Xpert® HCV VL Fingerstick*

Frequently Asked Questions: *Sales Relevant Information*

Q: What are the main differences between the Xpert® HCV Viral Load and the Xpert HCV VL Fingerstick tests?

A: The Xpert HCV VL Fingerstick test is intended for detection and quantification of Hepatitis C virus (HCV) RNA from 100µl human capillary fingerstick EDTA whole blood and venous whole blood collection. In contrast, the Xpert HCV Viral Load test is intended for quantification and detection from 1mL plasma or serum derived from venous collected blood for Xpert HCV Viral Load. The lower limit of quantification is 100IU/mL for Xpert HCV VL Fingerstick compared with 10IU/mL for Xpert HCV Viral Load.

Q: Is Xpert HCV VL Fingerstick a diagnostic test or a monitoring test?

A: Xpert HCV VL Fingerstick is intended to aid in the initial diagnosis in individuals at high risk of HCV infection or in anti-HCV positive individuals and to aid the management of HCV infected patients undergoing antiviral therapy. Detection of HCV RNA indicates that the virus is replicating and therefore indicates active infection

Q: Is Xpert HCV VL Fingerstick a laboratory or POC test?

A: Xpert HCV VL Fingerstick can be used for both near patient and laboratory settings.

Q: Can the test be used in a one step or two step algorithms?

A: The Xpert HCV VL Fingerstick test can be used as part of a 'one step' or 'two step algorithm'. The European Association for the Study of Liver (EASL) 2018 recommendations for HCV support the use of a direct (one step) test strategy for HCV RNA. In addition the Xpert HCV VL Fingerstick test can be used for confirmation in HCV antibody positive patients.

Q: Will the compassionate price be available for Xpert HCV VL Fingerstick?

A: Compassionate pricing will be available for the locations which meet the HBDC eligibility criteria. These are handled by our HBDC dedicated teams. This pricing will be subject to normal terms and conditions.

Q: Will Xpert HCV Viral Load be discontinued?

A: No. Xpert HCV Viral Load will continue to be supplied by Cepheid.

Q: Why is the test called "Fingerstick" even though venous blood is also a validated sample?

A: The Xpert HCV VL Fingerstick was validated for venous whole blood to support users who are collecting blood by venipuncture for multiple pathology tests. These users can use the Fingerstick test without having to take an additional capillary sample from the patient.

Q: Will Xpert HCV VL Fingerstick work for blood donor/transplant screening?

A: Xpert HCV VL Fingerstick is not intended for blood donor or organ transplant screening.

Q: Do you plan to make the Xpert HCV VL Fingerstick assay available on the Omni system?

A: Yes, we have plans for Xpert HCV VL Fingerstick to eventually be available for the Omni system after that system is launched.

Q: Can I purchase the Minivette® POCT sample collection devices from Cepheid?

A: Yes. The minivette cuvettes are available to purchase from Cepheid. The description is Sarstedt Minivette POCT 100µl and Cepheid product code – Minivette 100E-100.

* CE-IVD. *In Vitro* Diagnostic Medical Device. Not available in all countries. Not available in the United States.

FAQs: *Sales Relevant Information (continued)*

Q: Can I purchase Safety Lancets from Cepheid?

A: No. However the customer support team can recommend lancets that will provide appropriate sample collection for the assay.

The reason we do not provide the lancet is that it is considered an invasive sampling device, falls under the 93/42/EEC directive for medical devices and not the IVD directive 98/79/EC.

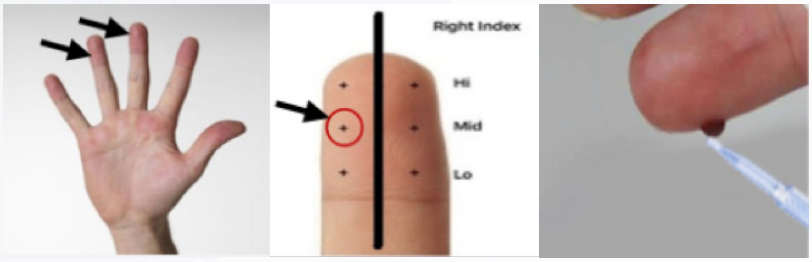
Q: My customer has a GX System already running the Xpert® HCV Viral Load test. Will my customer have to buy new system or modules?

