# HCV TRI-DOT

***Rapid Visual Test for the Qualitative Detection of Antibodies to HEPATITIS C Virus in Human Serum/Plasma***

**HCV Antigens for CORE, NS3, NS4 & NS5**

## 1. INTENDED USE

The **4th Generation** HCV TRI-DOT is a rapid, visual, sensitive and qualitative *in vitro* diagnostic test for the detection of antibodies to Hepatitis C Virus in human serum or plasma.

The **4th Generation** HCV TRI-DOT has been developed and designed with increased sensitivity for core and NS3 antibodies using a unique combination of modified HCV antigens. They are for the putative core (structural), protease/helicase NS3 (non-structural), NS4 (non-structural) and replicase NS5 (non-structural) regions of the virus in the form of two test dots "$T_1$" & "$T_2$" to provide a highly sensitive and specific diagnostic test.

## 2. INTRODUCTION

Hepatitis C Virus was identified in 1989 as the main aetiological agent of non-A, non-B hepatitis (NANBH) accounting for greater than 90% of post-transfusion hepatitis cases. HCV is a spherical virus of about 30-60 nm in diameter with single positive stranded RNA and is related to the family flaviviridae. It is considered to be the major cause of acute chronic hepatitis, liver cirrhosis and hepatocellular carcinoma throughout the world. It is therefore necessary to correctly diagnose Hepatitis C infection.

The test for antibodies to HCV was proved to be highly valuable in the diagnosis and study of the infection, especially in the early diagnosis of HCV after transfusion. The diagnosis of hepatitis C can be easily made by finding elevated serum ALT levels and presence of anti-HCV in serum/plasma (Fig.1).

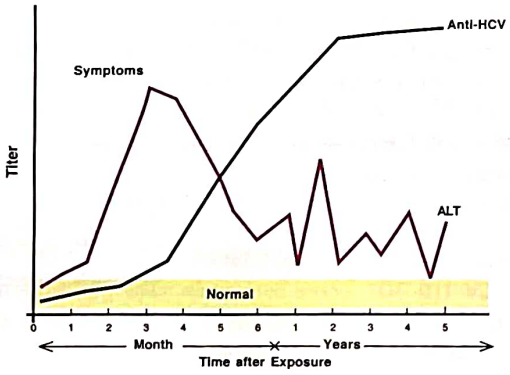



**Fig.1 Hepatitis C Virus Infection**
Typical Serologic Course

Recently recombinant DNA techniques have been used to encode the genome of HCV. The genome encodes for structural proteins (capsid protein) and several non-structural proteins (NS3, NS4 & NS5) (Fig.2).

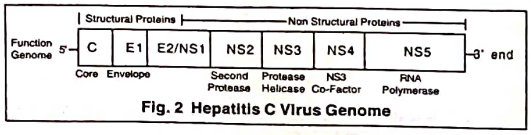



**Fig. 2 Hepatitis C Virus Genome**

The first generation anti HCV assay used C100-3 peptide where as the second generation assay used several recombinant viral proteins and peptides typically C-22 from the core region, C33-C from the non-structural (NS3) region and 5-1-1 & C100-3 from the NS4 region. They were associated with a high rate of both false positive and false negative results.

This led to the development of third generation anti-HCV assay which uses a greater range of antigens from core, NS3, NS4 & NS5 regions of the HCV genome, thus providing greater sensitivity and better specificity.

Recently the 4th generation assay for testing of anti-HCV has been established. The **4th Generation** HCV TRI-DOT utilizes a unique combination of modified HCV antigens from the putative core, NS3, NS4 & NS5 regions of the virus to selectively identify all subtypes of Hepatitis C Virus in human serum/plasma with a high degree of sensitivity and specificity.

The antigens used are chemically treated and unfolded in a special way to make them more reactive & specific to different epitopes of core & NS3 region thereby minimizing the chances of crossreactivity & enhancing the specificity.

Also,the superior sensitivity of the test allows for the significantly earlier detection of antibodies during sero-conversion following HCV infection, thereby reducing the incidence of post transfusion hepatitis and providing a safer blood supply.

