ENGLISH

HCV Antibody Test

Single-use rapid assay for the detection of antibodies to Hepatitis C Virus

REF **90-1127** – One INSTI® HCV Antibody Test with support materials (for POC use)

90-1157 – 50 INSTI® HCV Antibody Tests with support materials (for POC use)

Read the Instructions for Use completely before using the product. Although the assay is designed to be simple to use, conformance with the test procedure is necessary to ensure accurate results.

INTENDED USE - Not for Donor Screening

The INSTI® HCV Antibody Test (referred to as INSTI HCV Test) is a single use, rapid, flow-through in vitro qualitative immunoassay for the detection of antibodies to Hepatitis C Virus in human fingerstick whole blood, venous whole blood, serum and EDTA plasma. The test is intended for use as an aid in diagnosis of HCV infection in individuals at risk who are over 18 years of age by trained personnel in medical facilities, clinical laboratories, emergency care situations, and physicians' offices as a test capable of providing results in one minute. Although suitable for near-patient or point-of-care (POC) testing, the INSTI HCV Test is not suitable for home testing.

SUMMARY

Hepatitis C virus (HCV), a single-stranded, positive-sense RNA virus belonging to the Flaviviridae family, is the causative agent for most, if not all, non-A, non-B hepatitis[1]. Discovered in 1989 and with over 60 subtypes identified that are classified into 7 genotypes (1-7), HCV is a leading cause of liver disease and can cause both acute and chronic hepatitis. HCV is a blood-borne virus and is generally transmitted through contact with contaminated blood or blood products, such as may happen through injection drug use, unsafe injection practices, transfusion of unscreened blood and blood products, and sexual practices that lead to exposure to blood. New HCV infections are usually asymptomatic and as a result, few people are diagnosed when the infection is recent. While around 30% of infected persons spontaneously clear the virus without any treatment, the remaining 70% of persons will develop chronic HCV infection which can progress to cirrhosis or liver cancer. Chronic HCV infection is also often undiagnosed as it may remain asymptomatic for decades until symptoms develop that are associated with serious liver damage. Globally, an estimated 71 million individuals have chronic HCV infection worldwide and thus HCV infection is a major healthcare concern[2].

The presence of antibodies to HCV indicates that the individual may be currently infected and capable of transmitting the virus. HCV antibody tests are used in combination with other tests (e.g. HCV RNA) to detect HCV infection.

PRINCIPLES OF THE TEST

The **INSTI HCV Antibody Test** is a manual, visually read, flow through immunoassay for the qualitative detection of HCV antibodies. The assay is packaged as a kit containing a single-use Test Device, Sample Diluent (Solution 1), Colour Developer (Solution 2), and Clarifying Solution (Solution 3) with support materials (lancet, pipette, and alcohol swab). The test consists of a synthetic filtration membrane positioned atop an absorbent material within a plastic cartridge, referred to as the Test Device. The membrane contains a recombinant chimeric protein derived from the core, NS3 and NS4, and NS5 regions of the HCV genome (test spot) and a protein-A treated spot (procedural control). The protein-A at the control spot reacts with IgM and IgG antibodies normally present in blood and blood components and acts as in-built quality control. Results are visualized in as little as 60 seconds following reactions with proprietary INSTI solutions during the test procedure.

HCV Antibody Detection: The INSTI HCV assay utilizes a recombinant chimeric protein derived from the core, NS3, NS4, and NS5 regions of the HCV genome. The antigen when used in combination with the INSTI Colour Developer will detect antibodies specifically directed against the Hepatitis C virus.

Test Complexity: The INSTI HCV assay was designed to reduce protocol complexity. The INSTI HCV assay does not require sample preparation, accurate timing, or several steps, which include multiple washes and reagents. These requirements increase the complexity of an assay and lead to procedural errors which may adversely affect sensitivity and specificity. Total test time may vary slightly depending on specimen type, but results of valid tests are always clearly readable within one minute.

SPECIMEN COLLECTION AND STORAGE

1. For whole blood, plasma, or serum specimens, follow venipuncture blood collection procedure using lavender-top EDTA anticoagulant tubes for whole blood; lavender-top EDTA anticoagulant, light blue-top sodium citrate, or green-top sodium heparin tubes for plasma; or red-top (no anticoagulant) tubes for serum.
2. If plasma or serum is used, separate from the blood cells by centrifugation.
3. Serum or plasma may be stored at 2-8°C for up to 5 days, stored frozen at ⩽ -20°C for 3 months, or stored frozen at ⩽ -70°C for one year.
4. Whole blood specimens collected in EDTA anticoagulant may be stored at 2-8°C and should be tested within 48 hours. **Do not heat or freeze whole blood specimens.**
5. Do not dilute prior to testing.

