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 μl) 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 :

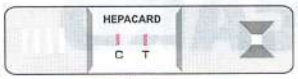

Fig. 1

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 :

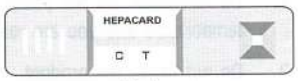

Fig. 3

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.

in vitro diagnostic reagent, not for medicinal use

R-05

Manufactured & Marketed By:

DIAGNOSTIC ENTERPRISES

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MN/HPC/003

HCV TRI-DOT

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

HCV Antigens for CORE, NS3, NS4 & NS5

1. INTENDED USE

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

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

2. INTRODUCTION

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

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

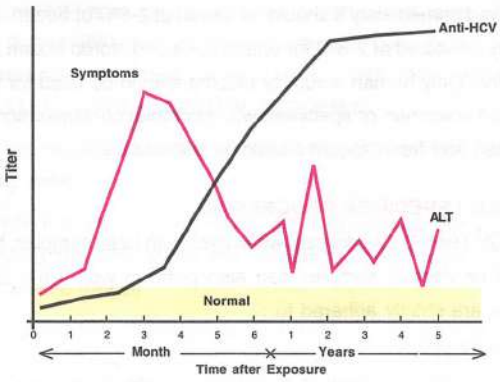


Fig.1 Hepatitis C Virus Infection
Typical Serologic Course

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

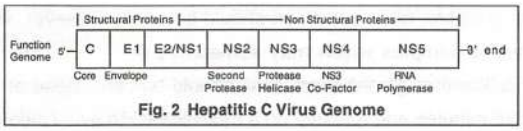


Fig. 2 Hepatitis C Virus Genome

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

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

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

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

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

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

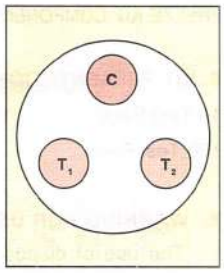


Fig. 3 Test Device

3. PRINCIPLE OF THE ASSAY

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

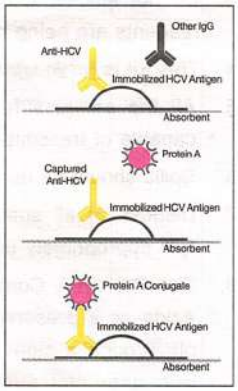


Fig. 4 Principle of the Assay

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

4. KIT COMPONENTS

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

5. STORAGE OF THE KIT

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

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

6. KIT PRESENTATION

10 Test Pack 50 Test Pack
100 Test Pack

7. WARNING FOR USERS

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:

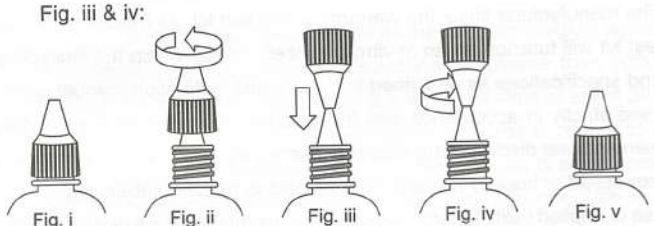


Fig. i — close vial provided in the kit
Fig. ii — open the cap
Fig. iii — invert the cap & press on the closed nozzle
Fig. iv — give a half turn twist to puncture the nozzle
Fig. v — screw the cap tightly after use

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.

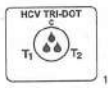
   However drops of each solution should be added in continuous stream to wet the entire area of membrane.
5. If the solution does not soak-in within 40-60 seconds; observe the sample for any suspended particulate matter. If it is present, centrifuge the sample at 10,000 r.p.m. for 15 min. and use a fresh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

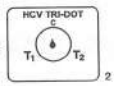
13. TEST PROCEDURE

Step No. 1
Add 3 drops of Buffer Solution to the centre of the device.



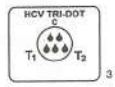
1

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).



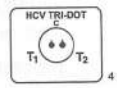
2

Step No. 3
Add 5 drops of Buffer Solution.



