Wherever possible, both germline and somatic testing approaches should be designed to minimise these risks, and the testing laboratory must have a policy on how to approach and report unsolicited findings.

No. and topic	Standard and commentary	
S 1.01 Testing as a clinical service	The laboratory provides medical genetic testing in the context of clinical service provided by a: a. qualified medical practitioner; or b. midwife; or c. nurse practitioner; or d. paramedical person working for the Australian Red Cross LifeBlood under the clinical supervision of a qualified medical practitioner who takes responsibility for the test.	
S 1.02 Guidance on testing	The laboratory must provide its workforce with access to guidance on genetic tests and the ethical implications for each level of test conducted.	
	C 1.02a	The distinction between Levels (see Appendix A) should be clear from the clinical information in the referred test and the testing approach used by the laboratory.
	C 1.02b	If the laboratory cannot determine the Level, the test should be deferred, and the requesting medical practitioner contacted to resolve the Level of the test requested.
S 1.03 Informed consent	The laboratory must ensure consent forms for testing comply with best practice and jurisdictional requirements.	

No. and topic	Standard and commentary	
	C 1.03a	Consent for Level 1 testing can be implied by the patient presenting for testing and does not require additional evidence or documentation of informed patient consent.
	C 1.03b	Consent should explicitly include: i. expected results, including variants of uncertain significance ii. unsolicited findings, including unexpected familial relationships iii. data sharing of potentially re-identifiable data for clinical care iv. data sharing for ethically approved research v. ability to recontact with new or updated information vi. opt out if results are not to be included in the My Health Record.
S 1.04	The laboratory must have a process to be assured informed consent for testing has been obtained by the referring clinician for Level 2 testing.	
	C 1.04a	Level 2 testing consent forms do not need to be sighted or retained.
S 1.05	For Level 3 tests, the laboratory must document and act in accordance with the patients' decisions regarding the items below: a. relationships b. data sharing of potentially re-identifiable data for clinical care c. data sharing for ethically approved research d. opt out if results are not to be included in the My Health Record.	
	C 1.05a	This is most easily done by retaining a copy of the consent form.
S 1.06 Testing Policy	The laboratory must provide access to its policy on testing genetic material available to patients and clinicians, on request.	

No. and topic	Standard and commentary	
S 1.07	The laboratory must use its policy for testing genetic material to: a. minimise the possibility of unsolicited findings when defining the scope and designing each test to be performed b. determine which, if any, unsolicited findings should be reported c. ensure only tests which are consistent with the clinical request are performed.	
	C 1.07a	The laboratory should consider providing guidance to requesting clinicians for tests with a high probability of unsolicited findings. This guidance should include which unsolicited findings are possible and how they will be communicated.
	C 1.07b	The laboratory performing Level 1 testing using technologies with significant potential to reveal unsolicited findings, including comprehensive gene panels, exomes, or genome scale assays, should address this risk in their unsolicited findings policy. See Appendix A definitions of level 1,2 and 3.
	C 1.07c	The policy should also address action to be taken by the laboratory if Level 2 unsolicited findings, such as constitutional variants are identified.
S 1.08 Prenatal and predictive testing	The laboratory must include in its policies: a. Requirements for requests for prenatal and predictive testing. b. Guidance on the acceptability of prenatal and predictive testing requests.	
	C 1.08a	Testing of variants that are not in accordance with ethical guidelines or lack a medical context should be rejected. Further information is available from NHMRC ART guidelines.
	C 1.08b	Written guidelines should consider when to report findings, including any minimum thresholds for prenatal versus postnatal versus neoplasia findings, regions of homozygosity, or unsolicited findings.
	C 1.08c	Written guidelines should consider thresholds for reporting autosomal regions of homozygosity, which may reflect suspected consanguinity. Consideration should include the finding of increased levels of autosomal homozygosity that may reveal a suspected close parental relationship (1st or 2nd degree), and potential abuse of a disabled or underage individual.

No. and topic	Standard and commentary
Somatic	Some of the ethical considerations pertinent to constitutional variants do not apply to somatic variants. Germline tests will more often have complex associated ethical issues and be categorised into higher levels. However, this does not exclude the possibility of such complex issues arising during somatic testing, as these may detect constitutional variant – either as a primary finding, or as an incidental finding. Further information relating to the ethics of human genetic testing is available from: • NHMRC Medical Genetic Testing: Information for health professionals (NHMRC 2010)[3] • Joint Australian Law Reform Commission – NHMRC Essentially Yours — The Protection of Human Genetic Information in Australia (ALRC–NHMRC 2003)[4]

2. Specimen Collection

Errors in specimen collection while infrequent, do occur.[5] The significance of a sampling error varies according to the probability of the result being clinically significant and the availability of other evidence to corroborate or refute the result.

Errors in genetic testing are of particular concern when a genetic test identifies a healthy person as being at high risk of developing an illness in the future, without there being corroborating evidence. Such a prediction may also carry significant medical implications for genetic relatives. The context in which a genetic test is performed dictates the level of risk that may be acceptable in specimen collection.

No. and topic	Standard and commentary
S 2.01 Specimen Collection Policy	The laboratory must use and monitor compliance with it's policies on: a. specimen collection, processing, and transport b. acceptance criteria to ensure specimen are suitable for testing c. specimen transport collection and transport procedures d. management of contamination of specimens e. maintaining sample provenance.
C 2.01a	This includes, in the appropriate clinical context, consideration of gestational age, tissue type, tumour proportion, assay type, collection and transport conditions for viable versus non-viable tissue.
C 2.01b	cfDNA is more labile than genomic DNA and may be contaminated by genomic DNA from cell lysis during specimen collection and transport. Factors affecting cfDNA stability and cell lysis must be considered when validating assays based on cfDNA (e.g., NIPS, circulating tumour DNA testing). Examples of such factors include type of blood collection tube; transport time and conditions; plasma isolation method and plasma storage conditions.

No. and topic	Standard and commentary
	C 2.01c For RNA specimens the collection and transport processes should include use of specialised specimen collection containers and stabilising reagents.
S 2.02 Contamination	The laboratory amplifying nucleic acids must: a. have policies and procedures that identify and mitigate risks of cross-contamination of specimens and samples b. establish mechanisms for detecting errors associated with contamination. C 2.02a Contamination may include sample cross contamination and background nucleic acid contamination. C 2.02b Other than for self-collection specimens, specimens should be collected by a relevant trained professional. C 2.02c Wherever possible, genetic tests should be performed on dedicated specimens or portion of a larger sample taken before other tests are performed. C 2.02d Where it is necessary to perform genetic tests on specimens that have already been used for other purposes and there is a significant risk of cross-contamination, the report should be annotated accordingly. Where possible, reported results should be confirmed on a dedicated specimen (if one is available) or methods to exclude contamination should be used.
S 2.03 Patient Collection Information	The laboratory must ensure patients collecting samples have access to clear, contemporary, comprehensive, and relevant written instructions. C 2.03a Specimens collected by the patient are not ideal for nucleic acid tests due an increased risk of pre-analytical errors and specimen contamination.
S 2.04	The laboratory's policy must include guidance on measures to ensure specimen and sample provenance and ensure these measures are commensurate with level of risk C 2.04a This is particularly important in the collection for Level 2 and Level 3 risk tests.

