NATIONAL PATHOLOGY ACCREDITATION ADVISORY COUNCIL

LABORATORY ACCREDITATION STANDARDS AND GUIDELINES FOR NUCLEIC ACID DETECTION AND ANALYSIS

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Contents

Abbreviations **iv**

Introduction **1**

Definitions **2**

Scope of the document **2**

1 Diagnostic molecular testing for human genetic disease **3**

1.1 General 3

1.2 Categorisation of Nucleic Acid-Based tests for human genetic disorders 3

1.3 Ethical responsibilities of laboratories providing molecular testing for human genetic disorders 6

1.4 Laboratory services 7

1.5 Reports and records 19

1.6 Retention of specimens and records 20

1.7 Quality systems 21

1.8 Staff 21

1.9 Laboratory facilities 22

2 Diagnostic molecular testing of microorganisms causing disease in humans **26**

2.1 General 26

2.2 Laboratory services 27

2.3 Reports and records 40

2.4 Retention of specimens and records 41

2.5 Quality systems 41

2.6 Staff 41

2.7 Laboratory facilities 43

Glossary **48**

References **51**

NATIONAL PATHOLOGY ACCREDITATION ADVISORY COUNCIL

The National Pathology Accreditation Advisory Council (NPAAC) was established in 1979 to consider and make recommendations to the Commonwealth, States and Territories on matters relating to the accreditation of pathology laboratories and the introduction and maintenance of uniform standards of practice in pathology laboratories throughout Australia. An ongoing function of NPAAC is to formulate standards, and to initiate and promote guidelines and educational programs relating to the performance of 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.

Abbreviations

BSC	biological safety cabinet
DNA	deoxyribonucleic acid
HEPA	high-efficiency particle arrest
IVD	in vitro diagnostic devices
NHMRC	National Health and Medical Research Council
NPAAC	National Pathology Accreditation Advisory Council
PCR	polymerase chain reaction
RNA	ribodeoxynucleic acid
TTP	trusted third party

Introduction

Human molecular genetics and laboratory microbiology have been revolutionised by the availability of deoxyribonucleic acid (DNA) and ribodeoxynucleic acid (RNA) sequences from humans and microorganisms, and by the development of methods for their detection and characterisation. It is now possible to identify nucleic acid sequences, pathogens and gene mutations rapidly and with a high degree of sensitivity and specificity, through a variety of nucleic acid detection techniques. As a result, these techniques are replacing many conventional laboratory methods such as cell and pathogen culture, immunoassays and protein biochemistry.

The aim of this publication is to provide consensus standards and guidelines for using nucleic acid analysis. It is directed at:

- laboratories that are either using nucleic acid detection techniques in medical diagnosis, or intending to establish a testing program using these techniques
- accreditation authorities such as the National Association of Testing Authorities, Australia, so that laboratories using nucleic acid detection techniques may be assessed for compliance.

Other applications, such as testing for paternity, kinship and identity and forensic analysis of samples for use by law enforcement authorities, are not considered in this document. 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 areas of testing.

This document must be read in conjunction with the following NPAAC documents:

- *Standards for Pathology Laboratories*
- *Requirements for Supervision of Pathology Laboratories*
- *Guidelines for Quality Systems in Medical Laboratories*
- *Retention of Laboratory Records and Diagnostic Material*
- *Requirements for the Validation of In-House In-Vitro Diagnostic Devices (IVDs)*
- *Guidelines for Cytogenetic Laboratories*

and the following international standards:

- AS ISO/IEC 17025:1999, General requirements for the competence of testing and calibration laboratories
- ISO 15189:2003, Medical laboratories — Particular requirements for quality and competence.

Definitions

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

- A **standard** is the minimum standard for a procedure, method, staffing resource or laboratory facility that is required before a laboratory can attain accreditation.
- A guideline is a consensus recommendation for best practice and should be used if a higher standard of practice is appropriate, particularly when setting up or modifying a laboratory, or when contamination problems have occurred.

The use of the words '**shall**' or '**must**' in each standard within this document indicates a mandatory requirement for pathology practice; '**should**' or '**may**' is used to indicate guidelines or recommendations where compliance would be expected for good laboratory practice.

Scope of the document

Principles set out in this document are intended to apply to tests used for the detection, characterisation and quantification of nucleic acids. In medical laboratories, there are two main applications of these techniques:

- testing for human genetic conditions (inherited disorders and acquired somatic cell disorders)
- testing for microorganisms that can or may cause disease in humans.

This document has two major sections:

- **Section 1** describes NPAAC standards and guidelines for nucleic acid detection and analysis in human genetics.
- **Section 2** describes NPAAC standards and guidelines for nucleic acid detection and analysis in medical microbiology.

Laboratories that perform nucleic acid amplification testing in human genetics **and** medical microbiology **must ensure** that they comply with the standards set out in **both sections** of this document.

1 Diagnostic molecular testing for human genetic disease

1.1 General

Standard

S1.1.1 **The laboratory director shall ensure that laboratory practice is consistent with consensus documents on the ethical issues involved in nucleic acid detection produced by relevant organisations, such as the National Health and Medical Research Council (NHMRC); professional organisations with standing in the field, such as the Human Genetics Society of Australasia, the Australian Medical Association, the Royal College of Pathologists of Australasia; and the relevant state or territory health department.**

S1.1.2 **Laboratories shall not provide genetic testing for any patient-initiated services outside the context of a clinical service, such as mail-order testing or shopfront testing.**

Commentary

C1.1.1 NPAAC is approaching the Australian Health Minister's Advisory Council regarding the establishment of a cross jurisdictional committee to advise on ethical laboratory practice.

Guideline

G1.1.1 To facilitate uniform procedures nationally, laboratory practice should be consistent with the policies of all jurisdictions.

1.2 Categorisation of Nucleic Acid-Based tests for human genetic disorders

Commentary

C1.2.1 Nucleic acid-based diagnosis of human disease arising from gene mutations falls into two major categories:

a) germline inherited disorders (eg cystic fibrosis) or

b) somatic cell disorders (eg the bcr-abl translocation in leukaemia).

Inherited disorders

Standard

S1.2.1 **In relation to human inherited disorders, laboratories must provide guidance (relevant for their particular community and circumstance) to the Level 1 and Level 2 grouping of DNA tests that they perform.**

Commentary

C1.2.2 DNA testing for inherited disorders has an added complexity compared with many other areas of laboratory testing because the information generated has relevance not only for the person being tested but also for other family members. Some DNA tests for human inherited disorders simply require the patient's verbal consent after the provision of appropriate information by a qualified practitioner. For other DNA tests involving inherited disorders, however, formal pre and post-test professional genetic counselling, as well as formal written consent and additional confidentiality procedures, are appropriate parts of the testing process and are required for optimal patient care.

C1.2.3 For the purpose of this document, a distinction is drawn between two broad classes of medical DNA testing for inherited genetic disorders, as shown in Table 1.1.

Table 1.1 Levels of DNA testing

Type of DNA test for an inherited genetic disorder	Explanatory notes[a]
Level 1 DNA test (standard)	Included here would be: a) DNA testing for diagnostic purposes (eg 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) Neonatal screening programs.
Level 2 DNA test (ie 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 presymptomatic DNA testing for conditions for which there are no simple treatment, would usually be included in this grouping. Specific written consent and counselling issues are associated with this grouping.

a 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.

C1.2.4 The previous description of DNA testing for inherited genetic disorders — which used Class A and Class B categories — has not been followed in this version of the NPAAC standards and guidelines. Although some type of categorisation is necessary, feedback indicated that Class A and Class B were confusing (particularly when examples were given) because there were inconsistencies in reasons for choosing a particular class. Also, an example could move from one class to the other depending on use and circumstances. It is not possible to rigidly assign disorders to either Class A or Class B, and the definition of a 'serious genetic disorder' can vary, depending on the circumstances (Wertz and Koppers 2002).

C1.2.5 In using the above Level 1 (standard DNA test) versus Level 2 DNA test (complex issues) categorisation, no examples are given because the implication of the test will vary. Thus, a test may be standard in one particular circumstance but complex in another. This confusion will only be resolved as knowledge about DNA genetic testing and its implications increases in the health profession.

C1.2.6 Despite the above open-ended approach, this NPAAC document requires laboratories to review the categorisation of DNA tests for human inherited disorders. This determination is best undertaken with input from representative professional bodies. In this way, some guidance will be provided as to what constitutes a Level 1 or Level 2 DNA test. This guidance will take into consideration resources, current knowledge, circumstances, the type of condition being tested for and the implications of the DNA test result for the patient and family.

C1.2.7 The review should include input from groups such as the professional colleges (eg the Royal Australasian College of Physicians, the Royal Australian College of General Practitioners, and the Royal Australian and New Zealand College of Obstetricians and Gynaecologists) and professional expert associations (eg the Human Genetics Society of Australasia). Rather than the review being undertaken by each individual laboratory, the process will be organised on a broader front. For example, public hospital laboratories might coordinate this process through their state health departments, utilising the broad experience and input described above. Large private laboratories may have the necessary resources to undertake a similar exercise. Smaller private laboratories could adopt the policy promulgated by larger laboratories or state health departments.

Somatic cell disorders

C1.2.8 DNA testing for somatic cell disorders is usually undertaken to confirm a clinical diagnosis or to follow progress of a disease, and would be considered to be standard DNA testing (Level 1)

1.3 Ethical responsibilities of laboratories providing molecular testing for human genetic disorders

Informed consent

C1.3.1 With Level 2 DNA tests, the primary health professional attending the person seeking genetic advice is responsible for ensuring that informed written consent is obtained and for providing or referring to appropriate pre-test and post-test professional genetic counselling.

