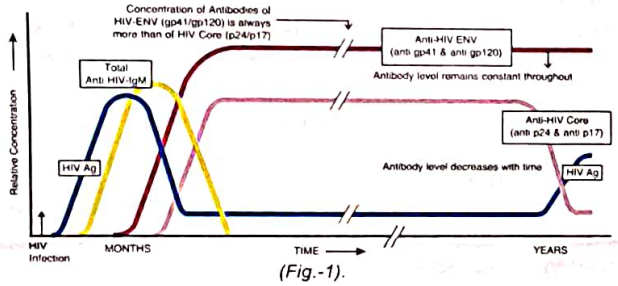
# HIV TRI-DOT

*Rapid Visual Test for the Qualitative Detection of Antibodies to HIV-1 & HIV-2 in Human Serum/Plasma*
*Separate Dots for HIV-1, HIV-2 & Control*

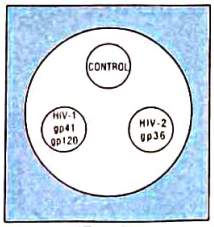
## I. HISTORICAL REVIEW AND AETIOLOGY OF AIDS (Acquired Immuno Deficiency Syndrome)

First confirmed case of AIDS was identified in 1983 and by 1984 the etiologic agent, the Human Immunodeficiency Virus (HIV), subsequently named HIV-1 was isolated. Shortly afterwards in 1985 another retrovirus subsequently named HIV-2 was isolated in Africa. These two viruses belong to the retrovirus group and are slow viruses. The structure, gene organisation and serological behaviour of HIV-1 & HIV-2 and their complete nucleotide sequence has been determined. This knowledge has laid a foundation for the development of a new assay based on Recombinant DNA technology leading to the differential detection of antibodies to HIV-1 & HIV-2 (if present) in Human Serum or Plasma. Research has shown that antibodies produced against envelope gene are found in infected people as shown in graph, (Fig.-1).




*(Fig.-1).*

HIV TRI-DOT has been developed and designed using gp41, C terminal of gp120 & gp36 representing the immunodominant regions of HIV-1 & HIV-2 envelope gene structure respectively. The device (an immunofiltration membrane) includes a "Built-in Quality Control DOT" which will develop colour during the test, thereby, confirming proper functioning of the device, reagents and correct procedural application. This CONTROL DOT is the "Built-in Quality Control." (Fig.2)




*(Fig.-2).*

HIV TRI-DOT has been specially researched, developed and engineered using several thousands of serum/plasma specimens. It has also been evaluated by UNAIDS (WHO) Geneva, using samples of European, Asian, Latin American & African origin. The Sensitivity and Specificity has been extremely high in these samples of diverse origin.

The panel used for evaluation of HIV TRI-DOT by Institute of Tropical Medicine, WHO Collaborating Centre in AIDS, Belgium also included HIV-O Virus, which was found reactive with HIV TRI-DOT.

## 2. INTENDED USE

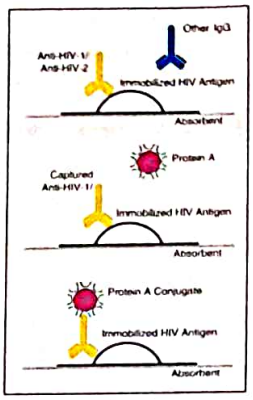
The HIV TRI-DOT Test is a visual, rapid, sensitive and accurate immunoassay for the differential detection of HIV-1 & HIV-2 antibodies (IgM, IgG & IgA) in Human Serum or Plasma using HIV-1 & HIV-2 Antigens immobilized on an immunofiltration membrane. The test is a screening test for anti-HIV-1 & anti-HIV-2 and is for *in vitro* diagnostic use only.

## 3. PRINCIPLE OF THE TEST

HIV antigens are immobilized on a porous immunofiltration membrane. Sample and reagents pass through the membrane and are absorbed into the underlying absorbent.

As the patient's sample passes through the membrane, HIV antibodies, if present, bind to the immobilized antigens.

Conjugate binds to the Fc portion of the HIV antibodies to give distinct pinkish purple DOT(s) against a white background. (Fig.-3)




*(Fig.-3).*

## 4. KIT DESCRIPTION

| COMPONENTS | CONTENTS | PREPARATION |
|---|---|---|
| 1. HIV TRI-DOT Test Device | Packed individually. Device has membrane with 1 Control & 2 Test Dots, one each for HIV-1 & HIV-2. | 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. | |

Store the kit at 2-8°C in the driest area available.
Bring all reagents and test components to room temperature (20-30°C) before use. Return entire kit at 2-8°C when not in use. DO NOT FREEZE TEST COMPONENTS.

## 5. MATERIAL REQUIRED BUT NOT PROVIDED

The kit contains all the items required to perform this test. But if the sample is viscous/turbid/contains particulate matter, a centrifuge will be required, to separate off the suspended matter. Since the test is completed in less than 5 minutes a timer or stop watch is not essential.

## 6. STORAGE

Store the entire kit at 2-8°C in the coolest and driest area available. The components are stable for 24 months from the date of manufacturing, when stored at 2-8°C. Do not use the kit beyond the expiry date. DO NOT FREEZE THE KIT COMPONENTS.

## 7. KIT PRESENTATION

50 Test Pack
100 Test Pack
200 Test Pack

## 8. 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.

## 9. 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 after use.
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.

## 10. SPECIMEN/SAMPLE COLLECTION & STORAGE

Collect blood in a clean dry sterile vial and allow to clot or separate the serum by centrifugation at room temperature. It is recommended that fresh sample should be used if possible. If serum is not to be assayed immediately it should be stored at 2-8°C or frozen at minus 20°C (-20°C). 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.

## 11. SPECIMEN/SAMPLE PROCESSING

### (A) FROZEN SAMPLE:

The HIV TRI-DOT Test is best when used with fresh samples that have not been frozen and thawed. However, most frozen samples will perform well if the following suggested procedure is followed.

1. Allow the sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample can be centrifuged at 10,000 r.p.m. for 15 min.
2. Insert the dropper just below the top surface of the sample and withdraw one drop of sample. If the above procedure still yields a high background, dilute 1 drop of sample with 2 drops of normal saline. Use 1 drop of this diluted sample in the test.

### (B) THICK OR VISCOUS SAMPLES:

Whenever possible, clear specimens 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 min. and retested on a fresh device to avoid inconsistent results.

### (C) TRANSPORTATION

If the specimen is to be transported it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

## 12. BEFORE YOU START

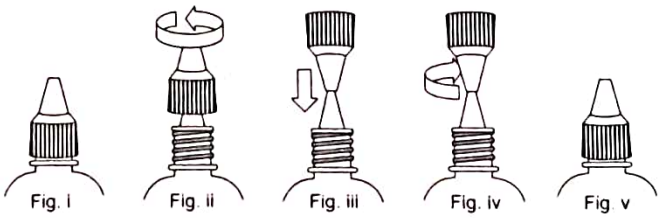


Fig. i Fig. ii Fig. iii Fig. iv Fig. v

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:

## 13. 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.

**R.T. 20-30°**

2. Place the required number of HIV TRI-DOT test devices at the working area.

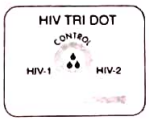
3. Tear off the pouch and take out the device for performing the test. Write the sample number to be tested on the device.
4. While adding sample/reagents to the device, be sure to ALLOW EACH SOLUTION TO SOAK IN BEFORE ADDING THE NEXT SOLUTION.
   However drops of each solution should be added in continuous 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.

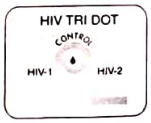
7. For consistent results, ensure FREE FALLING OF DROPS on the membrane.
8. Do not use kit components beyond the expiration date.
9. The liquid conjugate should not be subjected to frequent temperature fluctuations.
10. The procedural sequence of reagent addition should be strictly adhered to avoid any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

## 14. TEST PROCEDURE

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




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




3. Add **5** drops of Buffer Solution.


4. Add **2** drops of Protein-A Conjugate directly from the conjugate vial.


5. Add **5** drops of Buffer Solution and read results.


***Read results immediately and discard the device 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.*

## 15. INTERPRETATION OF RESULTS

### NON-REACTIVE

1. If only One DOT (only the Control Dot) appears as shown in fig., the specimen is non reactive for antibodies either to HIV-1 or HIV-2. Interpret sample as non-reactive.