Specimen collection for somatic testing

The critical importance of pre-analytic factors in preservation of nucleic acids and proteins for biomarker analysis is well known. However, best practice is not evident in many laboratories, in part due to factors beyond the control of the laboratory. Suboptimal pre-analytic processes have the potential to significantly impact on treatment-defining assays. This particularly occurs as more sophisticated genomic testing is being requested on routine formalin-fixed paraffin-embedded biopsy samples. Guidance on best practice tissue acquisition, fixation and processing protocols is available.[6]

S 2.05 Policies for somatic testing	The laboratory conducting somatic testing should use policies for: a. ensuring requestors have access to information on specimen or sample characteristics, storage conditions and transportation for successful testing b. exhaustion of tissue specimens and samples c. return of tissue to custodial laboratories.	
	C 2.05a	Factors to consider include tissue area, cellularity, fixation protocols, decalcification of bony specimens and sample age.
S 2.06 Biomarker testing	The laboratory involved in the acquisition of samples for biomarker testing must use and monitor compliance with its processes to minimise the impact of pre-analytic variables on the integrity of nucleic acid and protein targets.	
	C 2.06a	For other pre-analytical variables, fixation greater than 24-hours should be noted to testing laboratories if the specimen request is for any molecular assay, and decalcification conditions should be explicitly described.

3. Contamination Risk

The design of laboratories undertaking nucleic acid amplification should minimise the risk of contamination of specimens and reagents from other specimens in the laboratory or by amplified nucleic acid.

Nucleic acid detection techniques are usually designed to maximise sensitivity and can detect very small amounts of nucleic acid. Contamination may occur during:

- specimen collection or transport
- handling and testing the laboratory or referring laboratory before nucleic acid detection
- reagent preparation and use of reagents
- extraction of nucleic acids from the specimen
- amplification
- product detection.

The sources of potential contamination include:

- other specimens (cross-contamination)
- nucleic acid (e.g., contamination of stock reagents or equipment, or in aerosol droplets). The risk is increased for amplified nucleic acid.
- operator-derived nucleic acid.

No. and topic	Standard and commentary
S 3.01 Contamination risks	The laboratory must: a. have processes to prevent and detect contamination b. monitor compliance with these processes c. ensure the processes are commensurate with the risk of contamination.
S 3.02 Separation of workspace	The laboratory must ensure the physical separation of workspaces for each specific stage of testing: a. is adequate to minimise the risks of contamination b. takes into consideration instruments capable of producing aerosols and use of robotic equipment.
S 3.03	For nucleic acid amplification, the laboratory must have a separate area for: a. preparation of reagents including dispensing of master mixes b. nucleic acid extraction, preparation, and handling before testing c. amplification and product detection d. manipulation of specimens prior to a second round of amplification.
S 3.04	The laboratory preparing reagents and specimens in a single room must use a risk-based approach to: a. maintain the separation of these activities b. implement control mechanisms to detect contamination.

No. and topic	Standard and commentary
	C 3.04a The laboratory can achieve this by having processes to ensure: i. specimens (pre- and post-amplification), reagents and equipment are held in separate areas ii. specimens, reagents, and equipment are labelled correctly iii. patient specimens are not taken into the reagent preparation area iv. equipment from other areas is not taken into the reagent preparation area without prior decontamination v. post-PCR area is separate to the reagent or specimen preparation areas and positioned to minimise contamination of pre-amplification areas vi. movement of specimens and containers is unidirectional from pre-amplification to post-amplification areas vii. PCR amplification tubes are sealed when carried between the pre-and post-amplification areas viii. containers returned against the flow must first be decontaminated ix. the risk of contamination is assessed and minimised when a single instrument is used in both pre and post amplification processes.
Laboratory hygiene	
S 3.05 Preventing contamination	The laboratory must comply with evidence-based guidelines for the decontamination of work surface, considering: a. case load b. type of activity of the laboratory c. level of risk.

No. and topic	Standard and commentary
S 3.06 Personal protective equipment	The laboratory must: a. have processes for the use, training, testing, and fitting of personal protective equipment by the workforce which uses a risk-based approach b. align their processes with the current edition of the *Australian Guidelines for the Prevention and Control of Infection in Healthcare*[7], jurisdictional requirements, and relevant jurisdictional laws and policies, including work health and safety laws.
S 3.07	The laboratory has a risk-based approach to the use of PPE that ensures: a. movement of PPE is unidirectional from pre- to post-amplification areas b. PPE used in post-amplification area is not used in other areas c. PPE is changed whenever there is evidence of contamination.
S 3.08 Reporting contamination events	The laboratory must have processes to document: a. contamination events b. the source of the contamination c. measures taken to reduce the risk of future similar contamination events.

4. Specimen preparation and storage

No. and topic	Standard and commentary	
S 4.01 Documented extraction procedures	The laboratory performing testing on extracted nucleic acids must have documented procedures for extracting nucleic acid of suitable quantity and quality for the intended testing.	
S 4.02 Validated extraction and isolation methods	The laboratory must ensure that nucleic acid extraction methods are validated and there are quality controls for all relevant sample types and associated downstream testing methodologies used by the laboratory.	
S 4.03	The laboratory must have processes to monitor the extracted nucleic acid quality and quantity and ensure it is sufficient for downstream testing methodologies used by the laboratory.	
	C 4.03a	Cell-free DNA is of low molecular weight and is typically present at low concentration in plasma. Extracted cell-free nucleic acids should contain minimal cellular (or cell-based) genomic nucleic acid.
	C 4.03b	For RNA, interfering substances that can interfere with downstream analysis include DNA and organic chemical compounds used during the extraction.

No. and topic	Standard and commentary	
S 4.04	The laboratory must have processes to minimise degradation of stored nucleic acids and assess integrity of stored nucleic acids prior to testing.	
	C 4.04a	Quality control checks should be fit for purpose. For example, spectrophotometry may not be sufficiently accurate or sensitive for intended uses.
	C 4.04b	Specimen types should be assessed for potential sources of interference.
	C 4.04c	As RNA is less stable than DNA, and the level of gene expression may vary markedly between different tissues and developmental stages, manipulation of RNA requires specific consideration, such as dedicated pipettes and equipment, regular cleaning of work areas to reduce contaminating DNAses and RNAses, and assessment of sample degradation and concentration before testing.
S 4.05 Handling and storage of samples	The laboratory must have processes to: a. ensure storage procedures minimise the risk of degradation, contamination and misidentification of samples b. monitor compliance with its storage and handling processes.	
	C 4.05a	This includes appropriate storage temperatures and labelling systems for both RNA and DNA and other samples, including plasma.
S 4.06 Methods for chromosome preparations	Where the laboratory is performing chromosome fragility studies, it must have processes for documenting: a. the conditions for inducing culture stress b. the use of control specimens.	
	C 4.06a	Various approaches are required for karyotype analysis of a range of specimen types and may involve unsynchronised versus synchronised cultures, unstimulated and stimulated cultures with overnight, 24, 48, 72-hour culture times.
S 4.07 Chromosome stress testing	The laboratory undertaking chromosome fragility “stress” testing must include in their policies the conditions for inducting culture stress and the use of appropriate control specimens.	
S 4.08 Chromosome stress testing	The laboratory must ensure evidence-based, best practice guidelines are followed for the preparation of specimens for prenatal diagnosis, including minimising the risk of maternal cell contamination.	