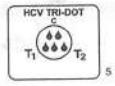
3

Step No. 4
Add 2 drops of Protein- A Conjugate.



4

Step No. 5
Add 5 drops of Buffer Solution.



5

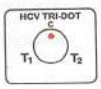
Step No. 6
Read result immediately and discard the device immediately considering it to be potentially infectious.

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

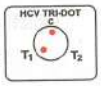
1. Appearance of only one dot at the control region **"C"** indicates that the sample is NON-REACTIVE for antibodies to HCV. (Fig:a)



a

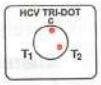
REACTIVE RESULT

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)



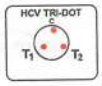
b

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)



c

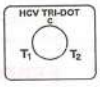
3. Appearance of all the three dots, one each at **"C" "T_1"& "T_2"** region indicates that the specimen is REACTIVE for antibodies to HCV. (Fig:d)



d

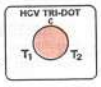
INVALID RESULT

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).



e

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



f

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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For *in vitro* diagnostic use only, not for medicinal use

R-07

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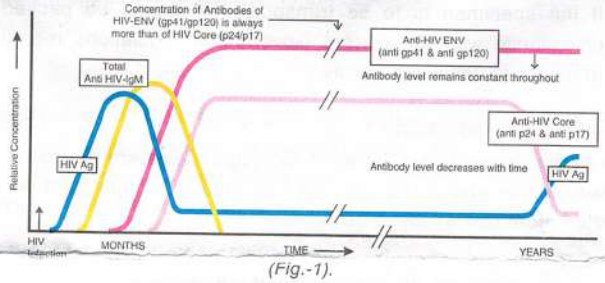
MN/HCD/002

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

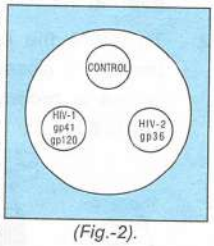
I. HISTORICAL REVIEW AND AETIOLOGY OF AIDS (Acquired Immuno Deficiency Syndrome)

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

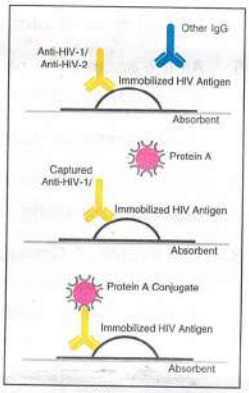
The HIV TRI-DOT Test is a visual, rapid, sensitive and accurate immunoassay for the differential detection of HIV-1 & HIV-2 antibodies (IgM, IgG & IgA) in Human Serum or Plasma using HIV-1 & HIV-2 Antigens immobilized on an immunofiltration membrane. The test is a screening test for anti-HIV-1 & anti-HIV-2 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 15 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

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

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:

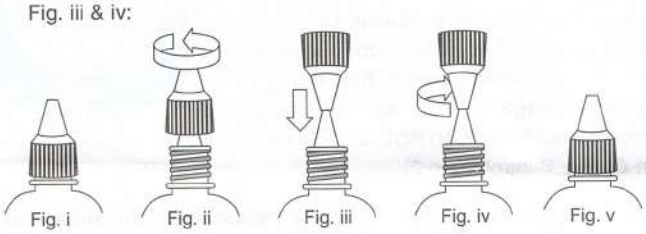


Fig. i Fig. ii Fig. iii Fig. iv Fig. v

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.

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 tested on a new device.

(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

1. Ajaka Z.L. et.al. (1994). HIV-1/HIV-2 seronegativity in HIV-1 subtype O infected patients. The Lancet., 343, 1393-1394.
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R-06

in vitro diagnostic reagent, not for medicinal use

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DIAGNOSTIC ENTERPRISES

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MNHTD/001

ASPEN® Syphilis

Syphilis Rapid Test Strip (Serum/Plasma/WB) Package Insert

A rapid test for the diagnosis of Syphilis to detect antibodies (IgG and IgM) to Treponema Pallidum (TP) qualitatively in serum/plasma/whole blood. For professional in vitro diagnostic use only.