### REACTIVE

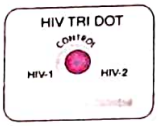
1. If two DOTS, one for the control and the other for HIV-1 appear as shown in Fig., the specimen is reactive for antibodies to HIV-1.


2. If two DOTS, one for the control and the other for HIV-2 appear as shown in Fig., the specimen is reactive for antibodies to HIV-2.


3. If all the three DOTS, one each for control, HIV-1 & HIV-2 appear as shown in Fig., the specimen is reactive for antibodies to HIV-1 & HIV-2.


### INVALID TEST

If no DOT appears after the test is complete, either with clear background or with complete pinkish/purple background the test indicates ERROR. This may indicate a procedural error or deterioration of specimen/reagens or particulate matter in the specimen. The specimen should be centrifuged at 10,000 rpm for 15 minutes and re-run the test using new device (Refer Specimen/ sample processing).


*(If the problem persists, please call our Technical/ Customer service cell, Parwanoo, Himachal Pradesh, Phone: 01792-232253).*

### IMPORTANT

1. All initially reactive samples should be subjected to centrifugation at 10,000 r.p.m. for 15 min. It is recommended that this centrifugation step should be carried out prior to sending the sample for the Western Blot. The test should be repeated with supernatant collected after centrifugation. If no dot appears on repetition, it indicates a falsely reactive sample. A truly reactive dot will not show much change in its colour intensity after centrifugation. The false reactivity of the sample is generally due to the presence of suspended particulate matter in the serum which may or may not be visible to the naked eye.
   This critical step of centrifuging a reactive sample should be faithfully followed. Its correct application makes the test EXTREMELY SENSITIVE and completely eliminates the possibility of false reactivity.
2. 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 min. Use a fresh device to re-run the test.
3. Test dots HIV-1 and HIV-2 either dark or light in pink colour should be considered reactive.
4. ***Sample found to be reactive by the above screening test must be confirmed by standard supplemental assay, like Western Blot.***

## 16. LIMITATIONS OF THE TEST

1. The kit works best when used with fresh samples. 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.
2. 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 results.
3. HIV-1 and HIV-2 viruses share many morphological and biological characteristics. It is likely that due to this, their antibodies have a cross reactivity of 30-70%. Appearance of dots for HIV-1 and

HIV-2 antibodies on the test device does not necessarily imply co-infection from HIV-1 & HIV-2.

4. Some samples show cross reactivity for HIV antibodies. **Following factors are found to cause false positive HIV antibody test results:** Naturally occurring antibodies, Passive immunization, Leprosy, Renal Disorders, Tuberculosis, Myco-bacterium avium, Herpes simplex, Hypergamma-globulinemia, Malignant neoplasms, Rheumatoid arthritis, Tetanus vaccination, Autoimmune diseases, Blood Transfusion, Multiple myeloma, Haemophelia, Heat treated specimens, Lipemic serum, Anti-nuclear antibodies, T-cell leukocyte antigen antibodies, Epstein Barr virus, HLA antibodies and other retroviruses.
5. **This is only a screening test. All samples detected reactive must be confirmed by using HIV Western Blot.** 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.

## 17. PERFORMANCE CHARACTERISTICS

Performance of the HIV 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 1325 nos. of known serum/ plasma samples including cross reacting samples. The results of all the samples with a defined HIV status were fully comparable with those of HIV TRI -DOT. The results of the in-house study done are as follows:

| No. of Samples | Status | HIV TRI-DOT | HIV TRI-DOT |
|---|---|---|---|
| | | + ve | - ve |
| 50 | ELISA +ve | 50 | - |
| 1275 | ELISA -ve | 1 | 1274 |

**Sensitivity: 100%**

**Specificity: 99.84%**

**Precision**: Within-run and between-run precisions have been determined by testing 10 replicates of 10 samples: 7 HIV-1 positive (1 strong, 1 moderate & 5 weak), 1 HIV-2 positive and 2 HIV negative. The C.V.(%) of all the samples were within 10%.

## 18. DISPOSAL

Discard the test device immediately after reading result. Before discarding it, add few drops of disinfectant on device membrane and on all other items used for handling serum. Put all items to be disposed in Disposable Bags and dispose off accordingly.

## 19. 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.

## 20. REFERENCES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In vitro diagnostic reagent, not for medicinal use


*Manufactured & Marketed By:*

**DIAGNOSTIC ENTERPRISES**

Plot No.: 26, Indl. Estate, Sector-1, Parwanoo - 173 220, (H.P.)

Phone: 01792-232253 E-mail: do@diagnosticenterprises.com

# 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).

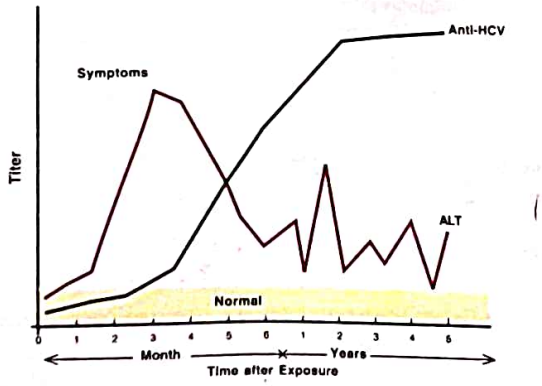



**Fig.1 Hepatitis C Virus Infection**
Typical Serologic Course

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).

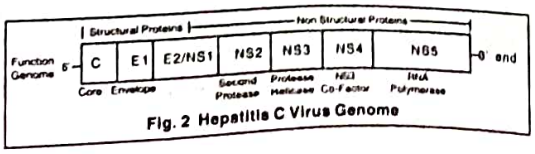



**Fig. 2 Hepatitis C Virus Genome**

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."

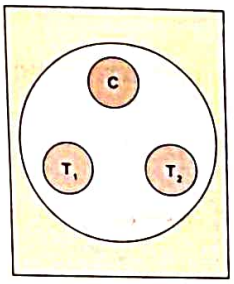



Fig. 3 Test Device

## 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).

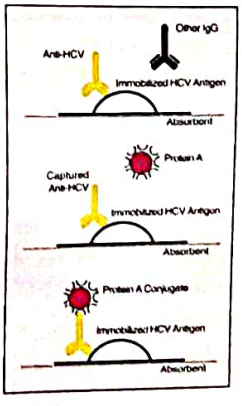



Fig. 4 Principle of the Assay

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 24 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:

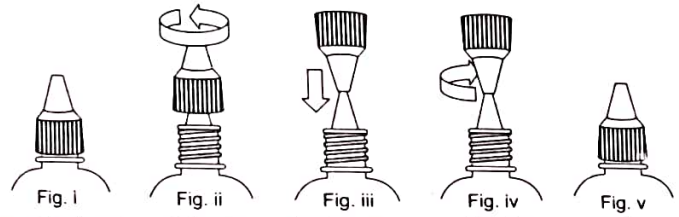


Fig. i close vial provided in the kit | Fig. ii open the cap | Fig. iii invert the cap & press on the closed nozzle | Fig. iv give a half turn twist to puncture the nozzle | Fig. v screw the cap tightly after use

### 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.**

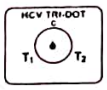
### 13. TEST PROCEDURE

**Step No. 1**

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

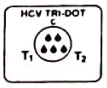

**Step No. 2**

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).



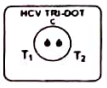

**Step No. 3**

Add 5 drops of Buffer Solution.



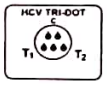

**Step No. 4**

Add 2 drops of Protein- A Conjugate.




**Step No. 5**

Add 5 drops of Buffer Solution.




**Step No. 6**

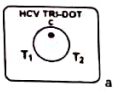
Read result immediately and discard the device immediately considering it to be potentially infectious.