No. and topic	Standard and commentary
	C 4.08a Best practice guidelines require: i. chorionic villus specimens to be dissected free from decidua, blood and blood clots to reduce maternal cell contamination ii. chorionic villus karyotype studies to include analysis of long-term cultures iii. establishment of duplicate or independently established cultures for all specimen types where stand-alone karyotype analysis is performed, provided there is adequate specimen available. The specimen should be divided and cultured in two separate incubators, running on different electrical circuits (if possible), and maintaining the cultured cells with independent cell culture media and other reagents iv. recognition of the possibility of maternal cell contamination, pseudo mosaicism, true mosaicism and in-vitro derived chromosome [artefact] aberrations. The systems of culture and analysis used should be designed to detect and differentiate these.
S 4.09 Minimising contaminants in prenatal testing	The laboratory must minimise the risk of maternal cell contamination by supporting the implementation and monitoring compliance with evidence-based, best-practice guidelines for the preparation of prenatal samples:
Specimen preparation for somatic testing	
S 4.10 Tumour purity	The laboratory must implement and monitor: a. procedures for estimating and recording tumour purity / cellularity b. the acceptance limits for tumour purity / cellularity.
	C 4.10a The specimen submitted for testing should be assessed for purity and cellularity.
	C 4.10b Acceptance limits for tumour purity / cellularity should be based on the limit of detection established during assay validation experiments.

No. and topic	Standard and commentary	
	C 4.10c	An adjacent Haematoxylin and Eosin-stained tissue section slide cut from a formalin-fixed paraffin embedded tissue block submitted for testing or stained from the slide series submitted for testing should be used to assess tumour purity. Use of previous diagnostic slides is not acceptable. Nucleic acid quantity and quality may be affected by sample type (for example cytology smears, cell blocks, formalin-fixed paraffin embedded tissue blocks, fresh tissue, cell-free DNA from plasma), sample age, fixation conditions, and decalcification conditions.
	C 4.10d	Preanalytical quality control measures should be designed to ensure amplifiable nucleic acids are sufficient to generate a reliable result.

Analytical

5. Testing Methodologies

No. and topic	Standard and commentary	
General Considerations		
S 5.01 Assay quality controls	The laboratory must implement and monitor processes to mitigate the risk of assay failure or sample contamination and ensure the assay performance characteristics remain in control.	
	C 5.01a	This may include the use of positive, negative and 'no-template' controls where required. 'No template' controls are intended to alert the laboratory to the existence of external contamination. 'No template' controls may not be required if the laboratory has alternative validated methods for measuring contamination, for example bio-informatic approaches.
Sequencing to detect unspecified variants Nucleic acid sequencing provides nucleotide-by-nucleotide analysis of a region and is usually able to identify variants regardless of pre-test probability. A sequencing assay may target thousands of nucleotides. The resulting complexity of the assay and its analysis raises particular issues in relation to analytical consistency and accuracy, and the performance characteristics of analytical software.		
S 5.02 Assay Target region	The laboratory must implement processes for: a. clearly defining the target region of interest for analysis b. considering the clinical significance of the target region c. providing the requesting clinician with access to information on the target region.	
S 5.03	The laboratory must implement processes to:	

No. and topic	Standard and commentary
Sequence Quality	a. produce a quantitative assessment of sequence quality b. establish acceptance limits for sequence quality score C 5.03a Limitations in the sequence quality may be resolved by sequencing of both DNA strands. This also allows prompt identification of some sequencing artefacts.
S 5.04	The laboratory must implement processes to: a. identify a sequence change using systematic comparison of the sample sequence to a reference sequence b. interpret a nucleic acid sequence change in the context of the clinical request.
S 5.05	The laboratory must perform sequence comparison using computerized analysis C 5.05a Unassisted visual inspection of sequence data is potentially unreliable (particularly for homozygous mutations).[8]
Non-sequencing assays to detect unspecified variants This section addresses methods that screen for the presence of variants in a specified locus without precisely defining the nature of the variant. This includes assays such as high-resolution melt analysis.	
S 5.06 Assay sensitivity	The laboratory must implement processes to document the analytical and clinical sensitivity of assays used for detecting pathogenic variants.
S 5.07 Variants quality controls	The laboratory must implement processes to confirm the genotype of a variant detected by the assay using a second method such as sequencing or another genotyping assay
S 5.08	The laboratory must implement processes for: a. assessing the risk of false negatives from assays with reduced sensitivity for homozygous or hemizygous variants b. reducing the risks of false negatives. C 5.08a This applies to technologies such as high-resolution melting and denaturing high performance liquid chromatography where wild-type spike-ins may be used.

No. and topic	Standard and commentary
Non-assays to detect specified variants	
This section addresses assays for variants that have a high prior probability of being detected in a defined clinical situation, including assays for recurrent pathogenic variants and for family-specific variants. Such assays may be performed as a targeted single variant or multiplexed assay.	
S 5.09 Variant detection	The laboratory must implement processes to: a. establish control procedures and defined positive controls to validate assays that detect known variants, and for use in ongoing assay quality control b. ensure other variants that interfere with the detection of specified variants are addressed in the validation of the assay and documented in the limitations of the report
S 5.10	The laboratory testing for a family-specific variant must implement processes to test a positive control for the familial variant.
	C 5.10a Where a positive control is not available, and the assay has the potential for allele dropout, laboratories should document the absence of a familial control in any negative report.
	C 5.10b For large panels, laboratories may use a collection of positive controls comprised of samples harbouring known variants. At least one control sample should be tested in each analytical batch or reagent lot, or a schedule established to ensure controls are tested regularly over a given period.
S 5.11 Validate multiplex assays	The laboratory must implement processes to validate multiplexed assays for analytical specificity
S 5.12 Allelic drop out	The laboratory must implement processes to mitigate the risk of an allelic drop out.
	C 5.12a The mitigation of allelic drop out is particularly important when the laboratory has designed the assay and for family-specific testing.
	C 5.12b The sequence of primers or probes should be assessed for interference from single nucleotide variants on a regular basis as part of the ongoing monitoring of the assay.
S 5.13 Reference intervals and controls	The laboratory must implement processes to define reference intervals and measurement uncertainty and use relevant controls for the reference ranges in each batch of assays of instability variants.
	C 5.13a Unstable variants include triplet repeat variants.

