NRL EQAS Science Architect

Supplementary Information for all NRL EQAS Final Reports

NRL Science of Quality
4th Floor, Healy Building
41 Victoria Parade
FITZROY VICTORIA 3065
AUSTRALIA
T: +61 3 9418 1111
F: +61 3 9418 1155
www.nrlquality.org.au
A.B.N. 52 004 705 640
SVI ST VINCENT'S INSTITUTE MEDICAL RESEARCH
NATA Accredited for compliance with ISO/IEC 17043 Accreditation number: 14253

Supplementary information prepared by NRL EQAS

Reviewed: 2024-06-06

1 INTRODUCTION

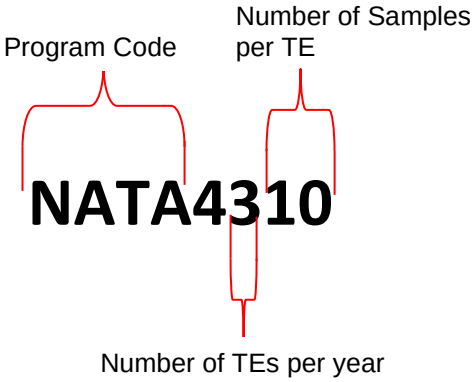
NRL provides panels of samples for each of the three Test Events (TEs) per calendar year for the External Quality Assessment Schemes (EQAS) NRL offers.

The Schedule of TEs can be found on OASYS dashboard or in the NRL EQAS Catalogue 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 1WA will communicate with affected participants before the original closing dates.

EQAS panels are manufactured and managed by NRL. The NRL EQAS 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;
- 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;
- TTIM Transplant-Transmitted Infections Molecular.

EQAS Program Codes contain information about each Program:



2 PROGRAMS

The composition of the 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;
- Phosphate buffered saline (PBS);
- MEM (1x) with Earle's salts.
- PreservCyt Solution;
- Stabilised whole blood.

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 Storage and Handling Instruction, which can be obtained from the OASYS Test Event Dashboard. EQAS panel samples are also required to be processed and tested according to the manufacturer’s IFU or your laboratory’s working instructions as routine clinical samples. For some Programs where multiple clinical sample types can be used, of which may require different testing processes, a specified clinical sample type may be suggested in the Storage and Handling Instruction.

DTS Programs are provided to participants as dried sample tubes (DTS). DTS material is shipped along with reconstitution buffer consisting of sterile PBS. These DTS Programs are instructed to be reconstituted with supplied PBS by the participants according to the Program’s Storage and Handling Instruction.

All panel samples are tested in one or more assays for the target analyte(s) to confirm the positivity before distribution. The agreed assay interpretations or mean viral load values are also 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 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 two or more donations 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.

Due to the true nature of clinical plasma samples stored for extended periods of time for EQAS purposes, fibrin microclots or other precipitates may be present in the samples. NRL EQAS endeavoured to improve sample quality. However, all precipitates in the samples cannot be completely eliminated. Participants are reminded that samples should be processed according to the Storage and Handling Instructions which are located on the OASYS Test Event Dashboard. If after centrifugation, there are still precipitates present, please try to avoid or remove when sampling.

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 extraction kit, the same amplification kit and the same detection kit.
- For molecular quantitative analytes, a peer group shares the same detection kit.
- For serology Programs, a peer group shares the same detection kit and the same neutralisation kit if used.

Therefore, selecting the correct test kits at every step of assay registration is very critical.

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 assessed by being 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 rare 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 submitted for a particular analyte achieve consensus, the consensus result is regarded as the reference result.

In addition, NRL encourages participants to submit measurable values along with the assay interpretations for qualitative evaluation, as this can provide extra statistical information and assist with

troubleshooting if required. However, **the measurable values are Not Evaluated for qualitative analytes**.

3.4 Quantitative (viral load) evaluation

For molecular viral load Programs, the **log_{10} transformed** viral load values reported by participants are assessed.

1. <u>Peer Group Results n≥5</u>

- When a peer group includes equal to or more than five results (n⩾5), the Peer Group Mean is determined and results that exceed ± 0.5 log_{10} from the Peer Group Mean are identified as **Unacceptable**.

2. <u>Peer Group Results n<5</u>

- If the peer group includes less than five results (n<5), the participant result is evaluated at NRL EQAS' discretion, according to the statistical reliability of the relevant dataset.
- The "Peer Group Mean" and/or "Overall Mean" of all results of the same unit may be used to assist with result evaluation in these small peer groups, when possible. Most commercial viral load assays are calibrated against the International Standard and provide similar results.