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

### 14. INTERPRETATION OF RESULTS

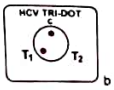
**NON REACTIVE RESULT**

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

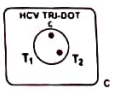



**REACTIVE RESULT**

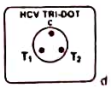
1. Appearance of two dots, one at the control region **"C"** & other at the test region **"$T_1$"** indicates that the sample is REACTIVE for antibodies to HCV. (Fig:b)




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)

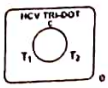



3. Appearance of all the three dots, one each at **"C" "$T_1$"& "$T_2$"** region indicates that the specimen is REACTIVE for antibodies to HCV. (Fig:d)

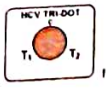



**INVALID RESULT**

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 centrifuged at 10,000 rpm for 15 minutes and re-run the test using new device (Refer Specimen/ sample 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 bleeds 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 Poel, C.L. etal (1971) J. of Hepatology, 13, 5.15.
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3. Sarin, S.K. & Hess. G. (1998). Transfusion associated Hepatitis.
4. Sayers, M.H. & Gretch D.R. (1993). J. Transfusion 30,809-13.


In vitro diagnostic reagent, not for medicinal use


*Manufactured & Marketed By:*

**DIAGNOSTIC ENTERPRISES**

Plot No.: 26, Indl. Estate, Sector-1, Parwanoo - 173 220, (H.P.)
Phone: 01792-232253 E-mail: de@diagnosticenterprises.com

# HEPACARD

***One Step Rapid Visual Test For the Qualitative Detection of HBsAg in Human Serum/Plasma***

## INTENDED USE

HEPACARD is a visual, rapid, sensitive and accurate one step immunoassay for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum or Plasma. The assay is intended to be used as an aid in the recognition and diagnosis of acute infections and chronic infectious carriers of the Hepatitis B Virus (HBV).

## INTRODUCTION

The antigenic determinant of the HBsAg protein moiety is antigenically heterogenous and it determines specific HBV serotypes and provides a basis for immunodetection. The principal antigenic determinant is "a" which is common to all HBV serotypes. In addition, two pairs of subspecific determinants have been identified, d/y & w/r, which are apparently mutually exclusive. Four antigenic combinations are therefore possible: adw, adr, ayw and ayr.

## PRINCIPLE

HEPACARD is a one step immunoassay based on the antigen capture, or "sandwich" principle. The method uses monoclonal antibodies conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. The test sample is introduced to and flows laterally through an absorbent pad where it mixes with the signal reagent. If the sample contains HBsAg, the colloidal gold-antibody conjugate binds to the antigen, forming an antigen-antibody-colloidal gold complex. The complex then migrates through the nitrocellulose strip by capillary action. When the complex meets the line of immobilized antibody (Test line) "T", the complex is trapped forming an antibody-antigen-antibody collidal gold complex. This forms a pink band indicating the sample is reactive for HBsAg. To serve as a procedural control, an additional line of anti-mouse antibody (Control line) "C", has been immobilized at a distance from the test line on the strip. If the test is performed correctly, this will result in the formation of a pink band upon contact with the conjugate.

## KIT CONTENTS

a) Hepacard Test Device b) Sample Dropper

c) Instruction Manual

## KIT PRESENTATION

100 Test Pack 200 Test Pack

## STORAGE AND SHELF LIFE

HEPACARD should be stored at 2-30°C in the coolest and driest area available. Expiry date on the kit indicates the date beyond which the kit should not be used. **The HEPACARD should not be frozen and must be protected from exposure to humidity.**

## 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 and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with suitable disinfectant.
9. Take out the Cards from the pouch just before performing the test to avoid denaturation of antisera due to atmospheric exposure.

   **Optimal test performance requires strict adherence to the test procedure described in the insert.**

## PRECAUTIONS

1. Do not open the foil pouch to remove the product until it attains room temperature and you are ready to perform the test.
2. Do not freeze the product.
3. Interpret the result at the end of 20 minutes only.
4. Take out the Cards from the pouch just before performing the test to avoid denaturation of antisera due to atmospheric exposure.

   **Optimal test performance requires strict adherence to the test procedure described in the insert.**

## SAMPLE / SPECIMEN COLLECTION & STORAGE

a) HEPACARD should be performed on human serum or plasma only immediately after collection.

b) If not tested immediately, specimen should be refrigerated at 2-8°C upto 3 days following collection.

c) If testing within 3 days is not possible, specimen should be stored frozen at -20°C.

d) Specimen containing visible precipitates or cloudy specimens may give inconsistent test results. Such specimens should be clarified prior to testing by high speed centrifugation i.e. 10,000 rpm for 15 minutes before testing.

e) Haemolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

## TEST PROCEDURE

1. Bring the required number of **HEPACARD** foil pouches and specimen to room temperature prior to testing.
2. Take out **HEPACARD** device from the foil pouch.
3. Label the test card with patient's name or identification number.
4. Add 2 drops (70 $\mu$l) of human serum/plasma specimen into the sample well using the dropper provided (use separate dropper/microtip for each specimen).
5. Allow reaction to occur during the next 20 minutes.
6. Read results at 20 minutes.
7. Discard the HEPACARD immediately after reading result at 20 minutes, considering it to be potentially infectious.

## INTERPRETATION OF RESULT

**REACTIVE :**

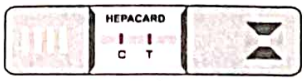

Fig 1

As shown in Fig.1, appearance of pink coloured line, one each in test region "T" and control region "C" indicates that the sample is REACTIVE for HBsAg. A difference of intensity in colour may occur between the Test line & Control line depending on the concentration of the HBsAg in the serum but this does not affect interpretation of the result. Faint test line also should be considered HBsAg reactive.

Depending on the concentration of HBsAg, positive results may be observed within 60 seconds. However, to detect concentration around 0.5 ng to 1ng/ml and to confirm a negative result, the test result should be read only at 20 minutes. If the conc. of HBsAg in the sample is very high, only test line may be observed. This is due to Hook's effect. Such samples should be diluted 1:10 or 1:20 in normal saline & again re-run the test, Diluted sample should show both control & test line. In case, if control line does not appear or is faint dilute the sample further.

**NON-REACTIVE :**

Fig. 2

As shown in Fig.2 appearance of one distinct pink line in the control region "C" only, indicates that the sample is "NON REACTIVE" for HBsAg.

**INVALID :**

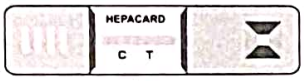

Fig. 3

When neither control line nor test line appears on the membrane as shown in Fig.3, the test should be treated as invalid which may be because of following reasons:

a) Improper storage at temperature other than the recommended temperature.

b) Wrong procedure.

c) Long atmospheric exposure of the test device after opening the pouch.

The test should be repeated using a new HEPACARD and test sample.

## LIMITATIONS OF THE PROCEDURE

1. The HEPACARD is for *in vitro* diagnostic use only.
2. The test should be used for the detection of HBsAg in serum or plasma only and not in other body fluids.
3. **This is only a Screening test.** All reactive samples should be confirmed by confirmatory test. Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serologica data, should be considered. The results should be reported only after complying with above procedure.
4. Additional follow up testing using available clinical methods (along with repeat HEPACARD test) is required, if HEPACARD test is non-reactive with persisting clinical symptoms.
5. False positive results can be obtained due to the presence of other antigens or elevated levels of RF factor. This occurs in less than 1% of the samples tested.

## PERFORMANCE CHARACTERISTICS

The performance of HEPACARD 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 1400 nos. of known serum/ plasma samples including cross reacting samples. The results of all the samples with a defined HBsAg status were fully comparable with those of HEPACARD. The results of the in-house study done are as follows:

| No. of Samples | Status | HEPACARD | HEPACARD |
|---|---|---|---|
| | | + ve | - ve |
| 125 | ELISA +ve | 125 | - |
| 1275 | ELISA -ve | 8 | 1267 |

**Sensitivity : 100%** **Specificity : 98.75%**

**Precision** : Within-run and between-run precisions have been determined by testing 10 replicates of seven HBsAg positive samples : 4 weak, 2 moderate positive, 1 strong positive and 2 HBsAg negative. The C.V.(%) of negative, weak, moderate positive and strong positive samples were within 10% of the time.

## ANALYTICAL SENSITIVITY :

a) HEPACARD can detect Hepatitis B Surface Antigen in serum or plasma at a concentration of as low as 0.5 ng/ml at 20 minutes. It shows overall agreement of 99.8% with EIA techniques for sample having conc. 0.5 ng/ml or more.

b) All the eleven HBsAg subtypes can be detected positive with HEPACARD.

