AUSTRALIAN COMMISSION ON SAFETY AND QUALITY IN HEALTH CARE

NPXC

Key changes: Requirements for medical testing for human genetic variation

Contents

Table 1 contains a mapping of significant changes between the second edition of the Requirements for medical testing of human nucleic acids, the third edition of the Requirements for cytogenetic testing and the 2022 release of the Requirements for medical testing for human genetic variation (third edition). Noting, this does not include minor spelling and/or grammatical changes or changes to references.

Table 1: Key changes between the second edition of the Requirements for medical testing of human nucleic acids, the third edition of the Requirements for cytogenetic testing and the 2022 release of the Requirements for medical testing for human genetic variation (third edition)

Requirements for medical testing of human nucleic acids (Second Edition, 2013) and Requirements for cytogenetic testing (Third Edition, 2013)	**Requirements for medical testing for human genetic variation (Third Edition, 2022)**
Personnel (Requirements for cytogenetic testing, third edition 2013)	
Personnel The following ranges can be considered as a basis for calculating annual workloads: a) 250–350 lymphocyte cultures, or b) 250–350 prenatal cultures, or c) 250–350 solid tissues, or d) 150–250 haematological malignancy cultures, or e) 100–200 solid tumour cultures, or f) 400–500 metaphase/interphase fluorescence in situ hybridisation (FISH) tests, or g) 150–220 specialised FISH tests (e.g. multiple subtelomere).	Deleted.

Requirements for medical testing of human nucleic acids (Second Edition, 2013) and Requirements for cytogenetic testing (Third Edition, 2013)	Requirements for medical testing for human genetic variation (Third Edition, 2022)
Ethical responsibilities	
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.	1.02 The laboratory must provide its workforce with access to guidance on genetic tests and the ethical implications for each level of test conducted
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(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.	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. 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.
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.	1.03 The laboratory must ensure consent forms for testing comply with best practice and jurisdictional requirements 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. Consent should explicitly include: a. expected results, including variants of uncertain significance b. unsolicited findings, including unexpected familial relationships c. data sharing of potentially re-identifiable data for clinical care d. data sharing for ethically approved research e. ability to recontact with new or updated information f. opt out if results are not to be included in the My Health Record

Requirements for medical testing of human nucleic acids (Second Edition, 2013) and Requirements for cytogenetic testing (Third Edition, 2013)	Requirements for medical testing for human genetic variation (Third Edition, 2022)
This is a new addition.	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 Level 2 testing consent forms do not need to be sighted or retained.
This is a new addition.	1.05 For Level 3 tests, the laboratory must document and act in accordance with the patients' decisions regarding the items below: a. return of unsolicited findings, including unexpected familial 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 This is most easily done by retaining a copy of the consent form.
This is a new addition.	Testing policy 1.06 The laboratory must provide access to its policy on testing genetic material available to patients and clinicians, on request 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 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. 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. The policy should also address action to be taken by the laboratory if Level 2 unsolicited findings, such as constitutional variants are identified.

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This is a new addition.	Prenatal and predictive testing 1.08 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 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. 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. 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.
This is a new addition.	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) • Joint Australian Law Reform Commission – NHMRC Essentially Yours — The Protection of Human Genetic Information in Australia (ALRC–NHMRC 2003)

D23-10847

Requirements for medical testing of human nucleic acids (Second Edition, 2013) and Requirements for cytogenetic testing (Third Edition, 2013)	Requirements for medical testing for human genetic variation (Third Edition, 2022)
Specimens and referral types (Requirements for cytogenetic testing)	
Prenatal (general)	Relevant actions incorporated into 4. Specimen preparation and storage and 5. Testing methodologies.
Chorionic villus Fetal blood	Relevant actions incorporated into 4.08 implementation advice.
Bone marrow and tumour	Relevant actions incorporated into section 5. Testing methodologies.
Chromosome instability syndromes	Relevant actions incorporated into 4. Specimen preparation and storage.
Chromosome analysis (Requirements for cytogenetic testing)	
General Numbers of cells to be studied Prenatal studies Acquired disorders	Relevant actions incorporated into section 6. Interpretation under Cytogenetic analysis.
Banding methods	Relevant actions incorporated into Appendices C and D
Verification of chromosomal analysis	Relevant actions incorporated into section 6. Interpretation under Cytogenetic analysis.
Karyotyping	Relevant actions incorporated into 4. Specimen preparation and storage.
Use of molecular techniques	Relevant guidance incorporated into Examples
Fluorescence in situ hybridisation (Requirements for cytogenetic testing)	
Fluorescence in situ hybridisation (FISH) techniques FISH analysis	Relevant actions incorporated into 6. Interpretation under Cytogenetic analysis and 7. Reporting Standards.
Laboratory performance (Requirements for cytogenetic testing)	
Measures of performance	Deleted.

