

**NATIONAL PATHOLOGY ACCREDITATION ADVISORY COUNCIL**

**REQUIREMENTS FOR MEDICAL  
TESTING OF HUMAN NUCLEIC ACIDS  
(Second Edition 2013)**

**NPAAC Tier 4 Document**

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Australian Government Department of Health

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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 laboratories and the introduction and maintenance of uniform standards of practice in pathology laboratories throughout Australia. A function of NPAAC is to formulate Standards and initiate and promote education programs about pathology tests.

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

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

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## Scope

The *Requirements for Medical Testing of Human Nucleic Acids* is a Tier 4 document which 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.

The *Requirements for Medical Testing of Human Nucleic Acids* document sets out the Standards that apply to medical tests for heritable and non-heritable variants in human nucleic acids. These tests involve the detection, characterisation and quantification of these nucleic acids.

The Standards in this document are the minimum requirements for the accreditation of Laboratories performing human nucleic acid testing where the testing is in response to a request by or on behalf of a medical practitioner. This document cannot be used for conferring or inferring accreditation status in relation to direct-to-consumer (DTC) human nucleic acid testing.

Tests for paternity, kinship, and identity and forensic analysis of Specimen for use by law enforcement authorities are not considered in this document.

The Standards for testing for microorganisms that can or may cause disease in humans are described in a separate NPAAC document *Requirements for Medical Testing of Microbial Nucleic Acids*.

## Abbreviations

AS	Australian Standard
ISO	International Organization for Standardization
IVD	<i>in vitro</i> diagnostic device
MLPA	multiplex ligation-dependent probe amplification
NHMRC	National Health and Medical Research Council
NPAAC	National Pathology Accreditation Advisory Council
OGTR	Office of the Gene Technology Regulator
RCPA	Royal College of Pathologists of Australasia

## Definitions

<p>Requirements for Medical Pathology Services (RMPS)</p>	<p>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.</p> <p>The standard headings are set out below –</p> <p>Standard 1 – Ethical Practice</p> <p>Standard 2 – Governance</p> <p>Standard 3 – Quality Management</p> <p>Standard 4 – Personnel</p> <p>Standard 5 – Facilities and Equipment</p> <p style="padding-left: 40px;">A – Premises</p> <p style="padding-left: 40px;">B – Equipment</p> <p>Standard 6 – Request-Test-Report Cycle</p> <p style="padding-left: 40px;">A – Pre-Analytical</p> <p style="padding-left: 40px;">B – Analytical</p> <p style="padding-left: 40px;">C – Post-Analytical</p> <p>Standard 7 – Quality Assurance</p>
<p>Separate area</p>	<p>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.</p>
<p>Validation* ISO9000:2005 3.8.4</p>	<p>means the confirmation by examination and the possession of objective evidence that the particular requirements for a specific intended use are fulfilled.</p>
<p>Variant</p>	<p>means any alteration in a gene or genes from a normal reference sequence. It may cause disease or be benign. In the case of "copy number" variants, such an alteration can often involve several genes, contiguously located.</p>

<p>Verification<sup>*</sup> ISO9000:2005 3.8.5</p>	<p>means the application of the validation process only to a nonconforming aspect of an otherwise validated IVD. Verification can comprise activities such as:</p> <ul style="list-style-type: none"> <li>• performing alternative calculations</li> <li>• comparing a new design specification with a similar design specification</li> <li>• undertaking tests and demonstrations</li> <li>• reviewing documentation prior to issue.</li> </ul>
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<sup>\*</sup> Definition from ISO/FDIS-WD 14971:2005, *Medical Devices – Application of Risk Management to Medical Devices*

# Introduction

National Pathology Accreditation Advisory Council (NPAAC) accreditation material is designed for the accreditation of pathology Laboratories under the *Health Insurance Act 1973*. Paternity testing is in the province of the *Family Law Act 1975*. Identity testing and forensic analysis are within the jurisdiction of the various crimes acts, while kinship testing falls within immigration and inheritance matters. However, many of the principles described here are also applicable to these additional arenas.

Testing for microorganisms is in the NPAAC *Requirements for Medical Testing of Microbial Nucleic Acids*.

Human nucleic acid testing is not necessarily provided by a specialist genetics Laboratory. Any medical Laboratory which provides testing which falls within the scope of this document should make reference to this document, together with the Tier 2 document *Requirements for Medical Pathology Services*.