KIT COMPONENTS AND STORAGE

30°C / 2°C Store INSTI HCV Antibody Test unopened at 2 to 30°C (35.6° to 86°F).

Components	90-1127	90-1157
Membrane Unit	x 1 unit	x 50 units
Sample Diluent	x 1 vial	x 50 vials
Colour Developer	x 1 vial	x 50 vials
Clarifying Solution	x 1 vial	x 50 vials
Lancet	x 1	x 50
Alcohol Swab	x 1	x 50
Pipette	x 1	x 50

Each test contains the following materials:

1. **Membrane Unit**, individually packaged, prepared with control (antibody capture) and test (recombinant chimeric HCV antigen) reaction spots. For single use only in the INSTI procedure.
2. **Sample Diluent** Bottle 1 containing 1.5 mL of a proprietary Tris-Glycine buffered solution containing cell lysis reagents.
3. **Color Developer** Bottle 2 containing 1.5 mL of a blue-coloured borate-buffered proprietary indicator solution designed to detect IgG in the control spot and specific HCV antibodies in the test spot.
4. **Clarifying Solution** Bottle 3 containing 1.5 mL of a proprietary Tris-Glycine buffered clarifying solution designed to remove background staining from the Membrane Unit prior to reading the result.

⚠ All solutions contain 0.1% sodium azide as a preservative.

SUPPORT MATERIALS

The following materials are required when testing fingerstick whole blood and are included with each kit:

1. Single-use Alcohol Swab
2. Single-use Lancet STERILE R CE 0050 Becton, Dickinson and Company Limited located at Pottery Road, Dun Laoghaire, Co. Dublin, Ireland
3. Single-use Pipette, 50 µL

MATERIALS REQUIRED BUT NOT PROVIDED

- Personal protective equipment such as gloves, lab coat or gown
- Biohazard waste containers
- Absorbent cotton balls for fingerstick or venipuncture wound closure or bandage

For venipuncture blood collection and testing:

- Venipuncture apparatus if collecting blood samples.
- Appropriate blood collection tubes.
- Appropriate shipping containers.
- Pipette capable of delivering 50 µL of sample.

MATERIALS AVAILABLE AS AN ACCESSORY TO THE KIT: INSTI HCV Test Controls

WARNINGS

For *in vitro* diagnostic use only. IVD

Read the Instructions for Use completely before using the product. Although the assay is designed to be simple to use, conformance with the test procedure is necessary to ensure accurate results.

1. Do not mix reagents from different lots.
2. Do not use reagents or kits beyond the stated expiration date on the outer packaging.
3. Order of bottle use must be strictly followed as per the Instructions for Use. Any deviation may result in false or invalid results.
4. Do not use the Membrane Unit if the foil pouch has been previously opened or if the packaging integrity has been compromised. Once the test device has been opened, it must be used immediately.
5. Avoid microbial contamination and exercise care in handling the kit components.
6. ⚠ Sodium azide is present at 0.1% in all assay reagents. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with large amounts of water to prevent azide build-up. Check with local regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous waste.
7. This kit has been approved for use with fingerstick whole blood, venipuncture whole blood, plasma, and serum only. Use of this test kit with specimen types other than those specifically approved for this device may cause inaccurate test results.
8. Failure to use the recommended reagent and specimen volumes may result in leakage and/or overflow of liquids from the test device.
9. If the kit is refrigerated, ensure it is brought to room temperature before performing the test. Use the INSTI HCV Test Controls to ensure proper kit performance.
10. There is a risk of false negative results if testing is performed in the 'window period'. Window period for an HCV test refers to the time between HCV exposure and when a test can detect antibodies to HCV in your body.
11. Cross-reactivity can occur in tuberculosis and gonorrhea infected samples.
12. Auto-immune diseases (i.e., ANA positive samples) can interfere with the test result.
13. If a negative result is obtained within three months of a high-risk event, repeat testing at two to three months to confirm the initial negative result.
14. A negative result does not necessarily mean that the person is not infectious. If symptoms persist, seek medical assistance.