A: No, there is no change in the instrument needed. Please simply check whether your customer has the latest version installed (GX DX 4.7b or higher / INF Xpertise 6.4b or higher). For running the assay on an existing instrument, your customer should make sure that the Xpert HCV VL Fingerstick assay definition file (ADF) is installed.

Frequently Asked Questions: *Technical Information*

Q: What is the best practice for sample collection? Should I perform more fingerpricks to collect enough blood?

A: The sample should be collected from the middle or ring finger of non dominant hand and the fingerprick should be performed on the side of the finger where the skin is thinner and avoiding the finger pads where the skin is thicker. Once the fingerprick is performed wipe away the first drop of blood before collecting the sample in the Minivette POCT.



From user feedback it is not often that multiple fingersticks are required to fill Minivette POCT. This may happen if collecting additional spots for dried blood spot testing at the same time. In general collection from multiple fingersticks is not always as successful as collecting from one good fingerstick, simply due to clotting of the blood on the end of the Minivette POCT. Good patient preparation, lancet type and technique, plus minivette technique will help.

Tips include:

1. Patient

- Massaging the hand/lower arm to warm it up helps a lot. Opening and closing hand a few times can help.
- Offer the patient some hydration before sample collection. A warm drink will help both these.
- Washing hands in warm water and asking the patient to stand up and hold hand in downward position so gravity will help blood flow.

2. Lancet type and technique

- We recommend to use the Sarstedt Safety lancet super (Sarstedt Prod Number 85.1018), violet, with a blade width of 1.5 mm, penetration depth 1.6 mm and which is suitable for collecting at 100-500µl of blood from finger, heel, ear. Other lancets with similar features and that collect at least 100 µl may also be considered.
- When collecting the sample hold the lancet at 45 degree angle with reasonable pressure to ensure good fingerprick to collect sufficient sample. When possible, placing the hand on a stable surface will help having a good cut, as this avoids the hand going backward during the fingerstick.

3. Minivette technique

- Collect the drop of blood by touching the droplet, and not the skin. **This is important.** The minivette relies on the surface tension on the droplet of blood to begin. Make sure form a big enough blood drop on the finger. Three to 4 blood drops should be enough to fill the Minivette POCT. Scraping/wiping the skin with the Minivette POCT tends to clot the cut further — so the formation of a droplet start is important. Also aim to either hold the Minivette POCT horizontal as per the manufacturer's instructions, although a 45 degree angle with the hand held downwards will encourage sample to be drawn up into the device.
- Do not hold the minivette completely vertical as this will introduce air in the minivette collection device. If the blood begins to clot or spread all over the finger, wipe away with a gauze, then form a new blood drop.

FAQs: *Technical Information (continued)*

Q: Why is there a difference of 50 minutes between Xpert® HCV Viral Load and Xpert® HCV VL Fingerstick?

A: The test is using lower sample volume (100 µl blood) compared to 1 mL plasma. By doing that it is possible to scale down the liquids in the cartridge and the movement and mixing could be shortened.

Q: Is a software upgrade required to run Xpert® HCV VL Fingerstick on the GeneXpert® and Infinity Systems?

A: Customers will need to upgrade to GeneXpert Dx software version 4.7b and for Infinity to Xpertise version 6.4b. Customers using Cepheid Link will need to upgrade to GeneXpert software version 4.8 and/or Xpertise version 6.5. If customers are connected to a Laboratory Information System (LIS), please note they will have to contact their LIS provider to implement the test settings as the result reporting will be different from that of the current Xpert HCV VL Fingerstick assay.

Q: Which collection devices are validated to use with Xpert HCV VL Fingerstick?

A: The only validated collection device is the Minivette® POCT 100 µL K3E (P/N: MINIVETTE 100E-100, 100 per box).

Q: What is the smallest sample size I can use with Xpert HCV VL Fingerstick?

A: To ensure correct quantification, 100µL should be used by customers. An insufficient sample volume (≤70 µL) will cause an error 2097 due to the non-acceptation of the criteria to pass the Sample Volume Adequacy control (SVA).

Q: Does the test have an Early Assay Termination (EAT) feature?

