NRL EQAS
Science of Quality

Supplementary Information for all Final Reports

NRL is:

- **Accredited by NATA as a Medical Testing Laboratory compliant with ISO 15189;**
- **Accredited by NATA as a Proficiency Testing Provider compliant with ISO 17043, with NATA Accreditation Number 14253;**
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- **Certified by BSI for Quality Management, as compliant with ISO 9001;**
- **Certified by BSI for Safety Management, as compliant with AS 4801;**
- **A World Health Organization (WHO) Collaborating Centre for Diagnostics and Laboratory Support for HIV and AIDS and Other Blood-borne Infections.**

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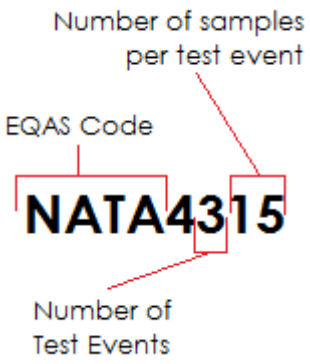
1 INTRODUCTION

NRL generally provides three test events per calendar year for each of its External Quality Assessment Schemes (EQAS), with the only exceptions being the HIV Drug Resistance Genotyping and HIV Co-receptor Tropism schemes. These schemes have two test events scheduled per calendar year.

The EQAS provided by NRL are listed below according to their EQAS code:

- CMVN Cytomegalovirus Qualitative and Viral Load Molecular
- CTNG *C. trachomatis* and *N. gonorrhoeae* Qualitative Molecular
- DTSB Dried Tube Hepatitis B Virus Viral Load Molecular
- DTSC Dried Tube Hepatitis C Virus Viral Load Molecular
- DTSI Dried Tube Human Immunodeficiency Virus Viral Load and EID Molecular
- HAPN Hepatitis A and Parvovirus Qualitative and Viral Load Molecular
- HBVL Hepatitis B Viral Load Molecular
- HCVG Hepatitis C Genotyping Molecular
- HCVL Hepatitis C Viral Load Molecular
- HCVN Hepatitis C Qualitative Molecular
- HEPM Hepatitis Comprehensive Multimarker Serology
- HIVC Human Immunodeficiency Virus Comprehensive Serology
- HIVG Human Immunodeficiency Virus Drug Resistance Genotyping Molecular
- HIVL Human Immunodeficiency Virus Viral Load Molecular
- HIVT Human Immunodeficiency Virus Co-receptor Tropism Molecular
- HPVN Human Papillomavirus Qualitative Molecular
- HSVN Herpes Simplex Virus types 1 & 2 Qualitative Molecular
- HTLV Human T-Lymphotrophic Virus Comprehensive Serology
- LEPN Leptospirosis Qualitative Molecular
- MMBS Multimarker Blood Screening Serology
- MTBN *Mycobacterium tuberculosis* Qualitative and Drug Resistance Molecular
- NATA Multimarker Blood Screening Molecular
- POCS Point of Care Comprehensive Serology
- STBV Sexually transmitted / Bacterial vaginosis Multimarker Qualitative Molecular
- TORC Toxoplasma, Rubella & Cytomegalovirus Comprehensive Serology
- TREP *Treponema pallidum* (Syphilis) Serology

The EQAS codes contain further information about each scheme:



2 METHODS

The composition of the samples provided for all schemes are shown in Appendix A of the relevant EQAS Final Report. All samples were manufactured according to NRL procedures, ensuring homogeneity. The storage and transport conditions for the EQAS samples have been extensively validated to assure sample stability for the duration of the test event.

2.1 Molecular Schemes

Positive samples provided for the molecular schemes were prepared by diluting the positive stock material in one of the matrices listed below:

- Normal human plasma (NHP)
- Human serum
- OptiMatrix, a matrix designed to mimic cerebrospinal fluid
- Phosphate buffered saline (PBS)
- Liquid based cytology (LBC) medium
- Ellinghausen-McCullough-Johnson-Harris (EMJH) medium
- Synthetic whole blood
- Synthetic respiratory material

Several molecular schemes (while diluted into one of the listed matrices) are presented to participants as either dried sample tubes (DTS) or dried swabs. DTS material will often be shipped with its own reconstitution buffer consisting of sterile PBS.