PRECAUTIONS

1. Wear disposable gloves while handling kit reagents or specimens. Change gloves and wash hands thoroughly after performing each test.
2. Avoid contact with skin and eyes. If contact occurs, wash affected areas with water.
3. Dispose of all specimens and materials used to perform the test in a biohazard waste container. The preferred method of disposal is sterilization by autoclaving for a minimum of one hour at 121°C. Disposable materials may be incinerated. Liquid waste may be mixed with sodium hypochlorite (bleach) in volumes such that the final mixture contains 1.0% sodium hypochlorite (using a freshly prepared solution containing 10% household bleach).
4. Spills should be cleaned up and decontaminated in accordance with the user facility's established procedures for handling biohazardous spills.
5. For additional information on biosafety, refer to "Universal Precautions for prevention of transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other bloodborne pathogens."[3] and in accordance with Local, or Federal Regulations. Follow standards biosafety guidelines for handling and disposal of potentially infective material.

INSTRUCTIONS FOR USE

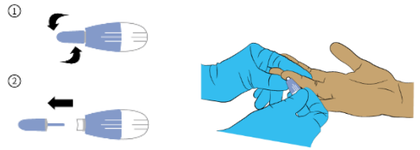
Workplace Preparations

- Gather the material you will need to perform the test.
- Allow the INSTI HCV Test to come to operating temperature before use.
- Refer to the *Quality Control* section in this Instructions for Use to determine when the Test Controls should be run.

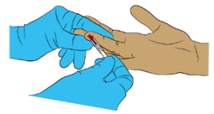
Specimen Collection and Test Procedure

Sampling Fingerstick Blood:

1. Massage the finger to allow the blood to move to the surface (fingertip will become pink). Use a heating pad if available to warm the hand. Hand must be positioned at waist level or lower.
2. Wipe the fingertip with the alcohol swab. Allow the finger to air dry.
3. Twist and remove the protective insert from the lancet. Press the finger firmly at the point just below where the lancet will be applied. With the other hand, place the lancet on the side of the fingertip and press hard until it clicks.



4. As the blood droplet forms, hold the pipette horizontally and touch the tip of the pipette to the blood sample. Capillary action automatically draws the sample (50uL) to the fill line and stops. If very little blood trickles out of the puncture, gently apply intermittent pressure below the puncture site to obtain the required blood volume. If blood is inadequate, perform a second skin puncture using a new lancet.



⚠ **CAUTION!** Filling is automatic: Never squeeze the tube while sampling.

5. Transfer the blood held in the pipette to the Sample Diluent vial (Solution 1). Align the tip of the pipette with the Sample Diluent vial and squeeze the bulb to dispense the sample. **NOTE:** If the sample will not expel, hold the pipette vertically and slide a finger over (without pressing) the vent hole, then squeeze the bulb. Recap the vial and mix by inversion. Follow the Test Procedure after Sampling, below.

Sampling Venipuncture Whole Blood, serum, EDTA-plasma, and Test Controls:

1. Bring specimens to room temperature and mix each specimen thoroughly prior to use. **Do not heat or repeatedly freeze/thaw specimens.**
2. Using a pipette, add 50 µL of whole blood, serum, plasma, or kit controls (see NOTE below) to the Sample Diluent vial. Recap the vial and mix by inversion. ⚠ Adding an excessive amount of specimen may cause the device to overflow or leak.

NOTE: For INSTI Test Controls, it is important to use a 50 µL pipette to add the control material to the Sample Diluent vial. Do not use the disposable single-use pipette provided for finger stick blood collection.

Test Procedure after Sampling:

1. Tear open the Membrane Unit pouch and remove the test device without touching the center well. Place the device on a level surface. For sample identification purposes the tab of the test device may be labeled with the patient's name or number.

NOTE: At this point, it is important that the following steps be performed immediately and in sequence.

2. Mix the Sample Diluent-specimen mixture by inverting several times and pour the entire contents to the center of the test device well. (**NOTE:** Do this within 5 minutes after the specimen has been added to the Sample Diluent vial). The sample should be absorbed through the membrane in less than 30 seconds; however, absorption times will vary slightly depending on sample type.

3. Resuspend the Colour Developer by slowly inverting to mix the solution thoroughly until the reagent is evenly suspended and add the entire contents to the center of the test device well. The coloured solution should flow through completely in about 20 seconds.

4. Open the Clarifying Solution and add the entire contents to the center of the test device well. This will lighten the background colour and facilitate reading. Results can be read immediately. **Do not read the results if more than 60 minutes have elapsed following the addition of Clarifying Solution.**

QUALITY CONTROL

Kit Controls:

The INSTI HCV Antibody Test has a built-in IgM/IgG capture procedural control that demonstrates assay validity and adequate sample addition. A blue colour on the control dot indicates that the proper specimen was added and that the assay procedure was performed correctly. The control dot will appear on all valid INSTI tests. Refer to Interpretation of Results of this Instructions for Use.

