NRL EQAS
Science of Quality

Supplementary Information for all NRL EQAS Final Reports

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Supplemental information prepared by NRL EQAS

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1 INTRODUCTION

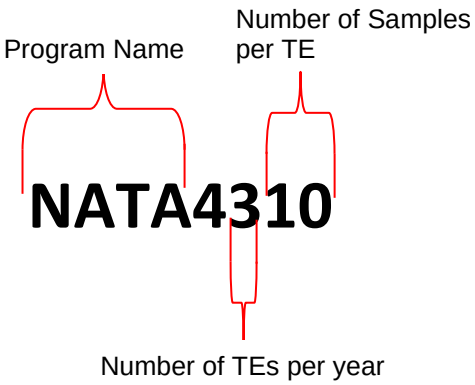
NRL provides panels of samples for each of the three Test Events (TEs) per calendar year for its External Quality Assessment Schemes (EQAS) it offers.

The Schedule of TEs can be found on OASYS dashboard or in the NRL EQAS Catalogue (https://www.nrlquality.org.au/wp-content/uploads/2022/03/2023-NRL-EQAS-Catalogue.pdf/). Occasionally, NRL may extend the TE timeframe for some or all programs. In these circumstances, NRL and 1WA will communicate with affected participants before the original closing dates.

The programs for which EQAS panels are manufactured and managed by NRL are listed below according to their program names:

- CMVN CMV Molecular;
- CNTP *C. trachomatis*, *N. gonorrhoeae* & *T. vaginalis* Molecular POC;
- COVS SARS-CoV-2 Antibodies;
- DTSB Dried Tube Sample HBV Molecular POC;
- DTSC Dried Tube Sample HCV Molecular POC;
- DTSI Dried Tube Sample HIV and Early Infant Diagnosis Molecular POC;
- HBVL HBV Molecular;
- HCVQ HCV Molecular;
- HEPM Hepatitis Serology;
- HIVL HIV Molecular;
- HPVN HPV Molecular;
- HTLD HTLV Molecular;
- LEPN Leptospirosis Molecular;
- MMBS Multimarker Blood Screening Serology;
- MMPF Multimarker Plasma Fractionation Molecular;
- MTBN Mycobacterium Molecular POC;
- NATA Multimarker Blood Screening Molecular;
- RASH Viral Exanthems Molecular;
- RESP Viral Respiratory Molecular;
- RESB Bacterial Plus Respiratory Molecular;
- RESV Extended Viral Respiratory Molecular;
- RVSS Retrovirus and Syphilis Serology;
- STIC Sexually-Transmitted Infections Molecular;
- TRCH TORCH and EBV Serology.

EQAS Program Codes contain information about each program:



2 PROGRAMS

The composition of the samples provided for all programs are presented in Appendix A of the relevant NRL written Final Report. All samples are 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 TE.

2.1 Molecular Programs

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

- Human plasma;
- 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;
- MEM (1x) with Earle's salts.

Samples which are determined to be “Negative”, consist of the dilution matrix on its own or with non-infected cells only.

The sample type of a certain program is listed in the program’s Storage and Handling Instruction, which can be obtained from OASYS dashboard or NRL website (https://www.nrlquality.org.au/products-services/eqas/storage-and-handling-instructions/). Please store and handle the EQAS samples according to the corresponding Storage and Handling Instruction, and also process and test the samples according to the manufacturer’s IFU or your laboratory’s working instructions.

DTS programs are provided to participants as dried sample tubes (DTS). DTS material is shipped with its own reconstitution buffer consisting of sterile PBS. Programs that are provided as dried tubes are instructed to be reconstituted by the participant according to the program’s Storage and Handling Instruction.

Stock material used for the Multimarker Blood Screening Molecular, HIV Molecular, HBV Molecular and HCV Molecular programs were calibrated against the WHO International Standards for HIV-1 (16/194), HBV (10/266) and HCV (18/184).

2.2 Serological Programs

Samples provided for the serological programs may have been prepared from either an individual plasma donation or a pool of multiple plasma donations. Pooled samples are prepared by mixing volumes of at least two donations with the same antibody and antigen profiles for the target analytes. NRL does not dilute samples for serology programs, except in rare circumstance which are stated specifically.