**4th generation** HCV TRI-DOT has been developed and designed using modified HCV antigens representing the immunodominant regions of HCV antigen. The device (an immuno-filtration membrane) includes two test dots "$T_1$" & "$T_2$" and a Built in Quality Control Dot "C" (Fig.3). The control dot will always develop colour during the test, thereby confirming proper functioning of the device, reagent and correct procedural application. This control dot is the "Built in Quality Control."

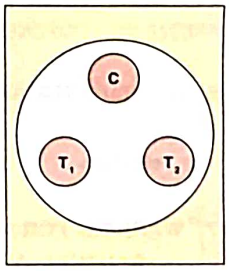



Fig. 3 Test Device

## 3. PRINCIPLE OF THE ASSAY

1. HCV antigens are immobilized on a porous immunofiltration membrane. Sample and the reagents pass through the membrane and are absorbed into the underlying absorbent pad (Fig. 4).
2. As the patient's sample passes through the membrane, HCV antibodies if present in serum/plasma, bind to the immobilized antigens. In the subsequent washing step, unbound serum/plasma proteins are removed (Fig. 4).

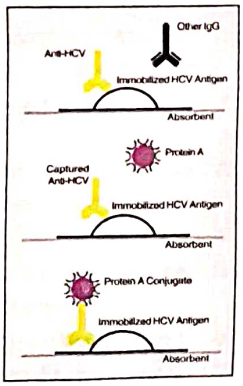



Fig. 4 Principle of the Assay

3. In the next step, the protein-A conjugate is added which binds to the Fc portion of the HCV antibodies to give distinct pinkish purple dot against a white background at the test region ("$T_1$"&/or "$T_2$"). At the control region ("**C**") a "Built-in Quality Control Dot" has been devised to confirm the proper functioning of the device, reagent and correct procedural application.

## 4. KIT COMPONENTS

| COMPONENTS | CONTENTS | PREPARATION |
|---|---|---|
| 1. HCV TRI-DOT Test Device | Packed individually. It is marked with "**C**" for Control Dot and "$T_1$" & "$T_2$" for Test Dots. | Cut open the pouch before use. |
| 2. Buffer Solution | Buffer containing BSA and sodium azide. | Ready to use. |
| 3. Protein-A Conjugate | Protein-A Conjugate in liquid form containing sodium azide. | Ready to use. |
| 4. Sample Dropper | Long Plastic dropper provided for adding the sample. | |

## 5. STORAGE OF THE KIT

Store the kit at 2-8°C in the driest area available. **The shelf life of the kit is 15 months from the date of manufacturing.**

Do not use the kit beyond the expiry date mentioned on it. Before running the test bring all the kit components to room temperature (20-30°C) for best results. Return the entire kit to 2-8°C when not in use. DO NOT FREEZE KIT COMPONENTS.

## 6. KIT PRESENTATION

10 Test Pack 50 Test Pack

100 Test Pack

## 7. WARNING FOR USERS

⚠ **CAUTION:** ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves is STRONGLY RECOMMENDED during the test.
2. In case there is a wound or cut in the hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. This Kit is for *in vitro* diagnostic use only.
5. All the samples to be tested should be handled as though capable of transmitting infection.
6. Spills should be decontaminated promptly with disinfectant.
7. Dispose of all specimens and materials used to perform the test appropriately using disinfectant.
8. The Protein-A Conjugate and Buffer Solution contain Sodium Azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amount of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to Remove Azide Salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).
9. Thoroughly wash hands with soap after the use of this kit. In case of a needle prick or other skin puncture or wounds, wash the hands with excess of water and soap.

## 8. PRECAUTIONS

1. Do not use kit components beyond the expiration date, which is printed on the kit.
2. Do not combine reagents from different batches during the same series, as they are optimized for individual batch to give best result.
3. Due to interchange of caps of the vials, the reagents may get contaminated. Care should be taken while handling the reagent caps to avoid cross contamination of the reagents. Place white nozzle cap on Buffer Solution vial and red cap on Protein-A Conjugate Vial.
4. Use a separate sample dropper for each sample and then discard it as biohazardous waste.
5. Avoid several times freezing and thawing of the sample to be tested.
6. Always allow each reagent to fall freely from the dropper tip. Do not touch the dropper tip to any surface; this may contaminate the reagent.
7. Avoid microbial and cross contamination of reagents.