INSTI HCV Test Controls are available separately for use only with the INSTI HCV Antibody Test. The controls are used to verify test performance and interpretation of results. Kit controls should be run under the following circumstances:

- for new INSTI operator verification prior to performing testing on patient specimen.
- when switching to a new lot number of INSTI test kits.
- whenever a new shipment of kits is received.
- when temperature during storage of the kit falls outside of 2°-30°C.
- when the temperature of the test area falls outside of 15°-30°C.
- at regular intervals as determined by the user facility.

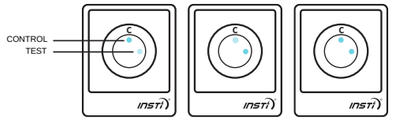
Refer to the INSTI HCV Test Controls instructions for use for additional information on the use of these reagents. It is the responsibility of each laboratory using the INSTI HCV Antibody Test to establish an adequate quality assurance program to ensure the performance under their specific locations and conditions of use.

INTERPRETATION OF RESULTS

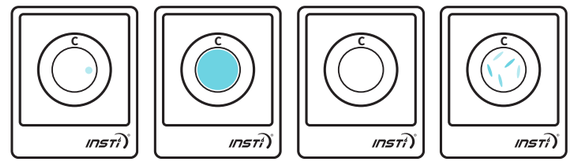
- **Do not read the results if more than 60 minutes have elapsed following the addition of Clarifying Solution.**
- **If using the control samples provided by bioLytical, all Positive Controls must be reactive with INSTI and all Negative Controls must be non-reactive with INSTI. Controls that produce incorrect or invalid results must be re-tested with INSTI. If results are still incorrect or invalid, inform bioLytical Laboratories immediately.**

NON-REACTIVE ► One blue dot that is clearly discernable above any background tint should appear on the membrane. This is the procedural Control Dot and shows that the test has been performed correctly. The Control Dot is located towards the top of the read frame furthest from the plastic tab on the Membrane Unit. No reaction should be visible at the test spot located to the right of the Control Dot. A non-reactive result indicates that HCV antibodies were not detected in the specimen.

REACTIVE ► Two blue dots (visible in the control and test area) indicate that the specimen contains HCV antibodies. One dot may be darker than the other. The presence of any visible blue dot on the test spot should be considered as reactive. A sample giving this pattern is considered a **preliminary reactive**. Individuals with a reactive result with the INSTI HCV Antibody Test should undergo appropriate clinical follow-up.



INVALID ► The test is invalid if any of the following occurs:
A. The test dot appeared without the control dot.
B. Uniform tint across the membrane.
C. There are no dots on the membrane.
D. Only blue specks appear on the membrane.



NOTE: An invalid test result means that the test was run incorrectly, or insufficient specimen was added. Invalid test results cannot be interpreted. Repeat the test with a fresh specimen using a new Membrane Unit, kit components and support materials. Contact bioLytical's Technical Support if you are unable to produce a valid result upon repeat testing.

LIMITATIONS OF THE TEST

- In some instances, samples may exhibit longer than normal flow times (from the time the Sample Diluent specimen mixture is poured in the membrane well to the time the Clarifying Solution has fully flowed through the membrane). This is due to variable factors such as cellular components, especially with whole blood.
- The INSTI HCV Antibody Test must be used in accordance with these Instructions for Use to obtain accurate results.
- Insufficient data are available to interpret tests performed on other body fluids, pooled blood or pooled serum and plasma, or products made from such pools; therefore, testing of these specimens is not recommended.
- This test is not to be used for monitoring.
- Intensity of the dot does not necessarily correlate with the HCV antibody titer in the specimen.
- Insufficient data is available to interpret tests performed on specimens from newborns and children, therefore testing on this population is not recommended.

PERFORMANCE CHARACTERISTICS

Precision

Within-Laboratory Precision

Within-laboratory precision was evaluated on one lot of INSTI HCV Antibody Test materials using an eight-member panel of blind-coded plasma and whole blood specimens. Each sample matrix was evaluated at four HCV antibody concentrations: Negative, High Negative, Weak Positive and Moderate Positive. Testing was carried out by three independent operators at n=5 replicates per specimen, one run per day per operator over 5 days, for a total of 600 samples tested. Results for all samples and all operators were 100% concordant with expected results and demonstrate a high level of precision and repeatability between operators, between runs and between days.

