NRL
EQAS
Science Architect

Supplementary Information for all NRL EQAS Final Reports

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Supplementary information prepared by NRL EQAS

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1 INTRODUCTION

NRL provides a panel of samples for each program for relevant Test Events (TEs) per calendar year as part of their External Quality Assessment Schemes (EQAS).

The schedule of TEs can be found on the OASYS dashboard or in the NRL EQAS Catalogue located on NRL's website (https://www.nrlquality.org.au/products-services/eqas/). Occasionally, NRL may extend the TE timeframe for some or all programs. In these circumstances, NRL and One World Accuracy (1WA) will communicate with affected participants before the original closing dates.

EQAS panels are manufactured and managed by NRL. The NRL EQA programs are listed below according to their program codes and 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;
MLIO	Melioidosis Serology;
MLRM	Malaria Molecular;
MMBS	Multimarker Blood Screening Serology;
MMPF	Multimarker Plasma Fractionation Molecular;
MPOX	Mpox Molecular;
MTBN	Mycobacterium Molecular POC;
NATA	Multimarker Blood Screening Molecular;
RASH	Viral Exanthems Molecular;
RESB	Bacterial Plus Respiratory Molecular;
RESP	Viral Respiratory Molecular;
RESV	Extended Viral Respiratory Molecular;
RVSS	Retrovirus and Syphilis Serology;
STIC	Sexually-Transmitted Infections Molecular;
TRCH	ToRCH and EBV Serology;
TTIM	Transplant-Transmitted Infections Molecular;
VECT	Vector-Borne Infectious Diseases.

EQAS program codes contain information about each program:

All details of TE frequency, sample numbers and shipping condition, etc, are located in the NRL EQAS Catalogue on NRL's website (https://www.nrlquality.org.au/products-services/eqas/).

2 PROGRAMS

The composition of samples provided for each panel is presented in the Appendix of the corresponding NRL EQAS Science Architect 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;
- Human whole blood;
- Stabilised human whole blood;
- Phosphate buffered saline (PBS);
- Minimum Essential Medium (MEM) (1x) with Earle's salts;
- PreservCyt Solution.

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

The NRL EQAS Catalogue lists the sample types included in the programs. EQAS panel samples are required to be stored and handled according to the corresponding 'Program Instructions', which can be obtained from the OASYS Test Event Dashboard. EQAS panel samples are required to be processed and tested according to the manufacturer's Instructions for Use (IFU) or your laboratory's validated protocol for routine clinical samples. Where multiple clinical sample types may be suitable and require different testing processes, the clinical sample type mimicked by the panel sample will be specified in the 'Program Instructions'.

Dried Tube Samples (DTS) are provided to participants for DTS programs. DTS material is shipped along with reconstitution buffer consisting of sterile PBS. DTS programs are instructed to be reconstituted with supplied PBS by the participants according to the 'Program Instructions'.

All panel samples are tested in one or more assays for the target analyte(s), where possible, to confirm positivity before distribution. The agreed assay interpretations or mean viral load values are used as reference results.

Stock material used for the Multimarker Blood Screening Molecular (NATA), HIV Molecular (HIVL), HBV Molecular (HBVL) and HCV Molecular (HCVQ) programs are calibrated against the prevailing WHO International Standards as specified in the NRL EQAS Science Architect Final Report.

Note for Plasma Sample Types:

For molecular programs that provide a plasma sample type, please be aware due to the nature of true clinical plasma stored for extended periods for EQAS purposes, fibrin microclots or other precipitates may be present. While NRL EQAS is committed to providing high-quality samples, the complete elimination of such precipitates is not always possible.

Participants are reminded to process samples according to the ‘Program Instructions’ available on the OASYS Test Event Dashboard. If precipitates remain after centrifugation, please avoid when sampling or remove where possible.

2.2 Serological Programs

Samples provided for serological programs may have been prepared from either an individual plasma specimen or a pool of multiple plasma specimens. Pooled samples are prepared by mixing volumes of two or more specimens with the same antibody and antigen profiles for all analytes of the target program. NRL does not dilute samples for serology programs, except in rare circumstance which will be stated specifically in the NRL EQAS Final Science Architect Report of the corresponding panel.

All panel samples are tested on a range of assays according to NRL’s testing algorithm for each analyte included in the program to confirm their reactivity before distribution. The test results are also used as reference results.