Guideline

G1.3.1 Clinicians who request genetic testing should be provided with information about which tests need specialised pre-test counselling and informed written consent before collection of samples. In these circumstances the clinician should indicate on the request form that this has been done.

Confidentiality of reports

C1.3.2 Laboratories must comply with Standard 1 of the NPAAC publication *Standards for Pathology Laboratories*.

New developments

C1.3.3 Because DNA testing in genetic disorders is advancing rapidly, the significance of a result may change with time and experience. Therefore, the DNA laboratory director should be prepared for this and (in consultation with relevant clinical colleagues and, if necessary, the human research ethics committee at the institution) should determine what action will be taken in respect of previous results. Generally, a change in the significance of a test should be reported to the ordering clinician, who will determine what the next step should be.

C1.3.4 Further information relating to the ethics of laboratory genetic testing is available in the NHMRC publication *Ethical Aspects of Human Genetic Testing: an Information Paper* (NHMRC 2000) and in the joint Australian Law Reform Commission – NHMRC publication *Essentially Yours — The Protection of Human Genetic Information in Australia* (ALRC–NHMRC 2003).

1.4 Laboratory services

Commentary

C1.4.1 The satisfactory application of nucleic acid detection techniques depends on the correct performance of all components of testing procedures, including patient preparation and consent (where appropriate), specimen collection, transportation, reagent preparation, nucleic acid isolation, amplification, product visualisation, data transcription, data interpretation, reporting, record keeping, sample storage and quality assurance.

Proficiency and competence

Standard

S1.4.1 **The laboratory shall maintain proficiency and competence by undertaking sufficient testing to maintain the knowledge, experience and expertise of staff.**

Commentary

C1.4.2 Achieving and maintaining standards of practice and expertise requires an adequate number of samples to be tested with a given method (McGovern et al 1999).

Guideline

G1.4.1 The benefits of centralisation versus those of developing local expertise and autonomy should be carefully assessed by laboratories intending to establish a new nucleic acid detection test.

G1.4.2 The performance of only a small number of tests with any given nucleic acid based method is discouraged.

G1.4.3 Where small-volume testing is necessary, appropriate training, assessment and ongoing education procedures should be implemented to maintain and document the proficiency of staff in conducting these tests, and the number of quality assurance samples should be increased.

Sample collection

Standard

S1.4.2 **Trained personnel shall be available at the facility where the sample collection is performed. Specimens shall be collected in accordance with written specimen collection protocols.**

S1.4.3 Where patient-collected samples are used for diagnosis, clear instructions shall be provided to the patient, to reduce the likelihood of sample contamination.

Commentary

C1.4.3 To minimise the risk of contamination in nucleic acid amplification techniques, some special sample collection and preparation is needed, in addition to the usual requirements for pathology testing.

C1.4.4 The precise method of sample collection, initial processing and transportation depends on the specimen concerned and the nucleic acid target (DNA or RNA). Specimens are to be collected according to the principles outlined in the guidelines below, because sample contamination can occur at any stage of specimen collection and processing.

C1.4.5 The potential for false positive or false negative results to occur in genetic DNA testing, particularly for serious disorders, must be considered as part of the evaluation and setting up of diagnostic assays. One way in which this type of error can be minimised is to take two samples from a single patient at the clinical end, and process them at the laboratory end as if they were two distinct specimens. In some circumstances it may be appropriate to test a single sample by two methods, where two methods exist.

C1.4.6 Patients may require that samples be collected using a deidentification protocol, through a trusted third party (TTP) intermediary such as a gene trustee (see Essentially Yours, ALRC Report 96, Vol. 1, Chapter 18, pp. 492-4). In such cases, patient and sample identification must use the coded identifiers provided by the TTP, and appropriate registration of the patient and sample identification numbers must be made with the TTP. To comply with the patient's consent and with the TTP protocols, laboratories must not separately record any linkage between the actual physical identity of the patient and the identification codes provided by the TTP for the patient and any samples.

Guideline

G1.4.4 Wherever possible, nucleic acid detection tests should be performed on dedicated samples or on aliquots taken before other tests are performed. Where it is necessary to perform nucleic acid detection tests on samples 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 sample (if one is available).

G1.4.5 If samples are referred to another laboratory for testing, it is the responsibility of the referring laboratory to ensure that the sample conditions outlined above have been met, and to inform the receiving laboratory if they have not been met.

G1.4.6 Single-use disposable collection equipment should be used.

G1.4.7 Where nucleic acid detection tests are being performed for human genetic diseases that require written consent, additional procedures are strongly recommended to minimise the probability of errors through either:

a) the testing of two samples collected at different times, with both samples tested independently

or

b) where possible, splitting the samples on receipt in the laboratory and processing them in different batches.

G1.4.8 However, in some cases, such as prenatal genetic testing, duplicate samples will not be available.

Sample transport

C1.4.7 Guidelines for the appropriate transport of patient samples are contained in the NPAAC publication *Information on the Transport of Pathology Specimens.*

Standard

S1.4.4 Where there are specific requirements for the transport and handling of samples for nucleic acid detection, these must be documented and must be available to referring practitioners and laboratories.

Sample preparation

Standard

S1.4.5 Where nucleic acid extraction is required, nucleic acids should be extracted and purified using standard methods. The procedures used for nucleic acid isolation from the full range of sample types, collection methods (eg patient versus staff collection) and the condition of specimens received by the laboratory must be validated, and procedures must be detailed in the laboratory methods manual.

Commentary

C1.4.8 The quality of nucleic acid prepared from a specimen has a major effect on the subsequent probability of successfully performing the test.

Sample integrity

Commentary

C1.4.9 Care needs to be taken to ensure that DNA and RNA remain intact during sample storage, transport and preparation. If the number of target sequences in the sample is very small, a false negative may be obtained if degradation occurs. If the starting material for amplification is mRNA, the sample should be processed as rapidly as possible after collection to minimise RNA degradation by ribonucleases.

Guideline

G1.4.9 Specific instructions for handling samples to minimise nucleic acid degradation should be included in all relevant manuals and should be available to staff in collection centres.

G1.4.10 Where samples of marginal quality, quantity or integrity have been received, the laboratory should notify the referring clinician and seek recollection. Where only a single sample is available, the test is essential and recollection is not possible, the report should be annotated accordingly.

Methods

Commentary

C1.4.10 Laboratory directors are responsible for ensuring the analytic validity of tests before they make them available for use in clinical practice. This is particularly important for nucleic acid detection techniques because of the range of tests, the high sensitivity of the methodology and the potential for variable specificity of amplification and hybridisation procedures.

C1.4.11 The term ‘method’ includes kits, individual reagents, instruments, platforms and software. Elements of methods endorsed ‘research use only’ or ‘not for diagnostic use’ must be validated by the laboratory before use for diagnostic purposes, as outlined in the NPAAC publication *Requirements for the Validation of In-House In-Vitro Diagnostic Devices (IVDs).*

Test systems

Standard

S1.4.6 All test systems shall be appropriately validated before routine use.

S1.4.7 Laboratories that have modified kit components or the manufacturer's procedures must demonstrate equivalence or superiority of the modified procedure before putting the process into routine use. The modified procedure must be treated as an inhouse test for validation purposes.

Commentary

C1.4.12 Commercial kits have been developed for a number of nucleic acid detection tests for common human genetic disorders. A large number of nucleic acid detection techniques that are currently in use in Australia have been developed within individual laboratories, or adapted from published methods by laboratories.

Guideline

G1.4.11 Where a laboratory uses a commercial test kit in which the methodology and reagents are unchanged from the manufacturer's instructions, the kit does not need to be independently revalidated in the testing laboratory.

G1.4.12 Standards and guidelines for the validation of inhouse tests can be found in the NPAAC document *Requirements for the Validation of In-House In-Vitro Diagnostic Devices (IVDs).*

G1.4.13 Both commercial kits and inhouse test systems should be regularly reviewed to ensure that the currency of results is maintained, taking into account new discoveries relevant to the field.

Validation of methods

Standard

S1.4.8 In-house tests, or commercial tests endorsed by the manufacturer as 'for research use only' or 'not for diagnostic use', shall be validated in accordance with the NPAAC publication *Requirements for the Validation of In-House In-Vitro Diagnostic Devices (IVDs).*

S1.4.9 If any modification is made to a commercial IVD, it must be treated as an in-house IVD and it must be fully validated in accordance with the NPAAC publication *Requirements for the Validation of In-House In-Vitro Diagnostic Devices (IVDs).*

S1.4.10 Validation data shall be retained by the laboratory in sufficient detail to enable external review. The period for which laboratory records are to be retained is stipulated in the NPAAC publication *Guidelines for the Retention of Laboratory Records and Diagnostic Material.*

Commentary

C1.4.13 Laboratories shall only offer nucleic acid detection tests as routine tests if their technical validity has been established, either by the laboratory or the manufacturer.

Guideline

G1.4.14 Where a validated test is available, it should be used in preference to a nonvalidated test. If a nonvalidated test must be performed because of clinical necessity, the report should clearly indicate that the diagnostic validity of the test has not been established.