These Requirements are intended to serve as minimum standards in the accreditation process and have been developed with reference to current and proposed Australian regulations and other standards from the International Organization for Standardization 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 Document

- *Requirements for Cytogenetic Testing*

In addition to these Standards, Laboratories must comply with all 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. As a general rule, 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.

Please note that all NPAAC documents can be accessed at [www.health.gov.au/internet/main/publishing.nsf/Content/health-npaac-publication.htm](http://www.health.gov.au/internet/main/publishing.nsf/Content/health-npaac-publication.htm)

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

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## Pre-Analytical

### 1. Ethical responsibilities of Laboratories providing nucleic acid testing for human genetic conditions

(Refer to Standard 1, Standard 2 and Standard 3 in *Requirements for Medical Pathology Services*)

Further information relating to the ethics of Laboratory nucleic acid testing is available in the NHMRC publication *Medical Genetic Testing: Information for health professionals* (NHMRC 2010)<sup>1</sup> and in the joint Australian Law Reform Commission – NHMRC publication *Essentially Yours – The Protection of Human Genetic Information in Australia* (ALRC–NHMRC 2003)<sup>2</sup>.

**S1.1 The Laboratory must provide medical nucleic acid testing only in the context of a clinical service provided by a medical practitioner.**

**S1.2 The Laboratory must be able to provide guidance regarding the categorisation of the tests that they perform as Level 1 or Level 2 tests (see Appendix A) according to the ethical implications.<sup>†</sup>**

C1.2(i) The distinction between Level 1 (standard DNA test) and Level 2 (DNA test with potential complex issues) would preferably be made by the medical practitioner 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.

C1.2(ii) Laboratories are not required to sight copies of the consent for Level 2 testing but an indication that consent has been obtained should be documented.

C1.2(iii) If there is concern in the Laboratory that a Level has not been correctly assigned, the clinical scientist/pathologist in charge of the Laboratory should arrange for the test to be deferred and the requesting medical practitioner to be contacted so that the uncertainty about the Level of the test request is resolved.

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<sup>†</sup> See Normative Appendix A - *Classification of human genetic testing*

## 2. Specimen collection

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

### S2.1 For Specimens collected by the patient, clear and appropriate written instructions must be available.

- C2.1(i) Specimens collected by the patient are not ideal for nucleic acid tests due to the greater risk of incorrect identification of the patient, labelling and contamination of Specimen.
- C2.1(ii) Wherever possible, nucleic acid detection tests should be performed on dedicated Specimens or on aliquots taken before other tests are performed.<sup>‡</sup> Where it is necessary to perform nucleic acid detection 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 and the results confirmed on a dedicated Specimen (if one is available).
- C2.1(iii) To minimise the risk of contamination in nucleic acid amplification techniques, some special Specimen collection and preparation is needed, in addition to the usual requirements for pathology testing. The precise method of Specimen collection, initial processing and transportation depends on the Specimen concerned and the nucleic acid target (DNA or RNA).
- C2.1(iv) The potential for false positive or false negative results to occur in nucleic acid testing, particularly for serious conditions, should be considered as part of the evaluation and setting up of diagnostic assays.
- C2.1(v) Patients may require that Specimens be collected using a de-identification protocol, through a trusted third party (TTP) intermediary such as a gene trustee<sup>2,3</sup>. In such cases, patient and Specimen identification should use the coded identifiers provided by the TTP, and appropriate registration of the patient and Specimen identification numbers must be made with the TTP. To comply with the patient's consent and with the TTP protocols, Laboratories should not separately record any linkage between the physical identity of the patient and the identification codes provided by the TTP for the patient and any Specimens.

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<sup>‡</sup> Where Specimens are referred to another Laboratory for testing, see also Appendix B

## Specimen collection for Level 2 tests

For **Level 2 tests**<sup>§</sup>, **additional** procedures are strongly recommended to minimise the possibility of errors. The issue of Specimen collection for Level 2 tests is discussed in the RCPA document *Sample requirements for medical genetic testing: Do genetic tests demand a different standard?*<sup>4</sup>. Procedures that could be considered include the following options:

- (a) the testing of two Specimens collected at different times, with both Specimens tested independently
- (b) splitting the Specimen on receipt in the Laboratory and processing in different batches
- (c) the Specimen tube is signed by the patient (or appropriate delegate) to confirm the Specimen identity.