INTENDED USE

The Aspen Syphilis Rapid Test strip (Serum/Plasma/Whole blood) is a rapid chromatographic immunoassay for the qualitative detection of antibodies (IgG and IgM) to Treponema Pallidum (TP) in serum, plasma or whole blood to aid in the diagnosis of Syphilis.

SUMMARY

Treponema Pallidum (TP) is the causative agent of the venereal disease Syphilis. TP is a spirochete bacterium with an outer envelope and a cytoplasmic membrane.[1] Relatively little is known about the organism in comparison with other bacterial pathogens. According to the Center for Disease Control (CDC), the number of cases of Syphilis infection has markedly increased since 1985.[2] Some key factors that have contributed to this rise include the crack cocaine epidemic and the high incidence of prostitution among drug users. One study reported a substantial epidemiological correlation between the acquisition and transmission of the HIV virus and Syphilis.

Multiple clinical stages and long periods of latent, asymptomatic infection are characteristic of Syphilis. Primary Syphilis is defined by the presence of a chancre at the site of inoculation. The antibodies response to the TP bacterium can be detected within 4 to 7 days after the chancre appears. The infection remains detectable until the patient receives adequate treatment.[3]

The Syphilis Rapid Test strip (Serum / Plasma / whole blood) utilizes a double antigen combination of a Syphilis antigen coated particle and Syphilis antigen immobilized on membrane to detect TP antibodies (IgG and IgM) qualitatively and selectively in serum / plasma / whole blood.

PRINCIPLE

The Aspen Syphilis Rapid Test strip (Serum /Plasma /Whole blood) is a qualitative membrane based immunoassay for the detection of TP antibodies (IgG and IgM) in whole blood, serum or plasma. In this test procedure, recombinant Syphilis antigen is immobilized in the test line region of the test. After specimen is added to the specimen well of the test strip, it reacts with Syphilis antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized Syphilis antigen. The double antigen test format can detect both IgG and IgM in specimens. If the specimen contains TP antibodies, a colored line will appear in the test line region, indicating a positive result. If the specimen does not contain TP antibodies, a colored line will not appear in this region, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENT

The test contains Syphilis antigen coated particles and Syphilis antigen coated on the membrane.

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date. Do not use test if pouch is damaged.
- Do not eat, drink or smoke in the area where the specimens or test strips are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing, disposable gloves and eye protection when specimens are assayed.
- The used tests, specimens and potentially contaminated materials should be discarded according to the local regulations.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

The test is stable through the expiration date printed on the sealed pouch 2-30°C. DO NOT FREEZE. Do not use after the expiration date.

SPECIMEN COLLECTION AND PREPARATION

- The Aspen Syphilis rapid test strip can be performed using Serum / Plasma / Whole blood.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimen at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.
- Bring specimen to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimen should not be frozen and thawed repeatedly.

MATERIAL PROVIDED

- Test strips
- Droppers
- Strip support
- Buffer
- Package insert

Material required but not provided

Specimen Collection containers, Centrifuge, Timer, Test tubes

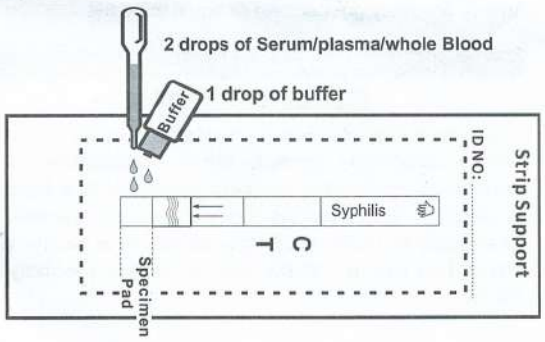
DIRECTIONS FOR USE

Note: Bring the test device, specimen and buffer to the room temperature if stored at 2-8°C.