G1.4.15 The procedures and methods used in nucleic acid detection techniques for diagnostic purposes should be validated for clinical use according to the following principles:

a) Evaluation should use known positive and negative samples.

b) Evaluation should be by comparison with proficiency test material, if available.

c) Evaluation should be by comparison with an existing validated method in the laboratory.

d) Evaluation should be with all specimen types and conditions that will be used to make laboratory diagnosis.

e) If a significant modification to an analytical procedure has been made, the modified procedures should be compared to the original using either identical samples or identical types of samples.

f) Reproducibility should not be determined by repetitive analysis of the same sample.

g) The sensitivity of a test, or cutoff values, should be set at a level that is relevant to the diagnostic use of the test.

Contamination control

Commentary

C1.4.14 Nucleic acid detection techniques are usually designed to maximise sensitivity and are capable of detecting very small amounts of nucleic acid.

C1.4.15 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 sample

d) during amplification

e) during product detection

f) by contamination from reagents used for the test.

C1.4.16 The sources of potential contamination include:

a) positive samples (cross-contamination)

b) amplified nucleic acid (eg contamination of stock reagents or equipment, or in aerosol droplets)

c) operator-derived nucleic acid.

C1.4.17 Mathematical methods can be used to assess the likelihood of contamination in the use of polymerase chain reaction (PCR)-based methods (Shapiro 1999).

Measures to control contamination

Standard

S1.4.11 Laboratories shall retain records documenting contamination events, the identified source of the contamination and measures taken to reduce the risk of future similar contamination events.

Guideline

G1.4.16 The greatest protection a laboratory has against contamination arises from:

a) the competence of the staff in performing laboratory tasks

b) the design of the laboratory

c) the routine use of controls to detect contamination.

G1.4.17 These issues are addressed in detail in the relevant sections of this document.

G1.4.18 For single round PCRs, the contamination risk may be reduced by replacing thymidine with uracil. The amplified product can then be destroyed by uracil-N-glycosolase, which is added to new samples. This is not sufficient to deal with heavy contamination and is not a substitute for the other measures. Also, it cannot be used in nested PCR, a technique that poses additional contamination risks due to the large amounts of second-round product, which is smaller and more resistant to decontamination procedures. Probe amplification methods (eg branched-chain DNA) have low contamination potential and may be performed in routine laboratory areas, provided those areas are not used for specimen processing.

Laboratory hygiene

Standard

S1.4.12 Spills shall be cleaned up promptly. Those containing amplified nucleic acids shall be covered with absorbent paper soaked in 2–10% sodium hypochlorite and left for 10 minutes. The absorbent paper shall then be discarded and the area wiped over with 2–10% sodium hypochlorite.

S1.4.13 The level of laboratory hygiene shall be high. Staff shall be careful to avoid contamination of their gowns and gloves. These shall be changed promptly if they have potentially been contaminated or if they become soiled.

Guideline

G1.4.19 High standards of laboratory hygiene are required, including the measures below. All work areas, especially those used previously for other testing, should be cleaned thoroughly.

G1.4.20 Gowns and gloves are to be worn by laboratory staff in the work areas. Gloves are to be discarded and hands washed before leaving the area. Gowns should be dedicated to each area. Gowns or gloves should be changed whenever there is evidence of soiling.

G1.4.21 Tube or pack racks used for holding tubes or plates containing amplified nucleic acid should be decontaminated in 2–10% sodium hypochlorite (or similar reagent) for a minimum of four hours.

Equipment

Standard

S1.4.14 All equipment shall be maintained in working order and shall be subject to a regular schedule of maintenance in line with the manufacturer's recommendations.

S1.4.15 Centrifuges used for preparation of biological samples shall have either sealed buckets or a sealed rotor.

Controls

Standard

S1.4.16 All assays shall include appropriate negative controls that are subject to the whole test process, including the extraction.

S1.4.17 Maternal cell contamination controls shall be used for prenatal diagnosis of human genetic disease.

S1.4.18 Where a negative result is obtained, there must be confirmation that DNA has been extracted.

S1.4.19 In assays using mRNA as the starting material, control measures must be incorporated to detect the success of mRNA extraction and of reverse transcription on that particular sample.

Commentary

C1.4.18 The types of controls used in nucleic acid detection techniques will vary with specific assays.

C1.4.19 The exact number of controls required for PCR-based systems depends on the number of samples in each run. A negative patient sample is essential, and laboratories may also include a 'no nucleic acid' sample (ie all reagents but no nucleic acid) to determine whether reagent contamination is the cause of reactions in the negative patient sample, should they occur. A negative control should be placed after the last patient samples.

C1.4.20 Where tests are undertaken for genetic variations, appropriate positive and negative controls are required. However, it is necessary to investigate all negative results as these may represent a failure of the test.

Guideline

G1.4.22 Where test runs are expected to contain a large proportion of positive results, additional 'no nucleic acid' controls should be interspersed among the patient samples at an appropriate frequency, as validated by the particular laboratory.

G1.4.23 Positive controls should be selected to verify that the test would detect significant variants.

G1.4.24 Positive controls should be just above the limit of sensitivity of the test.

G1.4.25 Assurance that successful mRNA extraction and reverse transcription has occurred can be achieved by testing the sample for a housekeeping gene such as beta-actin.

Additional testing

Commentary

C1.4.21 Laboratories performing nucleic acid amplification testing should be aware that erroneous results may occur for reasons other than contamination. These may occur through:

a) nonspecific primer binding and amplification of other sequences, which are then misidentified as the target sequence

b) amplification of similar or identical target sequences found in other portions of the eukaryotic genome

c) nonspecific, usually low-level, reactivity in the detection system

d) primer design and binding

e) limitations of the methodologies of detection

f) presence of deletions.

C1.4.22 The frequency and significance of these events will vary with the test technique, the target sequence chosen, the detection system used and the patient population.

Guideline

G1.4.26 It is strongly recommended that, where the implications of a positive result are substantial, and where the specificity of the test is known to be suboptimal or has not been established by extensive validation, one or more of the following methods be performed to verify positive results:

a) use of a nested PCR or other techniques that require the binding of more than one set of primers to generate a positive result

b) repeat testing of all positives using another set of primers directed at a different target sequence

c) identification of the product using methods such as specific probes, sequencing or restriction enzyme analysis.

G1.4.27 It may be necessary to use several of these methods to achieve the desired specificity.

Nucleic acid sequencing

Standard

S1.4.20 **DNA sequencing used primarily to detect an unknown mutation must be undertaken with particular care. Efforts must be made to confirm that the sequencing methodology is appropriate (eg limited BLAST search or comparison with a known standard sequence, or a second independent assay).**

Commentary

C1.4.23 DNA sequencing is now an alternative approach to the detection of DNA mutations or DNA changes in human genetic disorders. The benefits of DNA sequencing are that it is a robust technology that allows for high-throughput mutation detection and characterisation in a single assay. The major problem with the technology is that the signal from each base pair needs to be individually considered and compared with one or more controls. Software packages are being marketed that allow rapid screening of DNA sequence data, but in their current form are not sufficiently robust to allow fully automated analysis.

C1.4.24 The reasons a laboratory may wish to sequence a PCR product may include:

a) mutation screening, which is the detection of an unknown mutation in a length of DNA

b) confirmatory testing

c) genotyping.

C1.4.25 In some cases, the laboratory to which the sample is referred for DNA testing undertakes to have the sequencing carried out in a DNA sequencing facility. By their nature, centralised DNA sequencing facilities carry out a broad range of activities that, in the main, are research oriented. Diagnostic-quality DNA sequencing must be distinguished from research-based DNA sequencing, and must conform to standards which ensure that false positive and false negative results are minimised. Therefore, the DNA sequencing facility that is used to perform a DNA diagnostic test should be accredited by the National Association of Testing Authorities and the Royal College of Pathologists of Australasia or ISO certified.

C1.4.26 The DNA sequencing facility may not always receive sufficient information to distinguish between research work and service diagnostic work. Therefore, the onus is on the referring laboratory to ensure that the DNA sequencing facility undertakes the necessary controls and standards that would normally be expected for a more traditional DNA laboratory. Included in this would be quality assurance, quality control, appropriate retention of records, standards with each run and published criteria on what is acceptable for the particular DNA sequencing run (including such criteria as peak intensity, baseline fluctuations and signal-to-noise ratio).

C1.4.27 A valuable document for DNA sequencing of genetic disorders is the Clinical Molecular Genetics Society's *Best Practice Guidelines for DNA Sequencing Analysis and Interpretation.*[1]

C1.4.28 A guide to what standards would be expected in sequencing for human leukocyte antigen typing may be found in the American Society for Histocompatibility and Immunogenetics standard protocol for human leukocyte antigen typing. For new technologies, appropriate validation must be performed.

[1] Available from http://www.cmgs.org

1.5 Reports and records

Standard

S1.5.1 **Reports containing test results shall be provided to the person requesting testing with a minimum of delay, commensurate with good laboratory practice and patient care.**

S1.5.2 **Records of test requests, equipment history and service, testing procedures, results of test procedures on patient specimens, material for quality control and proficiency testing for consistency (quality assurance) shall be kept in a readily accessible form.**

S1.5.3 **Laboratory databases that contain information on patients or test results must be secure, password coded and backed up at regular and frequent intervals. Appropriate measures shall be in place to prevent unauthorised physical or electronic access to the databases, especially if the databases are located in nonsecure premises or are stored on network computers.**

S1.5.4 **Where a test is referred to another laboratory, the report shall be sent to the referring laboratory. A copy of the original report may be provided to the referring practitioner but the referring laboratory must be advised.**

Commentary

C1.5.1 Testing may sometimes be performed on deidentified samples using a trusted third party (TTP) intermediary such as a gene trustee (see *Essentially Yours*, ALRC Report 96, Vol. 1, Chapter 18, pp. 492–4). In such cases, patient and sample identification must use the coded identifiers provided by the TTP. Where a report arises from such patients or samples (or from multiple patients or samples) all with only a coded identifier assigned by the TTP, the report should still conform in other respects with the other Standards and Guidelines in this section.