Intermediate Precision

Intra-lot, inter-day and inter-operator variability was evaluated on three lots of INSTI HCV Antibody Test materials using an eight-member panel of blind-coded plasma and whole blood specimens. Each sample matrix was evaluated at four HCV antibody concentrations: Negative, High Negative, Weak Positive and Moderate Positive. Testing was carried out by two operators with each panel member run in duplicate (n=2) on three lots of materials, two runs per day over 20 days for a total of 1921 samples tested. Results for all samples and all operators were 100% concordant with expected results and demonstrate a high level of precision intra-day, inter-day, inter-operator, inter-lot and within-laboratory.

Sample	Replicates	Total Reactive	Total Non-Reactive	Invalid
Negative Plasma (C_0)	240	0	240	0
Negative Whole Blood (C_0)	241	0	240	1
High Negative Plasma (C_5)	240	0	240	0
High Negative Whole Blood (C_5)	240	0	240	0

Sample	Replicates	Total Reactive	Total Non-Reactive	Invalid
Weak Positive Plasma (C_{95})	240	240	0	0
Weak Positive Whole Blood (C_{95})	240	240	0	0
Moderate Positive Plasma (C_{100})	240	240	0	0
Moderate Positive Whole Blood (C_{100})	240	240	0	0
All Samples	1921	960	960	1

Hook Effect
20 high titer HCV specimens (confirmed reactive by Abbott Anti-HCV and Siemens ADVIA Centaur) were tested on one lot of INSTI HCV Test materials at three concentrations in both a plasma-based matrix and whole blood, at n=2: neat (undiluted), 1/10, and 1/20. Out of the 20 unique specimens tested, all 20 were reactive on the INSTI HCV Test, therefore demonstrating the absence of a hook effect on the INSTI HCV Antibody test.

Limit of Detection
The overall LoD was calculated through an analytical sensitivity and traceability study conducted by bioLytical. In this study, among two INSTI HCV Antibody Test kit lots tested, determined by the least sensitive lot of INSTI HCV Antibody test, the LoD was in the range of 1.4 to 5.15 s/co when tested with a 3rd generation immunoassay.

Endogenous Interferences
To evaluate the effect of elevated levels of various endogenous substances on the INSTI HCV Antibody Test, whole blood samples were spiked with the following levels of endogenous interferences and assayed at four HCV antibody levels: negative, high negative, low positive, and moderate positive. All samples were tested in triplicate. No interference was observed at the following tested concentrations:

Interferent Tested	No Interference up to
Hemoglobin	10 mg/mL
Bilirubin, Conjugated	0.40 mg/mL
Bilirubin, Unconjugated	0.40 mg/mL
Cholesterol	4.0 mg/mL
Total Protein/Albumin	60 mg/mL
Triglycerides/Intralipid	20.5 mg/mL
Immunoglobulin	6 mg/mL
Anti- *E.coli* antibody	0.5 mg/mL

To evaluate the effect of various autoimmune conditions, pregnancy, blood transfusion and hemolysis on INSTI HCV Antibody Test performance, 91 serum or plasma samples obtained from international biorepositories were tested as is (negative) and spiked with HCV antibodies to a weak positive level. All samples were tested in triplicate on one lot of INSTI HCV Antibody Test materials. The results are presented in the following table:

	Negative			Weak Positive		
Condition	**N**	**INSTI Reactive**	**INSTI Non-Reactive**	**N**	**INSTI Reactive**	**INSTI Non-Reactive**
Anti-Nuclear Antibody	24[1]	3[1]	21	24	24	0
Autoimmune Hepatitis	13[2]	7[2]	6	9	9	0
Rheumatoid Factor	10	0	10	10	10	0
Pregnancy (1st Trimester)	9	0	9	9	9	0
Pregnancy (2nd Trimester)	10	0	10	10	10	0
Pregnancy (3rd Trimester)	10	0	10	10	10	0
Multiparity	5	0	5	5	5	0
Multiple Blood Transfusions	5	0	5	5	5	0
Hemolyzed Plasma	5	0	5	5	5	0

[1]Two of the three ANA samples were confirmed HCV antibody positive by external comparator testing.
[2] One of the seven autoimmune hepatitis samples was confirmed HCV antibody positive by external comparator testing.