Remove the strips from the sealed pouch.

- Peel off the tape from the strip support and stick the test strip in middle of the strip support as shown in below picture.

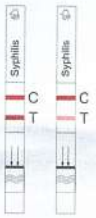
1. Add **2 drops** (50µl) of **Serum/ Plasma / Whole blood** to the specimen pad of the test strip using dropper/ pipette.
2. Add **1 drop** of **buffer** (40µl). Read result at **10 minutes**. (Do not interpret the result after 30 minutes).



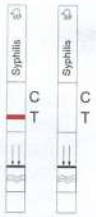
INTERPRETATION OF RESULTS

NEGATIVE : Pink/Purple line at **C** only

POSITIVE : Pink/Purple lines at **C & T**



INVALID : If control line does not appear, the test is invalid. In this case, please repeat the test using another device following the test procedure correctly.



QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms adequate membrane wicking.

LIMITATIONS

1. The Aspen Syphilis Rapid Test strip (Serum /Plasma /Whole blood) is for in vitro diagnostic use only. The test should be used for the detection of TP antibodies in serum, plasma or whole blood specimens only. Neither the quantitative value nor the rate of increase in TP antibodies can be determined by this qualitative test.
2. The Aspen Syphilis Rapid Test strip (Serum /Plasma /Whole blood) will only indicate the presence of TP antibodies in the specimen and should not be used as the sole criteria for the diagnosis of TP infection.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of TP infection.

EXPECTED VALUES

The Aspen Syphilis Rapid Test Strip (Serum/Plasma/Whole blood) has been compared with a leading commercial TPPA Syphilis test, demonstrating an overall accuracy greater than or equal to 99.8%.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The Aspen Syphilis Rapid Test strip (Serum /Plasma /Whole blood) has correctly identified specimens of a performance panel and has been compared to a leading commercial TPPA Syphilis test using clinical specimens. The results show that the relative sensitivity of the Syphilis Rapid Test strip is >99.9% and the relative specificity is 99.7%.

Method	TPPA			Total Result
Aspen Syphilis Rapid Test Strip(serum/Plasma/WB)	Results	Positive	Negative	
	Positive	130	1	131
	Negative	0	299	299
Total Result		130	300	430

Relative sensitivity: >99.9% (95%CI*: 97.7%~100.0%);
Relative specificity: 99.7% (95%CI*: 98.2%~100.0%);
Accuracy: 99.8% (95%CI*: 98.2%~100.0%).
*Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 15 replicates of four specimens: a negative, a low positive, a medium positive and a high positive. The negative, low positive, medium positive and high positive values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 15 independent assays on the same four specimens: a negative, a low positive, a medium positive and a high positive. Three different lots of the Syphilis Rapid Test strip (Serum/Plasma/WB) have been tested over a 3-day period using negative, low positive, medium positive and high positive specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

The Aspen Syphilis Rapid Test Strip (Serum/Plasma/WB) has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb,HCV, HIV, H. Pylori, MONO, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Syphilis negative and positive specimens.

Acetaminophen: 20 mg/dL	Caffeine: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL
Ascorbic Acid: 2g/dL	Albumin: 2 g/dL
Creatin: 200 mg/dL	Hemoglobin 1.1 mg/dL
Bilirubin: 1g/dL	Oxalic Acid: 600mg/dL

None of the substances at the concentration tested interfered in the assay.

BIBLIOGRAPHY

1. Fraser CM. Complete genome sequence of Treponema Pallidum, the Syphilis spirochete, Science (1998); 281 July: 375-381.
2. Center for Disease Control. Recommendations for diagnosing and treating Syphilis in HIVinfected patients. MMWR Morb. Mortal Wkly Rep. (1988); 37: 601.
3. Johnson PC. Testing for Syphilis. Dermatologic Clinic (1994); 12 Jan: 9-17.

