Public Health Ontario | Santé publique Ontario

LABSTRACT – Released July 2017, updated April 2020

Hepatitis C Virus (HCV) RNA detection using Dried Blood Spots (DBS) – Update

Audience

Clinicians assessing patients for the presence of current/active HCV infection and need for treatment

Overview

The submission of DBS samples must meet the criteria described below. Any request that does not meet these criteria requires approval from a Public Health Ontario (PHO) Laboratory microbiologist prior to collection and submission. PHO Laboratory will accept appropriately collected DBS samples for the detection and quantification of HCV RNA in the following circumstances:

- Clients that have had a confirmed HCV antibody result from a test conducted at PHO Laboratory ,
- Clients that have had a confirmed HCV antibody result from a Point of Care (POC) test and this information is clearly indicated on the requisition

AND

- Clients meet at least one of the following conditions:

    1. Are from remote and/or isolated areas where access to laboratory facilities may not be available or, if available, may have limited capacity for the processing and transportation of frozen EDTA plasma or serum; OR
    2. Unable to provide a venous-collected sample

DBS samples are not a replacement for venous-collected samples as the sensitivity for detecting HCV RNA from DBS is lower than from EDTA plasma- and serum-collected samples. Use of DBS for HCV RNA testing should only be considered when standard methods are not possible.

Testing will be performed using the Roche cobas® 6800 *System.*

Ontario Agency for Health Protection and Promotion
Agence de protection et de promotion de la santé

Background information

PHO Laboratory began accepting DBS specimens for HCV RNA testing in July 2017. The purpose of this Labstract update is to provide clarification of requirements for HCV RNA testing from DBS.

SPECIMEN COLLECTION INSTRUCTIONS AND SUBMISSION REQUIREMENTS

Appropriate specimen types:

- Capillary blood from a finger-prick or heel-prick spotted onto a blood collection filter card; OR
- Venous blood collected in EDTA that has been spotted onto a blood collection filter card

Required supplies:

- Whatman® 903 blood collection filter card
- Heavy duty and moisture-resistant sealable biohazard bags for individual DBS cards
- MiniPax® absorbent packets (desiccant)
- Supplies for performing a finger prick
- Sealable biohazard specimen bag
- PHO Laboratory Hepatitis PCR Requisition (Part A: HCV RNA) available at publichealthontario.ca/requisitions

Preparation and submission of DBS:

- Follow the fingerprick procedure.
- Apply gentle pressure to the finger to allow a large drop of free flowing blood to collect at the puncture site.
- Working quickly, hold the filter paper by the edges and touch the filter paper gently against the large drop of blood and in one step allow a sufficient quantity of blood to soak through and completely fill or saturate a circle. A completely saturated spot will contain 70 to 100 ul of blood and will cover to the edges of the circle. Improperly collected DBS will be cancelled and not tested for HCV RNA due to inadequate sensitivity.
- Repeat until you have collected enough blood to fill completely at least 4 circles on the blood collection card.
- If collecting spots using a pipette (i.e. venous sample), collect 100ul of blood and gently apply to the paper.

- Do not press the paper against the puncture site.
- While the collection card contains 5 circles, only one patient's blood may be collected on one card.
- Do not layer successive drops of blood or apply blood more than once in the same collection circle.
- Allow blood spots to fully air dry horizontally for at least 3 hours before submitting to PHO Laboratory for testing; however, do not allow sample to dry for more than 24 hours.
- Label filter paper with at minimum two identifiers (including the patient's full name), and the date of DBS collection.
- Insert into a sealable plastic biohazard bag; each patient DBS must be packaged individually, with one card per bag with a desiccant sachet in each bag. Bags containing multiple patient DBS samples will not be tested for HCV RNA due to risk of cross contamination.
- Complete **all** fields of the PHO Laboratory Hepatitis PCR Requisition (Part A: HCV RNA) available at http://www.publichealthontario.ca/requisitions. Include, the patient's full name, date of birth, Health Card Number (must match the specimen label), enter test submitter's name and address, reason for testing, and other relevant clinical information.
- Place individually packaged DBS specimen into a sealable biohazard bag for transport and seal bag.
- Insert the completed requisition in the pocket on the outside of the sealed biohazard bag.
- Deliver to PHO Laboratory at 2-25^{0}C.

Important notes/limitations for the use of DBS for HCV RNA detection:

1. When DBS samples are appropriately prepared, they will be stable at room temperature for up to 30 days.

2. Based on internal validation studies performed at PHO Laboratory, HCV RNA testing conducted on DBS is less sensitive compared to venous-collected samples. The lower limit of detection (LLOD) of HCV RNA using two DBS per test (10^3 IU/mL) is approximately 1.6 to 2.0 logs higher than a concomitantly tested EDTA plasma or serum sample (LLOD = 15 IU/mL); thus DBS samples should NOT be used to rule out active HCV infection or to determine whether a patient on treatment has achieved an undetectable HCV RNA level.

3. HCV genotyping will be performed on all first time samples submitted to PHO Laboratory with HCV RNA levels ≥500 IU/mL, (provided there is sufficient sample to complete the genotype testing).

Testing frequency and turnaround time (TAT):

Testing is performed once per week. TAT may be up to 10 days.

Results Interpretation:

Hepatitis C RNA Viral Load Reported Result	Interpretation
Target Not Detected	***HCV RNA Not Detected**
≤1.00 E+3 IU/mL	**HCV RNA detected below the linear range of the assay. Unable to quantify.** **(Note: The result for HCV RNA is below the linear range of the assay which is 1000 IU/mL and thus the exact value cannot be calculated)**
Quantitative value reported for detections >1.00 E+3 IU/mL	**Viral Load will be provided**

*** Note:** An undetectable HCV RNA viral load result from DBS does not rule out active HCV infection. Based on internal validation studies performed at PHO Laboratory, HCV RNA testing conducted using DBS is less sensitive than venous-collected samples. Therefore patients with an undetectable HCV RNA viral load tested using DBS should have an EDTA plasma or serum venous sample collected and submitted for follow-up testing.

References

Greenman J1, Roberts T, Cohn J, Messac L. Dried blood spot in the genotyping, quantification and storage of HCV RNA: a systematic literature review. J Viral Hepat. 2015 Apr;22(4):353-61

For further information

- Contact the PHO Customer Service Centre at 416-235-6556 or 1-877-604-4567 (toll-free), or by email at customerservicecentre@oahpp.ca
- For PHO Laboratory specimen collection information and previous Labstracts, refer to publichealthontario.ca/test directory
- The current version of the PHO Laboratory General Test Requisition and other forms are available at publichealthontario.ca/Requisitions
- To subscribe to future Labstracts, register on our website
- To register for Autofax and receive laboratory reports by fax directly from our laboratory information system as soon as they are released, contact the PHO Laboratory Customer Service Centre.