Quality Control in Infectious Disease Serology
NRL is a:

- NATA-accredited proficiency testing provider, complying with ISO 17043: 2010
- World Health Organization (WHO) Collaborating Centre for Diagnostics and Laboratory Support for HIV and AIDS and Other Blood-borne Infections

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and distributed in the week beginning 2011/June/14
1 INTRODUCTION

Australian medical testing laboratories are required to conform to the Australian Standard AS 4633 (ISO 15189) if they are to obtain reimbursement from the Australian Government Medicare Benefits Schedule. The standard is supplemented by several National Pathology Accreditation Advisory Council (NPAAC) guidelines. These documents outline the requirements of medical testing laboratories and include the requirements for quality control (QC) testing and result monitoring. This document reviews the Australian regulations, and provides additional information to those laboratories introducing QC testing and monitoring.

Appendix 1 is an extract from the National Association of Testing Authorities (NATA) AS 4633 (ISO 15189) Field Application Document (FAD). Section 5.6.1 relates to QC. In documents published by NATA, the term “should” is used where guidance is provided but does not preclude other acceptable practices. Section 5.6.1 (ii) states “Controls independent of those produced by the manufacturer of the test or analyser should be used.”

There are two main sources of independent controls to facilitate compliance with this requirement. Laboratories may purchase QC materials from commercial suppliers or manufacture their own. In either case, it is important to understand the issues relating to QC manufacture and use, and to ensure that these are considered in the selection of QC materials.

QC samples are manufactured by diluting a stock material in an appropriate matrix. Stock materials ideally have high concentrations of the markers in question and are available in sufficient volume to enable production of QC samples for several years. For the purposes of serology, the matrix in which a stock material is diluted is usually pooled normal human plasma or serum.

2 Commercial QC materials

There are a number of suppliers of QC materials suitable for use in infectious disease serology. A list of known QC suppliers is attached (Appendix 2). When choosing a QC sample the following should be considered:

2.1 Physical Attributes:

a. Storage temperature and stability:

Manufacturers may direct the user to store the QC liquid samples refrigerated or frozen. Users should follow the manufacturers’ instructions. On receipt of the QC sample, note the expiry date and the period of time after opening during which the sample can be used (open vial dating). A vial should be labelled with the date when it is opened and discarded after the expiry date or when the open vial dating is exceeded.

Occasionally QC samples may supplied be in a lyophilised form. These samples should be re-constituted carefully as specified by the manufacturer, using the correct reconstitution matrix. Allow the lyophilised sample to dissolve completely. It is useful to invert the reconstituted, sealed vial to ensure any lyophilised material in the lid is also dissolved.
b. Sample volume:

QC samples are often available in various volumes. When choosing a QC sample consider the volume your laboratory may use in the context of the QC sample’s shelf life. If the QC sample will not be used by the time of expiry, the manufacturer may allow for the sample to be aliquotted into smaller volumes and stored frozen. Follow the manufacturer's instructions if this is the case. Otherwise the stability of the QC sample under conditions other than that specified by the manufacturer should be validated.

c. Tube type and labelling:

If an automated testing system is used in the laboratory, QC samples provided in vials that fit into the instrument and that are bar-coded are convenient. If a QC sample needs to be aliquotted into a new container for use on the instrument, care must be taken to label the new container appropriately and to ensure that it is adequately sealed if the sample is to be stored between uses.

2.2 QC Sample Characteristics

a. Reactivity:

A QC sample should be chosen based on the reactivity of the sample in the assay(s) in which it will be used. The reactivity of the QC sample must fall in the linear part of the dose - response curve that is produced when serial dilutions containing the marker are tested in the assay in question. At this level changes in the assay system are able to be detected. Targeting the linear part of the curve ensures that a change in reactivity reflects a proportional change in concentration of the marker. If the reactivity is too high or low, then the reactivity may not change proportionally to the concentration of the marker. Ideally, the QC sample reactivity should be about 2 to 4 times the cut-off of the assay.

It should not be assumed that if a sample reacts appropriately in one assay that it will show similarly appropriate reactivity in another.

b. Multimarker samples:

Many QC samples are manufactured as multimarkers (MM), i.e. multiple analytes included in the same sample. This is advantageous because:

- Only one QC sample needs to be loaded when tests for multiple analytes are conducted on the same instrument;
- MM QC sample volume is used more quickly reducing waste associated with expired reagent; and
- Generally, purchasing MM QC samples is less expensive than purchasing individual QC samples.