Number: RP5155505
Effective date: 2017-05-26

ADVANTAGE **TYPHI IgM & IgG CARD**

Rapid visual test for the differential and simultaneous detection of S. typhi IgM & IgG antibodies in Human Serum / Plasma

INTRODUCTION

Typhoid caused by Salmonella enterica, serovar Typhi remains a major healths problem. The organisms are non-capsulated, non sporulating, facultative anerobic bacilli. The outer membrane protiens (OMPs) of salmonella have been considered possible candidates for causing typhoid.

A febrile condition, typhoid fever is a bacterial infection caused by salmonella serotypes including S. typhi, S. paratyphi A, S paratyphi B and S. Sendai.

Accurate diagnosis of typhoid fever at an early stage is not only important for etiological diagnosis but to identify and treat the potential carrier and prevent acute typhoid fever outbreaks.

INTENDED USE

Advantage Typhi IgM & IgG Card is a rapid solid phase immuno-chromatographic test for the qualitative differential and simultaneous detection of salmonella typhi (S. typhi) IgM and IgG antibodies in human serum / plasma. This test is for *in vitro* diagnostic use only and is intended as an aid in the earlier diagnosis of typhoid infection and in the determination of recent and past infection.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)

Advantage Typhi IgM & IgG Card is a lateral flow immunochromatographic assay. The test uses monoclonal anti-human IgM antibody (test line M) and monoclonal anti-human IgG (test line G) immobilised on a nitrocellulose strip. The conjugate contains colloidal gold conjugated to S. typhi antigen which is prepared from culture of micro-organisms. When a specimen followed by assay buffer is added to the sample well S. Typhi specific IgM &/or IgG antibodies if present, will bind to S. Typhi antigen gold conjugate making antigen antibodies complex. This complex migrates through nitrocellulose membrane by capillary action. When the complex meets the line of the corresponding immobilized monoclonal antibody (anti-human IgM &/or anit-human IgG) the complex is trapped forming a purplish pink band which confirm a reactive test result. Absence of a coloured band in the test region indicates a non reactive test result. A red procedural control line should always develop in the test device window to indicate that the test has been performed properly.

KIT CONTENTS

a) Advantage Typhi IgM & IgG Card
b) Assay Buffer
c) Sample Dropper
d) Instruction Manual

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number		Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight
2	Single Time use only		Keep Dry

KIT PRESENTATION

25 Test pack 50 Test pack

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 test and samples should be disposed off in accordance with established safety procedures/ guidelines.
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 Sodium Hypochlorite or any other suitable disinfectant.
9. Assay Buffer contains Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts 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).
10. **Optimal test performance requires strict adherence to the test procedure described in the instuction manual.**

PRECAUTION

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 reuse test cards.
3. Do not use kit beyond the stated expiry date mentioned on the kit.
4. Do not mix components from different lot numbers.

KIT STORAGE & STABILITY

The kit should be stored at 2-30°C in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. Advantage Typhi IgM & IgG Card should not be frozen and must be protected from exposure to humidity.

SPECIMEN COLLECTION AND PREPARATION

1. Advantage Typhi IgM & IgG Card test should be performed with human serum or plasma only immediately after collection.
2. If specimens cannot be tested immediately, they should be refrigerated at 2-8°C. For storage for more than 3 days, freeze the specimen at -20°C or below.
3. Repeated freezing and thawing of the specimen should be avoided.
4. Specimens containing precipitate or particulate matter may yield inconsistent test results. Such specimens must be centrifuged at 10,000 rpm for 15 minutes and the clear supernatant should only be used for testing.
5. The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous results.