Drug Interferences
A drug interference study was performed with ten common therapeutic drugs representing common over-the-counter anti-inflammatory drugs and anti-bacterial and anti-viral drugs used in HCV treatment. Each drug was evaluated at approximately 3x the highest concentration reported followed a drug therapeutic dosage as recommended by EP07-A3 and EP37 and spiked into whole blood samples at four HCV antibody levels: negative, high negative, low positive and moderate positive. Each test condition was evaluated in triplicate. No interference was observed at the following tested concentrations:

Compound	Concentration
Acetaminophen	0.156 mg/mL
Acetylsalicylic acid	0.03 mg/mL
Ibuprofen	0.219 mg/mL
Ampicillin	0.075 mg/mL
Erythromycin	0.138 mg/mL
Tetracycline Hydrochloride	0.024 mg/mL
Gentamicin Sulfate	0.03 mg/mL
Ribavirin	1.2 mg/mL
Interferon alpha 2a	6,000 IE/mL
Caffeine	0.108 mg/mL

Matrix Equivalency
A sample type equivalency study was carried out using matched sets of serum, EDTA whole blood and EDTA plasma specimens from 50 individual donors. Each specimen was tested in singlicate (n=1) at four HCV antibody concentrations – Negative, High Negative, Weak Positive, and Moderate Positive, for a total of 600 samples tested. Results demonstrated that performance is equivalent for all sample types tested.

A second sample type equivalency study was performed on capillary (fingerstick) whole blood, venous whole blood (no anticoagulant), venous EDTA whole blood, EDTA plasma and serum samples, tested in a routine testing environment at a single study site in Africa. Matched sets of specimens were obtained from 25 HCV positive and 25 HCV negative donors; each sample was run in duplicate on one lot of INSTI HCV Antibody Tests. A sub-study to evaluate the impact of complement-related influence on INSTI HCV Antibody Test results was also run on 25 HCV positive "same day" fresh serum samples (tested within 8 hours of blood draw). Results demonstrated that performance is equivalent for all sample types tested.

An anticoagulant equivalency study was carried out using matched sets of EDTA whole blood, EDTA plasma, sodium heparin plasma, and sodium citrated plasma from 25 individual donors. Each specimen was tested in singlicate (n=1) at four HCV antibody concentrations – Negative, High Negative, Weak Positive, and Moderate Positive, for a total of 400 samples tested. Results demonstrated that performance is equivalent for all anticoagulants tested.

These studies support the use of the INSTI HCV Antibody Test on fingerstick (capillary) whole blood, EDTA whole blood, EDTA plasma, sodium heparin plasma, sodium citrated plasma, and serum.

Analytical Specificity
A study was conducted to evaluate the INSTI HCV Antibody Test for potential cross-reactivity in specimens from individuals with various medical conditions. Specimens were evaluated in singlicate (n=1) on one lot of INSTI HCV Antibody Test materials. The results are summarized in the following table:

Category	N	INSTI HCV Antibody Test	
		Reactive	Non-Reactive
Viral Infection			
Hepatitis A Virus (HAV) - IgM	5	0	5
Hepatitis A Virus (HAV) - IgG	5	0	5
Hepatitis B Virus (HBV)	10	0	10
Cytomegalovirus (CMV) -IgM	4	0	4
Cytomegalovirus (CMV) -IgG	4	0	4
Epstein-Barr virus (EBV)	10	0	10
Human Immunodeficiency Virus (HIV)	18[1]	0	18[1]
Herpes Simplex virus 1 (HSV-1)	9	0	9
Herpes Simplex virus 2 (HSV-2)	6	0	6
Parvovirus - IgM	3	0	3
Parvovirus - IgG	3	0	3
Varicella-zoster virus (VZV)	8	0	8
Hepatitis E Virus (HEV)	5	0	5
Human T-cell lymphotropic virus type 1 (HTLV-1)	3	0	3
Human T-cell lymphotropic virus type 2 (HTLV-2)	3	0	3
Dengue virus - IgM	2	0	2
Dengue Virus - IgG	5	0	5
Non-Viral Infections			
Syphilis - IgM	3	0	3
Syphilis – IgG	2	0	2
Toxoplasma - IgM	5	0	5
Toxoplasma - IgG	5	0	5
Malaria	5	0	5
Tuberculosis	5	3[2]	2
Trichomonas	5	0	5
Gonorrhea	5	1[2]	4
Leishmaniasis	3	0	3
Candida albicans	5	0	5
Non-Viral Liver Disease			
Non-alcoholic fatty liver disease (NAFLD)	5	0	5
Alcoholic Liver Disease (ALD)	5	1[3]	4
Primary Biliary Cholangitis (PBC)	5	0	5
Vaccination			
Influenza Vaccine	5	0	5
Human papillomavirus (HPV) Vaccine	5	0	5
Other			
Sickle Cell Anemia	5	0	5
Total	176	5	171

[1] Two samples found to be negative for HIV antigen and antibodies.
[2] The assay may cross react with samples positive for antibodies to tuberculosis and gonorrhea.
[3] Sample found to be positive for HCV antibodies by external comparator testing.