All samples are tested on a range of assays according to NRL’s algorithm for each analyte included in the Program to confirm their reactivity.

3 EVALUATION METHODS

3.1 Peer group

Results reported by participants using the same test method are grouped for analysis. This group of participants is known as a **peer group**. For molecular program, a peer group shares the same extraction kit, the same amplification kit and the same detection kit. For serology programs, a peer group shares the same detection kit.

3.2 Grading symbols

The grading symbols and their meaning are shown below.

 Acceptable

 Unacceptable

 Not Evaluated

Each participant should review their Performance Report for any results that have been identified as Unacceptable (designated with a ) and sometimes also as Not Evaluated (designated with a ).

3.3 Qualitative evaluation

For qualitative analytes, results reported by participants for **assay interpretations** are compared with the relevant reference results.

An **Unacceptable** assay interpretation is one that did not agree with the relevant reference result. False positive results (positive results reported for a negative sample) and false negative results (negative results reported for a positive sample) are defined as **Unacceptable**.

If there are no reference results for a particular analyte, the results submitted may be marked as "**Not Evaluated**". On some occasions, if deemed appropriate by NRL EQAS, consensus results from participants are used to evaluate results. The criterion for consensus is when at least 80% of the two largest peer groups with minimum five results in total submitted for a particular analyte achieve consensus.

Please note, NRL encourages participants to submit measurable values along with the assay interpretations, as this can provide extra statistical information and assist troubleshooting if required. However, **the measurable values are Not Evaluated for qualitative analytes**.

3.4 Quantitative (viral load) evaluation

In molecular viral load programs, the $\log_{10}$ transformed viral load values reported by participants are analysed.

When a peer group includes equal to or more than 5 results ($n \geq 5$), the peer group mean is determined and results that exceed ± 0.5 $\log_{10}$ from the peer group mean are identified as **Unacceptable**.

If the peer group includes less than five results ($n < 5$), statistical analysis is performed for information only but not assessed, and all results are marked as **Not Evaluated**.

Viral load results that are not $\log_{10}$ transformed are identified as **Unacceptable** and removed from statistical analyses.

Viral load results that were reported as "0" (zero) for non-reactive samples were also identified as **Not Evaluated**. In instances where a numerical viral load result is not obtained for a quantitative assay, participants should report either "Not Detected" or "Below Linear/Detection Limit" according to the results printout from the instrument used to perform the testing and manufacturer's Instructions for Use (IFU) or for in-house assays, according to the participant's reporting procedures.

False positive results (viral load values reported for a negative sample) and false negative results ("Not Detected" or "Below Linear/Detection Limit" for samples with a nucleic acid concentration above the limit of detection, where known, for the relevant assay) are identified as **Unacceptable,** unless a low concentration panel sample is included for educational purposes, in which case, the false negative results is identified as **Not Evaluated**.

In addition, viral load results may also be assessed for reproducibility and/or linearity (samples in a ten-fold dilution series). These assessments are not graded, but notes are added if any results exceed the criteria range on the Performance Reports. The criteria are:

- viral load difference between two replicate samples should be within ± 0.3 $\log_{10}$;
- viral load difference between two samples that form a ten-fold dilution series should be within 1 ± 0.3 $\log_{10}$.
- viral load difference between two samples that form a hundred-fold dilution series should be within 2 ± 0.3 $\log_{10}$.

3.5 Statistical analyses

ISO 13528 Robust Statistics is used for the Peer Group Statistics. The statistical analyses are displayed in both tables and graphs on the Performance Reports.

For qualitative analytes and programs, statistical analyses from measurable values are provided but **Not Evaluated.** The statistical analyses are provided for extra information and only the assay interpretations are evaluated.

For quantitative (viral load) analytes and programs, statistical analyses from peer groups with fewer than five participants are displayed but **Not Evaluated** as statistics based on a small datasets may not be reliable.

3.6 Troubleshooting notes, analyte notes and program notes

The Performance Report may include custom-tailored notes for individual samples, analytes or peer groups if any concerns or other significant observations are identified during analysis.