Note for Plasma Sample Types:

Please be aware that due to the nature of true clinical plasma stored for extended periods for EQAS purposes, fibrin microclots or other precipitates may be present. While NRL EQAS is committed to providing high-quality samples, the complete elimination of such precipitates is not always possible.

Participants are reminded to process samples according to the ‘Program Instructions’ available on the OASYS Test Event Dashboard. If precipitates remain after centrifugation, please avoid when sampling or remove where possible.

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 qualitative analytes, a peer group shares the same detection kit and, if applicable, the same extraction kit and amplification kit.
- For molecular quantitative analytes, a peer group shares the same detection kit. When there are insufficient participants to form a meaningful peer group, results are grouped and analysed under an “overall” peer group, which shares the same reporting unit, as detailed in **Section 3.4**.
- For serology analytes, a peer group shares the same detection kit.

Selecting the correct test kits for each process of assay registration is essential for accurate peer group allocation.

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/or Not Evaluated (designated with a ⊖).

3.3 Qualitative evaluation

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

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

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** or if appropriate, consensus results may be used as the reference results. The criterion for consensus is when at least 80% of the two largest peer groups, if available, submitted for a particular analyte achieve consensus. Smaller data sets may be evaluated at NRL EQAS' discretion.

3.4 Quantitative (viral load) evaluation

For molecular viral load analytes and programs, **$\log_{10}$ transformed** viral load results are assessed.

1. **Peer Group Results $n \geq 3$**

- When a peer group includes at least three results ($n \geq 3$), the Peer Group Mean is determined and results that exceed ± 0.5 $\log_{10}$ from the Peer Group Mean are identified as **Unacceptable**.

2. **Peer Group Results $n < 3$**

- When a peer group includes fewer than three results ($n < 3$), the participant's result is evaluated at NRL EQAS' discretion, according to the statistical reliability of the relevant dataset.
- The "Overall Mean" of all results of the same reporting unit may be used to assist with result evaluation in these small peer groups, when possible, to provide more meaningful evaluation for participants.

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

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.**

Viral load results that are reported as "0" (zero) for negative samples are 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 or the assay manufacturer's Instructions for Use (IFU) (or for in-house assays, according to the participant's reporting procedures).

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

3.5 Statistical analyses

Peer group statistics are performed by OASYS (provided by 1WA) according to ISO 13528 Robust 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 provided are **Not Evaluated.** Only the assay interpretations are evaluated with statistical analyses provided for extra information.

For quantitative (viral load) analytes and programs, statistical analyses are calculated at either the peer group level or overall results level depending on the size and statistical reliability of the peer group.

3.6 Troubleshooting notes, Analyte notes and Program notes

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

The notes are applied when deemed applicable, to assist participants with their troubleshooting. Each participant should review notes that may be included in their reports. The notes may be in **blue** and/or **black** font and located after the graph analysis and/or at the end of the report.

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, the symbol is in the middle of the blue section.

Please note, the bars do not show reference results. Please refer to the reference results in the Performance Report or the NRL EQAS Science Architect Final Report.

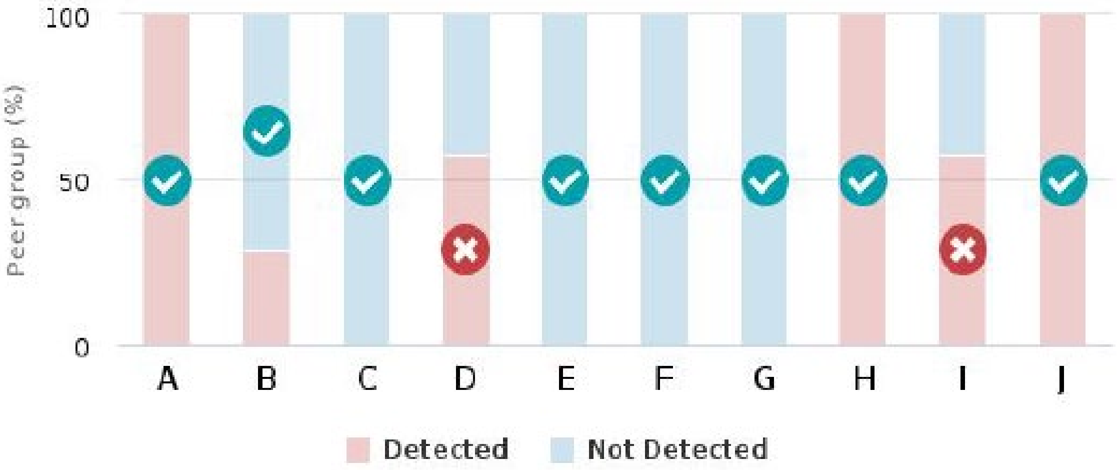

Figure 1. Example of a Bar Graph

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 (Mean ± 0.5, see also section 3.4).