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

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 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.** When a low concentration panel sample, the concentration of which is close to the limit of detection for some assays, is provided for educational purposes, NRL EQAS may decide to **Not Evaluate** the negative results submitted.

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

Peer Group Statistics is 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 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 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 introduced to assist participants with their troubleshooting. Each participant should review the notes if they are in their reports. The notes are written in **blue** and/or **black** font.

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 NRL EQAS Science Architect Final Report for the reference results.

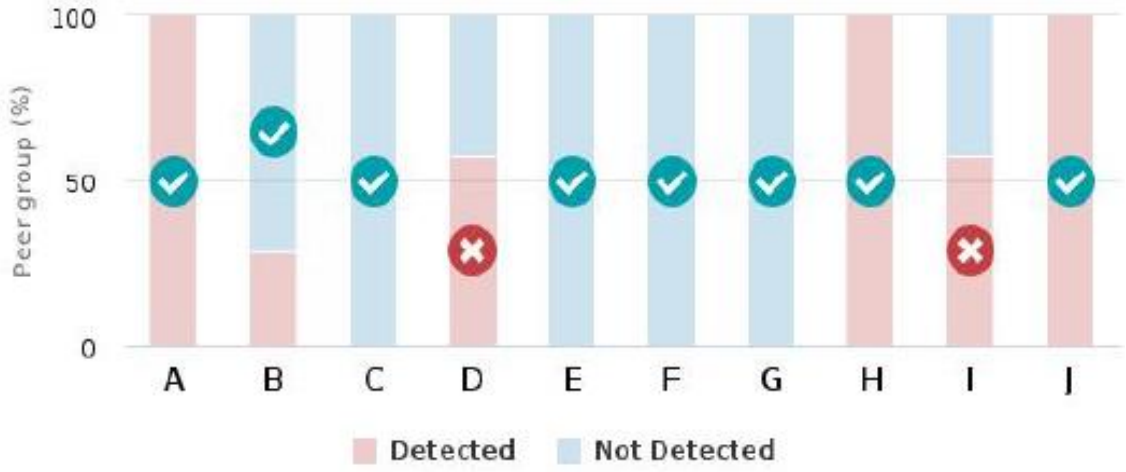


Figure 1. Example of a Bar Graph

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 (Mean ± 0.5, see also section 3.4).

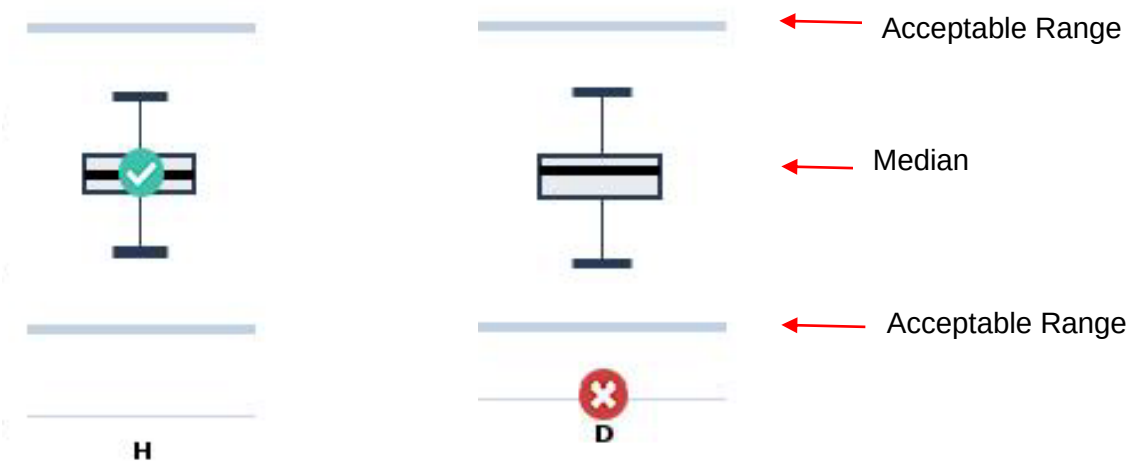


Figure 2. Examples of box and whisker plots

Note: When peer group n<5 for viral load analytes, the graph is populated based on overall results of the same unit.

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.
- For qualitative analytes, if an assay has more than three result options (e.g. RPR titres), no graph will be displayed.

3.8 Participation Statistics Reports (qualitative and/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 NRL EQAS Science Architect Final Report.

4 Reports

After each TE, a Final Report including an OASYS-generated individual Performance Report and NRL EQAS Science Architect Final Report is 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 that a participant has 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 NRL EQAS Science Architect Final Report, 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).