## 9. SAMPLE / SPECIMEN COLLECTION & STORAGE

Collect blood in a clean dry sterilized vial and allow it to clot. Separate the serum by centrifugation at room temperature.

It is recommended that FRESH samples should be used. If serum is not to be assayed immediately it should be stored at 2-8°C or frozen at -20°C. Serum may be stored at 2-8°C for upto 3 days and stored frozen at -20°C for 3 months. Only human serum or plasma should be used for the test. Haemolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

## 10. SAMPLE / SPECIMEN PROCESSING

Though HCV TRI-DOT works best when used with fresh samples, however the frozen or viscous samples can also perform well if the following instructions are strictly adhered to :

**A. Frozen samples**

(i) Allow the sample to thaw in a vertical position in the rack. Mix the sample thoroughly. If particles are seen, allow them to settle at the bottom or if a centrifuge is available, the sample can be centrifuged at 10,000 r.p.m. for 15 minutes.

(ii) Insert the dropper just below the top surface of the sample and withdraw one drop of the sample.

**B. Thick or viscous samples**

Whenever possible, clear specimen should be used. However, viscous, thick or turbid samples which may sometimes take more than 40-60 seconds to flow through the membrane should be centrifuged at 10,000 r.p.m. for 15 minutes and retested on a fresh device to avoid inconsistent results.

### C. Transportation

(i) The WHO guidelines for the safe transport of specimen (WHO/EMC/97.3) should be read carefully by the laboratory staff as these guidelines hold equally good for Hepatitis samples.

(ii) If the specimen is to be transported, it should be packed in compliance with the current Government regulations on transport of aetiologic agents.

### 11. BEFORE YOU START

The Buffer Solution and Protein-A Conjugate vials are provided with closed nozzle and screw cap with pin(outside), then punture the nozzle before use as given below:

1. Before using reagents, keep the vial vertically straight and tap down gently on the working platform, so that reagents come down at the bottom of the vial.
2. To orifice the closed nozzle, press the inverted cap on the respective closed nozzle and give a half turn twist to ensure nozzle is properly orificed/ punctured as illustrated below in Fig. iii & iv:

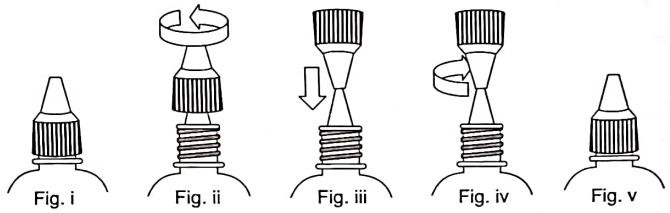


Fig. i — close vial provided in the kit

Fig. ii — open the cap

Fig. iii — invert the cap & press on the closed nozzle

Fig. iv — give a half turn twist to puncture the nozzle

Fig. v — screw the cap tightly after use

### 12. ASSAY PROCEDURE

Take care of the following points before starting the test.

1. Bring all the reagents and specimens to room temperature (20°C-30°C) before beginning the test. The immunological sequence of reactions which take place during different procedural steps shows best performance at room temperature. DO NOT HEAT OR REPEATEDLY FREEZE/THAW SPECIMEN.


2. Place the required number of HCV TRI-DOT test devices at the working area.
3. Tear off the pouch and take out the device for performing the test. Write the sample number to be tested on the device.

4. While adding sample/reagents to the device, be sure to ALLOW EACH SOLUTION TO SOAK IN BEFORE ADDING THE NEXT SOLUTION.

   However drops of each solution should be added in continuous stream to wet the entire area of membrane.
5. If the solution does not soak-in within 40-60 seconds; observe the sample for any suspended particulate matter. If it is present, centrifuge the sample at 10,000 r.p.m. for 15 min. and use a fresh device to re-run the test. Refer to "SPECIMEN / SAMPLE PROCESSING".


6. All solutions and sample should be added to the CENTRE OF MEMBRANE.

7. For consistent results, ensure FREE FALLING OF DROPS on the membrane.
8. Do not use kit components beyond the expiration date.
9. The liquid conjugate should not be subjected to frequent temperature fluctuations.
10. **The procedural sequence of reagent addition should be strictly adhered to avoid any discrepant results.**

### 13. TEST PROCEDURE

**Step No. 1**

Add 3 drops of Buffer Solution to the centre of the device.