Most errors in medical testing are not analytical but reflect events that occur outside the Laboratory, including errors in Specimen collection. Studies of pre-transfusion testing have revealed that 0.5-0.8 Specimens per thousand are blood from the wrong patient.<sup>5,6</sup> There is a similar rate of non-analytical errors in genetic testing.<sup>7</sup> 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 because a genetic test may identify 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. A single unsigned Specimen (the usual practice in medical testing) is appropriate for tests that carry few implications for genetic relatives e.g. tests for somatic variants, pharmacogenetic tests, population-based carrier testing.

Duplicate sampling and testing is warranted for tests which carry major implications for genetic relatives and for which there is little or no evidence to corroborate the result e.g. unexpected or abnormal diagnostic tests for heritable disorders, and pre-symptomatic or carrier testing of genetic relatives.

A single Specimen signed by the collector or a parent is appropriate for fetal Specimens collected for prenatal testing.

The clinical significance of a sampling error varies with the clinical context, and the pathology Laboratory will not necessarily be aware of this context. Hence the decision to utilise different sampling protocols to reduce the risk of Specimen errors rests with the medical practitioner requesting the test. Laboratories should be able to advise medical practitioners of the appropriate sampling strategy in different settings, and should make reference to such recommendations in reporting results. The risk of a Specimen being incorrectly identified is increased if Specimens are collected from genetic relatives simultaneously, or in operative settings such as during prenatal diagnosis.

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<sup>§</sup> Refer to Appendix A

### 3. Laboratory facilities and the risk of contamination

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

Laboratories undertaking nucleic acid amplification need to be configured to minimise the risk of contamination of Specimens and reagents by other Specimens in the Laboratory or by amplified material.

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

- (a) during Specimen collection or transport
- (b) during handling or testing in the testing or referring Laboratory before nucleic acid detection
- (c) during extraction of nucleic acids from the Specimen
- (d) during amplification
- (e) during product detection
- (f) by contamination from reagents used for the test.

The sources of potential contamination include:

- (a) positive Specimens (cross-contamination)
- (b) amplified nucleic acid (e.g. contamination of stock reagents or equipment, or in aerosol droplets)
- (c) operator-derived nucleic acid.

Mathematical methods can be used to assess the potential significance of contamination in the use of PCR-based methods<sup>8</sup>.

The wording of the following sections is intended to allow flexibility of Laboratory layout without compromising the guiding principle that Laboratories undertaking nucleic acid amplification should be configured to minimise the risk of contamination.

#### **S3.1 Laboratories using genetically modified organisms must comply with the relevant standards set by the Office of the Gene Technology Regulator<sup>\*\*</sup>.**

- C3.1 The use of genetically modified organisms poses a potential risk of contamination of studies of human genetic material. Appropriate precautions and monitoring **must** be implemented.

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<sup>\*\*</sup> <http://www.ogtr.gov.au>

## Minimum standards for a nucleic acid amplification facility

- S3.2 The layout of the Laboratory areas must be designed to minimise the potential for contamination.**
- S3.3 The clinical scientist/pathologist in charge of the Laboratory must ensure that the degree of separation is adequate for the specific stage. Care must also be taken to ensure that co-location of research and diagnostic activities do not compromise this Standard.**
- C3.3 Instruments capable of producing aerosols (e.g. vortex mixers, PCR machines and microcentrifuges) and robotic equipment **must** be considered when assigning the separate areas.
- S3.4 In order to reduce the risk of contamination, there must be a separate area for each of the following activities:**
- (a) preparation of reagents (including dispensing of master mixes)**
  - (b) nucleic acid extraction, preparation and handling before amplification**
  - (c) amplification and product detection**
  - (d) manipulation of Specimens prior to a second round of amplification.**
- C3.4(i) Where the areas for preparation of reagents and Specimen preparation are located within a single room, wide separation of these activities must be maintained and procedures and controls **must** be implemented to detect contamination.
- C3.4(ii) Specimens (pre- and post-amplification), reagents and equipment **must** be held in their respective areas and labelled accordingly. In particular, patient Specimens must not be taken into the reagent preparation area.
- C3.4(iii) Post-PCR analysis **must not** be incorporated into areas where reagent preparation or Specimen preparation occurs. The post-PCR area must be contained and positioned so as to minimise the possibility of contamination from pre-amplification areas.
- C3.4(iv) The movement of Specimens and used equipment **must** be unidirectional; that is, from pre-amplification to post-amplification areas. PCR amplification tubes **must** be sealed when carried between the pre-amplification area and the post-amplification area.
- C3.4(v) Where equipment (such as tube racks) is returned against the flow, it **must** first be decontaminated before being moved from the post-amplification area back into a pre-amplification area.