Requirements for medical testing of human nucleic acids (Second Edition, 2013) and Requirements for cytogenetic testing (Third Edition, 2013)	Requirements for medical testing for human genetic variation (Third Edition, 2022)
Reports (Requirements for cytogenetic testing)	
Reporting times	Relevant actions incorporated into section 7. Reporting standards
Records of images and worksheets Records of FISH analysis	Relevant actions incorporated into section 7. Reporting standards
Specimen collection	
S2.1 For Specimens collected by the patient, clear and appropriate written instructions must be available.	Patient collection information 2.03 The laboratory must ensure patients collecting samples have access to clear, contemporary, comprehensive, and relevant written instructions
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).	2.02 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
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.	Specimen collection policy 2.01 The laboratory must use and monitor compliance with its 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 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.

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This is a new addition.	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. For RNA specimens the collection and transport processes should include use of specialised specimen collection containers and stabilising reagents.
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. 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.	Deleted.
This is a new addition.	Sample provenance 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 This is particularly important in the collection for Level 2 and Level 3 risk tests.

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For Level 2 tests, 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?. 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. There is a similar rate of non-analytical errors in genetic testing. 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	Errors in specimen collection while infrequent, do occur. 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.

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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.	
This is a new addition.	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.

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This is a new addition.	Policies for somatic testing 2.05 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 Factors to consider include tissue area, cellularity, fixation protocols, decalcification of bony specimens and sample age.
This is a new addition.	Biomarker testing 2.06 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 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.
Laboratory facilities and the risk of contamination	
Mathematical methods can be used to assess the potential significance of contamination in the use of PCR-based methods. 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.	Deleted.
S3.1 Laboratories using genetically modified organisms must comply with the relevant standards set by the Office of the Gene Technology Regulator. 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.	Deleted.

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S3.2 The layout of the Laboratory areas must be designed to minimise the potential for contamination. 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.	Contamination risks 3.01 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
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.	Separation of workspace 3.02 The laboratory must ensure the physical separation between 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 robotic equipment

NPXXC

D23-10847

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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 preamplification 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.	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, including amplification c. amplification and product detection d. manipulation of specimens prior to a second round of amplification 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 The laboratory can achieve this by having processes to ensure: a. specimens (pre and post-amplification), reagents and equipment are held in separate areas b. specimens, reagents, and equipment are labelled correctly c. patient specimens are not taken into the reagent preparation area d. equipment from other areas is not taken into the reagent preparation area without prior decontamination e. post-PCR area are separate to the reagent or specimen preparation areas and positioned to minimise contamination of pre-amplification areas f. movement of specimens and containers is unidirectional from pre-amplification to post-amplification areas g. PCR amplification tubes are sealed when carried between the pre-and post-amplification areas h. containers returned against the flow must first be decontaminated i. the risk of contamination is assessed and minimised when a single instrument is used in both pre and post amplification processes Single-use aerosol-resistant pipette tips or single-use positive displacement pipettes are strongly recommended to minimise contamination and should be used routinely.

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Laboratory hygiene	
S3.6 Work surfaces and equipment must be decontaminated at a frequency that is appropriate for the case load of the Laboratory.	Preventing contamination 3.05 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
S3.7 Equipment from other areas must not be taken into the reagent preparation area without prior decontamination.	Deleted.
This is a new addition.	3.06 The laboratory must have: a. Processes for the use, training, testing, and fitting of personal protective equipment by the workforce which uses a risk-based approach b. Align their process with the current edition of the Australian Guidelines for the Prevention and Control of Infection in Healthcare, jurisdictional requirements, and relevant jurisdictional laws and policies, including work health and safety laws
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.	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
S3.9 Spills involving Specimens in pre- or post-amplification phases must be cleaned up and decontaminated promptly.	Deleted.
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.	3.08 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

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Specimen preparation and storage	
This is a new addition.	Documented extraction procedures 4.01 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
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.	4.02 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 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 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. For RNA, interfering substances that can interfere with downstream analysis include DNA and organic chemical compounds used during the extraction. Specimen types should be assessed for potential sources of interference. 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.
S4.2 Nucleic acids must be stored and labelled in a way that minimises degradation, contamination, misidentification and loss of identification of the Specimen.	4.04The laboratory must have processes to minimise degradation of stored nucleic acids and assess integrity of stored nucleic acids prior to testing Quality control checks should be fit for purpose. For example, spectrophotometry may not be sufficiently accurate or sensitive for intended uses. 4.05 The laboratory must have processes to:

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	a. Ensure storage procedures minimise the risk of degradation, contamination and misidentification of samples b. monitor compliance with its storage and handling processes This includes appropriate storage temperatures and labelling systems for both RNA and DNA and other samples, including plasma.
Testing methodologies	
S5.1 The sensitivity of the screening assay for detecting pathogenic variants must be assessed and be cited in reports.	5.06 The laboratory must implement processes to document the analytical and clinical sensitivity of assays used for detecting pathogenic variants
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.	5.07 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
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.	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 This applies to technologies such as high-resolution melting and denaturing high performance liquid chromatography where wild-type spike-ins may be used.
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.	Assay target region 5.02 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

NPXXC

D23-10847

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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.	5.03 The laboratory must implement processes to: a. produce a quantitative assessment of sequence quality b. establish acceptance limits for sequence quality score Limitations in the sequence quality may be resolved by sequencing of both DNA strands. This 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).	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 5.05 The laboratory must perform sequence comparison using computerized analysis Unassisted visual inspection of sequence data is potentially unreliable (particularly for homozygous mutations).
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.	5.09 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
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.	5.11 The laboratory must implement processes to validate multiplexed assays for analytical specificity
This is a new addition.	5.12 The laboratory must implement processes to mitigate the risk of an allelic drop out The mitigation of allelic drop out is particularly important when the laboratory has designed the assay and for family-specific testing. 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.

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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.	Deleted.
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.	Deleted.
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.	5.13 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 Unstable variants include triplet repeat variants.
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.	5.14 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
This is a new addition.	5.15 The laboratory must implement processes to determine the lower limit of detection for each class of variant that is intended to be measured
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.	5.16 The laboratory must implement processes to establish measurement uncertainty across the reportable range This should include measurement uncertainty at the limit of detection

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This is a new addition.	5.17 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 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.
This is a new addition.	5.18 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
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.	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
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.	Deleted.

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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.	5.20 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
S5.19 Procedures for determining the adequacy of a Specimen in terms of amount of template and quality must be validated.	5.21 The laboratory must implement processes to validate procedures used for measuring the quality and quantity of input nucleic acid 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.
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.	5.22 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 Serial amplification of DNA includes nested PCR.
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.	5.23 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 This especially applies to variants classified outside of the testing laboratory.
S5.22 Appropriate liaison with external Laboratories that refer prenatal Specimens must be maintained.	5.26 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.

Requirements for medical testing of human nucleic acids (Second Edition, 2013) and Requirements for cytogenetic testing (Third Edition, 2013)	Requirements for medical testing for human genetic variation (Third Edition, 2022)
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.	Appropriate liaison with external laboratories that refer prenatal specimens must be maintained.
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).	5.27 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
This is a new addition.	Cell-free nucleic acid detection, including NIPS
This is a new addition.	5.28 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
This is a new addition.	5.29 The laboratory must ensure that pathologists are responsible for and use evidence-based protocols for identifying somatic alterations in solid tumours
This is a new addition.	5.30 The laboratory must ensure qualified members of the workforce are responsibility for assessing, documenting, and reporting on source specimens
This is a new addition.	5.31 The laboratory must implement processes to validate assays using RNA to determine the minimum transcript levels required for a valid test result
This is a new addition.	5.32 The laboratory must ensure qualified members of the workforce are responsible for reporting the limitations of an assay
This is a new addition.	5.33 The laboratory screening for allele specific variants, including splice variants, must use evidence based best practice protocols

Requirements for medical testing of human nucleic acids (Second Edition, 2013) and Requirements for cytogenetic testing (Third Edition, 2013)	Requirements for medical testing for human genetic variation (Third Edition, 2022)
Interpretation	
This is a new addition.	6.03 The laboratory must ensure instances when the tissue tested influences the applicability of the test result are documented in reports 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.
Appendix A	
This is a new addition.	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.
Box 1 Schema for classifying human genetics tests	Deleted.

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This is a new addition.	Examples • 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.

Requirements for medical testing of human nucleic acids (Second Edition, 2013) and Requirements for cytogenetic testing (Third Edition, 2013)	Requirements for medical testing for human genetic variation (Third Edition, 2022)
Appendix B	
Appendix B Send-away tests (Normative)	Deleted.
Appendix C	
Appendix A in *Requirements for cytogenetic testing*.	Assessment of banding quality of cytogenetic slide preparations
Appendix D	
Appendix B in *Requirements for cytogenetic testing*.	Recommended minimum banding quality