## 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.

## BIBLIOGRAPHY

1. Blumberg, B.S., (1964) Bull. N.Y. Acab Med., 40:377
2. Blumberg B.S. etal, (1965) J.A.M.A. 191:541.
3. Caldwell C.W. etal., (1977) Clin. Chem. Acta: 31:305.
4. Peterson, D.L. etal., (1982) J. Biol. Chem., 257(17): 10414.
5. Robin, E (1979) Fed. Proc. 33 (13) 2665.

**WARNING** : The "see Through Device" of HEPACARD has been developed as a result of intensive research. It's DESIGN IS REGISTERED and the WORLD PATENT INCLUDING INDIA has been applied for. Anyone copying the device design will render oneself liable for legal action.

R-05

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MN/HPC/003

CE

# ENTEROCHECK®-WB

## Rapid test for detection of IgM antibodies to *S. typhi* in serum/plasma/whole blood

DEVICE

### INTENDED USE

**ENTEROCHECK®-WB** is a rapid, qualitative, sandwich immunoassay for the detection of IgM antibodies to *S. typhi* in human serum/plasma or whole blood specimen.

### SUMMARY

A febrile condition, Typhoid fever, is a bacterial infection caused by Salmonella serotypes including *S. typhi, S. paratyphi A, S. paratyphi B* and *Salmonella sendai*. The symptoms of the illness include high fever, headache, abdominal pain, constipation and appearance of skin rashes. Accurate diagnosis of typhoid fever at an early stage is not only important for etiological diagnosis but to identify and treat the potential carriers and prevent acute typhoid fever outbreaks. The conventional WIDAL Test usually detects antibodies to *S. typhi* in the patient serum from the second week of onset of symptoms. Early rising antibodies to Lypopolysaccharide (LPS) O are predominantly IgM in nature. Detection of *S. typhi* specific IgM antibodies instead of IgG or both IgG & IgM (as measured by the Widal test) would serve as a marker for recent infection.
**ENTEROCHECK®-WB** qualitatively detects the presence of IgM class of Lypopolysaccharide (LPS) specific to *S. typhi* in human serum/plasma or whole blood specimens.

### PRINCIPLE

**ENTEROCHECK®-WB** utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. The conjugate pad contains two components - Agglutinating sera for human IgM conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test specimen flows through the membrane test assembly, the Agglutinating sera for human IgM -colloidal gold conjugate complexes with the *S. typhi* specific IgM antibodies in the specimen and travels on the membrane due to capillary action. This complex moves further on the membrane to the test region (T) where it is immobilized by the *S. typhi* specific LPS antigen coated on the membrane leading to formation of a pink to pink- purple coloured band. The absence of this coloured band in the test region indicates a negative test result.
The unreacted conjugate and unbound complex, if any along with the rabbit globulin - colloidal gold conjugate move further on the membrane and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated on the membrane at the control region (C), forming a pink to pink-purple coloured band. This control band acts as a procedural control and serves to validate the results.

### REAGENTS AND MATERIALS SUPPLIED

**ENTEROCHECK®-WB** kit contains:

A. Individual pouches, each containing :
   1. DEVICE : Membrane assembly pre-dispensed with Agglutinating sera for Human IgM - colloidal gold conjugate, rabbit globulin - colloidal gold conjugate, *S.typhi* LPS antigen and Agglutinating sera for rabbit globulin coated at the respective regions.
   2. PIPETTE : Disposable Plastic Sample Applicator.
   3. Desiccant pouch.

B. BUF : Sample running buffer in a dropper bottle.

C. Package Insert.

| REF | 501020010 | 501020025 | 501020050 |
|---|---|---|---|
| ⧖ | 10 | 25 | 50 |

### OPTIONAL MATERIAL REQUIRED

Calibrated micropipette capable of delivering 5 µl sample accurately.

### STORAGE AND STABILITY

The sealed pouches in the test kit & the kit components may be stored between 4°C to 30°C till the duration of the shelf life as indicated on the pouch / carton. DO NOT FREEZE. After first opening of the sample running buffer bottle, it can be stored between 4°C to 30°C for remaining duration of its shelf life.

### NOTES

1. Read the instructions carefully before performing the test.
2. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
3. Do not use the kit beyond expiry date and do not re-use the test device.
4. Do not intermix reagents from different lots.

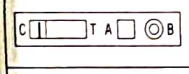
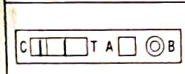
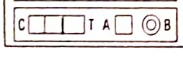
5. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
6. Handle all specimens as if potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infectious material.
7. If desiccant colour at the point of opening the pouch has turned from blue to pink or colourless, another test device must be run.
8. Sample running buffer contains Sodium Azide (0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxide. Flush with large volumes of water to prevent azide build-up in the plumbing.

## SPECIMEN COLLECTION AND PREPARATION

1. **ENTEROCHECK®-WB** uses human serum / plasma / whole blood as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. For whole blood, collect blood with a suitable anticoagulant such as EDTA or Heparin or Oxalate and use the freshly collected blood.
4. Whole blood should be used immediately and should not be frozen.
5. Though fresh specimen is preferable, in case of delay in testing, it may be stored **at 2°C to 8°C for maximum up to 24 hrs.**
6. If serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum.
7. Repeated freezing and thawing of the specimen should be avoided.
8. Do not use turbid, lipaemic and hemolysed serum/plasma.
9. Do not use hemolysed, clotted, contaminated, viscous/turbid specimens.
10. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only should be used for testing.
11. Refrigerated specimens must be brought to room temperature prior to testing.

## TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the kit components of **ENTEROCHECK®-WB** device to room temperature before testing.
2. Open a foil pouch by tearing along the "notch".
3. Remove the testing device and the sample applicator. ***Once opened, the device must be used immediately.***
4. Label the device with specimen identity.
5. Place the testing device on a flat horizontal surface.
6. Carefully dispense 5µl of whole blood / serum / plasma into the specimen port 'A' using a micropipette, OR using the 5µl sample applicator provided, dip the sample applicator in the sample container and blot the sample in the specimen port 'A'.
7. Add **five drops** of sample running buffer into the buffer port 'B'.
8. At the end of **15 minutes**, read results as follows:

| | |
|---|---|
|   | **Negative Result**<br>If IgM antibodies to *S.typhi* are not present, only one coloured band appears in the Control Window (C). |
|   | **Positive Result**<br>If IgM antibodies to *S.typhi* are present, two coloured bands appear in the Test (T) and Control Window (C). The intensity of the test band may be more or less than the Control band, depending upon the concentration of IgM antibodies in specimen. |
|     | **Invalid Result**<br>The test is invalid if no band is visible at fifteen minutes. The test should also be considered invalid if only test band appears and no control band appears. Verify the test procedure and repeat the test with a new device. |

## PERFORMANCE CHARACTERISTICS

*Internal Evaluation*

In an in-house study, the performance of **ENTEROCHECK®-WB** was evaluated using a panel of fifty specimens of WIDAL-positive (of varying reactivity) and WIDAL-negative sera in comparison with a commercially available DOT ELISA test kit. The results of the evaluation are as follows:

| SPECIMEN DATA | WIDAL | ENTEROCHECK®-WB | Commercially available DOT ELISA |
|---|---|---|---|
| No. of specimen tested | 50 | 50 | 50 |
| No. of positive specimens | 6 | 6 | 6 |
| No. of negative specimens | 44 | 43 | 44 |

Based on this evaluation:
Sensitivity of **ENTEROCHECK®-WB** : 100%
Specificity of **ENTEROCHECK®-WB** : 95.5%

*External Evaluation-I*

Seventy samples that were blood-culture positive, blood-culture negative sera and potentially cross-reacting sera were evaluated with **ENTEROCHECK®-WB** in University of Malaya, Malayasia. The results of the evaluation are as follows:

| SPECIMEN DATA | TOTAL | No. of Positives | No. of Negatives |
|---|---|---|---|
| Blood- culture positive sera | 29 | 23 | 6 |
| Blood- culture negative sera | 10 | 1 | 9 |
| Potentially cross-reacting negative sera | 31 | 3 | 28 |

The above evaluation report states that the Sensitivity and Specificity of **ENTEROCHECK®-WB** is 79.3% and 90.2% respectively.