However, the risk involved with the use of MM QC samples is that the reactivity of each marker may not be optimised for the assay with which it is used. Therefore appropriate reactivity of all markers should be confirmed before implementation of a MM QC sample. MM QC samples may be more expensive that single marker QC samples.
c. Preservatives:

To ensure sterility and stability, preservatives are often included during manufacture of QC samples. Many different preservatives can be used; however some factors should be considered. Sodium azide can be highly toxic and the heavy metal salts of azide pose a risk of explosion if allowed to crystallise. Some preservatives may interfere with the assay’s chemistry, e.g. the addition of sodium azide to QC samples has been known to interfere with QC sample reactivity when tested in some assays. It should not be assumed that the use of preservatives will affect reactivity in the same way from one assay to another. Some preservatives such as Merthiolate (Thiomerasol) contain mercury. Other preservatives that are used include Bronidox, ProClin 300 or antibacterial compounds such as Gentamicin sulfate. It is important to review the MSDS of any QC product introduced into the laboratory. Further, it is important to review the manufacturer’s instructions for the assay with which the QC sample is intended to be used to ensure its compatibility.

d. QC batch-to-batch variation:

One important use of QC results is to monitor batch-to-batch variation of assays’ reagents. To monitor these effectively, we must be confident in the homogeneity and stability of the QC sample. Each new batch of a QC sample is likely to have slightly different reactivity compared with the previous batch, although this should be minimised in commercial QC samples through compulsory compliance with manufacturing standards. Therefore it is important to identify a source of QC sample that has the same batch available for a long period of time. It is optimal to use a single batch of QC sample for periods up to, or more than, one year. This can be achieved by establishing a standing order with the QC sample supplier or choosing a supplier that can guarantee supply of the same batch of QC sample over that time period.

If new batches of QC sample cannot be avoided, then QC samples with minimal batch-to-batch variation are preferred. Part of the evaluation of prospective QC samples is to review data from multiple batches tested on the same assay over time.

e. Regulation of QC samples:

Until recently in Australia, QC samples were exempt from regulation by the Therapeutic Goods Administration. With the implementation in July 2010 of a new framework for regulating In Vitro Diagnostic Devices, all new, commercial QC samples now need to comply with the requirements of the Therapeutic Goods (Medical Devices) Amendment Regulations prior to their becoming available on the Australian market. Those QC samples that were already in the Australian market at the time of the implementation of the new framework, both commercial and in-house, will need to be assessed under the new regulations by July 2014. Details of the requirements can be found on the TGA website (http://www.tga.gov.au/regreform/regulations.htm).

Different jurisdictions have different regulatory requirements for QC samples. However, a CE marking or FDA clearance indicates that the manufacturer has complied with manufacturing regulations in Europe or the United States respectively. Note that compliance with these regulations does not indicate the quality of the performance of the material, rather the quality of manufacturing and safety of the product.
3 Non-Commercial QC materials:

The NATA FAD indicates that, if an independent, commercial QC material is unavailable then the laboratory can choose to i) use calibrator material supplied by the manufacture, or ii) use pooled patient serum/plasma. Generally option i) will not apply to infectious disease serology. Calibrator material is rarely available in sufficient quantities to guarantee availability of the same batch of material for an extended period of time.

Many laboratories use pooled patient specimens as QC samples, particularly when commercial materials are not available. This is typically the case when testing for local endemic diseases e.g Q fever, Ross River Virus, Barmah Forest Virus or when testing for a marker is specialised and/or infrequent e.g. *Bordetella pertussis* antibody, hepatitis E virus markers.

When developing an in-house QC sample, a written protocol should be developed. If the QC sample is manufactured by diluting a stock sample containing a high concentration of the marker, testing to determine the concentration of the marker and the dilution that will produce optimal reactivity in the assay in question should be undertaken, recorded and the results reviewed. Where possible, sufficient volume of the stock material should be stored to ensure sufficient QC sample production for extended periods of time. Access to an appropriate base matrix, negative for the marker and also for blood-borne viruses, is also required. The base matrix should not have interfering substances, be clear of fibrin and other particulate matter and have low levels of lipid and haemoglobin. Commercial base matrices are available and should be considered.

QC samples must be demonstrated to show stability and homogeneity. Commercial manufacturers of QC materials undertake both real-time and accelerated stability testing and must confirm the open vial dating and transport stability of the product. Stability testing of in-house QC samples is essential as variation in QC sample reactivity over time may be misinterpreted as being due to reagent or process variation. Laboratories that choose to store in-house QC samples at -20°C in single or multiple use aliquots should validate these storage conditions for each in-house QC sample produced.

In-house QC sample vials should be labelled appropriately and include the name of the QC sample, the date of manufacture and/or expiry date, the batch number and the required storage conditions. A written protocol for the storage and use of the sample should be available.

