# Stained Salmonella

## Antigen Set For Widal Test

IVD +2 °C +8 °C STERILE A

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

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

## 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 | – |
| Approximate 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.

## REFERENCES


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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 |

### 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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# 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 |
|---|---|---|---|
| Σ | 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.

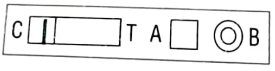
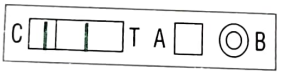
5. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
6. Handle all specimens as if potentially infectious. Follow standard biosafety guidelines for handling and disposal 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.

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## 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 |
| Σ 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

# ErbaQik

# HBsAg Test Card

*One Step HBsAg Rapid Test (Serum/Plasma)*

**Product Code: 133140 (10T) / 133156 (25T) /133157 (50T)/ 133158 (100T)**

### Intended Use

ErbaQik HBsAg test is one step *in vitro* Immunochromatographic assay designed for qualitative determination of Hepatitis B surface antigen (HBsAg) in Human serum or plasma.

### Introduction

Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease. The hepatitis B virus can survive outside the body for at least 7 days. The complex antigen found on the surface of HBV is called HBsAg. Previous designations included the Australia or Au antigen. The presence of HBsAg in serum or plasma is an indication of an active Hepatitis B infection, either acute or chronic. The incubation period of the hepatitis B virus is 120 days on average, but can vary from 45 to 160 days. Hepatitis B virus (HBV) is a global health problem. It is a major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.

### Principle of Test

The ErbaQik HBsAg test device contains, 1) A nitrocellulose membrane strip containing a test band (T) and control band (C).The T band is pre-coated with monoclonal antibody specific to HBsAg. 2) A conjugate pad containing monoclonal antibody specific to HBsAg and polyclonal antibody colloidal gold conjugate. The colloidal gold conjugate and sample moves along the membrane chromatographically to test region (T) and control region (C) and forms a visible band as the antibody-antigen-antibody gold particle complex forms.

The HBsAg test cassette has a letter of T and C as "Test band" and "Control band" on the surface of the cassette. Both the test and control band in result window are not visible before applying any sample. The control band is used for procedural control. The control band should always appear if the test procedure is performed properly and the reagents of control band are working.

### Kit Components

ErbaQik HBsAg Test Kit contains the following:

- Test device in a sealed pouch with a desiccant
- Assay Buffer
- Disposable dropper
- Instructions for use

### Kit Storage and Stability

For the reproducible results, users should be aware of the following:

1. ErbaQik HBsAg kit should be stored at room temperature (2°-30°C). Do not freeze.
2. The test device is sensitive to humidity as well as heat.
3. Perform the test immediately after removing the device from the sealed pouch.
4. Do not use the test kit if the pouch is damaged or the seal is broken.
5. Do not mix reagents of different lots.
6. Do not use the kit beyond the expiration date.
7. The shelf-life of the kit is as indicated on the outer package.
8. When transporting or storing the packages, avoid exposure to high temperature (over 30°C).

### Specimen Collection and Storage

1. **Plasma**
   Collect blood specimen into collection tube containing EDTA, Citrate or Heparin. Separate the plasma by centrifugation, 1500 rpm for 10 minutes. Carefully withdraw the plasma into a new labeled tube.
2. **Serum**
   Collect blood specimen into a collection tube containing no anticoagulants. Allow the blood to clot. Separate the serum by centrifugation, 1500 RPM for 10 minutes. Carefully withdraw the serum into a new Pre-labeled tube. Test the specimens as soon as possible after collection.
3. Stored serum/plasma specimens at 2-8°C up to 3 days can be used for testing. Serum/plasma specimens should be frozen at -20°C for storage longer than 2 weeks.

### Precautions

1. Anti-coagulants such as heparin, EDTA, and citrate do not affect the test result.
2. As known for relevant interference, haemolytic samples, rheumatoid factors containing samples and lipaemic, icteric samples can lead to impairment of the test results.
3. Use separate disposable dropper or pipette tips for each sample in order to avoid cross contamination of either samples which could cause erroneous results.

### Procedure of the Test

1. Allow the kit components and specimens to attain room temperature prior to testing.
2. Remove the test cassette from foil pouch and place it on flat dry surface.
3. With a 30 µL disposable dropper draw serum/plasma specimen and dispense one drop or with micropipette dispense 30 µL into the sample well (S).
4. Add 2 drops of Assay buffer into the well (S).Wait for 20 minutes and read the results.
5. Do not read the results after 30 minutes, as it may give false results.

### Interpretation of the Results

1. **Negative Result:** If only the control (C) band is developed, within the result window the test indicates that no HBsAg antigen is present in specimen. The result is negative for HBsAg.
2. **Positive Result:** The presence of both control band (C) and test band (T) within the result window indicates presence of HBsAg antigen in specimen. The result is positive for HBsAg.
3. **Invalid Result:** If the control (C) is not visible in the result window after performing the test, the result is considered invalid. The specimen must be tested using a new test device.