**Step No. 2**

Hold the dropper vertically downwards and add 1 drop of patient's sample (50 µl serum or plasma) using the sample dropper provided. (use a separate sample dropper for each specimen to be tested).

**Step No. 3**

Add 5 drops of Buffer Solution.

**Step No. 4**

Add 2 drops of Protein- A Conjugate.

**Step No. 5**

Add 5 drops of Buffer Solution.

**Step No. 6**

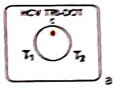
Read result immediately and discard the device immediately considering it to be potentially infectious.

***IMPORTANT: It is important to allow each solution to soak in the test device before adding the next solution.***

### 14. INTERPRETATION OF RESULTS

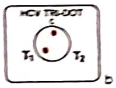
**NON REACTIVE RESULT**

1. Appearance of only one dot at the control region **"C"** indicates that the sample is NON-REACTIVE for antibodies to HCV. (Fig:a)

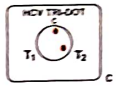


**REACTIVE RESULT**

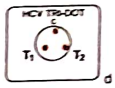
1. Appearance of two dots, one at the control region **"C"** & other at the test region **"$T_1$"** indicates that the sample is REACTIVE for antibodies to HCV. (Fig:b)



2. Appearance of two dots, one at the control region **"C"** & other at the test region **"$T_2$"** indicates that the sample is REACTIVE for antibodies to HCV. (Fig:c)

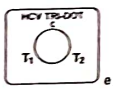


3. Appearance of all the three dots, one each at **"C" "$T_1$"& "$T_2$"** region indicates that the specimen is REACTIVE for antibodies to HCV. (Fig:d)

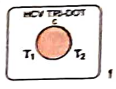


**INVALID RESULT**

If no dot appears after the completion of test, either with clear background or with complete pinkish/purplish background the test indicates ERROR (Fig.e&f).



This may indicate a procedural error or deterioration of specimen/reagents or particulate matter in the specimen. The specimen should be retested on a fresh device (Refer sample / specimen processing).



***IMPORTANT :***

(i) Test dots "$T_1$" & "$T_2$" either dark or light in colour (pink) should be considered reactive for antibodies to HCV.

(ii) Sometimes, if the sample solution does not soak-in within 40-60 seconds, the sample should be observed for any suspended particulate matter; if it is present, centrifuge the sample at 10,000 r.p.m. for 15 minutes. Use a fresh device to re-run the test.

(iii) Sample found to be initially reactive by the above screening test must be repeated, if the sample is repeatedly reactive it must be confirmed by standard supplemental assay test RIBA.

(iv) In case you have any problems in our HCV TRI-DOT, please call our Technical Customer Service Cell at Parwanoo, Himachal Pradesh, as per following details:
Ph: 0091-1792-232253

## 15. LIMITATIONS OF THE TEST

(i) The **4th Generation** HCV TRI-DOT detects anti-HCV in human serum or plasma and is **only a screening test**. All reactive samples should be confirmed by supplemental assays like RIBA. Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serological data, should be considered. The results should be reported only after complying with above procedure.

(ii) The assay is only validated for serum and plasma from individual bloods and not for pools of serum or plasma or other body fluids.

(iii) A non-reactive result does not exclude the possibility of exposure to or infection with HCV.

(iv) It should be noted that repeated false reactive results may occur due to non-specific binding of the sample to the membrane.

(v) The presence of anti-HCV does not imply a Hepatitis C infection but may be indicative of recent and / or past infection by HCV.

(vi) Patients with auto-immune liver diseases may show falsely reactive results.

(vii) The kit works best when used with fresh samples and when all the kit components are at room temperature (20-30°C). Samples which have been frozen and thawed several times contain particulates which can block the membrane, hence resulting in improper flow of reagents and high background colour which may make the interpretation of results difficult.

(viii) Rarely there may be an impression at the location T1&/or T2 where the antigens have been coated. These impressions will automatically get washed away & the membrane will be clear on addition of buffer solution in the first step of test procedure .However there will not be any impact on the test result.

(ix) Optimum test performance depends on strict adherence to the test procedure as described in this manual. Any deviation from test procedure may lead to erratic result.

