Validation and Proficiency Testing Plan for the Manual Abbott RealTime Assay

INTRODUCTION

The automated specimen processor is part of the FDA approved version of the kit. As defined by regulations, any modification of an FDA approved assay must be validated prior to use. The additional requirements for validating a 'modified' assay are dependent upon the modification being utilized. In the case of the Abbott RealTime HIV Assay, the exact same kit components are for both the manual and automated extraction methods. Because the chemistry of the assay is not different, and the assay contains internal controls that are co-amplified with the HIV target, additional validations for interfering substances are not necessary. However, since the extraction is being done manually, there is a possibility that accuracy, precision or sensitivity could be affected.

Laboratories must validate the installation of their Abbott assay. The VQA has developed a validation plan that has been approved by the clinical trial networks and is available to laboratories. Briefly, a set of VQA HIV RNA copy controls are combined with locally collected samples from HIV-infected and uninfected donors into templates that are assayed over five days. If the laboratory has a validated assay already installed, then they can use that existing assay as the comparator for the validation testing. Laboratories must validate the assay using the extraction method that shall be using for clinical trial and proficiency testing. If a laboratory switches extraction methods or plans on using the manual extraction method as a back-up extraction to the automated m2000sp then additional validation testing must be done as outlined below. If a laboratory plans on using the manual extraction method as a backup, then proficiency testing must be done using both the automated and manual extraction methods, on every round of testing, to ensure proficiency is maintained.

NOTE: the manual extraction requires additional equipment (heat blocks, magnetic racks, etc) that must be purchased separately by the laboratory before the manual extraction can be performed. A full revalidation is not required by laboratories that switch from the manual extraction to the automated extraction, or for laboratories that would like to use the manual extraction for a back-up to the automated assay, but extra clinical sample testing and/or control testing is required depending on which extraction method was originally validated.

Switching from Manual to Automated Extraction

If a laboratory decides to switch from a validated manual extraction procedure to the automated processor, they must test 20 clinical samples using both the manual and automated extraction methods. These 20 samples may be left-over specimens from the manual extraction validation if sufficient volumes remain. In this case, the retesting need only occur using the automated extractor. If insufficient volumes of plasma are available, then 20 new samples (HIV+ samples with >80% detectable virus loads) must be tested using both the manual and automated extractions. These data may be sent to the VQA for

analysis. A report will be sent to the laboratory director for final approval. A copy of the final, signed report should be sent to the PNL for that laboratory. Ongoing proficiency testing will be done with the automated extraction – no requalification will be required.

Opting to use the Manual Extraction as a Back-up Plan

A laboratory that performs the assay installation validation using the automated specimen processor must do additional testing to demonstrate proficiency before they will be permitted to use the manual extraction as a back-up plan. The laboratory must test 20 clinical samples and a panel of 21 VQA HIV RNA copy controls (as defined by the VQA) to further validate the use of the manual extraction prior to beginning VQA HIV RNA proficiency testing and/or clinical trial testing. The additional control testing is required to further demonstrate the laboratory's ability to do the manual extraction. The data may be submitted to the VQA for analysis. A report will be sent to the laboratory director for final approval. Ongoing proficiency testing must be done using both the manual and automated extraction methods. The laboratory must run each VQA HIV RNA proficiency panel using both the automated and manual extraction methods. Both sets of data must be sent to the VQA for analysis; both sets of data will be scored officially. Both sets of data will be tracked and reviewed by the VQAAB and network leadership.

Summary

- The installation of the Abbott RealTime HIV-1 Assay must be validated using a plan that has been approved by the networks (VQA HIV RNA validation or equivalent)
  - Validation testing must be done using the extraction method to be used for clinical sample testing
  - Validation testing must include both well characterized control samples as well as locally collected clinical samples

- Switching extraction methods requires additional validations
  - Switching from **manual to automated** extractions requires testing 20 clinical samples using both extraction methods.
  - Switching from **automated to manual** extractions requires testing 20 clinical samples and a panel of 21 VQA HIV RNA controls (as defined by the VQA) using both extraction methods.

- Proficiency testing (PT) requirements include:
  - Running VQA HIV RNA PT using the validated extraction method every round.
  - Running VQA HIV RNA PT using both automated and manual methods if you intend to use the manual method as a back-up extraction every round.
  - All PT testing shall be scored officially.