### Performance Characteristics:

ErbaQik HBsAg test has been tested using in-house panel of positive and negative clinical samples confirmed by leading commercial HBsAg ELISA and lateral flow test and the correlation between these two systems was found to be 100%.

| Status | Positive | Negative | Total |
|---|---|---|---|
| Positive | 166 | 0 | 166 |
| Negative | 0 | 560 | 560 |
| Total | | | 726 |

**Sensitivity- 100%** **Specificity- 100%**

### Limitations and Interferences

1. The HBsAg test will indicate the presence of HBsAg in the specimen (Serum/Plasma) and should not be used as sole criteria for the diagnosis of Hepatitis infection.
2. 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 serological data, should be considered. The results should be reported only after complying with above procedure
3. A negative result can occur if the quantity of the analyte of interest present in the specimen is below the detection limits the assay or the analyte of interest that are not present during the stage of disease i

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B-11, OIDC, Ringanwada, Daman - 396 210, India.

which a sample is collected.

4. The test is designed for use with serum or plasma samples only. Use of other body fluids, including urine or saliva or whole blood has not been established.
5. False positive results can obtain due to the presence of other antigens or elevated levels of RF factor. This occurs in less than 1% of the samples tested.

**Warning**

1. For *In-vitro* diagnostic use only. Do not re-use the test device.
2. The instruction must be followed exactly to get accurate results. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.
3. Do not eat or smoke while handling specimens.
4. Wear protective gloves while handling specimens. Wash hands afterwards.
5. Avoid splashing or aerosol formation.
6. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials in a biohazard container.

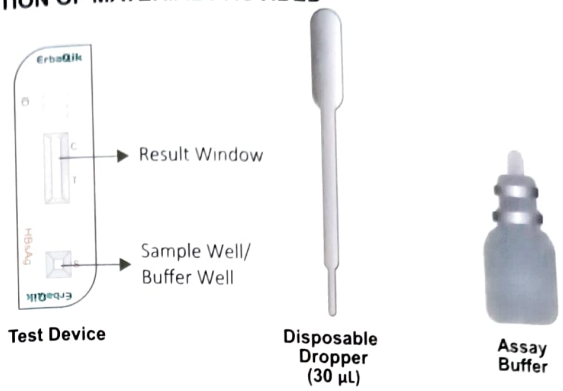
**Limited Express Warranty Disclaimer**

Transasia Bio-Medicals Ltd. Products are warranted to meet the applicable product specifications described. Notice of non-conforming products should be made to Transasia Bio-Medicals Ltd. for which liability is limited to either replacement 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. Transasia Bio-Medicals Ltd. disclaims any and all responsibility for any injury or damage or legal implications which may be caused by the fault of the user or buyer in accordance with the limitations and specifications herein. Due to continuous development, the manufacturer reserves the right to improve /change any specification /components without prior information /notice to the buyer.

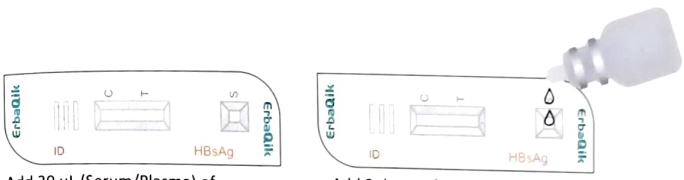
**References;**


1. Zampino, R., Boemio, A., Sagnelli, C., Alessio, L., Adinolfi, L. E., Sagnelli, E., & Coppola, N. (2015). Hepatitis B virus burden in developing countries. World journal of gastroenterology, 21(42), 11941.
2. Block, T.M; Guo, H; & Guo, J. T. (2007). Molecular virology of hepatitis B virus for clinicians. Clinics in liver disease, 11(4), 685-706.
3. Blumberg, B.; alter, H. J; & Visnich, S.(1984). A New antigen in Leukemia Sera. JAMA, 252 (2), 252-257.
4. Blumberg, B. S; Sutnick, A. I; & London' W. T. (1969). Australia antigen and hepatitis. Jama, 207 (10), 1895-1896.
5. Posthuma-Trumpie, G. A., & van Amerongen, A. (2012). Lateral flow assays. In Antibodies: Applications and New Developments (Vol. 2012, pp. 175-183). Bentham eBooks: Bussum, The Netherlands


## DESCRIPTION OF MATERIAL PROVIDED




## TEST PROCEDURE



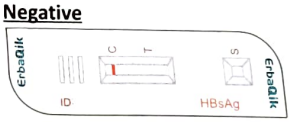

Add 30 µL (Serum/Plasma) of specimen into the sample well (S)

Add 2 drops of Assay Buffer into the well (S)


**Read the results at 20 minutes**

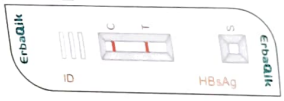
### Interpretation of the test

**Negative**



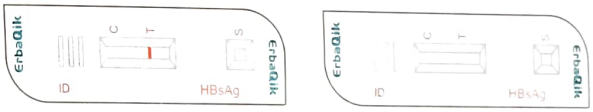

One band Control "C" in the result window

**Positive**




One band "T" and One band in "C" in the result window

**Invalid**




No Control band "C" in the result window

## KIT PRESENTATION

**Contents:**

- 10/25/50/100 Test devices packed individually, disposable droppers, Assay Buffer sufficient for provided number of test.
- 1 Instruction for use