## 16. PERFORMANCE CHARACTERISTICS

(I) The performance of **4th Generation** HCV TRI-DOT with reference to sensitivity and specificity has been evaluated in house with fresh as well as frozen samples from low risk as well as high risk groups by using a panel containing 1315 nos. of known serum/ plasma samples including cross reacting samples. The results of all the samples with a defined HCV status were fully comparable with those of **4th Generation** HCV TRI -DOT. The results of the in-house study done are as follows:

| No. of Samples | Status | HCV TRI-DOT + ve | HCV TRI-DOT - ve |
|---|---|---|---|
| 40 | ELISA +ve | 40 | - |
| 1275 | ELISA -ve | 5 | 1270 |

**Sensitivity : 100%** **Specificity : 99.21%**

**Precision:** Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of ten samples - three HCV negative and seven HCV Positive (1 strong positive, 1 medium and 5 weak positive). The C.V. (%) of all the ten samples were within 10%.

## 17. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

## 18. REFERENCES


1. Caypers, H.T.M. Wiakel, I.N. Vander Pool, C.L. etal (1971) J. of Hepatology, 13, 5.15.
2. Halfon, P. etal (1997) J. Medical Virology. 52:391-395.
3. Sarin, S.K. & Hess. G. (1998). Transfusion associated Hepatitis.
4. Sayers, M.H. & Grotch D.R. (1993). J. Transfusion 30,809-13.


In vitro diagnostic reagent, not for medicinal use


*Manufactured & Marketed By:*
**DIAGNOSTIC ENTERPRISES**
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Abbott

REF 02FK10CE, 02FK16CE, 02FK17CE

Bioline™

# HCV

Anti-HCV Test
Test de détection des anticorps anti-VHC
Prueba de VHC
Teste anti-VHC
TEST auf Anti-HCV

## About the test

**[Introduction]** Hepatitis C virus (HCV) is recognized as a major agent of chronic hepatitis, transfusion acquired non-A, non-B hepatitis and liver disease throughout the world. HCV is an enveloped positive-sense, single-stranded RNA virus. Testing for HCV infection begins serology testing with either a rapid or a laboratory-conducted assay for HCV antibody in blood. A reactive result indicates presumptive HCV infection. When to confirm the current HCV infection it is recommended nucleic acid testing (NAT) for the detection of HCV RNA be performed following HCV antibody reactive test result. Abbott Diagnostics Korea has constructed HCV genes for the expression of recombinant antigens in bacterium systems such as *E. coli* focused on structural and non-structural immunogenic regions of the HCV-encoded polyprotein. The major immunoreactive antigens of these proteins have been reported as core, NS3, NS4 and NS5 regions of HCV genome, which are known to be highly immunodominant. For detection of HCV infection, these recombinant proteins were used as capture materials in this immunochromatographic test. Improving on the first generation HCV serologic antibody test using a single recombinant antigen, recombinant proteins with multiple antigens have been used to minimize non-specific cross-reactivity and to increase the sensitivity in this assay.

**[Test principle]** The Bioline™ HCV test contains a nitrocellulose membrane strip, which is pre-coated with recombinant HCV capture antigen (core, NS3, NS4 and NS5) at the test line region (T). The protein A-colloid gold conjugate and the specimen moves along the membrane chromatographically to the test region. There the antigen-antibody protein A gold particle complex forms into a visible line with high degree of sensitivity and specificity. This test device has letter "T" and "C" representing "Test Line" and "Control Line" on the surface of the case. Both the test line and control line in result window are not visible before applying the specimen. The control line is a procedural control. The control line should always appear if the test procedure is performed properly and the reagents in the control line are working.

**[Intended use]** The Bioline™ HCV test is an *in vitro* immunochromatographic, rapid assay designed for the qualitative detection of antibodies specific to HCV, in human serum, plasma, venous whole blood or finger prick whole blood. The Bioline™ HCV test kit is intended only for professional use as the initial test, as an aid to diagnosis. Reactive specimens should be followed up with nucleic acid testing (NAT) for the detection of HCV RNA together with a HCV immunoblot testing to identify current HCV infection. This test may not be suitable for diagnosis of early infection or blood donation screening.