A: No, the test does not have an Early Assay Termination feature.

Q: What are the quality controls included in the Xpert HCV VL Fingerstick test?

A: Each test includes a Sample Volume Adequacy (SVA) control, Internal Quantitative Standard High and Low (IQS-H and IQS-L), which is also a sample processing control (SPC), a Probe Check Control (PCC) and Lot Specific Parameters (LSP) for quantification.

Q: What is the purpose of the Lot Specific Parameters (LSP) control?

A: Each kit lot has built-in LSP generated from an HCV calibration panel traceable to the 4th WHO International Standard for HCV NAT (NIBSC code 06/102)7 and the internal quantitative standards high and low, IQS-H and IQS-L. The Ct values of the IQS-H and IQS-L are included as parameters in the equation that forms the LSP of the kit lot. The LSP are unique for each kit lot and are used to ensure correct quantification.

Q: How do I have to store samples?

A: Whole blood specimens collected with the Minivette POCT may be held in the collection device for up to 15 minutes at 5-35°C.

Venous whole blood collected in EDTA as the anticoagulant may be stored for up to 72 hours at 2-8°C or for up to 24 hours at a maximum of 35°C. Also, venous whole blood collected in EDTA may be stored in a sterile vial for up to 6 months at -20°C.

Q: How long can samples be held in the Minivette POCT?

A: Samples can be held in the Minivette POCT for up to 15 minutes at 5-35°C.

Q: How long can samples be held in the cartridge before starting the test?

A: The test must be started within 4 hours of the sample being added to the cartridge.

Frequently Asked Questions: *Operational Information*

Q: Will there be any external control material for validation purposes only?

A: Cepheid is actively working to tailor a QC material panel for Xpert® HCV VL Fingerstick.

Q: How is Xpert HCV VL Fingerstick compared to Xpert HCV Viral load in terms of sensitivity and specificity?

A: The sensitivity and specificity of Xpert HCV VL Fingerstick was assessed using specimens collected from individuals determined to be at risk for HCV infection. Table 11 from the PI shows the performance of the Xpert HCV VL Fingerstick test using capillary and venous WB specimens, relative to an HCV RNA quantitative comparator method using EDTA plasma from the same specimen.

Table 11. Performance of the Xpert HCV VL FS Assay versus Two HCV RNA Comparator Methods in a HCV High Risk Population

	N	TP[a]	FP[b]	TN[c]	FN[d]	Sensitivity (%)	95% CI of Sensitivity (%)	Specificity (%)	95% CI of Specificity (%)
HCV VL FS Capillary vs Comparator (Plasma)	339	54	0	283	2	96.4	87.9 – 99.0	100	98.7 – 100
HCV VL FS Venous vs Comparator (Plasma)	352	55	0	295	2	96.5	88.1 – 99.0	100	98.7 – 100
HCV VL FS Capillary vs HCV VL (Plasma)	339	54	0	278	7	88.5	78.2 – 94.3	100	98.6 – 100
HCV VL FS Venous vs HCV VL (Plasma)	352	55	0	290	7	88.7	78.5 – 94.4	100	98.7 – 100

a. True Positive
b. False Positive
c. True Negative
d. False Negative

Q: How is Xpert HCV VL Fingerstick compared to Xpert HCV Viral Load in terms of Limit of Detection (LOD)?

A: The Limit of Detection for Xpert HCV VL Fingerstick is 13-35 IU/mL depending on the genotype, for full details please see the package insert; for Xpert HCV Viral Load, the LOD is 2-10 IU/mL dependant on genotype. Please see the package insert for detailed information.

Q: What is the Limit of Quantification (LOQ)?

A: HCV VL FS assay can quantify 100 IU/mL of HCV RNA with an acceptable trueness and precision; for plasma samples the LOQ is 10 IU/mL.

Q: Must I use a Safety Lancet 18G depth 1.8mm from Sarstedt, as mentioned in the Package Insert, or can use an alternate device?

A: Cepheid recommends disposable Safety-Lancet Super, 1.5 mm (Sarstedt, P/N: 85.1018 or similar). Alternative devices may be used. For callus fingers it is recommended to use a blade similar to what is recommended. The lancet mentioned is most likely sufficient for non-dehydrated individuals with normal fingers.

