ADDENDUM TO SEROLOGY EQAS REPORTS

1 METHODS

HEPM, HIV, HTLV and Serology EQAS: Panels with the programme codes XXXX4310 consisted of ten plasma samples. Panels with the programme codes XXXX435 consisted of five samples which were the same as the first five samples included in the panels with programme codes XXXX4310.

MMBS Serology EQAS: The panel with programme code MMBS4320 consisted of twenty plasma samples.

Syphilis and TORC Serology EQAS: Panels with the programme codes XXXX435 consisted of five plasma samples.

A panel sample may have been prepared from either an individual plasma donation or a pool of multiple donations. Each pooled sample was prepared by mixing volumes of at least two donations that had similar antibody and antigen profiles. The composition of panel samples is shown in Appendix A of the relevant EQAS report.

All samples were manufactured according to NRL procedures, ensuring homogeneity.

All samples were tested in a range of assays to confirm their reactivity. Syphilis Serology EQAS panel samples were not tested for anti-*Treponema pallidum* IgM.

The storage and transport conditions for NRL EQAS samples have been extensively validated to assure sample stability for the duration of the test event period.

1.1 Evaluation of results

Results reported by participants for assay interpretations and status 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 ISO 13528 method was used to identify outlying results.

An outlying test result is a numerical test result that is found to be statistically different from other test results reported by participants that tested the same sample in the same assay. Occasionally the EQAS coordinator may manually flag a result as outlying, which will cause it to be removed from the statistical analysis. This will only occur when inclusion of the result will erroneously bias the statistical analysis e.g. when the result is from the testing of an incorrect sample.

Participants were asked in the “Instructions” to test the samples in order according to their sample identification. When examining the results of the panel samples NRL identifies the potential of carryover having occurred when the results of the negative samples are:

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

1.2 Troubleshooting common causes of outlying and/or aberrant results

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

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); - 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.
Data entry	When samples are tested in an open system immunoassay (i.e. manual EIAs) and test results are obtained that are greater than the limit of detection of the reader that was used to quantify the reactions (e.g. all of the reactive test results had the same value) participants should either: - select Problem Code 22 (Above Linear/Detection Limit) when submitting results in OASYS rather than submitting test results for the samples or; - enter “>” before the relevant test value that was reported.
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 HBsAg tests of inadequate sensitivity: Simple rapid HBsAg 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 HBsAg.

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

Type of error	Possible cause(s)
Aberrant serology status This section applies to HEPM (HCV component), 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".
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.
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; - incubation time too long/short or temperature too high/low; - insufficient mixing/centrifuging before testing; - incorrect storage of test kits and/or reagents.