Specimen Dilution Plan for Real-Time HIV-1 RNA PCR Assays

Purpose:

The following is a plan to outline the proper procedure for running samples with low sample volume or for repeating samples that contain virus loads that exceed the upper limit of detection of the Abbott RealTime HIV-1 assay (AR) or the Roche COBAS AmpliPrep/COBAS TaqMan assay (RT). The RT assays require the use of EDTA plasma; the AR assay permits the use of either EDTA or ACD plasma. Ideally, samples should be diluted in the same matrix in which they were originally collected, but HIV-1 negative plasma is not provided in the kit in either assay for this purpose. Some laboratories may opt to use the negative kit control for diluting samples prior to running them, however the volume of control provided in each kit is limited. Therefore, a testing laboratory must identify the diluent they intend to use and then verify that the use of that diluent does not significantly affect the HIV-1 RNA result.

Plan Establishment:

The Virology Quality Assurance (VQA) Program has outlined a validation plan that can be used to verify the performance of a diluent used to dilute plasma prior to running the sample on either the RTv2 or AR assay. The VQA used historical data generated for HIV-1 RNA proficiency testing and actual dilution data generated using the Basematrix negative diluent (SeraCare material # 1805-0075, 1L) to establish the acceptability criteria for this validation plan. Basematrix negative diluent was selected for this testing because it provided the most consistent result across both AR and RT assays. The effect of dilution on assay results was evaluated using a linear model of $\log_{10}$ estimated RNA concentration as a function of dilution status (neat or diluted) and donor. In the absence of replication, a donor-dilution interaction was not evaluated. The acceptance window of [-0.42 to 0.59] and [-0.48 to 0.53] for AR and RT, respectively, was based upon kit specific estimates of the median difference (0.09 $\log_{10}$ for AR and 0.02 $\log_{10}$ for RT) and a robust ±3SD (±0.5 $\log_{10}$) window established using historical VQA data.

Diluent Selection and Handling:

A laboratory may opt to use other negative diluents, but the acceptability criteria will not change. The VQA did an extensive evaluation of different matrices, including PBS, SeraCon, SeraCon II, EDTA plasma, serum and Basematrix, and the results varied across diluents. On average, diluted plasma runs higher than neat EDTA plasma, presumably because the presence of EDTA in the extracted material affected PCR efficiency. Larger matrix effects are seen in data generated with the RT assay.

If a laboratory opts to use Basematrix, the negative diluent will be purchased in 1L bottles, but it should be aliquoted into 5 – 10mL volumes (or whatever volume is appropriate for the laboratory to consume within one week) and frozen at -20C or colder to avoid multiple freeze/thaw cycles or potential contamination. The material should be thawed, mixed well by inversion, and filtered prior to use with a 25mm sterile syringe filter (0.2um) such as Thermo Scientific Nalgene Syringe Filters, 09-740-35A; Thermo Scientific No. 190-2520.

The filtered material may be kept in the refrigerator for up to one week. The product should be re-filtered or discarded if any visible precipitate or flocculate forms.

Dilution Procedures:
Since the lower detection limit of an assay will be affected by the dilution factor used to dilute the sample, the decision was made to standardize the dilution factor across all HIV-1 RNA assay platforms. A 1:5 dilution was chosen as the standard dilution; 0.2 mL of plasma is mixed with 0.8 mL of diluent. This dilution factor should be used for diluting samples with low sample volume. A 1:20 dilution should be used for diluting samples with virus load result that exceeds the upper limit of detection for the assay used; 0.05 mL of plasma is mixed with 0.95 mL of diluent. If the diluted sample still yields a result above the upper limit of detection for that assay, a higher dilution will not need to be done. A higher dilution should not be used for low sample volume testing unless prior approval from the protocol team has been obtained. Viral load results from diluted samples must then be multiplied by the dilution factor (e.g. 5 or 20). Laboratories using LDMS should enter the dilution factor into LDMS and the system will perform this multiplication and calculate the final result automatically.

Dilution Matrix Validation:
A laboratory must validate the use of any matrix prior to using this matrix for clinical trial testing. Laboratories must notify the VQA of their intention to perform a dilution matrix validation by sending an email to vqa.dmg@fstrf.org, and should specify the assay platform, the lab #, and method of data submission (LDMS or DSS) in their message. Twenty HIV-1 positive samples should be tested; while the 20 unique samples may be assayed across multiple runs, the neat and diluted plasma for each unique sample must be assayed in the same run to reduce variability. The laboratory should choose samples that are known to contain greater than 1,000cp/mL and span as much of the linear range as possible. Only samples that yield a valid result for both the neat and diluted sample will be included in the analysis. No more than 4 samples (20%) should have an undetectable result to ensure there are sufficient data for the analysis. The results for the 20 samples may be submitted to the VQA for analysis. Dilution validation results may be submitted to through LDMS under the VQA group using the Specimen IDs RNA999RT.01A – RNA999RT.020A. Alternatively, results may be entered on the Basematrix Dilution Template Excel spreadsheet and submitted through the Data Submission System (DSS) on the VQA DMG website (www.frontierscience.org/VQA). A report will be generated by the VQA to document the study. The laboratory director and network laboratory leadership must sign off on the dilution validation prior to using the validation procedures in a clinical trial.

A summary of the specimen dilution plan is provided in Attachment I. SerCare contacts for domestic and international laboratories are provided in Attachment II.

Attachment I: The VQA Dilution Plan for HIV-1 RNA Testing

1. Obtain the dilution matrix
    a. Basematrix negative diluent is acceptable for either AR or RT assays. PBS can be used for testing on AR, but is not recommended for testing with RT.
    b. SeraCare (material # 1805-0075)
    c. Aliquot into appropriate volumes and store at -20C; filter prior to use (0.2um); stable at 4C for up to one week.
2. Dilution factors
    a. Low sample volume dilutions
        i. A 1:5 dilution should be used (0.2mL plasma + 0.8mL diluent)
        ii. Sample and diluent should be mixed by pipetting before diluting; avoid bubbles.
        iii. Final result must be multiplied by the dilution factor used (i.e. 5)
    b. High-titered sample dilutions
        i. A 1:20 dilution should be used (0.050mL plasma + 0.950mL diluent)
        ii. Samples and diluent should be well-mixed before diluting
        iii. Final result must be multiplied by the dilution factor used (i.e. 20)
3. Each laboratory must validate the dilution protocol
    a. 20 clinical samples
        i. Use samples with viral loads >1,000cp/mL and <5,000,000cp/mL.
        ii. Do not use samples with undetectable viral loads.
        iii. Try to select samples that span the linear range.
    b. Test neat and diluted (1:5) in the same assay
        i. Submit the data to the VQA for analysis
    c. Laboratory director and network laboratory leadership must sign off on dilution validation prior to use in a clinical trial.

Attachment II: SeraCare Contacts for International and Domestic Laboratories

Domestic laboratories
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