No. and topic	Standard and commentary
Quantitative assays	
	All molecular genetic assays are, to an extent, quantitative; quantifying the signal derived from an assay is an essential component of quality control in any assay. Nonetheless, the result of many genetic assays is reported only qualitatively, that is a variant is present or absent. Assays for constitutional variant are usually developed on the assumption that the genetic content of each of the cells under examination will be the same. This section deals with assays in which quantitation is an integral component of the outcome of the assay. Such assays are designed for situations either involving cells with differing genetic content in the specimens (mosaicism) or in which it is necessary to quantify the result to compare different specimens or different targets within the same specimen. This includes assays for determining variant, gene, or chromosome dosage (e.g. detecting constitutional mosaic variants, mitochondrial DNA variants or somatic variants, NIPS, circulating tumour DNA, Multiplex Ligation-dependent Probe Amplification, quantitative PCR or microarray).
S 5.14 Reporting on nucleic acid quality for quantitative assays	The laboratory must implement processes to assess the quality of the nucleic acids used as a substrate for quality assay and document this in the assay report if it is likely to affect assay performance characteristics.
S 5.15 Limits of detection	The laboratory must implement processes to determine the lower limit of detection for each class of variant that is intended to be measured.
S 5.16 Measurement uncertainty	The laboratory must implement processes to establish measurement uncertainty across the reportable range.
	C 5.16a This should include measurement uncertainty at the limit of detection
S 5.17 Fetal DNA	The laboratory carrying out NIPS must implement a process to ensure that adequate amounts of fetal DNA are present in the sample prior to data analysis and interpretation of results.
	C 5.17a The sensitivity limit determined for the assay should be considered when determining quality metrics for adequate fetal DNA, as different NIPT assays have different fetal fraction cut offs, and some do not measure fetal fraction. this may be justifiable, depending upon the required analytical or clinical sensitivity.

No. and topic	Standard and commentary
Microarrays	
Microarrays may include those for copy number variation, genotyping, methylation, or gene expression. The standards below relate to DNA microarrays primarily used to detect copy number variation. Other gene expression and methylation arrays are not specifically addressed as these are yet to have widespread diagnostic use in clinical laboratories. Where these technologies are being introduced in the diagnostic setting, best practice international guidelines should be consulted.	
S 5.18 DNA quality	The laboratory must implement processes to monitor the DNA quality of the sample being tested, intensity of labelling, and quality of hybridisation, scanning, and analysis
S 5.19	The laboratory must implement processes to: a. determine the mean effective resolution of the micro array design for determining a chromosomal aberration b. determine the minimum number of consecutive probes for calling copy number variants, loss of heterozygosity or regions of homozygosity.
Analysis of limiting amounts of template nucleic acid	
Most genetic tests involve the analysis of nucleic acids derived from many cells. The result reflects the majority genotype in the population of cells, and artefacts derived from individual cells or molecules (including low levels of contamination) are usually not evident. However, variants derived from a single cell can be relevant in analyses which use very small amounts of: • template DNA or RNA, for example in pre-implantation genetic diagnosis • specimens with limited DNA, for example in urine or plasma for somatic mutation detection or NIPS • low proportion of tumour cells when assessing for somatic variants • assays for low levels of tissue mosaicism, for example in minimal residual disease and mitochondrial testing.	
S 5.20 Templates	The laboratory must implement processes to: a. ensure the choice of method and the assay validation process address the range of template concentrations that could be experienced in clinical use b. specify for the input mass of template at the upper and lower thresholds c. address the risk of contamination and allelic bias during amplification in the validation of the assay.
S 5.21 Validation procedures	The laboratory must implement processes to validate procedures used for measuring the quality and quantity of input nucleic acid.

No. and topic	Standard and commentary	
	C 5.21a	This may include the method used to determine minimum fetal fraction in NIPS or number of cells analysed, or proportion of tumour cells in a somatic tissue.
S 5.22 Serial amplification	Where the laboratory uses serial amplification of DNA the reaction product from one round of amplification must be manipulated in an area separate from the areas used for single-stage PCR.	
	C 5.22a	Serial amplification of DNA includes nested PCR.

Invasive prenatal testing

Prenatal diagnostic tests are set apart from other tests because of the risk to the fetus associated with specimen collection, the small specimen size, the difficulty associated with repeat sampling, the requirement for a short turn-around time, and the significance of decisions arising from the test. The analytical context of testing can vary from testing for a pre-defined mutation, to seeking to identify an uncharacterised variant, or chromosomal aberration. The interpretation of a pre-defined variant is usually straightforward, but the interpretation of an uncharacterised variant may be difficult because of limited phenotypic information about the fetus and in data repositories.

S 5.23 Prenatal testing	The laboratory conducting prenatal testing involving the genotyping of a known familial variant must implement processes to: a. genotype the proband or other relevant family member(s) b. document in the report when proband or other relevant family member(s) cannot be tested c. address the issue of allele drop out d. review the classification of familial variants at regular intervals to ensure continued accuracy.	
	C 5.23a	This especially applies to variants classified outside of the testing laboratory.
S 5.24	The laboratory conducting prenatal testing must implement processes to determine risks associated with an incorrect test result and requirements for repeat testing.	
	C 5.24a	Subject to the risk assessment, repeat testing may be required to confirm the test result or when there is an unexpected test result.
S 5.25	The laboratory performing prenatal testing for unspecified variants must implement processes for handling and reporting on variants of uncertain significance and unsolicited findings.	
S 5.26 Reserve culture	The laboratory performing analysis on a specimen for invasive testing must implement processes to ensure a separate reserve culture is maintained until the results of the nucleic acid prenatal analysis is known.	
	C 5.26a	Appropriate liaison with external laboratories that refer prenatal specimens must be maintained.

No. and topic	Standard and commentary
S 5.27 Maternal cell contamination risks	The laboratory must implement processes to: a. assess the risks of maternal cell contamination in all prenatal tests b. mitigate identified risks c. determine the level of significance of maternal cell contamination for each of their assays and cut-off limits d. document any maternal cell contamination in the report.

No. and topic	Standard and commentary

Cell-free nucleic acid detection, including Non-Invasive Prenatal Screening

Cell-free DNA is a labile, typically low-level analyte. Similar pre-analytical and analytical considerations apply regardless of the clinical purpose of the assay, for example Non-Invasive Prenatal Screening (NIPS) or somatic variant detection.

NIPS, also sometimes referred to as Non-Invasive Prenatal Testing, is a screening test that employs either targeted or whole genome approaches to detect circulating cell-free DNA in maternal plasma, in order to identify fetal genetic variation. NIPS may be carried out by massively parallel sequencing, or other methods, for example microarray, real-time PCR, droplet digital PCR.

In addition to the pre-analytical and analytical requirements outlined above and in the *Requirements for human medical genome testing utilising massively parallel sequencing technologies*, NIPS has reporting considerations. These considerations are based on analytical and biological factors, for example the origin of the majority of cell-free 'fetal' DNA is the placental cytotrophoblast. These considerations apply regardless of the underlying variants targeted or methodology used for NIPS. Reporting considerations particular to NIPS are described in sections S8.17 to S8.21 of the *Requirements for human medical genome testing utilising massively parallel sequencing technologies*.