An additional study was conducted by an external testing laboratory on 501 stored anti-HCV negative samples (201 samples from hospitalized patients, 200 samples from pregnant women, 100 potentially cross-reactive samples) in a routine blood bank testing environment. All samples were confirmed anti-HCV negative on the Roche Anti-HCV II assay. Study results are summarized below:

Group	Samples (N)	INSTI HCV Antibody Test		Specificity
		Reactive	Non-Reactive	
Hospitalized patients	201	2	199	99.0%
Pregnant women	200	0	200	100%
Potentially Cross Reactive Samples[1]	100	0	100	100%
TOTAL	501	2	499	99.6%

[1] Samples were confirmed reactive for Anti-HIV (10), Anti-HBs (10), Anti-HBc (10), Anti-HTLV I/II (10), Anti-HEV (10), Anti-HAV (10), Anti-TP (5), Anti-EBV (5), Anti-CMV (5), Anti-VZV (5), influenza vaccine recipients (5), Anti-E.coli (5), HAMA+ (5) and RF+ (5).

Seroconversion Sensitivity
Seroconversion sensitivity of the INSTI HCV Antibody Test was evaluated by testing 32 commercially available seroconversion panels which demonstrated a range of antibody levels and antibody isotypes. The results of this study are presented in the table below and summarizes the INSTI HCV Antibody Test data compared to US licensed and European approved HCV antibody enzyme immunoassays (EIA). Overall the INSTI HCV Antibody Test has similar performance to commercially available anti-HCV EIA in the detection of HCV antibodies in seroconversion panels.

INSTI HCV Result	Number of Panels (n=32)
Detected the earliest bleed that was detected by an EIA	20
Detected 1 bleed earlier than the earliest EIA Positive	4
Detected within 1 bleed of earliest EIA positive	5
Detected within 2 bleeds of earliest EIA positive	2
Detected >2 bleeds after earliest EIA positive	1

Genotype Detection
Studies were performed to evaluate the ability of the INSTI HCV Antibody Test to detect antibodies to various known HCV genotypes and subtypes. 98 characterized HCV positive serum or plasma specimens were obtained from international biorepositories from the following genotypes and subtypes: 1 (33 samples),2 (16 samples),3 (15 samples),4 (23, including 4 non-A subtype samples),5 (6 samples) and 6 (5 samples). All samples were tested in singlicate (n=1) on two lots of INTI HCV Antibody Tests materials. The positive samples were all detected by the INSTI HCV Antibody Test.

Reproducibility
A study was conducted to evaluate the reproducibility of INSTI® HCV Test with a panel of 5 blind-coded whole blood and plasma samples. The panel members Negative whole blood, Weak Positive (1-2xLoD), Moderate Positive (2-3x LoD), HCV Positive Control, HCV Negative Control in plasma matrix were tested in randomized order on three lots of test kits. Testing was completed over 5 days by two operators over three testing sites (three kit lots per site) with 1 run per day (alternating morning and afternoon) and each panel member tested at n=5 per run for a total of 225 replicates per panel member. Overall, 1125 tests were performed to determine the reproducibility of the INSTI HCV Test. For the weak positive panel members, INSTI HCV test showed a reproducibility rate of 99.1 % (CI of 96.8% - 99.8%). All other panel members showed a reproducibility rate of 100% (CI of 98.3% - 100%) and the results indicate that the INSTI® HCV Test shows high levels of reproducibility across sites, days, operators, and lots for HCV Test.

SUMMARY OF CLINICAL PERFORMANCE
Clinical performance studies were performed at external testing laboratories in Europe to establish performance of the INST HCV Antibody Test as described below.

Diagnostic Sensitivity
A retrospective clinical performance study was conducted in the Netherlands by an external testing laboratory on a total of 304 confirmed positive HCV which included 212 confirmed anti-HCV positive samples, and 92 samples from individuals with confirmed infection of the following known HCV genotypes and subtypes 1 (21 samples), 2 (22 samples), 3 (22 samples), 4 (23 samples), 5 (2 samples) and 6 (2 samples).