BEFORE YOU START

The Assay Buffer Solution provided in the kit has closed nozzle and screw cap with pin (outside). Before using Assay Buffer, keep the vial vertically straight and tap down gently on the working platform, so that Assay Buffer comes down at the bottom of the vial. To orifice/puncture the closed nozzle, follow the instruction as illustrated below:

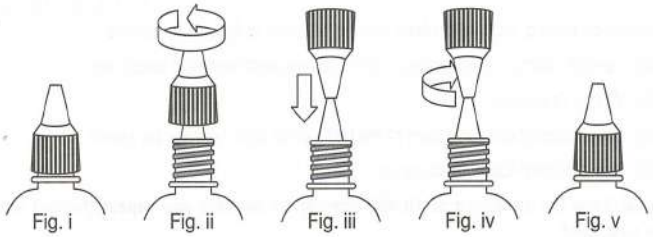


Fig. i Fig. ii Fig. iii Fig. iv Fig. v

TEST PROCEDURE

R.T. 20-30°

1. Bring the complete kit & specimen to be tested to room temperature prior to testing. Once the test card is opened, it should be used within one hour.
2. Remove the test card from the foil pouch prior to use and label the test card with patient name or identitification number.
3. Label the test card with patient's name or identification number.
4. Fill the lower circular part of the sample dropper with the specimen upto the mark provided on the dropper as shown in fig. (a-ii). Then add the specimen to the sample well of the device as shown in fig. (a). This will add 10 μl of specimen to the device. Dispose of the dropper considering it to be biohazardous.

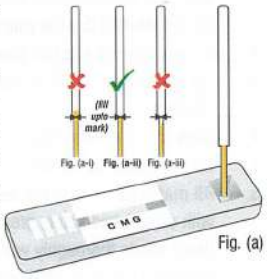


Fig. (a)

Alternatively, add 10 μl of sample using micropipette to the sample well of the antibody device.

5. Hold the Assay Buffer vial vertically and add 1 drop Assay Buffer to the well of the device as shown in fig. (b) and screw cap the vial after use.
   Note: Please ensure no air is entrapped.
6. Allow reaction to occur during the next 20 minutes.

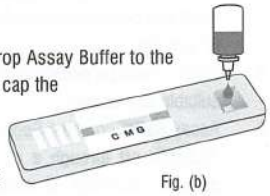


Fig. (b)

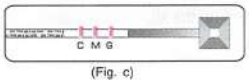
7. Read results at 20 minutes. Positive results may appear as early as 5-10 minutes. However, negative results must be confirmed only at 20 minutes.

Important Note: **Do not read result after 25 minutes.**

8. Discard the Advantage Typhi IgM & IgG Card immediately after reading result at 20 minutes, considering it to be potentially infectious.

INTERPRETATION OF THE TEST

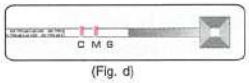
IgM & IgG REACTIVE



(Fig. c)

As shown in (Fig. c) appearance of red coloured line in the control region 'C' and Test region; IgM region 'M' and IgG region 'G' indicates that the sample is reactive for both S. typhi IgM & IgG antibodies.

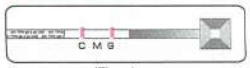
IgG REACTIVE



(Fig. d)

As shown in (Fig. d) appearance of red coloured line in the control region 'C' and Test region; IgM region 'M' indicates that the sample is reactive for S. typhi IgM antibodies.

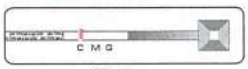
IgM REACTIVE



(Fig. e)

As shown in (Fig. e), appearance of red coloured line in the control region 'C' and Test region; IgG region 'G' indicates that the sample is reactive for S. typhi IgG antibodies.

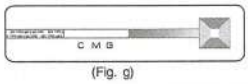
NON-REACTIVE



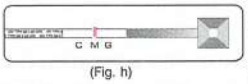
(Fig. f)

As shown in (Fig. f), appearance of one distinct red coloured line in the control region 'C' only (with no line in the IgM region 'M' & IgG region 'G') indicates that the sample is non-reactive for S. typhi antibodies.

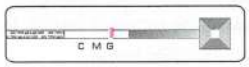
INVALID



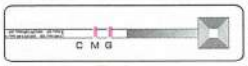
(Fig. g)



(Fig. h)



(Fig. i)



(Fig. j)

The test is invalid if no control line appear after completion of test either with clear background or pinkish background as shown in (Fig. g, h, i & j). 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 exprosure of the test device after opening the pouch.