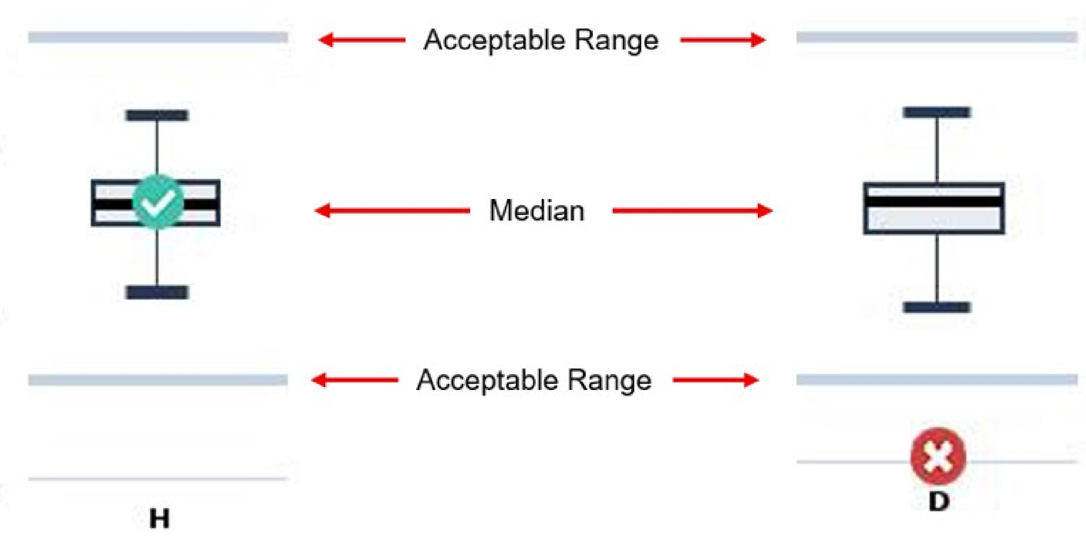

Figure 2. Examples of box and whisker plots

Note: When peer group n<3 for viral load analytes, the graph is populated based on overall results for the same unit of measure.

3.7.3 General rules for graphing

Graphs will not be displayed for samples in the following situations:

- No result is submitted;
- A Problem Code is submitted;
- If a qualitative analyte has more than three result options.

3.8 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** and a troubleshooting note is included in the Performance Report.

3.9 Educational samples

Occasionally, 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 may **Not Evaluate** some or all affected results and communicate with participants about this via comments on the Performance Reports or NRL EQAS Science Architect Final Report.

4 Reports

After each TE, Final Reports including an OASYS-generated individual Performance Report and NRL EQAS Science Architect Final Report are sent to each participant via email. All participants should receive their Final Reports within 15 working days after the TE 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 a participant enrolled in are available on the OASYS Dashboard.

5 Complaints and Appeals Process

Participants have the right to submit appeals, complaints or any other feedback regarding any aspect of their participation in NRL EQAS, including but not limited to samples, shipping, communication, assessment (grading and data analysis) and reports (comments provided in the individual Performance Reports, or the NRL EQAS Science Architect Final Report).

All appeals and complaints will be reviewed in accordance with NRL's documented procedures to ensure fair and timely resolution. Participants may submit their appeal, complaint or feedback in writing to NRL EQAS at **eqas@nrlquality.org.au**.

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. Panel samples from an incorrect TE may have been tested. Sample mix-up may occur during specimen reception or during testing.
Transcription error	Possible causes of transcription errors include: • interchanging the results for two or more samples; • 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 to avoid such errors.
Inappropriate testing strategy followed	Testing negative samples on confirmatory assays (immunoblots/neutralisation assays/avidity assays). Only samples reactive on screening assays should be tested on confirmatory assays to avoid possible non-specific reactivity.
Random error	Possible causes of random errors 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.
Systematic error	Possible causes of systematic errors include: • 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 or carryover; • ineffective extraction process; • degradation of master-mix components; • suboptimal primer design (in-house assays).