*External Evaluation-II (Specificity & Precision study)*

One blood-culture positive serum and thirty blood-culture negative sera were tested with **ENTEROCHECK®-WB** in a NABL-accredited reputed reference laboratory in India. The following are the results:

| SPECIMEN DATA | TOTAL | No. of Positives | No. of Negatives |
|---|---|---|---|
| Blood- culture negative sera | 30 | 0 | 30 |
| Blood- culture positive sera | 1 | 1 | 0 |

Based on this evaluation:
Specificity of **ENTEROCHECK®-WB** : 100%

*Intra-assay Precision study*

One blood-culture positive sample was assayed 10 times on the same day.
**Results:** No variation in results was observed indicating 100% correlation.

*Inter-assay Precision study*

One blood-culture positive sample was assayed 3 times on 3 different days.
**Results:** No variation in results was observed indicating 100% correlation.

## LIMITATIONS OF THE TEST

1. The membrane is laminated with an adhesive tape to prevent surface evaporation. Air pockets or patches may appear, which do not interfere with the test results. Presence of a band at the test region even if low in intensity or formation is a positive result.
2. The deliberate slow reaction kinetics of **ENTEROCHECK®-WB** is designed to maximize and enhance reaction time between sample capture and tracer elements to improve test sensitivity.
3. Most positive results develop within 15 minutes. However, certain sera sample may take a longer time to flow. Therefore, negatives should be confirmed only at 30 minutes. Do not read results after 30 minutes.
4. 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.
5. **ENTEROCHECK®-WB** should be used as a screening test in clinically suspected cases only, and its results should be confirmed by other supplemental method before taking clinical decisions.
6. In some studies, it has been reported that low titre IgM antibodies to *S.typhi* may persist for about 4 months post infection. Therefore, in endemic area, samples positive yet with low signal intensity should be interpreted with caution, preferably in light of patient history.
7. The following chart would explain the IgM seroresponse in *S.typhi* infected subjects after onset of fever.

| Detectable IgM Response | |
|---|---|
| **Onset of fever** | **Percent positive** |
| 4-6 days | 43.50 % |
| 6-9 days | 92.90 % |
| >9 days | 100 % |

8. A negative result, i.e., the absence of detectable IgM antibody does not rule out recent or current infection, as the positivity is influenced by the time elapsed from the onset of fever and immunocompetence of the patient. However, if *S. typhi* infection is still suspected, obtain a second specimen 5-7 days later and repeat the test.
9. Specific IgG may compete with the IgM for sites and may result in a false negative. Conversely, high titer Rheumatoid factor may result in a false positive reaction.
10. A low extent of cross reactivity may be observed with *S. paratyphi* infection.

## WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

## BIBLIOGRAPHY


1. Hatta M et al., Simple dipstick assay for the detection of Salmonella typhi-specific IgM antibodies and the evolution of the immune response in patients with typhoid fever. Am. J. Trop. Med. Hyg., 66(4), 2002, pp. 416-421.
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6. Data on file: Viola Diagnostic Systems.


## SYMBOL KEYS

| | | | |
|---|---|---|---|
| Temperature Limitation | Consult Instructions for use | Date of Manufacture | Do not reuse |
| Manufacturer | IVD — *In vitro* Diagnostic Medical Device | This side up | BUF — Sample Running Buffer |
| Use by | REF — Catalogue Number | DEVICE — Device | EC REP — Authorised Representative in the European Community |
| Contains sufficient for <n> tests | LOT — Batch Number / Lot Number | PIPETTE — Disposable Plastic Sample Applicator | |


Manufactured by:

**Viola Diagnostic Systems**

A Division of Tulip Diagnostics (P) Ltd.

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EC REP

CMC Medical Devices & Drugs S.L., Spain.

0920/VER-01

# Stained Salmonella

## Antigen Set For Widal Test

REF : 17SA428-05, 17SA427-05, 17SA429-05

**Pack Size :** 4 X 5 mL, 2 X 2 X 5 mL, 2 X 5 mL

IVD +2 °C +8 °C STERILE A

## INTENDED USE

Diagnostic reagents for the *in vitro* detection and quantitative estimation of specific antibodies to *Salmonella* present in serum by rapid slide and conventional tube tests.

## INTRODUCTION

Typhoid is an enteric fever caused by various species of Salmonella such as *S. typhi*, *S. paratyphi* A and *S. paratyphi* B. The disease is characterized by sustained high Fever, severe Headache, Nausea, Anorexia and Constipation initially and later Diarrhoea. Humans are the only reservoir of the bacteria. The bacteria is usually transmitted by five 'F's: flies, food, fingers, faeces and fomites.

Widal test is the most widely accepted, a century old, serodiagnostic technique used for diagnosis of Typhoid. The test uses "O" and "H" antigens of *S. typhi* and *S. paratyphi* "A" and *S. paratyphi* "B" to detect the high agglutinin titres of "O" and "H" antibodies in the serum of patients suffering from enteric fever.

"O" is somatic antigen shared by most of the enteric bacteria and thus of lower specificity for the diagnosis. "H" is flagellar antigen specific for *Salmonella* species. Antibodies against "O" disappear quickly, on the other hand, "H" antibodies persist for a longer period of time, lowering its diagnostic value. So, detection of "O" antigen alone or "H" antigen alone is not sufficient to prove Typhoid infection. While, a diagnostic "O" titre and a rising titre of both "H" and "O" agglutinins in two different samples, taken at the interval of 7-10 days assure Typhoid infection [1].

Stained Salmonella antigens are used for detecting, identifying and quantifying antibodies specific to "O" and "H" antigens in serum of patients suffering from enteric fever. These antigens are standardised, smooth suspensions of killed bacilli which have been stained using "supravital staining" technique that increases the sensitivity and specificity of the test. These antigens are suitable to be used in the standard Widal tube test as well as rapid slide test.

## PRINCIPLE

This test is based on the principle of direct agglutinations reaction. The smooth suspension of killed *Salmonella* bacilli carries homologous "O" and "H" antigens. When patient's serum (containing antibodies to *S. typhi* and *S. paratyphi*) is incubated with respective antigens, visible agglutination occurs. A rising titer of antibodies is indicative of enteric fever.

## REAGENTS

| Reagent No. | Reagent Name | REF 17SA428-05 Pack size: 4 X 5 mL | 17SA427-05 2 X 2 X 5 mL | 17SA429-05 2 X 5 mL |
|---|---|---|---|---|
| 1 | Stained Salmonella *S.typhi* "H" Antigen | 1 X 5 mL | 2 X 5 mL | 1 X 5 mL |
| 2 | Stained Salmonella *S.typhi* "O" Antigen | 1 X 5 mL | 2 X 5 mL | 1 X 5 mL |
| 3 | Stained Salmonella *S. paratyphi* "A(H)" Antigen | 1 X 5 mL | – | – |
| 4 | Stained Salmonella *S. paratyphi* "B(H)" Antigen | 1 X 5 mL | – | – |
| 5 | Positive Control Serum | 1 X 0.5 mL | 1 X 0.5 mL | 1 X 0.5 mL |

**ACCESSORIES** (*QS - Quantity Sufficient)
Glass Slide

## MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

1. *Applicator Sticks*
2. *Disposable Plastic Droppers*
3. Rubber Teats
4. Test Tubes.
5. Pipettes.
6. Normal Saline.
7. Disposable Gloves for specimen handling.
8. 5% Solution of Sodium or Calcium Hypochlorite to wipe and disinfect the spills.
9. Disposable Biosafety Bag to collect and dispose used accessories and waste.