## Materials provided and active ingredients of main components

1. The Bioline™ HCV test kit contains the following items to perform the assay:

| Catalog No. | Contents |
|---|---|
| 02FK10CE | 1. 30 Test devices with desiccant in individual foil pouches<br>2. Assay diluent (1 x 5 ml/vial)<br>3. 1 Instructions for use |
| 02FK16CE | 1. 25 Test devices with desiccant in individual foil pouches<br>2. Assay diluent (1 x 5 ml/vial)<br>3. 25 Capillary pipettes (10 µl), 25 Sterile lancets, 25 Alcohol swabs<br>4. 1 Instructions for use |
| 02FK17CE | 1. 25 Test devices with desiccant in individual foil pouches<br>2. Assay diluent (1 x 5 ml/vial)<br>3. 25 Capillary pipettes (10 µl), 25 Safety lancets, 25 Alcohol swabs<br>4. 1 Instructions for use |

2. Active ingredients of main components

- 1 Test device includes:
  - Gold conjugates: Protein A – gold colloid (1.0±0.2 µg)
  - Test line: Recombinant HCV antigen (core, NS3, NS4, NS5) (1.5±0.3 µg)
  - Control line: Goat anti-human Immunoglobulin (2.0±0.4 µg)
- Assay diluent includes: 50 mM Tris-HCl Buffer (5 ml), Sodium azide (0.02 %)

## Materials required but not provided

- Micropipette, Protective gloves, Timer, Biohazard container

## Kit storage and stability

1. The test kit should be stored at a temperature between 1 °C and 30 °C. Do not freeze the kit or its components.
2. Assay diluent may be opened and resealed for each assay. Cap should be firmly sealed between each use. Assay diluent is stable until expiration date if kept at 1 - 30 °C.
3. The test device is sensitive to both heat and humidity. Perform the test immediately after removing the test device from foil pouch.
4. Do not use the test kit beyond its expiration date. The shelf life of the kit is as indicated on the outer package.
5. Do not use the test kit if the pouch is damaged or the seal is broken.

ENGLISH

## Warnings

1. The test devices are for *in vitro* diagnostic use only. Do not reuse the test device.
2. The instructions must be followed exactly to achieve accurate results. Any individual performing an assay with this product must be trained in its use and must be proficient.
3. Do not use the pipette by mouth, smoke, drink, eat, apply cosmetics, or handle contact lenses in areas where specimens or kit components are being handled.
4. Wear protective gloves while handling specimens and wash hands thoroughly afterwards.
5. Clean up spills thoroughly using an appropriate disinfectant.
6. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials in a biohazard container as if they were infectious waste.
7. Do not mix or interchange different specimens.
8. Do not eat the desiccant from the foil pouch.
9. Avoid splashing or aerosol formation of specimen and assay diluent.
10. Do not mix or interchange components among different lots or those for other products.
11. Do not drink assay diluent.
12. Care should be taken to avoid contamination of the bottle nozzle when dropping assay diluent into the specimen well.
13. The assay diluent contains a proprietary anti-microbial agent, sodium azide, which presents no hazard to the user if normal laboratory safety precautions are followed. If contact with assay diluent to the eyes and/or skin, wash affected area with soap and water immediately. If irritation or signs of toxicity occur, seek medical attention.
14. The assay diluent contains sodium azide, which may react with lead or copper plumbing to form highly explosive metal azide compounds. When disposing of these reagents through plumbing fixtures, flush with a large volume of water to prevent azide build-up in drains.
15. Even though the color of assay diluent is changed to faint yellow, it doesn't affect the performance and stability of the kit.

## Specimen collection and storage

1. **Whole blood**

**[Collection by venipuncture]**

- Using venipuncture, draw the whole blood into the collection tube (containing anticoagulants including heparin, EDTA and sodium citrate).
- If the blood specimen is not immediately tested, it must be refrigerated at 2 - 8 °C.
- If stored at 2 - 8 °C, the blood specimen must be tested within 3 days of refrigeration.
- Do not use a blood specimen stored for more than 3 days; it can cause a nonspecific reaction.
- Bring blood specimens to room temperature (15 - 30 °C) prior to use.

**[Collection using a lancet]**

- Clean the area to be lanced with an alcohol swab.
- Squeeze the fingertip then prick the lateral side of the finger with a lancet provided. Wipe away the first blood drop. Then, safely dispose of the lancet immediately after.
- Immerse the open end of a new capillary pipette (10 µl) in the next blood drop and release the pressure to draw blood into the capillary pipette up to the fill line.