Samples determined to be “Negative” for a given analyte will consist of the dilution matrix on its own.

Samples provided for the Hepatitis C Genotyping scheme were further characterised by nucleic acid sequencing of the HCV core gene by an external laboratory. The nucleotide sequence obtained for each sample was assessed for homology to reference nucleic acid sequences stored in the online databases of the National Centre of Biotechnology Information. The samples were assigned the genotype/subtype of the reference sequence to which it displayed the greatest homology.

Samples provided for the Multimarker Blood Screening Molecular scheme were calibrated against the WHO international standards for HIV-1 (97/650), HBV (97/750) and HCV (06/102).

2.2 Serological Schemes

Samples provided for the serological schemes may have been prepared from either an individual plasma donation or a pool of multiple donations. Pooled samples were prepared by mixing volumes of at least two donations that had similar antibody and antigen profiles.

All samples were tested in a range of assays to confirm their reactivity. *Treponema pallidum* (Syphilis) serology samples were not tested for anti-*Treponema pallidum* IgM.

2.3 Evaluation of results

Results reported by participants using the same test method were grouped for analysis. This is known as a **peer group**.

2.3.1 Qualitative Evaluation

Results reported by participants for assay interpretations and status (where applicable) were compared with the relevant reference results.

In instances where an assay interpretation was not provided for a sample tested in a rapid or agglutination assay, the reactivity for the sample that was reported by the participant was taken to also be the assay interpretation.

An aberrant assay interpretation is one that did not agree with the relevant reference result. An aberrant status is one that did not agree with the relevant reference result or consensus status (if applicable).

False positive results (positive results reported for a negative sample) and false negative results (negative results for samples with a nucleic acid concentration above the limit of detection, where known, for the relevant assay) were defined as aberrant.

2.3.2 Viral Load Evaluation

The $\log_{10}$ transformed results reported by participants were analysed. The peer group mean was determined and results that differed by more than 0.5 $\log_{10}$ from the peer group mean were identified as aberrant.

False positive results (positive results reported for a negative sample) and false negative results (negative results for samples with a nucleic acid concentration above the limit of detection, where known, for the relevant assay) were also defined as aberrant.

2.3.3 Statistical analyses

An ISO 13528 method was used to identify outlying results.

Outlying test results are numerical values that are found to be statistically different from other test results reported by the peer group. Occasionally, results may be identified as outlying by the report author, causing it to be removed from analysis. This will only occur when result inclusion will erroneously bias the statistical analysis e.g. when the result is from the testing of an incorrect sample or submitted under an incorrect assay.

Statistical analyses from peer groups with fewer than five results were not evaluated as statistics based on very small numbers may not be reliable.

2.3.4 Sample carryover

Participants were asked in the “Handling and Reporting Instructions” to test the samples in order, according to their sample identification. Potential sample carryover may have occurred when the test results of negative samples were:

- reactive but with significantly lower signals than other reactive samples, or
- negative but with high signals identified as outlying.

2.4 Reviewing results

Each participant should review their Performance Report for any results that may have been identified as statistically outlying, aberrant and/or manually flagged as outlying (designated with ●, ♦ and ■ respectively). These participants should refer to the table in Section 3 TROUBLESHOOTING of this supplemental information document. These results may be clarified further in the DISCUSSION section of the relevant final reports.

Summaries of the performance of all the test methods used in each scheme are available as Participation Statistics Reports (Qualitative or Quantitative). These summaries may be useful in comparing the performance of all the test methods in each scheme.

2.5 Evaluation Appeals Process

A participant may appeal the data analyses or comments represented in Final Reports and/or individual laboratory Performance Reports should the participant have any concerns about them.

