



LABORATORY BULLETIN

2007- August - 22

Bulletin # 2007 - 11

To: Colleagues Ordering HIV Quantitative RNA Viral Load Test

Re: Changing the Assay for HIV Quantitative RNA Viral Load at ProvLab

PLEASE POST OR DISTRIBUTE WIDELY AS APPROPRIATE

Highlights of this change are as follows:

- Quantitative HIV RNA viral load at ProvLab will be performed with a new assay, Roche COBAS® AmpliPrep/COBAS® TaqMan®, after August 27, 2007 when the stock of the previous test, Roche COBAS® Amplicor®, is depleted.
- This new assay has comparable results with Roche COBAS® Amplicor®. However, we have observed more variation between the two assays (> 0.5 log difference) for samples with high viral load (>100,000 copies/ml) in a retrospective comparison and Roche COBAS® Amplicor® reported higher viral loads.
- It is **IMPORTANT** to always submit two full EDTA blood tubes for HIV viral load, as the new assay needs a higher volume of plasma (1 ml) for testing. Transport requirements remain the same as for the Amplicor assay.
- If HIV genotype resistance testing (GART) is ordered at the same time, please submit two **extra** EDTA blood tubes. It is possible that recollection of blood is needed for add-on GART request.
- For pediatric patients where the collection of two full EDTA is not possible, the plasma sample will be diluted before testing which will decrease the sensitivity of the assay.

The details:

As a technological advancement at ProvLab, the quantitative HIV RNA viral load test will be performed on a real-time RT-PCR platform, Roche COBAS® AmpliPrep/COBAS® TaqMan®, instead of an end-point conventional RT-PCR assay, Roche COBAS® Amplicor®. One of the advantages of the Roche COBAS® AmpliPrep/COBAS® TaqMan® assay is the broader linear range of 40 to 10,000,000 copies/ml, so there will be no need to distinguish between regular HIV viral load and ultra-sensitive HIV viral load request. The target of detection for the Roche COBAS® AmpliPrep/COBAS® TaqMan® assay is still in the *gag* region and is supposed to detect HIV-1 group M: A-H clades. However, for patients who have an endemic

source for the HIV infection, i.e. higher likelihood of non-B clade, it is still a good idea to submit a baseline sample for clade and b-DNA testing. For more information: <http://www.provlab.ab.ca/guide-to-services.pdf> page 66-67.

We have performed an evaluation and a retrospective comparison study between Roche COBAS® AmpliPrep/COBAS® TaqMan® and Roche COBAS® Amplicor®:

- The dilution series provided acceptable data on the detection limit and illustrated good linearity of the Roche COBAS® AmpliPrep/COBAS® TaqMan® assay. The precision on intra-run and inter-run for multiple repeats of the same sample was good.
- For 6 samples with quantifiable viral load <400 copies/ml by ultrasensitive Roche COBAS® Amplicor®, only 1 sample had a difference of > 0.5 log between the two methods, with Roche COBAS® AmpliPrep/COBAS® TaqMan® reporting higher viral loads (Range of log difference: 0.12 – 0.69, median = 0.24).
- For 17 samples with viral load from 400 to <100,000 copies/ml, only 1 sample had a difference of > 0.5 log between the two methods, with Roche COBAS® AmpliPrep/COBAS® TaqMan® reporting higher viral loads (Range of log difference: 0.04 – 0.73, median = 0.23).
- For 11 samples with viral load >100,000 copies/ml, Roche COBAS® Amplicor® tends to report higher viral loads and 6 samples had a log difference of > 0.5 log between the two assays (Range of log difference: 0.06 – 1.07, median = 0.51).

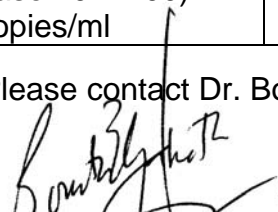
The Roche COBAS® AmpliPrep/COBAS® TaqMan® has an automatic platform for nucleic acid extraction and requires at least 1 ml plasma for the test. Thus it is very important to always submit sufficient blood sample for the test. If GART is ordered at the same time, please submit two extra EDTA blood tubes. For pediatric patients where blood collection is difficult, the plasma sample will be diluted before testing which will decrease the sensitivity of the assay and may affect the linearity of the assay. The transport requirements for HIV viral load samples remain the same:

- Within Calgary Health Region and Capital Health: **Two full** EDTA lavender top tubes (4 ml). **DO NOT** spin. **DO NOT** ship on ice packs or cold packs. Must be received within **6 – 8 hours** of collection.
- Outside of Calgary Health Region and Capital Health: **Two full** PPT (Plasma Preparation Tubes) (5 ml). Centrifuge. **DO NOT pour off**. Refrigerate until transport. Transport on ice packs or cold packs. Must be received within **24 hours** of collection. PPT can be ordered from ProvLab distribution center: 780-407-8971
- For pediatric collection: at least one 2 ml mauve top EDTA tube. **DO NOT** spin, **DO NOT** ship on ice packs or cold packs, must be received within **6 – 8 hours** of collection.

Below please find the new reporting format for the Roche COBAS® AmpliPrep/COBAS® TaqMan® assay:

If there is sufficient plasma for testing (at least 1 ml)	
When RNA viral load is < 40 (log base 10 <1.60) copies/ml	<p><u>Report statement:</u> HIV RNA NOT QUANTIFIABLE < 40 (log base 10 <1.60) copies/ml</p> <p><u>Report comment:</u> The linear range of the assay is between 40-10,000,000 copies/ml. Viral load was determined using COBAS AmpliPrep/TaqMan HIV-1 test (Roche Diagnostic Systems).</p>
When RNA viral load is 40-10,000,000 copies/ml	<p><u>Report statement:</u> HIV RNA DETECTED (viral load value and log base)</p> <p><u>Report comment:</u> Same as above</p>
When RNA viral load is > 10,000,000 (log base 10 >7.00) copies/ml	<p><u>Report statement:</u> HIV RNA DETECTED >10,000,000 (log base 10 >7.00) copies/ml</p> <p><u>Report comment:</u> Same as above</p>
If there is insufficient plasma (at least 250 ul for 1:5 dilution)	
When RNA viral load is < 200 (log base 10 <1.60) copies/ml	<p><u>Report statement:</u> HIV RNA NOT QUANTIFIABLE < 200 (log base 10 <2.30) copies/ml</p> <p><u>Report comment:</u> Insufficient blood was received thus the sample was diluted at 1:5 for testing. The lower limit of detection of the assay was changed to 200 copies/ml and the linearity and accuracy of the quantitation could be affected. Viral load was determined using COBAS AmpliPrep/TaqMan HIV-1 test (Roche Diagnostic Systems).</p>
When RNA viral load is 40-10,000,000 copies/ml	<p><u>Report statement:</u> HIV RNA DETECTED (viral load value and log base)</p> <p><u>Report comment:</u> Same as above</p>
When RNA viral load is > 10,000,000 (log base 10 >7.00) copies/ml	<p><u>Report statement:</u> HIV RNA DETECTED >10,000,000 (log base 10 >7.00) copies/ml</p> <p><u>Report comment:</u> Same as above</p>

Please contact Dr. Bonita Lee with any questions


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This bulletin was distributed to:

- Infectious Disease Physicians Edmonton
- Infectious Disease HIV pharmacists and nurse practitioners in Northern Alberta
- Dr. John Gill
- Dr Taj Jadavji
- STD Centre, Capital Health Region
- Regional Laboratory Managers of Alberta, Northwest Territories, Nunavut
- Regional Laboratory Directors of Alberta