C3.4(vi) Where a single instrument, such as a liquid handler, is used, the Laboratory **must** demonstrate functional separation of the steps of the assay by the use of suitable protocols and controls.

C3.4(vii) Aerosol-resistant pipette tips or positive displacement pipettes are strongly recommended to minimise contamination, and should be used routinely.

**S3.5 The clinical scientist/pathologist in charge of the Laboratory must ensure that procedures and controls are implemented to prevent and detect contamination. This must include the use of no-template controls in amplification assays.**

## **Laboratory hygiene**

**S3.6 Work surfaces and equipment must be decontaminated at a frequency that is appropriate for the case load of the Laboratory.**

**S3.7 Equipment from other areas must not be taken into the reagent preparation area without prior decontamination.**

**S3.8 Laboratory gowns and gloves must be worn and must be changed frequently enough to avoid contamination and in accordance with the Laboratory's protocols.**

C3.8(i) The movement of gowns and gloves **must** be unidirectional from pre- to post-amplification areas. Gowns and gloves that have been used in the post-amplification area **must** not be used in other areas.

C3.8(ii) Gowns and gloves **must** be changed whenever there is evidence of soiling.

**S3.9 Spills involving Specimens in pre- or post-amplification phases must be cleaned up and decontaminated promptly.**

**S3.10 Laboratories must retain records documenting contamination events, comment on the source of the contamination and measures taken to reduce the risk of future similar contamination events.**

## **4. Specimen preparation and storage**

**(Refer to Standard 6A and Standard 7 in *Requirements for Medical Pathology Services*)**

**S4.1 The procedures used for nucleic acid isolation from the full range of Specimen types used by the Laboratory must be validated and subject to quality control.**

C4.1 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.

**S4.2 Nucleic acids must be stored and labelled in a way that minimises degradation, contamination, misidentification and loss of identification of the Specimen.**

# Analytical

(Refer to Standard 2, Standard 3, Standard 4, Standard 6 and Standard 7 in *Requirements for Medical Pathology Services*)

## 5. Testing methodologies

### Screening for unknown pathogenic variants

This section addresses methods that screen for the presence of variants in a specified locus without precisely defining the nature of the variant. Screening methods are typically cheaper and quicker than direct sequencing of the entire region of interest and have an accuracy for detecting variants that is less than that provided by sequencing.

- S5.1 The sensitivity of the screening assay for detecting pathogenic variants must be assessed and be cited in reports.**
- S5.2 The genotype of a variant detected by a screening assay must be confirmed by a second method such as sequencing or other genotyping assay.**
- S5.3 If the screening method has limited sensitivity for variants which are homozygous or hemizygous, the Laboratory must assess the likelihood of clinically relevant variants being missed and, if necessary, implement methods to counter this.**

### Sequencing to detect unknown pathogenic variants

DNA sequencing provides nucleotide-by-nucleotide analysis of a region and is typically used to identify variants that have not been defined prior to the analysis. A sequencing assay may encompass 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. These issues will become more pressing with improvements in sequencing methodologies.

- S5.4 The region of interest that is to be sequenced must be clearly defined by the Laboratory.**
  - C5.4 The quality of sequencing is typically low at the extreme ends of the fragment being sequenced. To obtain high quality sequencing of a particular region of interest e.g. an exon, it may be necessary to sequence 30 or more nucleotides on each side of this region to ensure that the sequence quality in the region of interest is sufficient.
- S5.5 The Laboratory must provide a quantitative assessment of sequence quality, and establish quality score limits for acceptable sequence data.**
  - C5.5 Limitations in the quality of a sequence trace may be resolved by using bi-directional sequencing. Bi-directional sequencing also allows prompt identification of some sequencing artefacts.