Somatic variant testing of solid tumours

Genetic testing for somatic variants in solid tumours differs from other genetic tests in that the specimens invariably contain non-tumour tissue. There is a need for appropriate collaboration and input from an anatomical pathologist in selection of material to test and contextual interpretation of results.

For *in situ* somatic variant testing in solid tumours refer to the section on *in situ* hybridisation and FISH below.

S 5.28 **Tumour assessment**	**The laboratory must ensure an anatomical or general pathologist are given the responsibility for and use best practice guidelines to evaluate specimens for assessment of tumour area and percentage of tumour cells.**	
	C 5.28a	Solid tumour samples for somatic variant testing should be selected based on the greatest quantity and proportion of tumour cells.
	C 5.28b	Evaluating specimens for assessment of tumour area includes estimating the percentage of tumour cells in the specimen, or area selected for dissection.
	C 5.28c	Ideally, specimens should only be tested if estimated tumour cell percentage is above the sensitivity limits of the assay (and presuming all other quality control measures are met). Laboratories should have a policy in place that covers dealing with specimens that do not meet these criteria which includes an individualised risk assessment and consideration of repeat biopsy if clinically appropriate.

No. and topic	Standard and commentary	
S 5.29	The laboratory must ensure that pathologists are responsible for and use evidence-based protocols for identifying somatic alterations in solid tumours.	
	C 5.29a	Results of tumour assessment need to be interpreted in the context of the specific tumour type, as assessed by other techniques such as morphology, immunophenotype and clinical scenario.
	C 5.29b	When somatic testing is performed for the purpose of tumour profiling for patients who have exhausted all standard therapeutic options, clinical interpretation may be according to a broader disease context.
	C 5.29c	Comprehensive genomic profiling performed in these situations should follow internationally accepted recommendations for therapeutic, diagnostic, or prognostic variant classification.[9]

RNA

RNA is used as a template for testing in a variety of applications, from detection of sequence variants, splice isoforms and gene-fusions to the determination of expression levels both for diagnostic and functional impact studies. Use of RNA presents additional challenges due to its unstable nature, tissue and allele specific expression, different isoforms, nonsense mediated decay and the presence of contaminating DNA. Special precautions must therefore be taken when working with RNA as a specimen.

S 5.30 Source specimens	The laboratory must ensure qualified members of the workforce are responsibility for assessing, documenting, and reporting on source specimens.	
	C 5.30a	Assessing, documenting, and reporting on source specimens involves: i. assessing the RNA source specimen for its suitability to provide a relevant test result ii. assessing tissue specific expression patterns of the source specimen in the context of the disease specific tissue iii. documenting the test limitations arising from tissue specific expression patterns in the test interpretation and report iv. documenting in the method of the report when the source specimen has undergone cell culture and/or treatment before RNA extraction v. including in the report interpretation that representation of transcript levels and isoforms in cultured and treated specimens may differ from the source specimen.

No. and topic	Standard and commentary	
	C 5.30b	Wherever possible, disease relevant specimen sources should be used as the source specimen.
	C 5.30c	A source specimen may undergo cell culture and/or treatment to prevent nonsense mediated decay before RNA extraction.
S 5.31 Transcript levels	The laboratory must implement processes to validate assays using RNA to determine the minimum transcript levels required for a valid test result.	
S 5.32 Reporting assay limitations	The laboratory must ensure qualified members of the workforce are responsible for reporting the limitations of an assay.	
	C 5.32a	The report should include a statement that clarifies the absence of identified transcript in a given assay does not preclude low level presence of that transcript in the tested specimen type or presence of the transcript in the disease specific tissue.
S 5.33	The laboratory screening for allele specific variants, including splice variants, must use evidence based best practice protocols.	
	C 5.33a	When screening for allele specific variants, including splice variants practitioners should ensure assays contain measures to address levels of allele specific expression, degradation, and/or amplification bias for targeted transcripts, where possible.
	C 5.33b	This may be achieved by inclusion of informative SNPs in the assay or prevention of non-sense mediated decay through culture approaches.
	C 5.33c	Where allele specific expression and/or degradation cannot be excluded, this must be clearly stated in the interpretation of the report.

6. Interpretation

This section addresses key issues for laboratory professionals interpreting the biological significance of a variant.[10]

Data and images used for diagnosis must be retained in accordance with the *Requirements for the retention of laboratory records and diagnostic material | Australian Commission on Safety and Quality in Health Care*

No. and topic	Standard and commentary
S 6.01 Curation of Genetic variants	The laboratory must implement processes: a. for the curation of genetic variants b. to ensure the interpretation of genetic variants is evidence-based and documented c. to ensure interpretation applies relevant professional standards and guidelines (For constitutional variant this includes ACMG guidelines, ClinGen gene specific guidelines).
S 6.02 Statistical Methods	The laboratory must incorporate statistical methods, including linkage and Bayesian analyses, when relevant, when determining the clinical significance of an analytical result.
S 6.03 Documenting tissue tested	The laboratory must ensure instances when the tissue tested influences the applicability of the test result is documented in reports. C 6.03a Examples of this concept include mitochondrial testing where the tissue source is critical in determining the presence of pathogenic mitochondrial variants; RNA testing as the tissue used for RNA extraction should express the relevant gene.

No. and topic	Standard and commentary	
Cytogenetic analysis		
S 6.04 Sufficient assessment for analysis	The laboratory must implement processes for examining sufficient cells of adequate quality and resolution to address the clinical reason for referral, including investigation or exclusion of mosaicism or clonal abnormalities.	
	C 6.04a	Coordinates of analysed and counted cells, including images from digital analysis systems, should be recorded to enable review of cells as required.
	C 6.04b	The laboratory conducing karyotype analysis must routinely use G-banded chromosomes.
	C 6.04c	Ag-NOR, distamycin A and DAPI staining, and/or FISH should be available for further characterisation of a karyotype result, where appropriate.
S 6.05	The laboratory must implement a process to ensure an experienced cytogeneticist checks and verifies analytical results.	
	C 6.05a	An analyst is responsible for examining all chromosomes and documenting their provisional results. This work is checked by a more senior cytogeneticist, who reviews any abnormality and the analysis report to verify and authorise the report.
In situ hybridisation analysis (ISH) *In situ* hybridisation includes fluorescence and bright-field *in situ* hybridisation and RNA *in situ* hybridisation.		
S 6.06 Analysis of probes	The laboratory must have policies and procedures for the analysis of the repertoire of probes it holds for the range of constitutional and acquired somatic tumour (cancer) variants.	
	C6.06a	Where possible, use of hybridisation systems that include the target region of interest and a control probe to tag the chromosome should be used. For the detection of translocations in interphase nuclei, using fusion probe sets, an extra signal or a dual fusion strategy has greater specificity and should be used, whenever possible.
S 6.07 Cytogenetic specimens	The laboratory performing ISH on non-routine cytogenetic specimens must ensure the workforce has the skills, expertise, collaborative and supervisory arrangements to perform and fully interpret test findings.	
S 6.08	The laboratory assessing cytogenetic specimens must ensure sufficient numbers of metaphase and interphase cells are analysed to provide a statistically valid result.	

