

Labstract – October 2011

IMPORTANT NOTICE – Cobas Ampliprep/Cobas Taqman HIV-1 Qual Test - New test for HIV1 PCR Change in Testing Methodology/Sample Submission

To Health Care Providers

The Public Health Ontario (PHO) Public Health Laboratories (PHL) has replaced its current PCR test for Human Immunodeficiency Virus Type 1 with a new automated qualitative nucleic acid amplification test (also based on PCR) for the detection of HIV 1 RNA and Proviral DNA. This change has been prompted by the sole manufacturer of this test (Roche Diagnostics) which has replaced their former test with this new test called the *COBAS AmpliPrep/COBAS TaqMan HIV -1 Qual Test*.

As with the previous test, this test remains a research use only test. The reported lower limit of detection (LLD) of this test varies depending on the sample being tested. According to the literature, the following are approximate LLD values based on specimen type¹:

Specimen Type	Lower Limit of Detection (copies/ml)
Plasma	~500
Whole Blood	~700
Dried Blood Spot	~1100

This Labstract summarizes for submitters what remains the same and what has changed as a result of this new methodology.

WHAT REMAINS UNCHANGED:

How the test is applied and when is it appropriate to order this test?

This test detects the presence of virus in blood samples and is primarily used for the diagnosis of HIV infection in babies born to HIV positive mothers. Testing for HIV antibody in such patients is not an appropriate diagnostic tool because of the presence of maternal HIV antibody.

In this group of patients it is recommended that the first test be performed at or near the time of birth followed by repeat testing 1 and 2 months later. This should then be followed by an antibody test at 18 months of age to confirm that a baby with no evidence of HIV RNA/DNA by PCR has lost their maternal antibodies.

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This test may be used in other clinical situations such as needle stick injuries, sexual assault and other special circumstances. As this is a specialized test, notification and approval from the PHO HIV laboratory is required. Submitters should consult with one of the PHL medical/clinical microbiologists for more specific advice in these situations.

WHAT HAS CHANGED:

Test Methodology

According to the manufacturer, this new PCR test detects HIV-1 RNA **and**/or proviral DNA . The previous method only detected HIV-1 proviral DNA in lymphocytes. The new PCR test has also been validated by the manufacturer to include whole blood, plasma and dried blood spots (DBS). The previous method was only valid on whole blood.

IMPORTANT NOTE: The choice of sample type will dictate which type of nucleic acid is detected. If plasma is being tested RNA is primarily the sole analyte being detected. In the case of whole blood and DBS, both RNA **and** DNA are the analytes that can be potentially detected. The choice of sample type being submitted may have implications in the clinical diagnostics setting.

Sample submission

New sample submission and storage requirements are in effect immediately.

1. Appropriate specimens:
 - a. Human whole blood collected in EDTA anticoagulant only (This remains unchanged from what was recommended previously and is the preferred specimen type). Heparin has been shown to inhibit PCR and must not be used with this procedure.
 - b. Human plasma collected in the anticoagulant EDTA.
2. Specimen storage:
 - a. EDTA-anticoagulated whole blood may be stored for up to 12 hours at room temperature or for an additional 72 hours at 2-8 °C prior to testing or separation of plasma. Do not freeze.
 - b. Plasma must be separated from whole blood within 12 hours after collection if stored at room temperature and within 72 hours if stored at 2-8 °C.
3. Specimen volume:
 - a. A minimum of 500 uL of plasma or whole blood is required to complete testing. However, it is preferred to have greater than or equal to 1 ml of specimen to allow for additional testing if required.

The PHO HIV laboratory will attempt to validate the use of dried blood spots and will notify users once it has completed this evaluation.

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NB: An additional SST tube should be collected and submitted for standard HIV testing which is carried out with each PCR.

Results Interpretation:

HIV1 RNA/ Proviral DNA	Interpretation
Not Detected	If Plasma tested: <ul style="list-style-type: none"> No HIV1 RNA or Proviral DNA Detected If Lymphocytes tested (from whole blood specimen): <ul style="list-style-type: none"> No HIV1 Proviral DNA Detected
Detected	If Plasma tested: <ul style="list-style-type: none"> HIV1 RNA and/or Proviral DNA Detected If Lymphocytes tested (from whole blood specimen): <ul style="list-style-type: none"> HIV1 Proviral DNA Detected
Indeterminate	Indeterminate HIV1 RNA or Proviral DNA results. *See comment.

*Occasionally, samples may contain unknown substances which inhibit the PCR reaction resulting in an uninterpretable test result. Other factors may also affect the ability of the test to yield a valid result (e.g. extremely high viral load). These will be reported as “Indeterminate” and a repeat sample will be requested.

For further information:

- PHO Customer Service Centre in Toronto at **416-235-6556** or toll free at **1-877-604-4567**
- For the PHO Specimen Collection Guide and previous Lababstracts, refer to <http://www.oahpp.ca/services/public-health-laboratories.html>
- Subscribe to lababstracts, please e-mail lababstracts@oahpp.ca

Please note: Public Health Ontario is the new operating name for the Ontario Agency for Health Protection and Promotion.

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References:

1. Stevens W et al. Performance of a Novel Human Immunodeficiency Virus (HIV) Type 1 Total Nucleic Acid-Based Real-Time PCR Assay Using Whole Blood and Dried Blood Spots for Diagnosis of HIV in Infants. *J Clin Microbiol* 2008;46(12)
2. Product Insert – COBAS AmpliPrep/COBAS TaqMan HIV-1 Qual Test. Roche Molecular Systems, Inc. August 2009