**S5.6 The Laboratory must interpret the patient’s DNA sequence with reference to a standard DNA sequence.**

C5.6 The interpretation of a patient’s sequence **must** involve a systematic comparison of the patient and reference sequences, preferably using computerised analysis as unassisted visual inspection of sequence data is potentially unreliable (particularly for homozygous mutations).<sup>††</sup>

## **Assays to detect known variants**

This section addresses assays for variants that have been defined prior to the assay being performed e.g. assays for common pathogenic variants and for family-specific variants. Such assays may be performed as a single or multiplexed assay.

**S5.7 All assays designed to detect known pathogenic variants must be verified using positive and negative controls for each of the genotypes being assessed.**

C5.7 Where possible, positive and negative controls for each known pathogenic variant should be included in each batch of the assay.

**S5.8 Multiplexed assays must be validated for cross reactivity**

C5.8 Each batch of the assay **must** include control Specimens suitable to detect known or possible cross-reactivity.

**S5.9 If all the necessary controls cannot be run within a single batch of the assay, then they must be tested in a regular manner appropriate for the assay.**

C5.9 Common pathogenic variants should be tested in each batch of the assay.

**S5.10 The sequence of PCR primer sites must be assessed for polymorphisms on a regular basis as part of the ongoing monitoring of the assay.**

**S5.11 For assays of triplet repeat variants, the Laboratory must have defined a reference interval and measurement uncertainty, and use controls appropriate for each reference range in each batch.**

## **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 qualitative i.e. a variant is present or absent.

Assays for germline variants 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, i.e. quantitative information is included in the test report. Such assays are

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<sup>††</sup> Clinical Molecular Genetics Society (2009). *Practice guidelines for Sanger Sequencing Analysis and Interpretation.*, CMGS. <http://www.cmgs.org/BPGs/pdfs%20current%20bpgs/Sequencingv2.pdf>

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 with each other. This includes assays for determining gene dosage e.g. MLPA; proportion of mutated cells in a Specimen e.g. quantitative PCR; and proportion of mutant genes in cells e.g. assay for mitochondrial heteroplasmy.

**S5.12 The quality of the nucleic acids to be used as the substrate for a quantitative assay must be assessed and, if appropriate, reported.**

**S5.13 The Laboratory must have quality control processes to corroborate an abnormal result before it can be reported as being clinically significant.**

C5.13(i) If it is not practicable to corroborate the result, it **must** be reported as unconfirmed.

C5.13(ii) The result may be corroborated by:

- a) replication of the result using the same assay and a new dilution of the Specimen;
- b) replication of the result using a different assay;
- c) the Laboratory demonstrating proficiency in detecting the specific mutation in an external quality assessment program;
- d) the results of other clinical or Laboratory findings of the patient.

## Microarrays

Microarrays interrogate potentially millions of loci. Arrays can be designed and analysed for a variety of different purposes, including identifying copy number variants, genotyping and gene expression. The raw data is necessarily processed using software to enable analysis and reporting. The degree of multiplexing is orders of magnitude greater than for other assay formats, making test validation, replication between Laboratories, and quality control particularly challenging. For these reasons, particular care should be taken regarding the quality, reproducibility and interpretation of the assay.

**S5.15 The quality of the Specimen being tested, intensity of labelling, and quality of hybridisation, scanning, and analysis must be monitored.**

**S5.16 The Laboratory must determine the minimum number of consecutive probes that define a specific type of abnormality that might be detected by the array.**

C5.16 The average resolution (or comparable metric) of the microarray study should be included in the report.

**S5.17 For a variant to be reported as being clinically significant, both analytical and interpretive aspects of the result must be addressed.**

- C5.17(i) First, the analytical result **must** be corroborated e.g. by replication in an independent assay of the patient Specimen; analysis of a Specimen from a genetic relative; or consistency with clinical phenotype.
- C5.17(ii) Second, the clinical interpretation of the variant **must** be substantiated by reference to appropriate literature, resources or family studies.
- C5.17(iii) If it is not practicable to confirm the analytical and interpretive aspects of the result, the result **must** be reported as unconfirmed.
- C5.17(iv) Where appropriate, Laboratories should submit data about copy number variants and their associated phenotypes to curated publicly accessible repositories.

## **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, artefacts derived from a single cell can be relevant in analyses which use very small amounts of template DNA or RNA e.g. in pre-implantation genetic diagnosis, or Specimens with limited DNA (e.g. urine or plasma) or assays for low levels of tissue mosaicism (e.g. minimal residual disease & mitochondrial testing).