Understanding the DNA test result in genetic disorders

Commentary

C1.5.2 Apart from the actual performance of a DNA test, the interpretation of its result is critical. The laboratory must ensure that the way in which the result is given facilitates its interpretation; however, the clinical information provided will not always be sufficient for specific interpretation. Therefore, the report must not overinterpret the significance of a DNA test result. The clinician ordering the test must have some knowledge of its significance and interpretation.

Guideline

G1.5.1 Reports should be concise and unambiguous, and should include an appropriate interpretation of the results.

G1.5.2 Reports for predictive genetic tests should be available in as short a time as is practically possible and should be consistent with the recommendations of professional societies with expertise in the field (eg the Australian Cancer Network), unless alternative arrangements have been specifically agreed before sample collection.

1.6 Retention of specimens and records

Commentary

C1.6.1 National guidelines for sample and record retention times are detailed in the NPAAC publication *Retention of Laboratory Records and Diagnostic Material*.

Nucleic acid storage conditions

Standard

S1.6.1 Nucleic acids shall be stored in a way that minimises degradation and contamination of the sample.

Guideline

G1.6.1 DNA and RNA samples should be prepared and diluted into 'working stocks' and 'storage stocks'.

G1.6.2 Working stocks of DNA are often stable for several months if stored in sealed containers at 4°C.

G1.6.3 To minimise degradation, storage stocks of DNA should be held at –20°C and RNA should be held below –70°C. Positive control samples should be stored below –70°C.

G1.6.4 Stocks and controls should be aliquoted to minimise damage from freezing and thawing.

1.7 Quality systems

Commentary

C1.7.1 Guidelines for quality systems in pathology laboratories are detailed in the NPAAC publication *Guidelines for Quality Systems in Medical Laboratories*.

1.8 Staff

Standard

S1.8.1 **The person in charge of nucleic acid detection techniques in a laboratory shall be actively involved in determining methods and procedures, staff training and quality control procedures; in reviewing and interpreting laboratory data; and in providing laboratory reports and clinical consultation, as outlined in the NPAAC publication *Standards for Pathology Laboratories* (Standard 2 — Staffing, supervision and consultation).**

Commentary

C1.8.1 The level of education and training for pathologists and scientists has been established on a national basis for pathology laboratories in general by the NPAAC publications *Standards for Pathology Laboratories and Requirements for Supervision of Pathology Laboratories.*

Laboratory director

Standard

S1.8.2 **The director of the laboratory must be able to demonstrate by appropriate documentation that the procedures used and tests performed are within the scope of the education, training and experience of individual scientific or technical staff members.**

Commentary

C1.8.2 Nucleic acid amplification techniques are a relatively new and rapidly expanding discipline. Therefore, the laboratory director should ensure that the senior practitioners within the laboratory have wide training and competence appropriate to the complexity of testing undertaken.

Staff skills

Standard

S1.8.3 **At least one senior member of staff shall have significant diagnostic or research experience with nucleic acid detection methods, including their principles and design, and problem solving in their use.**

S1.8.4 **Staff shall have or acquire knowledge and understanding of human genetic disorders and the application of nucleic acid amplification techniques in the investigation of such disorders.**

S1.8.5 **Laboratories shall comply with the guidelines of the Office of the Gene Technology Regulator when using recombinant DNA probes.**

Commentary

C1.8.3 The performance of nucleic acid detection techniques is technically challenging and highly dependent on operator skills and facilities. Those working in the area need specific training, particularly in how to assess the validity of data and how to troubleshoot problems when they occur.

C1.8.4 Alternatively, those working in the area should undertake specific training in nucleic acid detection techniques in a laboratory with established proficiency and competence in nucleic acid detection and the development of inhouse testing.

C1.8.5 The presence of experienced supervisors and trainers is essential, given their critical involvement in error detection, error correction and problem solving.

Guideline

G1.8.1 Where a laboratory has only a limited number of staff involved in nucleic acid testing, particular attention should be paid to continuing education and the maintenance of knowledge and expertise in current techniques.

1.9 Laboratory facilities

Commentary and definitions

C1.9.1 Laboratories undertaking nucleic acid amplification should be configured to minimise the risk of contamination of samples and reagents by other samples in the laboratory or by amplified material.

C1.9.2 Laboratories undertaking nucleic acid detection from eukaryotic cells are viewed as being at significantly lower risk of contamination than those laboratories undertaking nucleic acid detection of microorganisms. In microbiology laboratories, microorganisms are present in samples in large numbers or are cultured at high concentrations. There is also greater potential for aerosol contamination because of the small size of microorganisms compared to eukaryotic cells.

C1.9.3 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 of samples and reagents by amplified material or other samples in the laboratory.

C1.9.4 The term 'separate areas' is used in the following sections to mean laboratory spaces that are used for nucleic acid based testing and are separated from other laboratory spaces by walls, 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. The term 'contained area' means a laboratory space that can be isolated from the rest of the laboratory either by walls and doors or within the working space of an instrument.

Minimum standards for a nucleic acid amplification facility

Commentary

C1.9.5 The standards listed below are the minimum standards for a PCR laboratory using exclusively eukaryotic cells, tissues or isolated DNA.

Standard

S1.9.1 Three physically separate areas are required in order to reduce the risk of cross-contamination or carry-over contamination.

S1.9.2 The three areas required in each nucleic acid amplification laboratory are:

a) a separate area for the extraction of nucleic acids from samples and for the addition of sample DNA to tubes containing master mix before PCR amplification

b) a dedicated clean area for the preparation of reagents (including dispensing of the master mix)

c) a dedicated, contained area for amplification and product detection.

S1.9.3 The normal airflow pattern of each of the regions shall be known and the layout of the laboratory areas designed to minimise the potential for aerosol cross-contamination.

S1.9.4 Where the areas for preparation of reagents and sample preparation are located within a single room, wide separation of these activities shall be maintained and appropriate procedures and controls shall be implemented to detect contamination.

S1.9.5 Post-PCR analysis shall not be incorporated into areas where reagent preparation or sample preparation occurs. The post-PCR area shall be positioned so as to minimise the possibility of cross-contamination of preamplification areas. Generally, this can be achieved by positioning the post-PCR area at an appropriate distance from the preamplification area.

S1.9.6 Reagents and equipment shall be limited to the appropriate sections. In particular, no nucleic acid samples shall be taken into the reagent preparation area. Samples shall be stored separately from reagents.

S1.9.7 Equipment from other areas shall not be taken into the reagent preparation area.

S1.9.8 The movement of specimens and equipment shall be unidirectional; that is, from preamplification to postamplification areas. Only sealed PCR amplification tubes and tube racks shall be carried between the preamplification area and the postamplification area.

S1.9.9 Where equipment (such as tube racks) is returned against the flow, it shall first be decontaminated in 2–10% hypochlorite or another noncorroding decontaminating agent for four hours before being moved from the postamplification area.

S1.9.10 Laboratory coats and gloves shall be changed before staff move to or from each area.

Guideline

G1.9.1 Sample processing and reagent preparation should occur in different rooms. Where the areas for preparation of reagents and sample preparation cannot be separated, the provision of a positive-pressure hood or Class II biological safety cabinet (BSC) for reagent preparation and a Class II BSC for specimen preparation is strongly recommended. The air outflow from the sample preparation BSC must have a high-efficiency particle arrest (HEPA) filter and must be directed away from the reagent preparation area.

G1.9.2 Equipment designated for a particular area should be marked (eg by colour) to clearly indicate to which area they belong.

G1.9.3 Aerosol-resistant pipette tips or positive displacement pipettes are strongly recommended to minimise contamination, and should be used routinely.

G1.9.4 Work surfaces should be regularly decontaminated by wiping with 2–10% hypochlorite or another similar decontaminating agent. Instrumentation such as microfuges, heating blocks and waterbaths should be cleaned regularly with 2–10% hypochlorite or another noncorroding decontaminating agent.

G1.9.5 Instruments capable of producing aerosols (eg vortex mixers, PCR machines and microfuges) should be placed at as great a distance from preparation areas as possible.

G1.9.6 While not specifically dealt with in these standards and guidelines, the use of robotic equipment in the laboratory should adhere to the principles outlined above.

2 Diagnostic molecular testing of microorganisms causing disease in humans

2.1 General

Sensitivity and contamination

Commentary

C2.1.1 Most nucleic acid tests for microorganisms are designed to detect the presence or absence of a particular organism. Usually, the target organism should not be present at all, although viral load testing seeks information about the levels of target organisms. Examples of detection tests are those for hepatitis C RNA in blood and for herpes simplex DNA in cerebrospinal fluid. Such tests are designed to maximise sensitivity and therefore have a greater risk of false positives caused by low-level contamination. Microorganisms are often present in samples in large numbers or are cultured at high concentrations in microbiology laboratories. The small size of microorganisms compared to eukaryotic cells creates greater potential for aerosol contamination. For these reasons, more stringent conditions are applied to microbiological testing in some circumstances.