4 Monitoring of QC reactivity

Sections iv) to vii) of the NATA FAD relate to the monitoring of QC test results. Systems for presenting the results graphically and documented procedures for reviewing the results are required. There are many tools available for this purpose including QC monitoring systems on immunoassay instruments, laboratory information systems, stand-alone QC packages, Microsoft Excel or web-based peer comparison QC monitoring systems.

It should be noted that the use of QC monitoring systems that record and monitor test results from a single laboratory provides the user with a measure of the imprecision in a test system. The graphical presentation and statistical analyses show the daily variation and can be useful to detect unexpected deviation.

To estimate the bias in a testing system using QC sample results, a peer comparison program should be considered. Bias can be determined by comparing the mean of results
reported by a given laboratory for a QC sample / assay combination with the mean reported by other laboratories using the same combination. Several peer comparison programs are available such as BioRad’s Unity and NRL’s EDCNet.

5 Review of QC test results

A laboratory should have a written procedure outlining the actions required when unexpected QC sample results are detected. There are many actions that may be undertaken in this event but the description of these is beyond the scope of this document. However, the procedure should state what constitutes an acceptable range into which the QC sample results should fall, what actions are required if the test results fall outside the range and which staff members are responsible for these actions.
Appendix 1. AS 4633 (ISO 15189) Field Application Document

Medical Testing

Supplementary requirements for accreditation

5.6 Assuring the quality of examination procedures

5.6.1 Internal quality control

Guidance on QC issues should be sought from publications of the relevant professional societies.

(i) The QC material used must cover the analytical concentrations encountered. Low/normal/high, normal/abnormal, positive/negative, reactive/non-reactive controls, as appropriate for the test, must be performed.

(ii) Controls independent of those produced by the manufacturer of the test or analyser should be used. Mean, standard deviations (SD) and ranges supplied by manufacturers may not always provide adequate control of assays. See (iv)

If independent commercial QC material is unavailable the following approaches should be considered:

a) where QC material is obtained from the manufacturer of the reagents or calibrator, information on the production of QC material should be sought from the manufacturer to determine the extent of independency from the kit calibration process. This should include the source of the QC material, traceability (including value assignment) and matrix matching.

b) pooled patient samples.

(iii) Where calibration of an assay is required, appropriate material must be used as a calibrator. If the material selected is not intended for use as a calibrator, assigned calibration values must be substantiated. Acceptable ranges (confidence limits) must be defined for internal quality control material. As far as practicable laboratories must define acceptable ranges based on the current analytical performance. Where acceptable ranges are set to limits other than +/- 2SD based on current analytical performance, the rationale for the limits must be documented.

(iv) Acceptable ranges (confidence limits) must be defined for internal quality control material. As far as practicable laboratories must define acceptable ranges based on the current analytical performance. Where acceptable ranges are set to limits other than +/- 2SD based on current analytical performance, the rationale for the limits must be documented.

(v) Numerical QC results should be presented graphically to assist in the early detection of trends.

(vi) The laboratory must have a system of long-term monitoring of internal quality control results to assess method performance.

(vii) There must be documented evidence of review of internal quality control results.

(viii) A protocol for action to be taken where QC results fall outside acceptable ranges must be documented. This must include consideration as to whether test results should be withheld and whether previously issued results should be recalled.

(ix) For tests which incorporate immunochromatographic methodology such as D-dimer and ß-hCG the use of additional controls should be considered. It is acknowledged that these assays
incorporate an internal check to validate the integrity of the reagent and cartridge, however, ambiguity may arise in the interpretation of faint bands.

Additional discipline-specific QC requirements are detailed below.

**Immunology**

Appropriate controls must be run with each ELISA plate. Optimally, non-kit controls should be included to monitor performance over time, and enable the determination of inter-lot batch variation. Appropriate negative controls should be included on each ELISA plate. This is also applicable to serological testing utilising this methodology.
Appendix 2. List of suppliers of infectious disease testing QC materials

Abacus ALS Australia
12 Mowbray Tce
East Brisbane
Brisbane QLD 4169
Free Call : 1800 222 287
Free Fax : 1800 287 222
Ph : +61 7 3391 9777
Fax : +61 7 3391 9799
Email : info@abacus-als.com

Bio-Rad Laboratories Pty. Ltd.
Level 5, 446 Victoria Road
Gladesville NSW 2111
Ph: +61 (2) 9914-2800
Freephone: 1-800-224 354 (within Australia only)
Fax: +61 (2) 9914-2889
E-mail: Sales.Australia@bio-rad.com

NRL
4th Floor Healy Building
41 Victoria Pde
Fitzroy, VIC 3065
Ph: +61 3 9418 1111
Fax: +61 3 9418 1155
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Accurun QC range
Virotrol QC range
BioRad QC range
PeliSpy QC range