SUPPLEMENTAL INFORMATION TO NAT EQAS REPORTS

1 METHODS

Clinical NAT EQAS Programmes: Positive samples were prepared by diluting the stock samples in one of several different materials as listed below:

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

HCV Genotyping EQAS Programme: HCV RNA positive plasma samples were obtained from blood donations. All stock samples were assigned a genotype/subtype 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 (www.ncbi.nlm.nih.gov). Each stock sample was assigned the genotype/subtype of the reference sequence to which it displayed the greatest homology.

Samples were prepared by dilution of the stock samples in NHP. Following preparation, the samples were tested to ensure their viral loads were adequate for genotyping.

Blood Screening NAT EQAS Programme: Positive samples were prepared by diluting HIV-1 RNA (group M, subtype B), HCV RNA (genotype 1) or HBV DNA (genotype A) positive plasma into NHP. All negative samples consisted of NHP and were identical in composition, being dispensed from the sample pool of NHP and were negative for HIV RNA, HCV RNA and HBV DNA using the Novartis Procleix Ultrio HIV-1/HCV/HBV TMA assay and the Roche COBAS TaqScreen MPX v.2.0 assay.

The samples were calibrated against the WHO international standards for HIV-1 (97/650), HBV (97/750) and HCV (06/102).

The composition of panel samples is shown in Appendix A of the relevant EQAS reports.

2 Evaluation of results

2.1 Qualitative evaluation

Qualitative interpretations reported were evaluated by comparing the results reported by each participant with the reference result.

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.

2.2 Viral Load evaluation

The $\log_{10}$ transformed results reported by participants were analysed. Results reported by participants using the same test method were grouped for analyses (peer group). 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.

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

2.3 Statistical analyses

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.

3 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 (eg. 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.
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).