Supplemental testing

Commentary

C2.1.2 Any single nucleic acid amplification test may be part of a testing algorithm that uses supplemental testing to improve specificity. A similar method is used in serological testing in which a sensitive but nonspecific screening assay is followed by supplemental tests (eg the Western blot for human immunodeficiency virus) to improve the specificity of the result. Currently, multiple tests are most commonly used in nucleic acid amplification testing for Neisseria gonorrhoeae, for which a single test is not specific enough to report a positive result, so that combinations of tests are used to yield a reliable result.

Standards and controls

Commentary

C2.1.3 Standards and controls are a routine part of the quality systems for all laboratory testing. However, these are not readily available for nucleic acid amplification testing for many organisms, especially uncommon pathogens. Under these circumstances, laboratories should seek or manufacture standards and controls that most closely mimic clinical samples. For example, a negative sample seeded with a synthetic target may be used to mimic a positive patient sample.

Detection of human DNA

Commentary

C2.1.4 Laboratories should be aware that when a nucleic acid amplification test is undertaken on human material, especially on samples such as tissues that have large amounts of human DNA, there may be nonspecific amplification of unrecognised human DNA sequences that are similar to the target organism sequences. Appropriate validation of the test should take into account this possibility.

2.2 Laboratory services

Commentary

C2.2.1 The satisfactory application of nucleic acid detection techniques depends on the correct performance of all components of testing procedures, including patient preparation and consent (where appropriate), specimen collection, transportation, reagent preparation, nucleic acid isolation, amplification, product visualisation, data transcription, data interpretation, reporting, record keeping, sample storage and quality assurance.

Proficiency and competence

Standard

S2.2.1 **The laboratory shall maintain proficiency and competence by undertaking sufficient testing to maintain the knowledge, experience and expertise of staff.**

Commentary

C2.2.2 Achieving and maintaining standards of practice and expertise requires an adequate number of samples to be tested with a given method (McGovern et al 1999).

Guideline

G2.2.1 The benefits of centralisation versus those of developing local expertise and autonomy should be carefully assessed by laboratories intending to establish a new nucleic acid detection test.

G2.2.2 The performance of only a small number of tests with any given nucleic acid based method is discouraged.

G2.2.3 Where small-volume testing is necessary, appropriate training, assessment and ongoing education procedures should be implemented to maintain and document the proficiency of staff in conducting these tests, and the number of quality assurance samples should be increased.

Sample collection

Standard

S2.2.2 **Trained personnel shall be available at the facility where the sample collection is performed. Specimens shall be collected in accordance with written specimen collection protocols.**

S2.2.3 **Where patient-collected samples are used for diagnosis, clear instruction shall be provided to the patient to reduce the likelihood of sample contamination.**

Commentary

C2.2.3 To minimise the risk of contamination, nucleic acid amplification techniques have some special sample collection and preparation needs, in addition to the usual requirements for pathology testing.

C2.2.4 The precise method of sample collection, initial processing and transportation depends on the specimen concerned and the nucleic acid target (DNA or RNA). Specimens are to be collected according to principles outlined below, as sample contamination can occur at any stage of specimen collection and processing.

C2.2.5 Samples that have been used for other tests before nucleic acid detection testing are at increased risk of contamination. This is a particular risk where the previous test performed was processed with

other samples containing the nucleic acid of interest. For example, if samples that have been tested for hepatitis C antibody are cross-contaminated by positive samples, they will yield a false positive result for hepatitis C RNA.

Guideline

G2.2.4 Wherever possible, nucleic acid detection tests should be performed on dedicated samples or on aliquots taken before other tests are performed. Where it is necessary to perform nucleic acid detection tests on samples that have already been used for other purposes and there is a significant risk of cross-contamination, the report should be annotated accordingly and results confirmed on a dedicated sample

G2.2.5 If samples are referred to another laboratory for testing, it is the responsibility of the referring laboratory to ensure that the sample conditions outlined above have been met, and to inform that receiving laboratory if they have not been met.

G2.2.6 Single-use disposable collection equipment should be used.

Sample transport

Standard

S2.2.4 **Where there are specific requirements for the transport and handling of samples for nucleic acid detection, these must be documented and available to referring practitioners and laboratories.**

Commentary

C2.2.6 National guidelines for the appropriate transport of patient samples are contained in the NPAAC publication *Information on the Transport of Pathology Specimens*.

Sample preparation

Standard

S2.2.5 **Where nucleic acid extraction is required, nucleic acids should be extracted and purified using standard methods.**

S2.2.6 **The procedures used for nucleic acid isolation from the full range of sample types, collection methods (eg patient versus staff collection) and the condition of specimens received by the laboratory must be validated and procedures detailed in the laboratory methods manual.**

Commentary

C2.2.7 The quality of nucleic acid prepared from a specimen has a major effect on the subsequent probability of successfully performing the test.

Guideline

G2.2.7 Where possible controls should be included which assess the adequacy of sample collection and extraction.

Sample integrity

Commentary

C2.2.8 Care needs to be taken to ensure that DNA and RNA remain intact during sample storage, transport and preparation. If the number of target sequences in the sample is very small, a false negative may be obtained if degradation occurs. If the starting material for amplification is mRNA, the sample should be processed as rapidly as possible after collection to minimise RNA degradation by ribonucleases.

Guideline

G2.2.8 Specific instructions for handling samples to minimise nucleic acid degradation should be included in all relevant manuals and should be available to staff in collection centres.

G2.2.9 Where samples of marginal quality, quantity or integrity have been received, the laboratory should notify the referring clinician and seek recollection. Where only a single sample is available, the test is essential and recollection is not possible, the report should be annotated accordingly.

G2.2.10 Class II biological safety cabinets should be used for specimen preparation.

Methods

Commentary

C2.2.9 Laboratory directors are responsible for ensuring the analytic validity of tests before they make them available for use in clinical practice. This is particularly important for nucleic acid detection techniques because of the range of tests, the high sensitivity of the methodology and the potential for variable specificity of amplification and hybridisation procedures.

C2.2.10 The term 'method' includes kits, individual reagents, instruments, platforms and software. Elements of methods endorsed 'research use only' or 'not for diagnostic use' must be validated by the laboratory before use for diagnostic purposes, as outlined in the NPAAC publication *Requirements for the Validation of In-House In-Vitro Diagnostic Devices (IVDs)*.

Test systems

Standard

S2.2.7 **Tests shall be validated in accordance with the NPAAC publication Requirements for the *Validation of In-House In-Vitro Diagnostic Devices (IVDs)*.**

S2.2.8 **Laboratories that have modified kit components or the manufacturer's procedures must demonstrate equivalence or superiority of the modified procedure before putting the process into routine use. The modified procedure must be treated as an inhouse test for validation purposes.**

Commentary

C2.2.11 Commercial kits have been developed for a number of nucleic acid detection tests, particularly for infectious agents. A large number of nucleic acid detection techniques that are currently in use in Australia have been developed within individual laboratories, or adapted from published methods by laboratories.

Guideline

G2.2.11 Where a laboratory uses a commercial test kit in testing in which the methodology and reagents are unchanged from the manufacturer's instructions, the kit does not need to be independently revalidated in the testing laboratory.

G2.2.12 The supply of some reagent kits is regulated by the Therapeutic Goods Administration under the *Therapeutic Goods Act 1989*. All kits used for human immunodeficiency virus (HIV) and hepatitis C testing, including nucleic acid detection tests for these agents, are registered on the Australian Register of Therapeutic Goods. Laboratories carrying these tests should ensure that they meet the requirements of these regulations. The range of registered tests and the requirements may change from time to time.

G2.2.13 Both commercial kits and inhouse test systems should be regularly reviewed to ensure that the currency of results is maintained, taking into account new discoveries relevant to the field.

Validation of methods

Standard

S2.2.9 **Inhouse tests, or commercial tests endorsed by the manufacturer as 'for research use only' or 'not for diagnostic use', shall be validated in accordance with the NPAAC publication *Requirements for the Validation of In-House In-Vitro Diagnostic Devices (IVDs)*.**

S2.2.10 **If any modification is made to a commercial IVD, it must be treated as an inhouse IVD and it shall be fully validated in accordance with the NPAAC publication *Requirements for the Validation of In-House In-Vitro Diagnostic Devices (IVDs)*.**

S2.2.11 **Validation data shall be retained by the laboratory in sufficient detail to enable external review. The period for which laboratory records are to be retained is stipulated in the NPAAC publication *Guidelines for the Retention of Laboratory Records and Diagnostic Materials*.**

Commentary

C2.2.12 Laboratories should only offer nucleic acid detection tests as a routine test if their technical validity has been established either by the laboratory or the manufacturer.

Guideline

G2.2.14 Where a validated test is available, it should be used in preference to a nonvalidated test. If a nonvalidated test must be performed because of clinical necessity, the report should clearly indicate that the diagnostic validity of the test has not been established.

G2.2.15 The procedures and methods used in nucleic acid detection techniques for diagnostic purposes should be validated for clinical use according to the following principles:

a) Evaluation should use known positive and negative samples.

b) Evaluation should be by comparison with proficiency test material, if available.

c) Evaluation should be by comparison with an existing validated method in the laboratory.

d) Evaluation should be with all specimen types and conditions that will be used to make laboratory diagnosis.

e) If a significant modification to an analytical procedure has been made, the modified procedures should be compared to the original using either an identical sample or identical types of samples.

f) Reproducibility should not be determined by repetitive analysis of the same sample.

g) The sensitivity of a test, or cutoff values, should be set at a level that is relevant to the diagnostic use of the test.