## REAGENT STORAGE AND STABILITY

All reagents are stable at +2 °C to +8 °C until the expiry date shown on the label. **Do not freeze.**

## SPECIMEN COLLECTION AND HANDLING

| Specimen | Storage at | Stability | Remarks |
|---|---|---|---|
| Serum | +2 °C to +8 °C | 48 hours | Freshly collected serum, free of visible turbidity and / or haemolysis should be used. In case of delay, serum should be stored at +2 °C to +8 °C. It should not be heated or inactivated. Grossly haemolysed and contaminated samples should not be used. |

## BIOSAFETY

1. Handle all the samples with care, as they can be potentially infectious.
2. Avoid splashing or spilling of reagents. In case of any spill, the contaminated area should be decontaminated immediately.
3. Do not pipette by mouth.
4. Place the used slides and tubes immediately in a disinfectant and leave overnight before using again or discarding.
5. Wear disposable gloves throughout the test procedure and dispose off the gloves as biohazard waste.
6. Wear protective laboratory clothing in laboratory areas.
7. Working space should be kept clean.
8. Do not smoke, eat or drink in the area where samples or kit reagents are handled.
9. Wash hands thoroughly after completion of the test.

## PRECAUTIONS

1. Use clean and dry glassware, free of detergents.
2. Use clear, fat-free and contamination free serum.
3. Bring all the reagents at room temperature (+15 °C to +30 °C) before use.
4. Take extreme care to prevent microbial contamination of reagents.
5. Antigen vials should be shaken before use to make the suspension homogenous.
6. Do not use kit reagents beyond expiry date.
7. Do not interchange vial caps as it will lead to cross-contamination and deterioration of reagents.
8. Do not interchange reagents from different lots.
9. Very rarely reagent may show fibric structure (appearance like fungus), which does not interfere with the specificity and sensitivity of the test.
10. Positive Control should always be included in the test for better interpretation of test results.
11. Normal saline should be used for serum dilution.
12. Incubation should be done at non-vibrating place.

## PROCEDURE

### A. RAPID SLIDE (SCREENING) TEST

1. Clean the glass-slide supplied in the kit properly and wipe it free of moisture.
2. Place one drop (50 µL) of undiluted test serum in each of the 1st four circles (1 to 4) and one drop of positive control serum in each of the last two circles (5 & 6).
3. Place one drop of antigens "O", "H", "A(H)" and "B(H)" in circles 1, 2, 3 and 4 respectively and "O" antigen in circle 5 and any one of the "H" antigens (H, A (H) or B (H)) in circle 6.
4. Mix the contents of each circle with separate applicator stick and spread it to the whole area of the individual circle.
5. Rock the slide for one minute and observe for agglutination. If agglutination is visible within one minute the test is positive, proceed for quantitative slide test or tube test for the quantitative estimation of the titre of the appropriate antibody.
6. If no agglutination is observed, the test is negative.

### B. SEMI-QUANTITATIVE SLIDE TEST

1. Clean the glass slide supplied in the kit well and wipe it free of moisture.
2. Place 0.005 mL, 0.01 mL, 0.02 mL, 0.04 mL, and 0.08 mL of undiluted serum in 1st, 2nd, 3rd, 4th and 5th circles respectively on the slide.
3. Add one drop of the appropriate antigen suspension which showed agglutination in screening slide test, to each of the above circles.
4. Mix the contents of each circle with separate applicator sticks.
5. Rotate the slide slowly for one minute and observe for agglutination.
6. The titre of the antibody is the highest dilution of serum upto which there is clear agglutination.
7. Repeat steps 1 to 6 with all the antigens, which showed agglutination in screening slide test.

The serum volumes in the quantitative slide test correspond approximately to the tube test titres as follows:

| Serum Volume | Approximate Tube Test Titre |
|---|---|
| 0.08 mL | 1 : 20 |
| 0.04 mL | 1 : 40 |
| 0.02 mL | 1 : 80 |
| 0.01 mL | 1 : 160 |
| 0.005 mL | 1 : 320 |

## C. QUANTITATIVE TUBE (WIDAL) TEST

1. Take a set of 8 clean, dry, 10 X 75 mm test tubes for each serum under test.
2. Dilute each serum sample and set up the test as follows.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| **Dilution** | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | Saline Control |
| Normal Saline, in mL | 1.9 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Patient's serum (undiluted) 0.1 mL | 0.1 | mix | mix | mix | mix | mix | mix | – |
| Transfer diluted serum, 1.0 mL | – | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | – |
| Appropriate Antigen, drop | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

Discard 1.0 mL

3. Mix well and incubate all tubes at 37 °C for 16-20 hours and examine for agglutination.
4. Alternatively, incubate "O" antigen at 50 °C for 4 hours, and all the "H" antigens at 50 °C for 2 hours, and take the reading.
5. The antibody titre is the highest dilution of serum showing distinct agglutination.

## INTERPRETATION OF RESULTS

1. Reading should be taken at least 30 minutes - 1 hour after removing the assay tubes from the incubator. "O" antigen shows disc like pattern whereas "H" antigen show the characteristic floccular appearance. In negative reactions and in saline control, the appearance of suspension remains unchanged with a minute button of deposit at the bottom of the tube which shows a typical swirl when the tube is flicked.
2. In addition to pattern of sedimented organisms, reduction in opacity of the supernatant as compared to saline control tube must be observed and considered to measure the degree of agglutination.
3. Agglutinin titer greater than 1:80 is considered significant and suggests infection, whereas low titers are found in normal individuals.
4. There should be a four fold rise in titre between two serum samples collected at the acute phase and the convalescent phase.
5. Other clinical features like "Rose spots", stepladder pattern of fever, relative Bradycardia and Leucopenia should also be taken into consideration while interpreting the result.
6. The past history such as previous attack of enteric fever or inoculation of TAB vaccine should be kept in mind while interpreting the result. In an inoculated person, the "H" titre should not be taken into account for diagnosis unless there is a rising titre of "H" antibody between two samples.

## QUALITY CONTROL

*Positive control serum, negative control serum* and saline should be used in parallel with the unknown test serum, to assure that the antigens used in the test are sensitive as well as specific and also to show the results that are to be expected in positive and negative samples.

## LIMITATIONS

1. Diagnostic titre is observed 7-10 days after the onset of fever. In first week of infection test is negative.[1]
2. High antibody concentration gives false negative result in the rapid slide test due to Prozone Phenomenon.[2]

## INTERFERENCE

1. Individuals vaccinated with Typhoid vaccine (TAB) may show moderately elevated titre of all three "H" antibodies.[1,3]
2. Repeated subclinical infection may give high titres due to previous antibodies.[1]
3. Treatment with antibiotic such as Chloramphenicol before the test gives false negative result for "O" agglutinins. In that case diagnosis should be based on the significant elevation of "H" agglutinins in the paired sera.[1]
4. Patients of chronic active Liver disease may give high titres due to failure of antigens in discriminating the specific antibodies from the Dysglobulinaemia of chronic active Liver disease.[1,4]
5. Infection with many non-Salmonella organisms like Malaria, Dengue, Miliary Tuberculosis, Endocarditis, Brucellosis, Influenza etc. may give anamnestic response.[3,5]
6. Potential carriers of the disease exhibit negative results due to high antibody concentration.[3]
7. Immunological disorders such as Rheumatoid Arthritis, Rheumatic Fever or Nephritic Syndrome demonstrate high titre of "O" and "H" agglutinin.[1]
8. Narcotic addicts demonstrate non-specific activity to the Widal test.
9. Vi antigen may block the "O" antigen from agglutinating with "O" antibody.
10. In endemic areas, people usually show moderately elevated level of "O" and "H" agglutinins.

## PERFORMANCE CHARACTERISTICS

Consistent sensitivity and specificity was observed with monospecific as well as polyspecific antisera for all the four antigens. Cross reaction was not observed amongst the antigens.

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## SYMBOL LEGENDS

| Symbol | Explanation of Symbol | Symbol | Explanation of Symbol |
|---|---|---|---|
| | Consult instructions for use | LOT | Batch code No. |
| IVD | In vitro diagnostic device | | Manufacturer |
| +2 °C +8 °C | Store at +2 °C to +8 °C | | Date of manufacture |
| | Keep away from sunlight | | Use by (date or month of expiry) |
| | Contains sufficient for < n > tests | | Do not use if package is damaged |
| STERILE A | Sterile A (sterilized using aseptic processing techniques) | EC REP | Authorized Representative |
| REF | Catalogue number | CE | The product meets all the legal requirements for CE marking as per directive 98/79/EC |

### LIMITED EXPRESSED WARRANTY DISCLOSURE

ARKRAY Healthcare Pvt. Ltd. (ARKRAY) limits the warranty to the test kit in as much as the said test kit will function only within the limitations and specifications as described and illustrated in the product insert. Any deviation there from by the purchaser or the end user shall not be the liability and/or responsibility of ARKRAY. ARKRAY shall not be liable and/or responsible for any misuse of the said test kit after the date of expiry. If any defect is proved in the manufacture of the test kit, ARKRAY shall be liable only to the extent of the replacement of the said test kit or the refund of its purchase price thereof and shall not be liable for any consequential loss arising there from.