The notes are introduced to assist participants with their troubleshooting. Each participant should review the notes if they are in their reports. The notes are highlighted in **blue** and/or **black**.

3.7 Graphing

3.7.1 Bar graphs for qualitative results

The Performance Reports include bar graphs for displaying the qualitative analysis of assay interpretations submitted by the peer group as demonstrated in Figure 1.

One bar represents a whole result set from the entire peer group (presented as 100%). The pink section indicates the percentage of Detected/Positive/Reactive results, while the blue section indicates the percentage of Not Detected/Negative/Non-reactive results.

The result submitted is presented as a symbol in the middle of the section in which your result corresponds to i.e. if you submitted a “Negative” assay interpretation, your symbol would be located in the middle of the blue section.

Please note, the bars do not show reference results. Please refer to the assay interpretation results tables in the Performance Report or the Final Summary Report for the reference results.

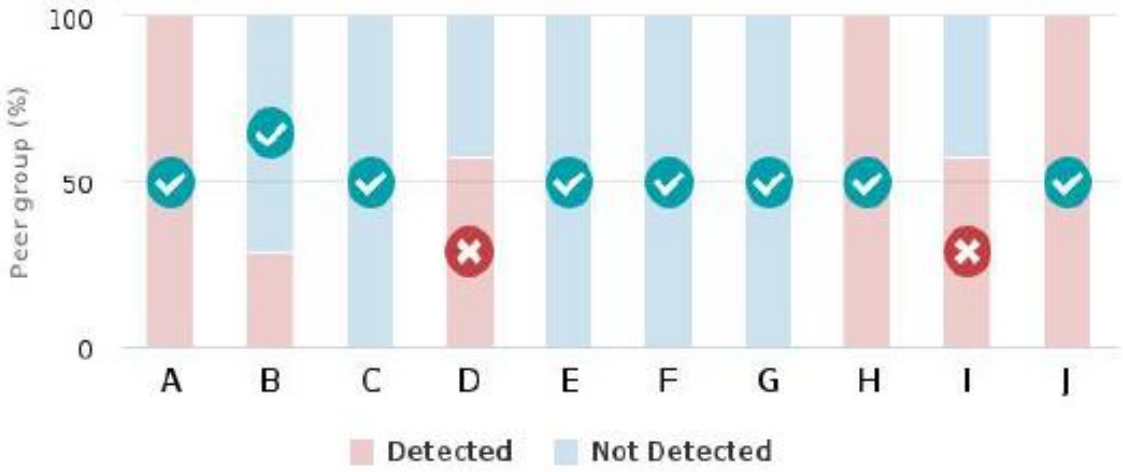


Figure 1. Example of a Bar Graph

3.7.2 Box and whisker plots for quantitative results

The Performance Reports include box and whisker plots for displaying quantitative results as demonstrated in Figure 2.

The boxplot features are as follows:

- the middle line of the box represents the peer group median also known as the second quartile (Q2);
- the top and bottom of the box represent the first and third quartiles (Q1 & Q3);
- the whiskers are calculated based on ISO 13528 Robust Statistics for outliers;
- for Viral Load programs, the long two grey lines represent the Acceptable Range (Peer Group Mean ± 0.5, see also section 3.4).