Q: Must I use a Disposable Minivette® POCT 100 µL K3E device, as mentioned in the Package Insert, or can I use an alternate device?

A: The only validated collection device by Cepheid is Minivette POCT 100 µL K3E. It is important to add 100 µl sample, not more, not less. If the customer does not have Minivettes (can be purchased directly from Cepheid) and a capillary connected to a tube is used, customer will have to ensure to collect >100 µl, i.e. in 150 µl-range and use a 200 µl-precision pipette with filter tip to transfer exactly 100 µl.

FAQs: *Operational Information (continued)*

Q: What are the recommended storage conditions for the kits?

A: Store the Xpert HCV VL Fingerstick test cartridges and reagents at 2-28°C. Bring the cartridges to room temperature prior to use if they have been stored cold.

Q: What are the recommended transport conditions for the samples?

A: Whole blood specimens collected with the Minivette POCT may be held in the collection device for up to 15 minutes at 5-35°C. Venous whole blood collected in EDTA may be stored in a sterile vial for up to 6 months at -20°C, 72 hours at 2-8°C or 24 hours at a maximum of 35°C.

Q: Does the test have potentially interfering substances?

Substance	Tested Concentration
Albumin	9 g/dL
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Human DNA	0.4 mg/dL
Triglycerides	3000 mg/dL

A: The susceptibility of the Xpert HCV VL Fingerstick test to interference by elevated levels of endogenous substances, by autoimmune disease markers, and by drugs prescribed to HCV infected patients was evaluated.

Pool	Drugs
1	Zidovudine, Abacavir sulfate, Saquinavir, Ritonavir, Interferon-alfa 2b, Ombitasvir, Paritaprevir, Dasabuvir
2	Fosamprenavir, Ribavirin, Ledipsavir, Sofosbuvir, Daclatasvir, Simeprevir, Peginterferon-alfa 2a, Peginterferon-alfa 2b
3	Tenofovir disoproxil fumarate, Lamivudine, Indinavir sulfate, Ganciclovir, Acyclovir, Valganciclovir HCl
4	Stavudine, Efavireniz, Lopinavir, Enfuvirtide, Ciprofloxacin, Clarithromycin, Maraviroc
5	Nevirapine, Nelfinavir, Azithromycin, Valacyclovir

The inhibitory effects were evaluated both in the presence and absence of 300 IU/mL HCV RNA reference material (HCV genotype 1b, calibrated against the WHO 4th International Standard, NIBSC code 06/102). Elevated levels of the endogenous substances listed in the table below were shown not to interfere with the quantification of the Xpert HCV VL Fingerstick test or impact the assay specificity.

The drugs as presented in the table below were shown to not interfere with the quantification of the Xpert HCV VL Fingerstick test or impact the assay specificity when tested at three times peak level concentration in five drug pools.

Testing of specimens from twelve individuals with autoimmune disorders which tested positive for the systemic lupus erythematosus (SLE) marker, of which seven were also anti-nuclear antibody (ANA) positive, and testing of specimens from eight individuals testing positive for rheumatoid factor (RF) showed no interference with either the quantification or specificity of the Xpert HCV VL Fingerstick.

FAQs: *Operational Information (continued)*

Q: Does the test have any limitations?

A: This test has been validated only for use with capillary and venous whole blood collected in EDTA. Testing of other specimen types may result in inaccurate results. The test is not for the screening of blood, plasma, serum, or tissue donations for HCV. A negative test result with the Xpert HCV Viral Load test does not preclude a patient from having an HCV infection. Rare mutations within the target region of the Xpert HCV VL Fingerstick test may affect primer or probe binding resulting in under quantitation or failure to detect the virus.

Q: Can I use blood collected in Sodium Citrate or is it mandatory to use blood collected in EDTA?

A: Use of sodium citrate as anticoagulant has not been validated by Cepheid.

Q: What will be the Test run Time (TRT) for this new test?

A: The TRT for the Xpert HCV VL Fingerstick test is significantly faster than the Xpert HCV Viral Load test: TRT for Xpert HCV VL Fingerstick is 57 minutes. The reduced TRT will increase the throughput of Xpert HCV VL Fingerstick on GeneXpert and Infinity Systems.