



April 14, 2005

Advisory limiting use of BD Vacutainer® PPT™ or other gel-barrier collection tubes for HIV-1 PCR testing.

Dear Valued Client,

BD Diagnostics has issued an advisory with specific recommendations on sample handling procedures when using the product listed above in conjunction with certain HIV-1 PCR viral load tests. **The relevant Esoterix test codes are 608881, 608882, 608884, 604315, 604312, and 604314.** The BD letter follows this note.

Our scientific and technical team has reviewed BD's "White Paper Report" detailing their internal studies using these products and test systems under various processing scenarios. Although not published in a peer-reviewed journal we believe the findings support BD's recommendations. Our recommendations and updated policies are as follows:

- Follow BD's instructions for using BD Vacutainer® PPT™ or other gel-barrier type tubes.
- HIV-1 PCR viral load samples collected in BD Vacutainer® PPT™ tubes can be processed as instructed by BD and shipped on a cold pack (4°C). Stability for HIV-1 PCR viral load measurements is 72 hours at 4°C. **Do not freeze and do not ship frozen.**
- HIV-1 PCR viral load samples collected in BD Vacutainer® PPT™ tubes can be processed as instructed by BD and then plasma can be transferred to another plastic tube for frozen storage and frozen shipping.
- Alternatively other EDTA vacutainer tubes can be used as instructed by the manufacturer and then plasma can be transferred to another plastic tube for frozen storage and frozen shipping.
- Effective May 16, 2005 we will no longer accept samples for HIV-1 PCR viral load testing when submitted in frozen BD Vacutainer® PPT™ or other gel-barrier type tubes.
- Until May 16, HIV-1 PCR viral load results from samples submitted frozen in these tubes will be appended with an advisory about the potential for falsely elevated HIV-1 PCR viral loads when samples are submitted in frozen BD Vacutainer® PPT™ or other gel-barrier type tubes.
- Our client services team will be calling many of you to communicate this advisory. This letter is also posted on our website <http://www.esoterix.com/>

For further assistance, please contact Esoterix Client Services 800-444-9111. We value you as a partner in healthcare delivery and thank you for your continued support.

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- To: Users of BD Vacutainer[®] PPT[™] and K₂EDTA tubes on the following assays and assay systems:**
- Roche AMPLICOR HIV-1 MONITOR[®] Test, v1.5 Standard and UltraSensitive Specimen Processing procedure
 - Roche COBAS AMPLICOR[™] HIV-1 MONITOR[®] Test, v1.5 Standard and UltraSensitive Specimen Processing Procedure

Subject: Recommended Sample Handling Procedures

Dear Valued Customer,

BD has become aware of reports of elevated HIV-1 viral loads with the use of the BD Vacutainer[®] PPT[™] tube in the Roche AMPLICOR HIV-1 MONITOR[™] Test, v1.5 when plasma is frozen *in situ* for patients with viral loads close to the assays' lower limit of detection. While BD does not recommend freezing plasma in PPT[™] tubes, results of an earlier study¹ indicated that overnight shipment of PPT[™] plasma frozen *in situ* had no effect on viral load results in patients with viral loads >5000 copies/mL. We have become aware of customers who have applied the results of this publication to samples with viral loads at or near the lower detection limit of the assays. BD has initiated an in-depth investigation concerning elevated HIV viral load values when using BD Vacutainer[®] PPT[™] and Plus K₂EDTA tubes processed and stored under various conditions. Under this investigation, studies were performed on specimens with viral loads ≤1000 copies/mL with most of the specimens at or near the lower limit of detection for both test procedures (<50 copies/mL for the UltraSensitive procedure; <400 copies/mL for the Standard procedure), and these studies are described in detail in the attached BD White Paper.

In summary, our investigation determined that apparent HIV-1 viral loads are elevated in some samples when plasmas are frozen in PPT[™] tubes *in situ* prior to testing. Furthermore, these increases were more pronounced when the Standard, rather than the UltraSensitive, sample processing procedure was used. Viral loads were not elevated when plasmas were stored in PPT[™] tubes at 4°C or removed to a secondary tube before freezing. Additionally, variations in sample handling parameters such as increased centrifugation time and speed and time stored as whole blood at ambient temperature were found to slightly increase viral loads in specimens collected in BD Vacutainer[®] K₂EDTA tubes.

When interpreting HIV-1 viral load results in patients with very low viral loads using BD Vacutainer[®] PPT[™] tubes and the Roche AMPLICOR or COBAS AMPLICOR HIV-1 MONITOR[®] Test, v1.5, you are advised to store plasma in PPT[™] without freezing and remove plasma from PPT[™] tubes to a secondary tube if the plasma samples are to be frozen prior to testing. Furthermore, in order to obtain consistent results, all sample handling conditions, regardless of collection tube type, must be validated by the laboratory and must be consistent throughout the viral load monitoring process.

* Holodniy M, Rainen L, Herman S, Yen-Lieberman B. Stability of Plasma HIV Viral Load in VACUTAINER[®] PPT[™] Plasma Preparation Tubes During Overnight Shipment. J Clin Micro 2000; 38(1):323-26.