Need for Procedural Details in the Protocol for Specimen Processing by the MagNA Pure LC Instrument

We read with interest the favorable findings of Hözl et al. (1) showing good agreement of plasma HIV-1 RNA measurements (by the ultrasensitive COBAS AMPLICOR HIV-1 Monitor test) by using the conventional (manual) RNA purification method and the fully automated method performed with the MagNA Pure LC instrument. Their findings would enable diagnostic laboratories to use a commercially available automated nucleic acid purification system for a complex molecular assay and to save considerable resources. The authors stated that they subjected the low-positive, high-positive, and negative controls provided with the COBAS AMPLICOR HIV-1 Monitor test to RNA purification and reverse transcription (RT)-PCR analysis in both the conventional and automated methods, but no further procedural details on this part of their evaluation were provided.

In the manufacturer-specified manual RNA purification procedures for the ultrasensitive assay, 12.5 µl of each control reagent is added to the respective tube containing 600 µl of lysis buffer and the centrifuged pellet of the normal human plasma provided in the assay kit. For processing clinical specimens, 500 µl of each patient plasma specimen is concentrated in a tube by centrifugation and then subjected to lysis (600 µl of lysis buffer). However, when using the MagNA Pure LC large-volume total nucleic acid protocol for automated nucleic acid purification, the instrument does not allow differential pipetting and processing of assay controls versus patient specimens during the same run. How did Hözl et al. use the MagNA Pure LC instrument to process the assay controls and patient specimens within the same run? Were modified procedures used for handling the controls so that they could be processed along with clinical specimens within the same run? If the controls were not processed on the same run as the clinical specimens, the validity of the automated nucleic acid purification process and the assay results obtained from the clinical specimens would be uncertain.

Procedural details from the authors regarding proper processing of assay controls by the MagNA Pure LC instrument are critically important to diagnostic laboratories planning to validate and adopt this fully automated nucleic acid purification method for use with the ultrasensitive COBAS AMPLICOR HIV-1 Monitor test.

REFERENCES

Authors’ Reply

We completely agree with Dr. Yao and colleagues that controls to diagnostic assays are required to be processed in the same way and within the same run or within the same string of runs as the patient samples. Undoubtedly, this also applies to complex molecular assays such as the COBAS AMPLICOR HIV-1 Monitor test.

The COBAS AMPLICOR HIV-1 Monitor test and its ultrasensitive procedure are provided with four controls (Roche instruction booklet, Roche Molecular Systems, Pleasanton, Calif.). An internal amplification control (quantitation standard) is added to each test sample (patient samples as well as run control samples), and quantification data derived therefrom are used for the calculation of the HIV copy number of each test sample. Additionally, the internal amplification control indicates whether a test sample is PCR inhibited or not. Two positive run controls (high and low) are analyzed in parallel to the patient samples in order to monitor RNA purification, RT-PCR, and the quantification procedure. A negative run control is analyzed in parallel and should not yield to any HIV-related RT-PCR amplification.
In the adaptation of the ultrasensitive COBAS AMPLICOR HIV-1 Monitor test for automated HIV-1 RNA purification on the MagNA Pure LC instrument, great care was applied to preserve volumes and especially concentrations, including those related to the controls. Thus, the amount of internal amplification control used was carefully determined in order to avoid any possible distortion of the sensitive quantification procedure of the COBAS AMPLICOR HIV-1 Monitor test (1). The three run controls (high, low, and negative) were prepared in keeping with the manufacturer’s instructions for the manual RNA purification procedure of the ultrasensitive modification of the assay (Roche instruction booklet). Since for the manual procedure 12.5 μl of each control reagent is added to the respective control samples, the same amount of each control reagent, i.e., 12.5 μl, was also administered for the automated procedure. In order to keep the volume of each run control equal to that for patient samples, they were brought to the volume of the patient samples, i.e., 550 μl, with the sample diluent provided with the test kit. This procedure ensured that the control samples were kept comparable to patient samples and to those for the manual RNA purification procedure. As reported, all control samples were analyzed in parallel with the patient samples within the same run on the MagNA Pure LC and on the COBAS AMPLICOR analyzer (1). Up to the present time, we have performed 118 runs with the described assay in our diagnostic routine. Four runs each returned one invalid positive run control. We assumed the cause was handling errors, since on repetition the respective controls turned out valid.

We regret that the slip in precisely describing the preparation of the run control samples may have generated any confusion or doubts.

The volumes of the control reagents and samples that Dr. Yao and colleagues refer to are used in the standard COBAS AMPLICOR HIV-1 Monitor test. These volumes do not, however, apply to the ultrasensitive COBAS AMPLICOR HIV-1 Monitor test that we have complemented with automated RNA purification and sample handling (1; Roche instruction booklet).

REFERENCES


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