(d) Use of turbid/ lipaemic specimen.

Centrifuge the specimen at 10,000 rpm for 15 minutes and repeat the test using a new card.

LIMITATIONS OF THE TEST

1. The test is for *in vitro* diagnostic use only.
2. This test detects the presence of S. typhi IgM & IgG antibodies to S. typhi antigen in the specimen and should not be used as the sole criteria for the diagnosis of typhiod infection.
3. In early infections and some secondary infections, detectable levels of IgM antibodies may be low. Some patients may not produce detectable levels of antibody within the first seven to ten days after infection. Where symptoms persist, patients should be re-tested 3-5 days after the first testing date.
4. High titre of Rheunetoid arthritis antibodies, SLE antibodies may show cross-reactivity.
5. As with all diagnostic tests, all results must be corelated with other clinical findings. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result at any time does not preclude the possibility of an early infection of typhiod bacteria.
6. This is only a screening test. Therefore, more specific alternative diagnosis method such as tube test and culture must be used in order to obtain a confirmation of typhiod infection.

PERFORMANCE CHARACTERISTICS

The kit has been evaluated in-house with the known panel of fresh as well as frozen S. typhi IgM & IgG antibody positive and negative samples and results are compared with licensced commercially available test. The samples included cross-reacting samples; HIV, HCV, HBV, Dengue, Chikungunya, Leptospira RA, ASO &,CRP. Following is the in-house evaluation:

Sample Type	No. of Samples tested	Result of licensed test	Advantage Typhi IgM & IgG Card
Negative for Ab to S. typhi	200	200	200
S. typhi IgM Positive	10	10	10

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

Precision: Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of four samples - two negative, two weak typhi IgM and/or IgG positive samples. The C.V. (%) of all the four samples were within 15%.

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 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. Choo KE et al., Longevity of antibody responses to a Salmonella typhi-specific outer membrane protein; interpretation of a dot enzyme immunosorbent assay in an area of high typhoid fever andemicity. Am J Trop Med Hyg. 1997 Dec; 57(6): 656-9.
2. Ismail A, Hai OK, Kader ZA. Demonstration of an antigenic protein specific for Salmonella typhi. Biochem Biophys Res Commun 1991;181(1):301-5.
3. Ivanoff BN, Levine MM, Lambert PH, Vaccination against typhoid fever: present status. Bulletin of the World Health Organization 1994; 72: 957-71.

in vitro diagnostic Reagent, not for medicinal use

VER-02 R-03

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MN/TAB/004
Rev. Date: Apr.-21

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	17SA427-05	17SA429-05
		Pack size: 4 X 5 mL	2 X 2 X 5 mL	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)
Σ	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

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CALIBRATION CERTIFICATE

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INSTRUMENT	INSTALLED ON	CALIBRATED ON
Mispa I3	**02/05/2021**	**09/11/2022**

EQUIPMENT DETAILS

Instrument name	Mispa I3
Serial No	21212211228
Environmental condition:	Temperature:
Equipment calibration	
Parameter calibration	In house (Calibrated IC card provided along with each kit)

This is to certify that the above mentioned product has been calibrated & tested for the performance of kits with Control.

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Equipment Test report

Checked the IC Card Reader	: Found Ok
Checked the Probe	: Found Ok
Checked Mother Board	: Found Ok
Checked the Wash Assembly	: Found Ok
	:
Channel-1 (with cartridge)	:
Channel-1 (without cartridge)	:
Channel-2 (with cartridge)	:
Channel-2 (without cartridge)	:
	:

Performance validation with Control

Protein Control **LOT NO:** **EXP:**

Parameter	Low	High	Result	Target
CRP				
RF				
ASO				

Next Calibration due on : 08-05-2023

For Agappe Diagnostics Ltd,

Authorized Signatory

AGAPPE DIAGNOSTICS LTD. 401 & 402, Jaising Business Centre, 119, Parshwada, Sahar Road, ANDHERI (E) MUMBAI-400069

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