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# RPR (Rapid Plasma Reagin) Test Kit

REF : 54FL200-50, 54FL200-10, 54FL200-60
Σ : 50, 10 mL, 100,

IVD +2 °C +8 °C STERILE A

## INTENDED USE

RPR (Rapid Plasma Reagin) test kit (18 mm circle card test) is an *in vitro* qualitative and semi quantitative test to diagnose Syphilis using human serum / plasma.

## INTRODUCTION

Syphilis is a curable venereal disease caused by the spirochete *Treponema pallidum*. *Treponema pallidum* is a fragile spiral bacterium, measuring 6-15 µm in length and 0.1-0.2 µm in diameter[1]. It is an obligatory human parasite and cannot survive outside human body. Hence its transmission is only possible by direct inoculation from one person to the other and therefore gets transmitted by sexual contact, transplacental route and infected blood transfusion[2]. The disease evolves through primary, secondary and tertiary stages. Tertiary manifestation of Syphilis may occur from one to thirty years after primary infection. It includes the development of gummatous lesions, Cardiovascular Syphilis and Neurological Syphilis. Latent Syphilis is a stage when patient shows positive serological test for Syphilis but does not show any sign and symptom for the same.

*Treponema pallidum* cannot be cultured *in vitro*[1] and therefore the diagnosis is dependent on clinical signs, direct observation of bacteria by microscopy from lesion and serology. Serological tests include specific and non-specific tests. Specific tests like *Treponema pallidum* haemagglutination assay (TPHA) and Fluorescent Treponemal Antibody Absorption (FTA-ABS) test detect specific anti-treponemal antibodies but are expensive and usually give positive results throughout the life even after successful treatment of a patient[3,4]. Non-specific tests detect non treponemal anti-lipoidal antibodies (Reagin / Wassermann antibody). Eventhough they show false positivity in some patients suffering from acute and chronic diseases causing tissue damage, they are of great value for rapid screening of the disease and assessing the effectiveness of therapy, as reagins disappear after successful treatment of Syphilis. Four fold decrease in titer in early Syphilis usually indicate adequate Syphilis therapy[5].

RPR (Rapid Plasma Reagin) test kit is a non treponemal test that detects "Reagin" (IgG and IgM) produced against the lipoidal material released by damaged host cells as well as to lipoprotein like material and possibly cardiolipin released from treponemas. This test uses a non treponemal antigen containing cardiolipin which has been modified by addition of choline chloride, ethylenediaminetetraacetate (EDTA) and charcoal. Choline chloride inactivates inhibitors and thereby eliminating need to heat inactivate the sample, EDTA enhancing the stability of the suspension and charcoal ease up the visualisation of the clumps.

## PRINCIPLE

The RPR reagent containing modified VDRL antigen and microparticulate carbon particles, flocculate when mixed with sample containing "reagin". A reactive specimen is indicated by macroscopically visible black clumps against white background, in contrast non reactive specimen appear to have smooth uniform light gray colour. This test is a screening test and if positive, further testing with specific confirmatory tests are required.

## KIT CONTENTS AND DESCRIPTION

### REAGENTS

| Reagent No. | Reagent Name | Content |
|---|---|---|
| Reagent 1 | RPR Antigen Suspension | Modified VDRL antigen with preservative |
| Reagent 2 | Positive Control Serum | Synthetic VDRL Positive Control serum |
| Reagent 3 | Negative Control Serum | Inactivated serum non reactive to VDRL antigen with preservative |

### ACCESSORIES (*QS-Quantity Sufficient)

Disposable Plastic Cards
Disposable Plastic Droppers
Disposable Applicator Sticks
Antigen Delivery Dropper
Rubber Teats

**Note : Negative Control and Synthetic Positive Control sera are free from anti-HIV, anti-HCV and HBsAg ( when tested by FDA approved procedures). Neverthless they must be handled with due care. Controls provided are sufficient for 4 Tests.**

## REAGENT STORAGE & STABILITY

All the reagent should be stored at +2 °C to +8 °C. **Do not freeze.** After opening the kit it is recommended to store the test cards at room temperature (+15 °C to +30 °C). Protect the kit from direct sunlight and elevated temperature.

## MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

1. High Intensity Incandescent Lamp (Optional)
2. Mechanical Rotator, fixed speed or adjustable to 100 ± 2 rpm, circumscribing a circle 3 / 4 inch in diameter in horizontal plane with humidifying cover
3. Micropipette and appropriate disposable tips to deliver 50 µL volume

## BIOSAFETY

1. Handle all the samples with care, as they can be potentially infectious.
2. Wear disposable gloves throughout the test procedure and dispose them off as biohazard waste.
3. Wear protective laboratory clothings in laboratory areas.
4. Do not smoke, eat or drink in area where samples are being handled.
5. Technicians with wound, cut or skin abrasions on the hand must refrain from performing the test without proper precautions.
6. Antigen and serum controls containing 0.1% sodium azide (preservative) may prove toxic on ingestion. Sodium azide reacts with lead & copper plumbing and forms highly explosive salts. Flush with large volumes of water for disposal.
7. Avoid spilling of samples or solutions containing samples. In case of spillage, immediately clean it with 1:10 dilution of 5% freshly prepared sodium hypochlorite and dispose off the cleaning material by a suitable method.
8. Remnants of samples and used test cards should be collected in a waste container. Discard them as biohazard waste in a suitable container. The containers should be finally incinerated or autoclaved at 121 °C for 1 hour.
9. Any accessories which come in direct contact with specimen, and used test cards should be considered as contaminated product and should be treated appropriately.
10. Wash hands thoroughly with disinfectant after completion of the test.

## SPECIMEN COLLECTION AND HANDLING

| Specimen | Storage at | Stable for | Remarks |
|---|---|---|---|
| Serum (heated or unheated) / Plasma (unheated) | +2 °C to +8 °C | 72 hours | Grossly haemolysed, contaminated or lipemic samples should not be used.<br>Samples containing clots or debris should be centrifuged prior use.<br>Centrifuge hazy samples and use the clear supernatant for testing.<br>The test shows best result with freshly collected sample, but perform well with frozen sample, if it is not repeatedly frozen and thawed<br>Frozen specimens should be thawed and well mixed before Testing<br>Heat inactivated sera may be used (56 °C for 30 minutes) however excessive time or temperature may cause non specific background activity. |
| | -20 °C | Long term | |

## PRECAUTIONS

1. For every new kit, check the intactness of the reagent bottle, do not use the reagents if they are opened or leaked.
2. Do not use reagents after expiry date.
3. Do not use the reagents from different lots of kits.
4. Do not use the reagents, if they appear turbid or discoloured.
5. Bring all the reagents and samples to room temperature (+15 °C to +30 °C)before starting the procedure. If the temperature of reagents / sample or testing area are less than 23 °C test activity decrease and at temperature greater than 29 °C the test reactivity increases.
6. After completing the whole day's work, clean reagent dropper (provided for dispensing the carbon antigen) with distilled water and air dry, do not wipe the needle.
7. Ensure thorough air drying after use to avoid contamination of reagent during subsequent use.
8. Avoid contact of test circles (finger touch) as the oily deposits formed thereof may invalidate the test results. Also avoid touching the dropper tip to the reagent / serum on the card.
9. If the moistened humidifying cover is not used while rotating, proper humidity will not be maintained and the test component may dry on the card giving rise to false reactive results.
10. Too fast or too slow speed of mechanical rotator may produce unpredictable test results due to improper antigen antibody interaction.
11. Rotation of the reaction mixture for longer than recommended time increases the test reactivity where as too short rotation period decrease the test reactivity.
12. Tilt the card gently by hand after removal from the rotator. Vigorous movements can hamper flocculation.
13. Read the test results immediately (after card rotation) under a high intensity lamp or strong day light.
14. While interpreting the results, if the light produces glare on the card may obscured the reaction.

