

Molecular diagnosis

Molecular Diagnosis of Infectious Diseases
Module 1: Introduction to Molecular Diagnosis at AAHL

CSIRO – Livestock Industries
Australian Animal Health Laboratory
Molecular Diagnosis

Quality system ensures confidence in diagnostic results

Laboratory accreditation standards and guidelines for nucleic acid detection and analysis

AS ISO/IEC 17025:1999, *General requirements for the competence of testing and calibration laboratories*

- 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.

Confidence in diagnostic results

Use multiple or combination of tests and don't rely on a single test

Manage risk of false positive or false negative results through appropriate controls and procedures

Follow guidelines for quality system and standard operating procedures (SOPs) for laboratory services and facilities

Sensitivity and contamination

Most nucleic acid tests for microorganisms are designed to detect the presence or absence of a particular organism and are intended to maximize 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 or gene fragments amplified by PCR in microbiology laboratories.

The small size of microorganisms or PCR fragments compared to eukaryotic cells creates greater potential for aerosol contamination. For these reasons, stringent conditions are applied to microbiological testing.

Standards and controls

Standards and controls are a routine part of the quality systems for all laboratory testing. However, if these are not readily available for nucleic acid amplification testing, 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 diagnostic sample.

Use of standard operating procedures (SOPs)

Laboratory services

Nucleic acid detection techniques depend on the correct performance of all components of testing procedures, including,

- specimen collection,
- transportation,
- reagent preparation,
- nucleic acid isolation,
- amplification,
- product visualization,
- data transcription,
- data interpretation,
- reporting,
- record keeping,
- sample storage and

Sample collection

Specimens shall be collected in accordance with written specimen collection protocols to reduce the likelihood of sample contamination. The precise method of sample collection, initial processing and transportation depends on the specimen concerned and the nucleic acid target (DNA or RNA).

Wherever possible, nucleic acid detection tests should be performed on dedicated samples or on aliquots taken before other tests are performed.

Samples that have been used for other tests before nucleic acid detection testing are at increased risk of contamination.

Where it is necessary to perform nucleic acid detection tests on samples that have already been used for other purposes the results should be confirmed on a dedicated sample (if one is available).

Single-use disposable collection equipment should be used.

Sample preparation

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 and the condition of specimens received by the laboratory must be validated, and procedures must be detailed in the laboratory methods manual. The quality of nucleic acid prepared from a specimen has a major effect on the subsequent probability of successfully performing the test.

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

Sample integrity

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. RNA sample should be processed as rapidly as possible after collection to minimize RNA degradation by ribonucleases.

Specific instructions for handling samples to minimize nucleic acid degradation should be included in all relevant manuals and should be available to staff in collection centers.

Where samples of marginal quality, quantity or integrity have been received, the laboratory should 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

- **Preparing reaction mixes**
- **Nucleic Acid Extraction Methods**
 - RNA, DNA, manual, robotics
- **cDNA Synthesis**
- **Conventional PCR**
 - one-step RT-PCR
- **Agarose gel electrophoresis**
 - Preparation, running and documentation
- **Real-Time PCR**
- **DNA sequencing**
- **Supplemental testing**

(More details in separate Modules)