Group	Samples (N)	INSTI HCV Antibody Test		Sensitivity [95% CI]
		Reactive	Non-Reactive	
EDTA Plasma – Known HCV Genotype	92	92	0	100% [96.3-100%]
Serum – Confirmed Anti-HCV	212	212	0	100% [96.3% -100%]

A retrospective study was conducted in Germany on 4 confirmed HCV Ab positive samples with genotype 5 and 100 HCV positive EDTA whole blood samples. All 4/4 and 100/100 samples were tested on both INSTI HCV Ab test and Roche Anti-HCV II assay resulting in 100% sensitivity.

Group	Samples (N)	INSTI HCV Antibody Test		Sensitivity [95% CI]
		Reactive	Non-Reactive	
Anti-HCV Genotype 5 – EDTA Plasma	4	4	0	100% [96.3-100%]
Hospital and rural laboratories – Fingerstick Blood	100	100	0	100% [96.3% -100%]

A prospective study was conducted in Republic of Congo and Uganda on 100 HCV Ab positive fingerstick samples which were tested on INSTI HCV Ab Test and then confirmed with anti-HCV ELISA 4.0 as a comparator method, the diagnostic sensitivity was found to be 100% [95% CI: 96.3% - 100%].

Diagnostic Specificity
A clinical performance study was retrospectively conducted by an external testing laboratory in Germany on 2000 anti-HCV negative blood donor samples (500 serum, 500 EDTA whole blood samples, 1000 EDTA plasma) collected in a routine blood bank testing environment.
The samples were investigated from two blood donation centers and consisted of consecutive blood donations including the first-time donors. Additionally, 201 and 200 anti-HCV negative samples from hospitalized patients and pregnant women, respectively, were tested on both INSTI HCV Antibody test and Roche Anti-HCV II. 199/201 of hospitalized patient samples and 200/200 of pregnant women samples were found to be non-reactive resulting in specificity of 99% (with 95% CI 96.4-99.7%) and 100 (with 95% CI 98.1-100%), respectively.

A prospective study was conducted on 100 HCV Ab negative fingerstick samples collected in a hospital and rural laboratories in Republic of Congo and Uganda. Samples were tested on INSTI HCV Ab Test and then confirmed with anti-HCV ELISA 4.0 as a comparator method and resulted in diagnostic specificity was found to be 100% [95% CI: 96.3% - 100%].

Group	Samples (N)	INSTI HCV Antibody Test		Specificity [95% CI]
		Reactive	Non-Reactive	
Blood donors – Serum	500	3	497	99.4% [98.3-99.8%]
Blood donors – EDTA Plasma	1000	5	995	99.5% [98.84-99.84]
Blood donors – EDTA Whole Blood	500	0	500	100% [99.2-100%]
Hospitalized Patients – EDTA Plasma	201	2	199	99% [96.4-99.7%]
Pregnant Women – EDTA Plasma	200	0	200	100% [98.1-100%]
Hospital and rural laboratories – Fingerstick Blood	100	0	100	100% [96.3-100%]
TOTAL	2501	10	2491	99.63%

BIBLIOGRAPHY

1. Q-L Choo, A.J. Weiner, L.R. Overby, G. Kuo, M. Houghton, and D.W. Bradley, Hepatitis C Virus: The Major Causative Agent of Viral Non-A, Non-B Hepatitis. British Medical Bulletin. 1990; Vol.46, No. 2:423-441.
2. WHO (15 May 2021). Hepatitis C. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c
3. Centers for Disease Control and Prevention (CDC) Universal Precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. MMWR 1988; 37(24):377-388.

TECHNICAL INFORMATION
For any serious incident that has occurred in relation to the device or for any further assistance, shall be reported to bioLytical's Technical Services at 1-604-644-4677.

Reference herein to any specific third party by name, trade name, trade-mark, manufacturer or otherwise does not constitute or imply an endorsement or recommendation of this Kit by such third party, or of the products or services of such third party by bioLytical or that such products or services are necessarily best suited for the intended purpose.

GLOSSARY

2°C 30°C	Store at 2°C to 30°C	STERILE R	Sterilization using irradiation
	Caution Harmful if swallowed	LOT	Lot number
IVD	*In Vitro* diagnostic medical device	REF	Catalogue Number
	Consult Instructions for Use		Manufacturer
	Do not reuse	Σ N	Contains sufficient for "N" tests
	Use by		This side up
	Keep dry		Keep away from direct sunlight
	Do not use if Damaged		

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