No. and topic	Standard and commentary	
S 6.09 Multiple assessments to confirm results	The laboratory must undertake a risk analysis to identify applications that require two or more independent analysts to confirm results.	
	C 6.09a	It is acknowledged that interphase FISH systems using automated digital image analysis are emerging in diagnostic pathology. Any data or results need independent visual inspection to verify the result.
	C 6.09b	If results are close to established cut-off thresholds, another independent analyst should be consulted.
	C 6.09c	The numbers of cells examined may differ depending on whether the assay is being used as a first-line test, to confirm or exclude a known abnormality, or to investigate mosaicism or malignant clones.
	C 6.09d	The number of interphase nuclei examined will depend on the analytical sensitivity of the probe(s) used and the confidence level required. It will also depend on the nature of the specimen examined, i.e., constitutional or oncology.[11]
S 6.10	The laboratory must ensure pathologists with the relevant scope of practice and experience are responsible for: a. ensuring the correct tumour cells are being assessed b. undertaking the assessment of solid tumour samples with specified-genetic rearrangements or gene copy number alterations c. deeming the specimen able to be interpreted without specific morphological expertise.	
S 6.11	The laboratory must ensure ISH and FISH analyses are documented, including the probe type, name and manufacturer, number of cells examined and detailed hybridisation results.	

No. and topic	Standard and commentary
Software	
S 6.12 Software	The laboratory using software in the analysis and interpretation of data must: a. ensure the software is fit for purpose and fully validated b. ensure there is a process for version control of the software c. ensure traceability by documenting databases accessed in analysis d. ensure on-line and cloud-based analysis of data meet all jurisdictional privacy and security requirements e. apply the principles of the bio-informatics pipeline.
	C 6.12a Laboratories should have a good understanding of the operation of their analytic software. As a minimum, laboratories should be able to identify when the pipeline is not working; and when 'edge cases', where a pipeline that is working (under control), may misidentify variants leading to a false positive or false negative result.

Post-analytical

7. Reporting standards

A genetic or genomic test report needs to include sufficient technical information to make it clear to the expert reader, perhaps in years to come, what method had been used to identify human genetic variation. On the other hand, the report must not obscure the key information for the non-expert reader who need to make medical decisions today.[12]

No. and topic	Standard and commentary	
S 7.01 Reporting	The laboratory must establish and monitor processes for the documentation and timely reporting of clinical results.	
	C 7.01a	The responsible pathologists or scientists, should ensure that the clinical report includes: i. their clinical interpretation and ensure this address the clinical question ii. relevant clinical details provided by the referring medical practitioner, or a statement of 'no clinical notes provided' iii. terms that are precise and unambiguous iv. the implications for genetic relatives and recommendations for genetic counselling.
	C 7.01b	When a nucleic acid variant is identified, the distinction between the patient having an abnormal genotype and the patient being affected should be made clear.

No. and topic	Standard and commentary	
	C 7.01c	When reporting genetic results, consider: i. the use of unambiguous quantifiers, for example “90% of people with this variant are affected” is preferable to “this variant is usually associated with disease”. ii. utilisation of positive statements, for example “the triplet repeat number is within the normal range” is preferable to “the triplet repeat number is not expanded”. iii. the terms “positive” and “negative” can be confusing and should be avoided. Use of “absent/ not detected” or “present/ detected” should be used, for example “MLH1 expression is absent” is preferable to the “MLH1 assay is positive”.
	C 7.01d	It is essential to reduce the potential for misinterpretation of the test result. For example, the purpose of testing an affected person is different to testing an unaffected person, and the identification of a pathogenic variant in diagnostic versus predictive testing carries different clinical implications. The wording of this statement could reflect the classification of human genetic testing.
	C 7.01e	Where the clinical details are illegible, absent, or ambiguous, and this affects interpretation of the result the laboratory should attempt to clarify the details with the referring medical practitioner.
	C 7.01f	In the case of simple reports, for example an assay for a recurrent variant, there may not be any need for further comment. If reporting a rare and/ or novel variant, there should be further interpretation of its biological and clinical significance. i. If this information has already been provided to the requesting medical practitioner in a report about a genetic relative, reference could be made to that report (referring to the laboratory identifier, not patient name) rather than repeating the information. ii. However, if this report is being provided to a different medical practitioner, the analysis should be provided again.
	C 7.01g	Where appropriate, laboratories should submit data about curated variants and their associated phenotypes to curated publicly accessible repositories.

No. and topic	Standard and commentary
S 7.02 Report requirements	The laboratory must ensure reports: a. specify when two independent specimens were collected for the purpose of reducing the risk of an erroneous result b. unambiguously identify the gene/s or genetic locus/loci assayed c. use standard gene names with synonyms or alternative names shown in brackets if considered necessary for effective communication d. includes information in the report on how to access unambiguous, version-controlled information when the number of loci tested is too large to be feasibly included on the report e. specify the genome reference sequence used in the assay.
C 7.02a	It is recognised that synonyms are in common use, but they are not specific and there is potential for confusion to both current and future readers of the report.
C 7.02b	The standard gene nomenclature as described by the Human Genome Organisation Gene Nomenclature Committee[13] should be used.
S 7.03	The laboratory must ensure reports state, in simple terms: a. the method and the scope of the analysis performed b. limitations of the test including germline predictive testing c. the system of variant nomenclature used in the report.
C 7.03a	Reports should include scope of the analysis performed, for example analysis of all exons by sequencing and dosage studies, or test for selected variants only.
C 7.03b	The technical characteristics of the assay are relevant for the interpretation by either the laboratory or a medical practitioner. When appropriate, the commercial kit, or specific primers and probes, should be specified.
C 7.03c	Reports for RNA test results must include sample collection and extraction method, any culture or treatment conditions, sample preparation, enrichment, and selection methods (whether mRNA or total RNA was used), and details of utilised analysis and interpretation methods, including bioinformatic tools where relevant.
C 7.03d	It is recognised that some recurrent variants are widely cited using non-standard nomenclature. This represents a hazard for consistent and accurate reporting in the future. Variants should be reported using standard nomenclature, with synonyms or common names shown in brackets until referrers become familiar with the correct terminology.

No. and topic	Standard and commentary	
	C 7.03e	The Human Genome Variation Society has established an international standardised nomenclature;[14] as has the International Society for Cytogenetic Nomenclature.[15]
	C 7.03f	The laboratory reporting on germline predictive testing must ensure reports include a statement on the limitations of the testing.
S 7.04 Reporting maternal contamination	The laboratory conducting prenatal testing must ensure reports include a statement on the levels of maternal cell contamination.	
S 7.05 Reporting on multiple family members	The laboratory testing a number of family members must implement a process to ensure: a. separate reports are provided regarding each person in the family, if clinically relevant b. the report does not reveal the identity of other relatives c. for trio testing where parents are included to assist in analysis of a child, a separate parental report is typically not required. The parental details should be provided in the child's report.	
	C 7.05a	The genetic and clinical interpretation of a patient's nucleic acid test may depend on the results of tests on genetic relatives. If necessary, the report can identify the clinical service which holds pedigree data or results of the entire family.
	C 7.05b	In cases in which a couple's results need to be considered together, reference must be made to the other report (using the laboratory identifier) but without naming the other party.
	C 7.05c	Where the intent of the assay is to perform couple testing or family-specific testing, the report may include all relevant family members; or if a couple provide explicit consent at the time of testing for their tests to be reported and stored together, and to be released at the request of either person.
S 7.06	The laboratory conducting cytogenetic and FISH testing must ensure reports include: a. the number of metaphase and interphase cells counted and analysed b. banding techniques applied and banding resolution, where applicable c. FISH probes used, including manufacturer.	