2. **Plasma or serum**

- [Plasma] Using venipuncture, draw the whole blood into the collection tube (containing anticoagulants including heparin, EDTA and sodium citrate) and then centrifuge blood to obtain the plasma specimen.
- [Serum] Using venipuncture, draw the whole blood into the collection tube (NOT containing anticoagulants) then leave for 30 minutes to allow blood coagulation to occur. Centrifuge the tube to generate a serum specimen.
- If plasma or serum specimens are not tested immediately, they must be refrigerated at 2 - 8 °C. For storage period longer than 2 weeks, freezing (below -20 °C) is required. Bring plasma or serum specimens to room temperature (15 - 30 °C) prior to use.
- Plasma or serum specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified by centrifugation prior to assaying.

3. **Precautions**

- Repeated frozen-thawed cycle for specimen should be avoided.
- Anticoagulants including heparin, EDTA and sodium citrate do not affect the test result. Use of other anticoagulants has not been validated. Their use may affect the test result.
- Use new pipette tips for each specimen in order to avoid cross-contamination of other specimens which could cause erroneous results.

## Test procedure

1. Bring all kit components and specimens to reach a temperature between 15 °C and 30 °C prior to testing.
2. Remove the test device from foil pouch and place it on a flat, dry surface. Label the test device with a patient identifier.
3. **[Using a micropipette]**
   Dispense 10 µl of plasma, serum or whole blood specimen into the specimen well marked "S".
   Or,
   **[Using a capillary pipette]**
   Dispense 10 µl of drawn whole blood specimen into the specimen well marked "S"
4. Dispense 4 drops of assay diluent into the specimen well "S".
   ⚠ **Caution:** If you do not hold the bottle vertically, it can lead to inaccurate results. Exactly, 4 drops should be added. Adding more than 4 drops may result in reddish color background or an invalid result.
5. As the test begins to work, you will see purple color move across the result window in the center of the test device.

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6. Interpret test results 5 - 20 minutes after adding assay diluent. Do not read after 20 minutes.
⚠ **Caution:** If the test result is not legible after 5 minutes due to high background color, read again later but within 20 minutes of adding the diluent. Reading outside of this time frame (before 5 min or after 20 min) may result in false results.

## Test interpretation

1. A colored control line will appear at "C" in the result window to show that the test is working properly.
2. The "T" section of the result window indicates the test result.

- **Non-reactive result:** The presence of only the control line (C) within the result window indicates a non-reactive result.
- **Reactive result:** The presence of the test line (T) and the control line (C) within the result window, regardless of which line appears first, indicates a reactive result.
⚠ **Caution:** The presence of any test line, no matter how faint, the result is considered reactive.
- **Invalid result:** If the control line (C) is not visible within the result window after performing the test, the result is considered invalid. Instructions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be retested using a new test device.

## Test limitations

1. A non-reactive result does not preclude the possibility of infection with HCV. Other clinically available tests are required if questionable results are obtained. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Due to the inherent design of qualitative IVD tests, a faint or absent test line (false non-reactive) may occur in specimens containing high antibody densities; the prozone effect. In order to obtain a definitive result, all clinical and laboratory findings should be evaluated.

## Internal quality control

The Bioline™ HCV test device has 2 pre-coated lines on the surface of the test: "T" (test line) and "C" (control line). Neither the test line nor the control line is visible in the result window before applying a specimen. The control line is used for procedural control and shows only that the diluent has been applied successfully and that the active ingredients of the main components on the strip are functional, but it is not an assurance that the specimen has been properly applied; it is not a reactive specimen control.

## Performance characteristics

Bioline™ HCV test kit has been evaluated in 3 different sites as below. The results of individual laboratories may vary from these data because the results can be unique to the population it serves depending upon geographical, patient, dietary, environmental and other factors.

1. Study 1 : European performance evaluation according to the common technical specification (2009/886/EC)

1) Diagnostic sensitivity for anti-HCV detection

- Anti-HCV positive specimens

410 anti-HCV positive specimens were tested by Sanquin in the Netherlands and the German Red Cross. The diagnostic sensitivity for anti-HCV antibody detection, calculated on 410 positive specimens, was 99.3 %.