## PROCEDURE

### QUALITATIVE TEST

1. Using disposable serum dropper, place one free falling drop of serum / plasma or control on the card and spread it to fill the entire circle with the help of disposable applicator stick.
   **Note: Unevenly spread serum in the circle results into improper mixing of the antigen and antibody.**
2. Gently shake the RPR Antigen Suspension bottle to resuspend the particles. From the dropper dispense few drops of the reagent back into the reagent bottle it self to remove air trapped in to the dropper.
3. Add one free falling drop of RPR Antigen Suspension using antigen delivery dropper. Keep the delivery dropper vertical, exactly at 90° while adding antigen suspension. Do not mix.
4. Place the card on the mechanical rotator under humidifying cover and rotate the card for 8 minutes at 100 ± 2 rpm.
5. After 8 minutes, immediately remove the card from the rotator; rotate and tilt the card gently by hand (three-four, to and fro motions).
6. Observe the result under a high intensity lamp or strong day light.

Perform the quantitative test for specimens exhibiting any degree of flocculation or "roughness".

### SEMI QUANTITATIVE TEST

1. Place 50 µL of 0.9% saline solution in 2nd, 3rd, 4th and 5th circles of the card by using micropipette. Do not spread the saline solution.
2. Using micropipette, add 50 µL sample to saline in 1st and 2nd circle.
3. Mix sample with saline in 2nd circle by drawing the mixture up and down for 8 times in the micropipette. Avoid bubble formation.
4. Aspirate 50 µL from 2nd circle and transfer to 3rd circle. Repeat the same successively upto 5th circle.
5. Aspirate 50 µL from the 5th circle and discard it.
6. Repeat step no. **2-6 of qualitative test** for each of diluted sample drop.
7. If the highest dilution tested (1:16) is reactive, continue as follows:
   (a) Prepare a 1:50 dilution of non reactive serum in 0.9% saline to be used for making 1:32 and higher dilutions of the specimen to be tested.
   (b) Prepare a 1:16 dilution of the test specimen by adding 0.1 mL of serum to 1.5 mL of 0.9% saline. Mix thoroughly.
   (c) Place 50 µL of the 1:50 non reactive serum diluent in circles 2 to 5 of an RPR card.
   (d) Using a safety pipetting device with disposable tip, place 50 µL of the 1:16 dilution of the test specimen in circle 1 and 50 µL in circle 2.
   (e) Using the same pipette and tip, make serial twofold dilutions. Complete test as described in qualitative test procedure. Use a clean tip for each specimen tested. Prepare higher dilutions if necessary in 1:50 nonreactive serum diluent.
8. The end point is the highest dilution showing visible black clumps.

## INTERPRETATION OF RESULTS

**Read the results in wet state under a high intensity incandescent lamp, without magnification.**

### FOR QUALITATIVE TEST

**REACTIVE**

Characteristic clumping ranging from marked and intense (reactive) to slight but definite (minimally to moderate reactive) clumps against white background indicates that the sample is reactive for reagin.

**NON REACTIVE**

Absence of clumps or slight roughness indicates that the sample is non reactive for reagin.

### FOR SEMI QUANTITATIVE TEST

Report the results in terms of highest dilution that has given a reactive result including a minimally reactive result, as follows.

| Undiluted (1:1) | Serum Dilutions | | | | Report |
|---|---|---|---|---|---|
| | 1:2 | 1:4 | 1:8 | 1:16 | |
| Rm | N | N | N | N | Reactive, undiluted 1:1, or R1 |
| R | R | N | N | N | Reactive, 1:2 dilution, or R2 |
| R | R | R | N | N | Reacitve, 1:4 dilution, or R4 |
| R | R | R | Rm | N | Reactive, 1:8 dilution, or R8 |

R = reactive, Rm = minimally reactive, N = nonreactive

## LIMITATIONS

1. RPR (Rapid Plasma Reagin) test kit is a rapid screening test. A positive result must be reconfirmed with tests employing specific treponemal antigen e.g. TPHA, FTA-ABS etc. as well as other clinical findings.
2. Biological false positive reactions (BFP) can be attributed to Malaria, Hepatitis, Mumps, Leprosy, Infectious Mononucleosis, Rheumatoid Arthritis and Collagen disease. When BFPs do occur, they may be of low titer (< 1:8 dilution).
3. Presence of dust, high heat, poor light and the drying of the reaction mixture before being read also affect results (particularly when the test is performed in field conditions).
4. A prozone effect may encountered occasionally, which prevent exhibition of positivity in undiluted specimen. So a specimen should be tested for prozone phenomenon when the clinician suspects syphilis but the qualitative test is non reactive.
5. Titers of some individuals will not decrease and these individuals may remain serofast retaining a low level reactive titer for life.[5]
6. This card test cannot be used to test spinal fluids.
7. Lipemic sample does not normally interfere with the test. However severe conditions obscure the state of antigen particles.

## QUALITY CONTROL

It is vital to deliver analytical services in useful, convenient and meaningful manner as it is first and foremost step on the way to correct treatment. To ensure the reliability of the final report, it is recommended

1. to follow kit's instructions meticulously.
2. to run kit controls periodically to check test validity.
3. to run third party controls as a part of internal and external quality control programme.

## PRESENTATION

| Reagent No. | Reagent Name | REF 54FL200-50 Σ 50, | 54FL200-10 10 mL, | 54FL200-60 100 |
|---|---|---|---|---|
| Reagent 1 | RPR Antigen Suspension | 1 X 1.6 mL | 1 X 10 mL | 1 X 3.2 mL |
| Reagent 2 | Positive Control Serum | 1 X 0.25 mL | 1 X 0.25 mL | 1 X 0.25 mL |
| Reagent 3 | Negative Control Serum | 1 X 0.25 mL | 1 X 0.25 mL | 1 X 0.25 mL |
| Accessories : (*QS-Quantity Sufficient) | | | | |
| Disposable Plastic Cards | | 5 | 40 | 10 |
| Disposable Plastic Droppers | | 50 | 400 | 100 |
| Disposable Applicator Sticks | | 50 | 400 | 100 |
| Antigen Delivery Dropper | | 1 | 1 | 1 |
| Rubber Teats | | 2 | 2 | 2 |

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3. SI Egglestone and AJL Turner, Serological Diagnosis of Syphills, *Communicable disease and Public health* (2000) **3**:158-162.
4. S.A. Larsen, B.M. Steiner and A.H. Rudolph, Laboratory diagnosis and interpretation of Test for Syphilis, *Clinical Microbiology Reviews* (1995) 8: 1-21.
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## SYMBOL LEGENDS

| Symbol | Explanation of Symbol | Symbol | Explanation of Symbol |
|---|---|---|---|
| | Consult instructions for use | Σ | Contains sufficient for < n > tests |
| IVD | *In vitro* diagnostic device | REF | Catalogue number |
| | Do not use if package is damaged | LOT | Batch code No. |
| +2 °C +8 °C | Store at +2 °C to +8 °C | | Manufacturer |
| | Keep away from sunlight | | Date of manufacture |
| | Keep dry | | Use by (date or month of expiry) |
| | Do not reuse | EC REP | Authorized Representative |
| STERILE A | Sterile A (sterilized using aseptic processing techniques) | CE | The product meets all the legal requirements for CE marking as per directive 98/79/EC |

## ACKNOWLEDGEMENT


RPR (Rapid Plasma Reagin) test kit has been manufactured using VDRL antigen licensed under Patent License Agreement with the Centers for Disease Control and Prevention, Atlanta, USA.


## LIMITED EXPRESSED WARRANTY DISCLOSURE

ARKRAY Healthcare Pvt. Ltd. (ARKRAY) limits the warranty to the test kit in as much as the said test kit will function only within the limitations and specifications as described and illustrated in the product insert. Any deviation there from by the purchaser or the end user shall not be the liability and/or responsibility of ARKRAY. ARKRAY shall not be liable and/or responsible for any misuse of the said test kit after the date of expiry. If any defect is proved in the manufacture of the test kit, ARKRAY shall be liable only to the extent of the replacement of the said test kit or the refund of its purchase price thereof and shall not be liable for any consequential loss arising there from.


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