8. Outsourced testing

No. and topic	Standard and commentary	
S 8.01 Reporting	The laboratory must ensure referral laboratories are qualified to conduct the required tests and the basis for selecting a referral laboratory is documented whenever a referral is made.	
	C 8.01a	Justification of laboratory should be based on the *Requirements for medical pathology services*. In general, the types of testing laboratory include in decreasing order of preference: i. Laboratories accredited to AS/ISO 15189 and NPAAC Requirements ii. Laboratories accredited to AS/ISO 15189 by an accreditation body which is a signatory to the ILAC mutual recognition arrangements for the referred activity iii. Laboratories accredited to ISO/IEC 17025 by an accreditation body which is a signatory to the ILAC mutual recognition arrangements for the referred activity.
	C 8.01b	When referring to research laboratories, it is important to communicate to the requesting doctor and patient that the result has been generated by a research laboratory. It is recommended the result is confirmed by an accredited laboratory before use in patient management, see action 8.02.

No. and topic	Standard and commentary	
S 8.02 Research results	The referring laboratory must ensure research results used to support interpretation of accredited test results are assessed in consideration of sample provenance, clinical utility, and quality of interpretation.	
	C 8.02a	The referring laboratory should consider sending unextracted specimens to minimise the risk of contamination if storage of DNA is not requested or likely to be required, and if specimen integrity is not compromised by transportation.
	C 8.02b	If the result from testing provided by an unaccredited laboratory will be used for clinical decision making, the analytical result should be confirmed in a laboratory accredited to NPAAC Requirements.
	C 8.02c	If a specimen has been forwarded to another laboratory for analysis, the referring laboratory remains responsible for ensuring that the test result is provided to the requesting medical practitioner. The transcription of complex nucleic acid test results by referring laboratories is discouraged.
	C 8.02d	The referring laboratory should seek to ensure the requesting medical practitioner is provided with a copy of the original report. This is feasible where requests are sent to accredited laboratories.

Appendix A — Classification of human genetic testing

The distinction between Level 1 (standard DNA test), Level 2 (DNA test with potential complex issues and Level 3 (genomic DNA/ RNA tests) would usually be made by the doctor 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.

Table 1 Levels of human genetic DNA and RNA testing

Level	Risk level and explanatory notes
Level 1	The risk of a clinically complex result is low. Included in this level are: • Testing for diagnostic purposes For example, 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 is conducted that is not assessed as Level 2 or 3. • Population-based screening programs • Other screening programs with low risk of clinically complex results For example, NIPS for cytogenetic variants or 3 condition (cystic fibrosis, Fragile X and spinal muscular atrophy) carrier screening. • Somatic testing for variants predictive of response to approved therapies • Targeted genotyping performed for red cell antigens (HEA), platelet antigens (HPA), neutrophil antigens (HNA) and tissue typing (HLA) performed on donor or patient samples, or their family members.
Level 2	The risk of a clinically complex result is moderate. Included in this level are: • DNA testing for which specialised knowledge is needed before the DNA test is requested, and for which professional genetic counselling and written informed consent should precede and accompany the test. For example, predictive or pre-symptomatic testing, including expanded carrier and couple reproductive risk screening, for conditions for which there is no specific treatment.

Level	Risk level and explanatory notes
Level 3	The risk of a clinically complex result is high. Included in this level are: Sequencing or genotyping based testing that has complex analysis and a high pre-test probability of unsolicited findings. Specialised knowledge relevant to the test is needed to request a Level 3 test. Professional genetic counselling, often from a health professional with formal training, and written informed consent must precede the test. The testing laboratory must document the consenting decisions (see Informed Consent, action 1.05). For example: • Whole genome analysis • Whole exome analysis • Whole transcriptome analysis • Large complex gene panels covering conditions associated with more than one organ system such as large gene panels for intellectual disability.

Counselling and consent

Counselling and consent for genetic testing is considered in the National Health and Medical Research Council publication, Medical Genetic Testing: Information for health professionals. Clinical scientists, pathologists in charge of a laboratory and their senior staff should be familiar with the issues addressed in this publication. This will allow meaningful discussions to occur between the laboratory and the requesting practitioner in cases where appropriate level classification of a request remains unresolved.

Classification of human genetic tests

Irrespective of the risk level of a test, the requesting medical practitioner should ensure the person or legal guardian gives informed consent for the investigation. The majority of requests for genetic testing, for example for diagnostic or medical screening purposes, will be Level 1. A test is classified as Level 2 and requiring professional genetic counselling and consent if it fulfils one or more of the criteria.

These criteria reflect the complexity of common genetic and counselling issues. The list of criteria is not exhaustive. In cases of doubt, the requesting clinician should manage the test process as a Level 2 genetic investigation or seek advice from the testing laboratory.

Level 3 tests will involve sequencing of large numbers of genes without any specific gene having a high prior probability of causative variants for the phenotype being investigated. Due to the number of genes being investigated there is a higher probability of an unsolicited findings being discovered.

Guidelines developed by the National Health and Medical Research Council, or a national medical specialty college recommend pre-test genetic counselling and written consent for certain conditions. For example, testing for a familial BRCA1 variant in a woman with breast cancer who is at high risk of having familial breast and ovarian cancer is defined as Level 2.

Examples

- A woman with an abnormal antenatal biochemical screening result for fetal Down Syndrome has an amniocentesis for fetal chromosome studies. The test is being done as part of a medical screening program and is categorised as a Level 1 risk.

  A separate informed consent process would be required for the invasive procedure. The fetus has an unbalanced chromosome translocation, and parental specimens are forwarded for chromosome studies to determine if either carries a balanced

translocation. The testing of healthy subjects with results not being predictive of disease in the subject is categorised as a Level 1 risk.

The paternal karyotype reveals a balanced translocation. The couple seek prenatal testing in the next pregnancy. The test is being done in a specific clinical context, and, other than a high prior risk of an unbalanced karyotype, there is no evidence that the fetus is affected. This is a Level 2 risk as categorised in Table 1.