Table 1. Result obtained with the Bioline™ HCV on all Anti-HCV positive specimens

| | Bioline™ HCV | |
|---|---|---|
| | **Reactive** | **Non-reactive** |
| **Anti-HCV positive serum/plasma (n=213)** | 211 | 2 |
| **Anti-HCV positive whole blood/plasma couples (n=100)** | 100 | 0 |
| **Anti-HCV with known genotype (n=97)** | 96 | 1 |
| **Total (n=410)** | 407 | 3 |
| **Sensitivity (95 % CI)** | 99.3 % (97.9 - 99.8 %) | |

- Anti-HCV positive serum/plasma

213 anti-HCV positive specimens confirmed with CHIRON HCV RIBA™ 3.0 SIA or INNO-LIA HCV Score without genotype information were tested at Sanquin. In two specimens the Bioline™ HCV obtained a non-reactive result.

- Anti-HCV positive whole blood/plasma couples (Paired specimens)

100 paired whole blood and plasma specimens anti-HCV positive with Abbott Architect anti-HCV, taken from HCV infected patients were tested at the German Red Cross. The Bioline™ HCV test was reactive on all 100 paired specimens.

- Anti-HCV with known genotype

94 specimens from patients with a known HCV genotype 1 to 6, based on the VERSANT HCV Genotype 2.0 Assay (LiPA) were tested by Sanquin, and 3 specimens of genotype 5 were tested by the German Red Cross.

Table 2. Anti-HCV positive specimens with known genotype

| | | | Bioline™ HCV | |
|---|---|---|---|---|
| **HCV genotype** | | **# Specimens** | **Reactive** | **Non-reactive** |
| **Genotype 1 (n=23)** | 1 | 1 | 1 | 0 |
| | 1a | 10 | 10 | 0 |
| | 1b | 12 | 11 | 1* |
| **Genotype 2 (n=22)** | 2a/2c | 13 | 13 | 0 |
| | 2b | 9 | 9 | 0 |
| **Genotype 3 (n=25)** | 3 | 1 | 1 | 0 |
| | 3a | 22 | 22 | 0 |
| | 3b | 1 | 1 | 0 |
| | 3k | 1 | 1 | 0 |
| **Genotype 4 (including non 4a) (n=20)** | 4a/4c/4d | 4 | 4 | 0 |
| | 4c/4d | 14 | 14 | 0 |
| | 4h | 2 | 2 | 0 |
| **Genotype 5 (n=5)** | 5a | 2 | 2 | 0 |
| | 5 | 3 | 3 | 0 |
| **Genotype 6 (n=2)** | 6 | 1 | 1 | 0 |
| | 6a | 1 | 1 | 0 |

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*One genotype 1b specimen was non-reactive on the Bioline™ HCV.

- Sensitivity on seroconversion panels

20 commercially available seroconversion panels (SeraCare Life Sciences and Zeptometrix) were tested with Bioline™ HCV by Sanquin.

In total 54 specimens were tested reactive with Bioline™ HCV and 47 with the competitor tests.

| Panel ID | Bioline™ HCV | Ortho HCV 3.0 or Ortho Enhanced SAVe Anti- HCV 3.0 (*) |
|---|---|---|
| PHV904 | 3/7 | 3/7 |
| PHV905 | 3/9 | 4/9 |
| PHV911 | 3/5 | 3/5 |
| PHV913 | 0/4 | 0/4 |
| PHV914 | 5/9 | 3/9 |
| PHV915 | 2/4 | 2/4 |
| PHV918 | 1/8 | 2/8 |
| PHV919 (°) | 7/7 | 3/7 |
| PHV920 | 6/10 | 6/10 |
| 6213 | 2/12 | 2/12 |
| 6214 | 6/13 | 4/13 |
| 6224 | 2/6 | 0/6 |
| 6226 | 4/12 | 3/12 |
| 6228 | 3/12 | 3/12 |
| 6229 | 3/8 | 3/8 |
| 9044 | 2/6 | 2/6 |
| 9045 | 2/8 | 1/8 |
| 9047 | 4/10 | 4/10 |
| 9054 | 1/10 | 1/10 (*) |
| 9058 | 2/5 | 1/5 (*) |
| **Total reactive bleeds (*)** | 54 | 47 |