Follow the steps outlined below:

1. Carefully review the Final Report in conjunction with all the reports and data that may influence what has been stated, including the Laboratory-specific Performance Report, participation statistics and copies of result printouts or worksheets;

2. Once step 1 has been completed and the conclusion remains that there are grounds for appeal, contact your local collaboration support representative or email oneworldaccuracy@nrlquality.org.au;

3. A support team member will reply as soon as practicable to confirm receipt of your request and give you details of who will be handling your request and a timeframe for formal response;

4. All matters will be addressed with either an amendment to the Final Report or Performance Report, or an erratum reported in the following test event where the error has been proven to be true. Otherwise, the participant will receive an explanation of the reason(s) why no amendment will be made.

3 TROUBLESHOOTING

Table 1. Troubleshooting common causes of outlying and/or aberrant results. Causes listed may be applicable to both Molecular and Serological assays

Type of error	Possible cause(s)
Sample mix-up	Two or more samples may have been interchanged, resulting in both outlying and aberrant results. Sample mix-up may occur during specimen reception or during testing.
Transcription	Common causes of transcription errors include: - Interchanging the results for two or more specimens; - Entering incorrect results; - Selecting the wrong assay or assay version in OASYS; - Entering values in the incorrect field (e.g. OD as S/Co); - Entering values in the incorrect unit (eg. IU/mL instead of $\log_{10}$ IU/mL); - Using a comma instead of a dot to denote a decimal point; - Selecting the incorrect assay interpretation. It is recommended that all results that are manually transcribed or entered via OASYS should be checked by a second individual in order to avoid such errors.
Inappropriate testing strategy followed	**Testing negative samples in an immunoblot:** Samples that are negative on screening should not be tested in an immunoblot as the samples may display non-specific reactivity and be reported indeterminate or falsely reactive unnecessarily. Only samples reactive on screening should be tested in an immunoblot. **Failing to conduct neutralisation testing on samples showing reactivity on antigen tests**: Participants that do not perform neutralisation testing may report falsely reactive test results. It is recommended that neutralisation be performed and reported on all samples screened reactive and results of other testing be reviewed to distinguish true from false reactivity. **Using simple rapid tests of inadequate sensitivity:** Simple rapid tests are often not as sensitive as EIA or instrument based tests. This can lead to falsely negative results in samples with low levels of analyte.
Aberrant serology status (Please note: This section refers to overall status of a sample after having tested in multiple tests. This section applies to HCV, HIV and HTLV Serology EQAS only.)	**Incorrect data entry:** When performing serological testing, participants use a variety of testing strategies that may involve testing with a combination of screening, supplemental and/or confirmatory assays. A final serology status (final interpretation) of "Positive", "Negative" or "Inconclusive/Indeterminate" should only be assigned to a sample after consideration of all test results (screening, supplemental and confirmatory). Participants that test samples using only one assay should refer any reactive samples for further testing in order to distinguish true from false reactivity. When reporting a final interpretation in OASYS, a participant that would not report a status/final interpretation but would refer a sample for further testing should submit their results as: Serology Status *Do not select any option* Select problem code *Would not report a status based on these results* Refer for Further Testing Yes. Otherwise, participants that would report a status/final interpretation should assign an appropriate status and select "No" for "Refer for Further Testing".

Table 1. Troubleshooting common causes of outlying and/or aberrant results (continued).

Type of error	Possible cause(s)
Outlying and/or aberrant test results due to random error	Sporadic test results identified as outlying and/or aberrant can be classified as random events. Possible causes of random outlying and/or aberrant results include: - Insufficient mixing of sample, especially following freezing; - Poor pipetting; - Ineffective or inconsistent washing; - Transcription errors; - Sample mix-up; - Cross-contamination or carryover; - Presence of inhibitors to the PCR.
Outlying and/or aberrant test results due to systematic error	A series of test results identified as outlying and/or aberrant may be due to a systematic problem. Systematic problems may be due to: - Reagents contaminated, expired or subject to batch variation; - Instrument error or malfunction; - Insufficient washing; - Incorrect wavelength used to read the assay result; - Cycling times too long/short or temperature too high/low; - Incubation time too long/short or temperature too high/low; - Insufficient mixing/centrifuging before testing; - Incorrect storage of test kits and/or reagents; - Contamination of master-mix, extraction areas or equipment; - Ineffective extraction process; - Degradation of master-mix components; - Suboptimal primer design (in-house assays).