**S5.18 The choice of method and validation of the assay using a limited amount of template must reflect the range of template concentrations that could be experienced. Threshold limits must be specified for both too little and too much template. Validation of the assay must address the potential for contamination and for preferential amplification of one allele.**

**S5.19 Procedures for determining the adequacy of a Specimen in terms of amount of template and quality must be validated.**

**S5.20 When using serial amplification of DNA e.g. nested PCR, the reaction product from one amplification must be manipulated in an area separate from the areas used for single-stage PCR.**

## **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 or seeking to identify an uncharacterised mutation. The interpretation of a pre-defined mutation is usually straightforward, but the interpretation of an uncharacterised mutation may be difficult because of limited phenotypic information about the fetus and in data repositories.

**S5.21 Where the prenatal testing involves the genotyping of a known familial variant, the Laboratory must have genotyped the proband or other relevant family member(s). If the Laboratory is not able to do this, it must be stated on the report.**

**S5.22 Appropriate liaison with external Laboratories that refer prenatal Specimens must be maintained.**

C5.22 Cytogenetics Laboratories that undertake chromosome analysis on the same prenatal Specimen should be requested to maintain a reserve culture until the results of the nucleic acid prenatal analysis is known.

**S5.23 Maternal cell contamination must be assessed in all prenatal tests.**

C5.23(i) The report **must** note the presence or absence of significant maternal cell contamination in the analysis.

C5.23(ii) Chorionic villus Specimens should be cleaned of contaminating maternal tissue or blood prior to nucleic acid extraction. This should be performed by experienced Laboratory personnel (usually by the receiving cytogenetics Laboratory).

## **Interpretation**

There may be a simple relationship between a genotype and its clinical relevance. Common variants may carry clearly-defined consequences for the patient. However, there are many genetic variants for which the relationship between genotype and clinical relevance is not straightforward. It may be necessary for the Laboratory professional to provide a qualitative interpretation of the biological significance of the variant before the requesting medical practitioner is able to determine the clinical relevance of the variant. For example, a missense variant may require careful consideration of the consequences for protein function before the clinical implications can be addressed.

For some assays, it may be necessary to perform complex calculations to determine the significance of the analytical result. The outcome of such an analysis may be qualitative e.g. “the patient is a carrier”, or quantitative e.g. “there is a 94% chance that the patient is a carrier”.

This section addresses some of the issues to be considered by the Laboratory professional in providing an interpretation of the biological significance of a variant.<sup>‡‡</sup>

**S5.24 Laboratories must be able to incorporate statistical methods, including linkage and Bayesian analyses (as appropriate), when determining the clinical significance of an analytical result.**

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<sup>‡‡</sup>Clinical Molecular Genetics Society (2007) Practice guidelines for the Interpretation and Reporting of Unclassified Variants (UVs) in Clinical Molecular Genetics.  
<http://www.cmgs.org/BPGs/pdfs%20current%20bpgs/UV%20GUIDELINES%20ratified.pdf>

# Post-Analytical

## 6. Reporting Standards

(Refer to Standard 6C in *Requirements for Medical Pathology Service and to Appendix C*)

A nucleic acid 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 nucleic acid variants. On the other hand, the report must not obscure the key information for the non-expert reader who needs to make medical decisions today.

### S6.1 The report must explicitly state the clinical question being addressed by the investigation.

- C6.1(i) Relevant clinical details provided by the referring medical practitioner **must** be stated.
- C6.1(ii) The clinical interpretation **must** be clearly identified in the report, and worded so as to address the question.

The use of unambiguous quantifiers e.g. “90% of people with this variant are affected” is preferable to “this variant is usually associated with disease”.

Utilisation of positive statements e.g. “the triplet repeat number is within the normal range” is preferable to “the triplet repeat number is not expanded”.

The terms “positive” and “negative” can be confusing and are best avoided e.g. “MLH1 expression is absent” is preferable to “the MLH1 assay is positive”.

- C6.1(iii) The interpretation **must**, if appropriate, identify implications for genetic relatives and include recommendations regarding genetic counselling.
- C6.1(iv) If a nucleic acid variant has been identified, the interpretation **must** reflect the distinction between the patient having an abnormal genotype and the patient being affected.
- C6.1(v) 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 nucleic acid testing<sup>§§</sup>.