Contamination control

Commentary

C2.2.13 Nucleic acid detection techniques are usually designed to maximise sensitivity, and are capable of detecting very small amounts of nucleic acid.

C2.2.14 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 sample

d) during amplification

e) during product detection

f) by contamination from reagents used for the test.

C2.2.15 The sources of potential contamination include:

a) positive samples (cross-contamination)

b) amplified nucleic acid (eg contamination of stock reagents or equipment, or in aerosol droplets)

c) operator-derived nucleic acid.

C2.2.16 Mathematical methods are available to assess the likelihood of contamination in the use of PCR-based methods (Shapiro 1999).

Measures to control contamination

Standard

S2.2.12 **Laboratories shall retain records documenting contamination events, the identified source of the contamination and measures taken to reduce the risk of future similar contamination events.**

Commentary

C2.2.17 The greatest protection a laboratory has against contamination arises from:

a) the competence of the staff in performing laboratory tasks

b) the design of the laboratory

c) the routine use of controls to detect contamination.

C2.2.18 These issues are addressed in detail in the relevant sections of this document.

C2.2.19 For single-round PCRs, the contamination risk may be reduced by replacing thymidine with uracil. The amplified product can then be destroyed by uracil-N-glycosolase, which is added to new samples. This is not sufficient to deal with heavy contamination and is not a substitute for the other measures. Also, it cannot be used in nested PCR, which poses additional contamination risks due to the large amounts of second-round product, which is smaller and more resistant to decontamination procedures. Probe amplification methods (eg branched-chain DNA) have low contamination potential and may be performed in routine laboratory areas, provided those areas are not used for specimen processing or for culture of target microorganisms.

Guideline

G2.2.16 Swabbing of work surfaces and including the swab in the assay can assist in assessing and investigating contamination problems.

Laboratory hygiene

Standard

S2.2.13 **Spills shall be cleaned up promptly. Those containing amplified nucleic acids shall be covered with absorbent paper soaked in 2–10% sodium hypochlorite and left for 10 minutes. The absorbent paper shall then be discarded and the area wiped over with 2–10% sodium hypochlorite.**

S2.2.14 **The level of laboratory hygiene shall be high. Staff shall be careful to avoid contamination of their gowns and gloves. These shall be changed promptly if they have potentially been contaminated, or if they become soiled.**

Commentary

C2.2.20 High standards of laboratory hygiene are required, including the measures outlined below. All work areas, especially those used previously for other testing, should be cleaned thoroughly.

Guideline

G2.2.17 Gowns and gloves are to be worn by laboratory staff in the work areas. Gloves are to be discarded and hands washed before leaving the area. Gowns should be dedicated to each area. Gowns or gloves should be changed whenever there is evidence of soiling.

G2.2.18 Tube or pack racks used for holding tubes or plates containing amplified nucleic acid should be decontaminated in 2–10% sodium hypochlorite (or similar reagent) for a minimum of four hours. For screening tests for transfusion or transplantation purposes, decontamination should be according to manufacturer's recommendations as a minimum or according to any licensing conditions imposed by the Therapeutic Goods Administration.

Equipment

Standard

S2.2.15 **All equipment shall be maintained in working order as part of a regular schedule of maintenance in line with the manufacturer's recommendations.**

S2.2.16 **Centrifuges used for preparation of biological samples shall have either sealed buckets or a sealed rotor.**

Controls

Standard

S2.2.17 **At least one of the negative controls and a weak positive control (wherever available) shall be subject to the whole test process, including the extraction.**

Commentary

C2.2.21 The types of controls used in nucleic acid detection techniques will vary with specific assays and according to whether microbiological or human molecular genetic testing is being undertaken.

C2.2.22 The exact number of controls required for PCR-based systems depends on the number of samples in each run. A negative patient sample is essential, and laboratories may also include a 'no nucleic acid' sample (ie all reagents but no human or organism nucleic acid) to determine whether reagent contamination is the cause of reactions in the negative patient sample, should they occur. A negative control should be placed after the last patient sample.

C2.2.23 Each run must include a positive control. Ideally, this should be a positive patient sample that has been diluted to a level that is just above the limits of detection for the test and is reliably positive when the test is performing adequately. Usually this control contains target that is at a concentration ten-fold higher than the limit of detection, ensuring that the result will be reliably positive if the assay is performing correctly, but will also detect a loss of sensitivity. If such samples are not available, a negative patient sample that has had target sequence added — either in the form of cultured organism or synthetic target — may be used. If these cannot be accessed and the test is clinically necessary, a weak extraction control should be added and the report should be annotated to indicate that the sensitivity of the assay could not be confirmed.

C2.2.24 Controls for inhibition are now used routinely in many laboratories in order to reduce the chance of false negatives due to the presence of inhibitory substances in the specimen. These may either be a human gene target that is expected to be present in the sample, a modified target sequence (eg a plasmid with a modified wild-type target sequence) that is added to the sample, or some other target (eg equine herpes virus) that is added to samples and will not yield false positive results. Choosing the appropriate control will vary with specimen type and the ease and availability of control material. For example, using a human gene target may also assess the adequacy of the sample. However some samples, such as cerebrospinal fluid, may not contain enough of that target. Also samples of blood, body fluids or tissues do not require assessment of specimen adequacy. Inhibition controls that are modifications of the wild-type target are theoretically the best but they may not be available. If used, they may compete for primers and reagents and lead to a reduction in sensitivity. Competition for reagents may also occur with the other types of inhibition controls if the inhibition control target is in marked excess compared to the diagnostic target. Laboratories need to consider the merits of the various forms of inhibition control and choose the most appropriate for their test.

Guideline

G2.2.19 Where test runs are expected to contain a large proportion of positive results, it is recommended that additional 'no template' controls be interspersed among the patient samples at an appropriate frequency as validated by the particular laboratory.

G2.2.20 Positive controls should be selected to verify that the test would detect significant variants.

G2.2.21 For microbial screening tests using a commercial manufacturer's reagents, additional controls should be sourced independently of the manufacturer, with levels of target sequence close to the sensitivity of the test but above 95% detection confidence intervals. These should be used in every run.

G2.2.22 Positive controls should be just above the limit of sensitivity of the test.

G2.2.23 Positive controls should be selected to verify that the test would detect significant known genetic variants.

G2.2.24 Inhibition controls should be used wherever possible, especially if particular test and specimen combinations are known to regularly experience problems with inhibition.

Additional testing

Commentary

C2.2.25 Laboratories performing nucleic acid amplification testing should be aware that erroneous results may occur for reasons other than contamination; for example, due to:

a) nonspecific primer binding and amplification of other sequences that are then misidentified as the target sequence

b) amplification of similar or identical target sequences found in other organisms

c) nonspecific, usually low-level, reactivity in the detection system

e) primer design and binding

f) limitations of the methodologies of detection

g) presence of deletions.

C2.2.26 The frequency and significance of these events will vary with the test technique, the target sequence chosen, the detection system used and the patient population.

C2.2.27 The use of supplemental testing rests on the positive predictive value of the test and the significance of the result. For those tests with a high positive predictive value, supplemental testing may not be indicated for all samples. For those tests with lower positive predictive value (eg screening tests) supplemental testing is required. Supplemental testing may take the form of further laboratory tests performed on the original sample (eg microscopy, biochemical testing), on the PCR product itself (eg nested PCR, DNA sequencing, diagnostic restriction enzyme digestion) or by further clinical examination and investigation.

Guideline

G2.2.25 It is strongly recommended that where the implications of a positive result are substantial (eg sexually transmitted diseases) and where the specificity of the test is known to be suboptimal or has not been established by extensive validation, one or more of the following methods be used to verify positive results:

a) nested PCR or other techniques that require the binding of more than one set of primers to generate a positive result

b) repeat testing of all positives using another set of primers directed at a different target sequence

c) identification of the product using methods such as specific probes, sequencing or restriction enzyme analysis.

G2.2.26 It may be necessary to use several of these methods to achieve the desired specificity.

Nucleic acid sequencing

Standard

S2.2.18 Laboratories performing sequence-based identification of microorganisms shall have staff who have received specific training in DNA sequencing, sequence editing and database interpretation.

Commentary

C2.2.28 Because nucleic acid sequencing is a rapidly developing area with very few standardised methods, its use requires an additional level of expertise to that needed for other nucleic acid detection methods. This is particularly so in regard to knowledge and expertise in sequencing methods, the editing of sequences, the use of databases for organism identification from sequences and the use of phylogenetic software.

Current databases are largely voluntary and the reliability of organism identification is variable, although the linking of submitted sequences to refereed publications is improving. However, specialised databases using sequences from well-characterised organisms are becoming more common. Interpretation of sequence data also requires knowledge of the natural mutation rates over time and the degree of sequence variability within the target population.

C2.2.29 As sequencing includes a number of additional procedures and transfers of data, laboratories need to be fastidious about maintaining the accuracy, traceability, integrity and security of data.

C2.2.30 The target chosen for sequencing may vary depending on the purpose for which sequencing is performed. The target should be found in all strains and, if the sequence is to be used to type the organism, it should also be sufficiently variable to provide the required level of organism and strain differentiation.

C2.2.31 Ideally, the sequencing should be performed on cloned DNA derived from cultured organisms, because such DNA does not require further amplification before sequencing. However, this is usually not possible or is impractical. If product of PCR or reverse transcription PCR is used for sequencing, variability in sequence may arise from incorporation errors by the DNA polymerase or reverse transcriptase, but this is usually minor. This risk can be reduced either by repeating the amplification and sequencing, or by sequencing both strands of amplified genomic DNA.