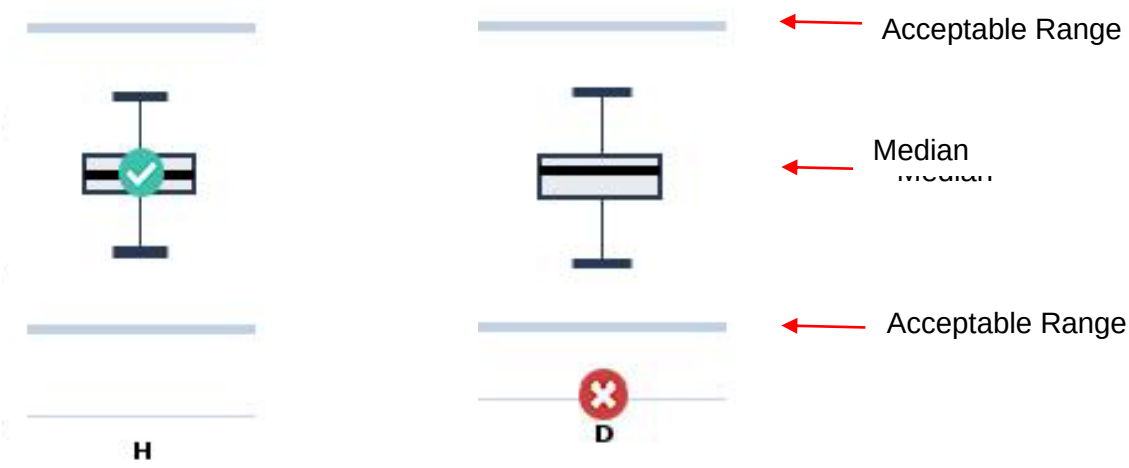


Figure 2. Examples of box and whisker plots

3.7.3 General rules for graphing

Several general rules are applied for graphing:

- If no result is received for a sample in an assay, a graph will not be displayed for this sample;
- If a Problem Code is submitted for a sample in an assay, the graphing will be displayed for this sample, but no symbol will be indicated for grading.

3.8 Participation statistics reports (qualitative or quantitative)

Summaries of the performance of all the test methods used in each program are available in the Participation Statistics Reports (Qualitative and/or Quantitative). These summaries may be useful in comparing the performance of all the test methods used by participants in each program. These summaries can be found on the OASYS Dashboard.

3.9 Biological false reactivity

In Serology programs, it is possible for biological false reactivity (BFR) to occur in any sample on any assay or test kit. In the instance that BFR is indicated, the panel sample is defined as **Not Evaluated** for the affected sample analyte in that peer group and a troubleshooting note is included in the Performance Report.

3.10 Educational samples

In rare circumstances, NRL EQAS includes or identifies educational samples in the panels. The educational samples may have a low concentration of a certain analyte close to the limit of detection or close to the cut-off for some assays. In this case, NRL EQAS makes the decision to **Not Evaluate** some or all affected results and to communicate with participants about this via comments on the Performance Reports or written Final Summary Reports.

4 Reports

After each TE, a Final Report including an OASYS-generated individual Performance Report and NRL written Final Summary Report is sent to each participant via email. All participants should receive their Final Reports within 15 working days after the Test Event closes.

Summaries of the performance of all the test methods used in each program are available in the Participation Statistics Reports (Qualitative and/or Quantitative) for a given TE. These summaries may be useful in comparing the performance of all the test methods used by participants in each program. These summaries can be found on the OASYS Dashboard.

Final reports from six previous TEs that a participant is enrolled in are also available on the OASYS Dashboard.

5 Evaluation appeals process

A participant may appeal the grading, data analyses or comments represented in the individual laboratory Performance Reports and/or EQAS written Final Summary Reports, should the participant have any concerns about them. A participant can contact NRL EQAS (eqas@nrlquality.org.au) for all queries and appeals.

6 TROUBLESHOOTING

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

Type of error	Possible cause(s)
Sample mix-up	Two or more samples may have been interchanged, resulting in both unacceptable results. Panel samples from an incorrect TE may have been tested. 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 (e.g. 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 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 on an immunoblot:** Samples that are negative on screening should not be tested on 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 on an immunoblot.
Unacceptable test results due to random error	Sporadic test results identified as unacceptable can be classified as random events. Possible causes of random outlying and/or unacceptable results include: • insufficient mixing of sample, especially following freezing; • not allowing samples or test kits to equilibrate to the instructed temperature prior to testing; • incorrect pipetting; • ineffective or inconsistent washing; • transcription errors; • sample mix-up; • cross-contamination or carryover; • presence of inhibitors or non-specific binding.
Unacceptable test results due to systematic error	A series of test results identified as unacceptable 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 at temperatures too high/low; • insufficient mixing/centrifuging before testing; • incorrect storage of samples or test kits prior to testing; • not allowing samples or test kits to equilibrate to the instructed temperature prior to testing; • contamination of master-mix, extraction areas or equipment; • ineffective extraction process; • degradation of master-mix components; • suboptimal primer design (in-house assays).