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<sup>§§</sup> Refer to Appendix A

- C6.1(vi) Where the clinical details are illegible, absent, or ambiguous, the Laboratory should attempt to clarify the details with the referring medical practitioner.
- C6.1(vii) In the case of simple reports e.g. assay for a common variant, there may not be any need for further comment. If reporting an uncommon variant, there should be further interpretation of its biological significance.
- 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.
  - However, if this report is being provided to a different medical practitioner, the analysis should be provided again.
- C6.1(viii) The Laboratory report should remind the requesting medical practitioner of relevant sampling recommendations. The wording could be along these lines:

*“Genetic tests results may have significant medical implications for both the patient and genetic relatives. Corroboration of this result by reference to other clinical or Laboratory information or by repeat testing may be warranted”<sup>4</sup>.*

**S6.2 If two independent Specimens were collected for the purpose of reducing the risk of an erroneous result, the report must indicate if such precautions were taken.**

**S6.3 The report must unambiguously identify the gene/s or genetic locus/loci being assayed.**

- C6.3(i) 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. Standard gene names **must** be used, with synonyms or alternative names shown in brackets until referrers become familiar with the correct terminology.
- C6.3(ii) Fusion genes should be shown as GENE1/GENE2, preferably in mechanistic order i.e. 5’ to 3’ in the fusion gene<sup>9</sup>.
- C6.3(iii) The standard gene nomenclature as described by the Human Genome Organisation Gene Nomenclature Committee<sup>\*\*\*</sup> should be used.

**S6.4 The reference sequence used by the Laboratory must be specified.**

- C6.4 The report should comply with international standards e.g. using genomic or gene sequence (cDNA) as recommended by the Human Genome Variation Society<sup>†††</sup>.

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\*\*\* [www.genenames.org/](http://www.genenames.org/)  
††† [www.hgvs.org](http://www.hgvs.org)

NCBI Reference Sequences, if available, are recommended<sup>†††</sup>. The Human Genome Organisation Gene Nomenclature Committee nominates a recommended NCBI Reference Sequence for most genes or loci with an approved name.

**S6.5 The report must state, in simple terms, the method and the scope of the analysis performed e.g. analysis of all exons by sequencing and dosage studies, or test for selected variants only.**

**S6.6 The system of variant nomenclature used in the report must be specified.**

C6.6(i) It is recognised that some common 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<sup>§§§</sup>, with synonyms or common names shown in brackets until referrers become familiar with the correct terminology.

C6.6(ii) The technical characteristics of the assay may be relevant for the interpretation of negative studies and for subsequent audits and reviews by either the Laboratory or a medical practitioner. When appropriate, the commercial kit, or specific primers and probes, should be specified.

C6.6(iii) The report should include a comment or explanation of the genotype. Information about family-specific or case-specific controls should be included.

**S6.7 In the case of prenatal testing, there must be a statement regarding the presence or absence of significant maternal cell contamination and, if relevant, parental genotypes.**

**S6.8 In the case of predictive testing, there must be a statement regarding the use of a positive control for a family-specific variant.**

**S6.9 If a number of family members are tested at the same time, a separate report must be issued for each person (irrespective of age).**

C6.9 The genetic and clinical interpretation of a patient's nucleic acid test may depend on the results of tests on genetic relatives. Nonetheless, the identity of relatives **must not** be revealed directly in the report. If necessary, the report can identify the clinical service which holds pedigree data or results of the entire family.

**S6.10 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.**

C6.10 This confidentiality requirement may be waived 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.

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<sup>†††</sup> [www.ncbi.nlm.nih.gov/RefSeq](http://www.ncbi.nlm.nih.gov/RefSeq).

# Appendix A Classification of human genetic testing (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 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.

## 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*.\*\*\*\* Clinical scientists/pathologists 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 practitioner 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.

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§§§ The Human Genome Variation Society has established an international standardised nomenclature,<sup>10</sup> as has the International Society for Cytogenetic Nomenclature for microarray results<sup>11</sup>.

\*\*\*\* <http://www.nhmrc.gov.au/publications/synopses/e99syn.html>

Irrespective of the classification of a test, the requesting medical practitioner 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]).