Guideline

G2.2.27 Where recommendations have been published by reputable organisations (eg recommendations for HIV and measles genotyping) these should be followed.

G2.2.28 The segment chosen for sequencing should be found in all strains, but should also be sufficiently variable to provide the required level of organism and strain differentiation. Sequences that show excessive natural variation, such as those in quasispecies of HIV and hepatitis C, should be avoided.

G2.2.29 Organisms should be identified based on matches with several sequences from different sources, and preference should be given to sequences supplied from known reputable sources.

G2.2.30 Identification should be based on sequencing of both strands, unless identification based on a single strand has been properly validated.

G2.2.31 Where sequencing is done on DNA amplified directly from patient samples, laboratories must ensure that they check for host DNA sequences that may match the organism.

G2.2.32 Identification should use only high-quality sequences.

G2.2.33 The laboratory should record the database used for the sequence identification and the degree of certainty.

G2.2.34 Regular quality checks should be performed using known organisms (See Fredericks and Relman 1996, Turenne et al 2001).

2.3 Reports and records

Standard

S2.3.1 **Reports containing test results shall be provided to the person requesting testing with a minimum of delay, commensurate with good laboratory practice and patient care.**

S2.3.2 **Records of test requests, equipment history and service, testing procedures, results of test procedures on patient specimens, material for quality control and proficiency testing for consistency (quality assurance) shall be kept in a readily accessible form.**

S2.3.3 **Laboratory databases that contain patient information or test results must be secure, password coded and backed up at regular and frequent intervals. Appropriate measures shall be in place to prevent unauthorised physical or electronic access to the databases, especially if the databases are located in nonsecure premises, or are stored on network computers.**

Guideline

G2.3.1 Reports should be concise, unambiguous and include an appropriate interpretation of the results.

G2.3.2 Where a test is referred to another laboratory, a copy of the original report (either electronic or hardcopy) should be retained by the referring laboratory.

2.4 Retention of specimens and records

Commentary

C2.4.1 National guidelines for sample and record retention times are detailed in the NPAAC publication *Retention of Laboratory Records and Diagnostic Material*.

Nucleic acid storage conditions

Standard

S2.4.1 **Nucleic acids shall be stored in a way that minimises degradation and contamination of the sample.**

Guideline

G2.4.1 DNA and RNA samples should be prepared and diluted into 'working stocks' and 'storage stocks'.

G2.4.2 Working stocks of DNA are often stable for several months if stored in sealed containers at 4°C.

G2.4.3 To minimise degradation, storage stocks of DNA should be held at –20°C and RNA should be held below –70°C. Positive control samples should be stored below –70°C.

G2.4.4 Stocks and controls should be aliquoted to minimise damage from freezing and thawing.

2.5 Quality systems

C2.5.1 Guidelines for quality systems in pathology laboratories are detailed in the NPAAC publication Guidelines for *Quality Systems in Medical Laboratories.*

2.6 Staff

Standard

S2.6.1 **The person in charge of nucleic acid detection techniques in a laboratory shall be actively involved in determining methods and procedures, staff training and quality control procedures; in reviewing and interpreting laboratory data; and in providing laboratory reports and clinical consultation as outlined in the NPAAC publication *Standards for Pathology Laboratories* (Standard 2 — Staffing, supervision and consultation).**

Commentary

C2.6.1 The level of education and training for pathologists and scientists has been established on a national basis for pathology laboratories in general by the NPAAC publications *Standards for Pathology Laboratories* and *Requirements for Supervision of Pathology Laboratories.*

Laboratory director

Standard

S2.6.2 **The director of the laboratory must be able to demonstrate by appropriate documentation that the procedures used and tests performed are within the scope of the education, training and experience of individual scientific or technical staff members.**

Commentary

C2.6.2 Nucleic acid amplification techniques are a relatively new and rapidly expanding discipline in diagnostic laboratories. Therefore, the laboratory director should ensure that the senior practitioners within the laboratory have wide training and competence appropriate to the complexity of testing undertaken.

Staff skills

Standard

S2.6.3 **At least one senior member of staff shall have significant diagnostic or research experience with nucleic acid detection methods, including their principles and design, and problem solving in their use.**

S2.6.4 **Staff shall have or acquire biological knowledge relevant to the discipline of microbiology. Specifically, this shall include adequate knowledge of pathogenic organisms and the technique for their correct handling and containment.**

S2.6.5 **Laboratories shall comply with the guidelines of the Office of the Gene Technology Regulator when using recombinant DNA probes.**

Commentary

C2.6.3 The performance of nucleic acid detection techniques is technically challenging and is highly dependent on operator skills and facilities. Those working in the area need specific training, particularly in how to assess the validity of data and how to troubleshoot problems when they occur.

C2.6.4 Alternatively, those working in the area should undertake specific training in nucleic acid detection techniques in a laboratory with established proficiency and competence in nucleic acid detection and the development of inhouse testing.

C2.6.5 The presence of experienced supervisors and trainers is essential, given their critical involvement in error detection, error correction and problem solving.

Guideline

G2.6.1 Where a laboratory has only a limited number of staff involved in nucleic acid testing, particular attention must be paid to continuing education and the maintenance of knowledge and expertise in current techniques.

2.7 Laboratory facilities

Commentary

C2.7.1 Laboratories undertaking nucleic acid amplification should be configured to minimise the risk of contamination of samples and reagents by other samples in the laboratory or by amplified material.

C2.7.2 Laboratories undertaking nucleic acid detection from eukaryotic cells are generally at significantly lower risk of contamination than those laboratories undertaking nucleic acid detection of microorganisms. In microbiology laboratories, microorganisms are present in samples in large numbers or are cultured at high concentrations. There is also greater potential for aerosol contamination because of the small size of microorganisms compared to eukaryotic cells.

C2.7.3 Because of the presence of high levels of cultured microorganisms in microbiology laboratories and the potential number of organisms present in aerosols, three physically separate and contained areas with known airconditioned or ventilated airflows are required in a laboratory undertaking PCR-based diagnosis of microorganisms, in order to reduce the risk of cross-contamination or carry-over contamination.

Definitions

C2.7.4 The term ‘separate areas’ is used in the following sections to mean laboratory spaces that are used for nucleic acid based testing and are separated from other laboratory spaces by walls, 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. The term ‘contained area’ means a laboratory space that can be isolated from the rest of the laboratory either by walls and doors or within the working space of an instrument.

Minimum standards for a nucleic acid amplification facility

Commentary

C2.7.5 Standards S2.7.1 to S2.7.8 are the minimum standards for a PCR laboratory working with microorganisms and using non-nested PCR techniques. Standards S2.7.9 to S2.7.13 provide additional standards and requirements for laboratories performing nested PCR techniques.

Standard

S2.7.1 Three physically separate areas are required to reduce the risk of cross-contamination or carry-over contamination.

a) A dedicated contained area shall be provided for the extraction of nucleic acid from samples. This area shall be physically separate from any region of the laboratory in which microorganisms are cultured and analysed, and shall be in a separate, contained area from the reagent and postamplification areas of the PCR facility. The normal airflow into this area shall not come from the amplification/detection area or from areas of the laboratory where the target pathogens are cultured. The exhaust air from this area shall not flow into the reagent preparation or the amplification/detection areas.

b) There shall be a dedicated, separate, clean and contained area for the preparation of reagents (including dispensing of the master mix) that is physically separate from all other areas of the laboratory. The air to this area must not come from the sample preparation or amplification/detection areas, or from any other area where potential target organisms may be present.

c) There shall be a dedicated, separate and contained area for amplification and product detection that is physically separated from any area of the laboratory in which microorganisms are cultured, analysed or stored. The normal airflow pattern from this area shall not pass into the sample preparation or reagent preparation areas.

S2.7.2 The normal airflow pattern of each of the regions shall be known and the layout of the laboratory areas designed to minimise the potential for aerosol cross-contamination.

S2.7.3 **Post-PCR analysis shall not be incorporated into areas where reagent preparation or sample preparation occurs. The post-PCR area shall be positioned so as to minimise the possibility of cross-contamination of preamplification areas by not allowing direct flow of air from this area back to either the reagent preparation or sample preparation area.**

S2.7.4 **Reagents and equipment shall be limited to the appropriate sections. In particular, no nucleic acid samples shall be taken into the reagent preparation area. Samples shall be stored separately from reagents.**

S2.7.5 **Equipment from other areas shall not be taken into the reagent preparation area.**

S2.7.6 **The movement of specimens and equipment shall be unidirectional; that is, from preamplification to postamplification areas. Only sealed PCR amplification tubes and tube racks shall be carried between the preamplification area and the postamplification area.**

S2.7.7 **Where equipment (such as tube racks) is returned against the flow, it shall first be decontaminated in 2–10% hypochlorite for four hours before being moved from the postamplification area.**

S2.7.8 **Laboratory coats and gloves shall be changed before staff move to or from each area.**

Guideline

G2.7.1 Sample processing and reagent preparation should occur in different rooms. Where the areas for preparation of reagents and sample preparation cannot be separated, the provision of a positive-pressure hood or Class II biological safety cabinet (BSC) for reagent preparation and a Class II BSC for specimen preparation is strongly recommended. The air outflow from the sample preparation BSC must have a high-efficiency particle arrest (HEPA) filter and must be directed away from the reagent preparation area.