- A boy with an intellectual disability has a genetic test for fragile X syndrome, which is a common cause of X-linked neurodevelopmental disorder. The diagnostic testing is in an affected subject and is categorised as a Level 1 risk. With the confirmation of this diagnosis, his 18-year-old sister wishes to have her genetic carrier status defined. If she has no evidence of intellectual disability, then the subject is unaffected and the test result is predictive of disease, including premature menopause in fragile X carriers, for which there is limited efficacy of treatment. Treatment focuses on correcting symptoms, not fertility. This is categorised as a Level 2 risk. If the sister has a mild intellectual disability, the test could be classified as Level 1 risk as the subject is affected.
- A child at 25% risk of inheriting the Huntington disease variant based on an affected grandparent presents with depressive symptoms and a facial tic at 14 years of age. The medical practitioner requests a genetic test for Huntington disease. The atypical clinical presentation of childhood Huntington disease is a Level 2 risk as categorised in Box 1. If the child had presented with typical features of juvenile-onset Huntington disease, such as developmental regression and increasing stiffness/rigidity, the test could be regarded as a diagnostic investigation and categorised as a Level 1 risk. However, the complexity of issues arising from an abnormal test result, including the possibility of revealing the genetic status of a parent, means this is categorised as a Level 2 risk.
- A couple are seeking to determine their risk of having an affected child for an autosomal recessive disorder. A large panel of recessive genes is offered, and an increased risk is reported on a couple basis. This is categorised as a Level 2 risk as it involves a large set of genes with potentially complex interpretation.
- An individual with retinal disease is seeking a genetic diagnosis for possible gene therapy. A large panel >200 genes associated with retinopathies is offered which is a Level 2 risk as a single organ system is primarily involved this is not classified as a Level 3 risk.
- A patient with renal failure is diagnosed with Alport syndrome and genetic testing, using massively parallel sequencing is ordered. This is a Level 1 risk as only three genes are involved in Alport syndrome. Following testing of the patient's asymptomatic sibling, they ask for genetic testing. This is a Level 2 risk as the sibling is not clinically affected.
- A child with hearing loss is referred by a paediatrician for genetic testing using a large panel of genes associated with isolated hearing loss, a Level 2 risk. Upon receiving a negative report, an expanded analysis is requested containing genes associated with syndromic hearing loss, that is involving other organ systems. This becomes a Level 3 risk.
- A clinical geneticist orders whole genome sequencing on a child with an intellectual disability. This is a Level 3 as testing generally involves genes associated with more than one organ system. The possibility of unsolicited findings is high, including de novo variants in the child and variants which may be inherited from a parent.
- A SNP array is ordered on an apparently normal fetus due to concerns over advanced maternal age. This is a Level 2 risk as it involves testing an apparently unaffected fetus for copy number variants, across large sets of genes.

- A couple seeks pre-conception testing for cystic fibrosis, Fragile X and spinal muscular atrophy genetic carrier status. The test is not predictive of disease in the subjects. This is categorised as a Level 1 risk. Both potential parents are found to be carriers. The parents conceive and prenatal testing is arranged at 12 weeks gestation. There is no evidence that the fetus is affected. This is a Level 2 risk as categorised in Table 1.

Appendix B — Report format

This section contains guidance on the structure of reports.

The presentation of data can enhance understanding and accuracy in interpretation. The key formatting features relevant to the presentation of reports in either hard or soft copy are as follows.

Provide consistent and informative headings

Headings should be meaningful for the reader, recognising that the reader may not be an expert in the subject matter.

Limit the information under each heading

The key information necessary for decision-making should be displayed on a single page or screen. This will require prioritisation, with key fields being included on the first page and supplementary information provided on subsequent pages.

Provide visual clues to the structure of the report

Reports should be structured so that there are fixed positions for fields, particularly for universal items such as patient identifiers and conclusions. For some fields, there may be a limited range of values that could be used, thereby making it possible to limit variation in report layout and facilitating comprehension by a medical practitioner. Structured reports can also facilitate the auditing of testing by one or more laboratories.

Set the context

The context of a medical investigation, that is indication and timing in relation to other events, determines the interpretation, hence the distinction between diagnostic and non-diagnostic (e.g., epidemiology) testing.

Meet the needs of multiple readers

The report should not include jargon in key fields which may be utilised by a non-expert for decision-making. Universally terms and abbreviations recognised may be acceptable, such as DNA and RNA however a key should be provided. Other abbreviations can be misleading and should not be used.

Jargon including non-technical phrases such, as “characteristic of”, “indicative of”, and “highly suggestive of” can imply different degrees of diagnostic certainty to different requestors. The report should provide readers with an explicit interpretation rather than requiring them to interpret the information. This might include an explicit statement regarding the uncertainty of the clinical significance of a particular variant.

Use simple formatting

Elaborate formatting can make the reader’s task more difficult. The key principles are:

- Keep the variety of fonts (including text colour) to a minimum.
- The ideal character size (using the letter “x” as the standard) is 2-6 mm. Maintain a clear space between lines.
- Use size and weight (boldness) to indicate emphasis rather than using a different font.
- Italicised and capitalised text is read more slowly.
- Underlining may obscure punctuation and parts of some letters (such as the tail of “g” or “y”).
- In an itemised report, text that is left-aligned is easier to read than centred or justified text.
- A line length of approximately 10 words is easier to read than shorter or longer lines.
- Separate long numbers (including record numbers) into readable and meaningful groups using spaces, hyphens, or commas. For example, “Record #234-567-8” is preferable to “Record #2345678”.
- Keep the report length to a single page whenever possible.

Note: There may be other reporting structures that are available for use for specific disciplines.

Appendix C — Assessment of banding quality of cytogenetic slide preparations

Table 2 International System for Human Cytogenetic Nomenclature Bands (ISCN)

ISCN bands per haploid set (bphs)	Examples
Solid stained	Unequivocal chromosome pairing is not possible
150	Can distinguish 8s from 9s Can distinguish 4s from 5s
400	Two distinct dark bands in 8p Two distinct dark bands in 9p Three distinct dark bands in 5q (5q14, 5q21, 5q23)
550	Four distinct dark bands in 18q 10q21, 10q23, 10q25 split 7q33 and 7q35 are clearly distinct 22q13.2 is visible
700	2p25.2 distinct 2q37.2 distinct 10q21.1 and 10q21.3 resolve 17q22-q24 resolves into 3 dark bands
850	4p15.3 splits 5p15.32 is clearly visible 10q11.22 is clearly visible 11p14.1 should resolve from 11p14.3 20p12.1 and 20p12.3 are clearly visible

Appendix D — Recommended minimum banding quality

Table 3 The recommended banding resolutions given below are defined as the lowest standard acceptable for a given reason for referral without issuing a qualified report

Reason for referral	Banding Resolution (bphs)
Routine prenatal diagnosis (e.g., for age or biochemical pre-screens)	400
Aneuploidies and known large structural rearrangements	400
Expected small structural rearrangements, including their prenatal diagnosis	400–550
Possible small unknown structural anomalies (e.g., recurrent abortion, dysmorphic features, delayed development)	550
Microdeletion syndromes (FISH or microarray is the preferred method of analysis where available)	700–850

In practice, a range in the banding resolution will be apparent for the cells examined and the minimum band resolution applied to a laboratory report should be based on more than one cell, the confidence in the analysis and clinical context.

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