### Examples

- A woman with an abnormal antenatal biochemical screening result for fetal Down syndrome has an amniocentesis for fetal chromosome studies (test being done as part of a medical screening program [*Level 1*]; separate consent 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 [*Level 1*]). The paternal karyotype reveals a balanced translocation. The couple seek prenatal testing in the next pregnancy (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 [*Level 2* — consistent with 2.3]).

- A couple seeks preconceptional testing for cystic fibrosis genetic carrier status (test is not predictive of disease in subject [*Level 1*]). Both are found to be carriers and prenatal testing is arranged at 12 weeks gestation (there is no evidence that the fetus is affected [*Level 2 — consistent with 2.3*]).
- A child at 25% risk of inheriting the Huntington disease mutation presents with depressive symptoms and a facial tic at 14 years of age. The medical practitioner requests a genetic test for Huntington disease (atypical clinical presentation of childhood Huntington disease [*Level 2 — consistent with 2.2*]). If the child had presented with typical features of juvenile-onset Huntington disease (developmental regression and increasing stiffness/rigidity), then the test could be regarded as a diagnostic investigation [*Level 1*] but the complexity of issues arising from an abnormal test result, including the possibility of revealing the genetic status of a parent, suggest that *Level 2 (consistent with 2.2)* is more appropriate.
- A boy with intellectual disability has a genetic test for the fragile X syndrome, a common cause of X-linked mental retardation (diagnostic testing in an affected subject [*Level 1*]). Once the diagnosis was confirmed, 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 (premature menopause in fragile X carriers) for which treatment is of limited efficacy (correcting symptoms, not fertility) i.e. *Level 2 (consistent with 2.4)*. On the other hand, if she has mild intellectual disability, then the test could be classified as *Level 1* (subject is affected) but the test also carries the same carrier implications as for an unaffected woman (premature menopause) and should be regarded as *Level 2 (consistent with 2.4)*.

## Appendix B Send-away tests (Normative)

If a Specimen is sent to another Laboratory for analysis, the scientist/pathologist in charge of the Laboratory **must** be able to provide justification for the choice of testing Laboratory as specified in AS ISO 15189. In general, the types of testing Laboratory include (in decreasing order of preference):

1. Laboratories accredited to AS ISO 15189 and NPAAC Requirements.
2. Laboratories accredited to AS ISO 15189 or ISO/IEC 17025 by an accreditation body.
3. Internationally recognised research Laboratories.

The referring Laboratory should ensure that the Specimen collection requirements outlined in Section 2 have been met, and inform the receiving Laboratory if they have not been met.

The referring Laboratory **must** ensure that the integrity of the Specimen, including packaging and labelling, is sufficient to allow the testing Laboratory to meet the Standards outlined in this document. The Laboratory should consider sending unextracted Specimens to minimise contamination if storage of DNA is not requested or likely to be required, and if Specimen integrity is not compromised by transportation.

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.

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, and a copy of the original report from the testing Laboratory should be provided to the requesting medical practitioner.

If the accreditation status of the testing Laboratory differs from that of the referring Laboratory, this **must** be stated in the report.

## Appendix C Report Format (Informative)

This section contains commentary only on formatting of reports which users may find helpful.

Careful presentation of data can enhance understanding and accuracy in interpretation. The key formatting features relevant to the presentation of reports on paper or a computer screen can be summarised as follows.

### *Provide consistent and informative headings*

Headings should be meaningful for the reader, recognizing 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 viewable on a single page or screen. This may require some prioritisation, with key fields being included on the first page and supplementary information being provided on a subsequent page.

### *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 consistent comprehension by medical practitioners. Structured reports can also facilitate the auditing of testing by one or more Laboratories.

### *Set the context*

The context of a medical investigation i.e. 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 other readers*

The report should not include jargon in key fields which may be utilised by a non-expert for decision-making. Some abbreviations, which are recognised as universal, may be acceptable e.g. DNA and RNA, but other less universal abbreviations can be misleading. Jargon can also include non-technical phrases such as "characteristic of", "indicative of", and "highly suggestive of" which can imply different degrees of diagnostic certainty to different groups of requestors. The report should provide readers with an explicit interpretation rather than requiring them to make their own interpretations. 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 as follows:

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 e.g. “Record #234-567-8” is preferable to “Record #2345678”.

If possible, keep the report length to a single page.

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

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## Further information

Other NPAAC documents are available from:

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