G2.7.2 Ideally, the airflow patterns in both the sample preparation area and the reagent preparation area should achieve a slight positive pressure so that air flows out of these areas.

G2.7.3 Normal airflow patterns to the product analysis area should achieve a slight negative pressure so that air flows into the area.

G2.7.4 Aerosol-resistant pipette tips or positive displacement pipettes are strongly recommended to minimise contamination.

G2.7.5 Work surfaces should be regularly decontaminated by wiping with 2–10% hypochlorite or another similar agent. Instrumentation such as microfuges, heating blocks and waterbaths should be cleaned regularly with 2–10% hypochlorite.

G2.7.6 Instruments capable of producing aerosols (eg vortex mixers, PCR machines and microfuges) are to be placed at as great a distance from preparation areas as possible.

G2.7.7 While not specifically dealt with in these standards and guidelines, the use of robotic equipment should adhere to the principles outlined above.

Additional standards and requirements for nested PCR

Commentary

C2.7.6 The standards and requirements below apply, in addition to those outlined above, when using nested PCR techniques.

Standard

S2.7.9 **Reagent preparation, specimen processing and amplification/product detection shall be carried out in three separate contained areas. Where material from the first-round PCR is aliquoted in an open environment, there shall be a fourth area.**

S2.7.10 **Normal airflow patterns in the laboratory shall direct air out of the reagent preparation area in order to avoid contamination of the reagents. If this cannot be achieved, reagent preparation shall be carried out in a positive-pressure hood or a Class II BSC within the contained area.**

S2.7.11 **Normal airflow patterns in the laboratory shall direct air out of the sample preparation area in order to avoid contamination of the samples. This is not necessary if the sample preparation area is distant from the other nucleic acid detection processing areas and has no airflow connections with the other areas.**

S2.7.12 **Normal airflow patterns in the laboratory must not direct air from the amplification/detection area into the reagent preparation or sample preparation areas.**

S2.7.13 **All manipulations of samples or of materials liable to contain amplified or unamplified nucleic acids shall be carried out in a Class I or II BSC with a HEPA filter on the exhaust.**

Commentary

C2.7.7 Because of the nature of the technique, nested PCR requires the most stringent guidelines. The most important aspects are:

a) adequately trained staff

b) use of aerosol-resistant pipette tips

c) constant vigilance against methodological causes of contamination.

Guideline

G2.7.8 Where a laboratory carries out large numbers of nested PCR reactions, which increases the potential for contamination, or where there are recurring or uncontrolled contamination problems, the following measures should be implemented:

a) The air conditioning/ventilation services to the reagent preparation and specimen processing areas should be at positive pressure in relation to adjoining areas, or they should be separated from adjoining areas by an anteroom that is at negative pressure to both its adjoining areas.

b) The air conditioning/ventilation services to the amplification and product detection areas should achieve a negative pressure in relation to adjoining areas, or should be separated from adjoining areas by an anteroom that is at negative pressure to both its adjoining areas.

c) All manipulations of material liable to contain amplified nucleic acids should be done in a dedicated, externally vented Class I or Class II BSC.

d) Ceiling-mounted ultraviolet light fixtures that are operated for 20–30 minutes after hours should be used, as these will assist in reducing environmental contamination.

Glossary

Allele-specific oligonucleotide hybridisation	Hybridisation of labelled allele-specific oligonucleotides to filter-bound target DNA or the converse.
Blotting	Methods for detecting and identifying genomes of microorganisms and mutations or polymorphisms in human DNA. The techniques employ the hybridisation of labelled DNA probes to filter-immobilised target nucleic acid sequences. Identification of variant sequences depends on differences in mobility or hybridisation intensity of DNA fragments hybridising with the probe DNA at a specific temperature and salt concentration.
Branched DNA (bDNA) assay	Method based on signal amplification rather than physical amplification of the nucleic acid target. The method uses a very large branched molecule to bind to the target DNA sequence. The branches of the large molecule are then detected using an enzymatic or chemiluminescent method.
Characterisation of DNA	To detect changes in DNA, there are four broad categories of tests, which are based on hybridisation, sizing, quantitation and determination of DNA sequence.
Competence	The ability of an individual to perform a specific job or task. (ISO 9000, 9001)
Denaturing high-performance liquid chromatography (DHPLC)	A method that relies on a reduced melting temperature of the heteroduplex compared with the homoduplex. DHPLC employs both heat and chemical denaturants to melt DNA. Heteroduplex molecules containing a mismatch will elute from an affinity column before homoduplex molecules, thereby indicating the presence of DNA sequence variation.
DNA microarrays (colloquially known as 'DNA chips')	Detection of DNA sequences by hybridisation to complementary oligonucleotides or DNA fragments fixed to a solid phase. At the time of publication these are not in routine use but are likely to be integrated into routine diagnostics over the next few years.
DNA sequencing	Sequencing uses a variant of the PCR technology with labelled nucleotides and polyacrylamide gel electrophoresis to determine the linear sequence of DNA bases in a PCR product.
Ligase chain reaction (LCR)	A method similar to PCR but involving joining DNA primers complementary to the target DNA sequence using a heat-stable DNA ligase enzyme.
Minisequencing	A method to detect single nucleotide variations in amplified DNA, based on the extension of a detection primer that binds immediately adjacent to a variable nucleotide position.
Multiplex ligation probe amplification	A quantitative PCR method involving hybridisation and ligation of oligonucleotide probes, followed by PCR amplification of the ligated probes rather than the sample nucleic acids.

Nested PCR	Nested PCR improves the sensitivity and specificity of PCR by carrying out two rounds of PCR amplification. The second round of amplification uses primers directed to sequences within the first-round product.
Nucleic acid amplification techniques	Include a range of very sensitive methods of detecting, identifying and quantifying minute amounts of nucleic acid (DNA or RNA). Using these techniques it is possible to detect and identify microorganisms, specific genes, mutations and polymorphic DNA, as well as to quantify RNA transcripts or DNA copy number. A number of different nucleic acid detection tests based on nucleic acid amplification are currently in use in Australian laboratories. These include PCR and other nucleic acid amplification techniques.
Nucleic acid hybridisation tests	Include a range of methods for the detection and identification of mutations and polymorphic DNA, and for the detection and quantification of microorganisms. A number of different nucleic acid hybridisation tests are currently in use in Australian laboratories.
Nucleic acid sequence based amplification (NASBA)	See transcription-mediated amplification (TMA) below.
Polymerase chain reaction (PCR)	The first nucleic acid amplification method to be developed. Using primers (short oligonucleotides) complementary to the ends of each strand of the DNA sequence of interest, a heat-stable DNA polymerase is used to extend these primers and create a copy of the DNA. This is cyclically repeated to amplify the sequence millions of times.
Postamplification techniques	A group of associated techniques that can identify or characterise amplified DNA or RNA. Techniques include hybridisation, characterisation (sizing and quantification), denaturing high performance liquid chromatography (DHPLC) and DNA sequencing.
Probe amplification techniques	These include the branched DNA and Q-beta replicase assays.
Protein truncation tests	The protein truncation test is based on the ability to amplify target DNA by PCR and at the same time incorporate into the PCR product a promoter sequence and Kozak consensus sequence. Together, the promoter and Kozak consensus sequence are required for an in vitro transcription and translation assay using the necessary protein machinery present in either rabbit reticulocytes or wheat germ lysates. If a protein truncating mutation is present within the target sequence of interest, the resultant protein will be shorter than expected. Usually, an internal control is present (the wild type sequence) that is used to determine the presence or absence of a premature stop codon by comparison of protein size on a polyacrylamide gel. This assay cannot be used for the identification of missense changes or silent polymorphisms.

Q-beta replicase assay	Involves using a modified RNA template, which contains sequences complementary to the target and the enzyme Q-beta replicase. The probe hybridises to the target and can be amplified millions of times.
Quantitative PCR (Q-PCR)	An application of PCR to determine the amount of a target sequence in a sample of DNA/RNA.
Real-time PCR	A modification in which the PCR product is measured as it is produced, using either binding of a dye or a fluorescent marker. As the rate of rise is proportional to the amount of target, it can be used to quantify the amount of DNA. Examples of this are the commercial LightCycler and TaqMan systems.
Reverse transcription PCR (RT-PCR).	As PCR is only able to amplify DNA, detection of RNA involves an initial step of using the enzyme reverse transcriptase to convert RNA into complementary DNA (cDNA) prior to PCR.
Strand displacement amplification (SDA)	An isothermal DNA amplification, using target DNA to make cDNA that lacks the restriction enzyme (RE) site. This cDNA is used as a template to bind a primer that contains the RE site, resulting in double-stranded DNA with an RE site at one end of one strand. The RE nicks that strand and the DNA polymerase binds at the nick and then moves along the strand. As it makes a copy of the complementary strand, it displaces the existing homologous strand. This is repeated to create the amplification.
Transcription mediated amplification (TMA) and nucleic acid sequence based amplification (NASBA)	Similar isothermal processes that make RNA copies of ribosomal RNA or DNA. A primer binds to target ribosomal RNA and reverse transcriptase is used to make a complementary DNA strand. The cDNA is used as a template to make double-stranded DNA, which is then transcribed by RNA polymerase to multiple copies. Each of these copies can then be used as target RNA to repeat the process.
Validation	Confirmation, through the provision of objective evidence, that requirements for a specific intended use or application are fulfilled. (ISO 9000)

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