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# ErbaQik

## HCV Test Card

*One Step HCV Ab Rapid Test*
*(Serum/Plasma/Whole Blood)*

**Product Code: 132824 (10T) / 132825 (25T) / 132826 (50T)/132827(100T)**

### Intended Use

ErbaQik HCV test card is a qualitative screening, *in-vitro* diagnostics immunochromatographic assay for detection of antibodies (IgG, IgM, IgA) specific to HCV in human serum, plasma or whole blood.

### Introduction

Hepatitis C Virus (HCV) is now recognized as a major agent of chronic hepatitis transfusion acquired non-A, non-B hepatitis and liver disease throughout the world. HCV is a positive sense single stranded RNA virus. The major immunoreactive antigens of its proteins have been reported as core, NS3, NS4 and NS5 regions of HCV genome, which are known as highly immunodominant regions.

HCV infection frequently progresses to chronic liver disease. On the basis of Phylogenetic analysis, HCV has been grouped into six major genotypes each of which contains one or more subtypes. The distribution of HCV genotypes varies in different geographical areas.

### Principle of Test

The ErbaQik HCV test card is based on a principle of double antigen sandwich immunochromatographic assay. The test card contains a membrane strip, which is pre-coated with recombinant HCV capture antigen (core, NS3, NS4 and NS5) on test band region. The HCV antigen-colloid gold conjugate and sample moves along the membrane chromatographically to test region (T) and forms a visible band as the antigen-antibody-antigen gold particle complex forms. The development of a colored band in the test region indicates the presence of antibodies to HCV in the specimen. The unreacted gold Conjugate and unbound complex move further on membrane and are subsequently immobilized by the control reagent coated on the membrane at the control region(C), forming a colored band. Control band is used for procedural control and should always appear if the procedure is performed correctly.

### Kit Components

ErbaQik HCV Test Kit contains the following:

- A test device in a sealed pouch with a desiccant
- Assay Buffer
- Disposable dropper
- Instructions for use

### Kit Storage and Stability

For the reproducible results, users should be aware of the following:

1. ErbaQik HCV should be stored at 2-30°C. Do not freeze.
2. The test device is sensitive to humidity as well as heat.
3. Perform the test immediately after removing the device from the sealed pouch.
4. Do not use the test kit if the pouch is damaged or the seal is broken
5. Do not mix reagents of different lots.
6. Do not use the kit beyond the expiration date.
7. The shelf-life of the kit is as indicated on the outer package.
8. When transporting or storing the kits, avoid exposure to high temperature (over 30°C).

### Specimen Collection and Storage

1. **Whole Blood**
   Collect the whole blood into the collection tube (containing EDTA, citrate or heparin) by venipuncture.
2. **Plasma**
   Collect blood specimen into collection tube containing EDTA, Citrate or Heparin. Separate the plasma by centrifugation, 1500 RPM for 10 minutes. Carefully withdraw the plasma into a new pre labeled tube.
3. **Serum**
   Collect blood specimen into a collection tube containing no anticoagulants. Allow the blood to clot. Separate the serum by centrifugation; 1500 RPM for 10 minutes. Carefully withdraw the serum into a new Pre-labeled tube. Test the specimens as soon as possible after collection.
4. Stored serum/plasma/ whole blood specimens at 2-8°C up to 3 days can be used for testing. Serum/plasma specimens should be frozen at -20°C for storage longer than 2 weeks.

### Precautions

1. Anti-coagulants such as heparin, EDTA, and citrate do not affect the test result.
2. As known for relevant interference, haemolytic samples, rheumatoid factors containing samples and lipaemic, icteric samples can lead to impairment of the test results.
3. Use separate disposable dropper or pipette tips for each sample in order to avoid cross contamination of either samples which could cause erroneous results.

### Procedure of the Test

1. Allow the kit components and specimens to attain room temperature prior to testing.
2. Remove the test device from foil pouch and place it on flat dry surface.
3. With a 10 µL disposable dropper draw **serum/plasma** specimen and dispense **one drop** or with micropipette dispense 10 µL into well(S).
   OR
   With a 10 µL disposable dropper draw **whole blood** specimen and dispense **2 drops** or with micropipette dispense 20 µL into well (S)
4. Add 3 drops of Assay buffer into the well (S).
5. Wait for 20 minutes and read the results. Do not read the results after 30 minutes.

### Interpretation of the Results

1. **Negative Result:** If only the control(C) band is developed, the test indicates that no detectable antibodies to Hepatitis C virus are present in the specimen. The result is non- reactive for Anti-HCV.
2. **Positive Result:** If the control(C), and HCV test bands (T) are developed the test indicates for the presence of antibodies to HCV in the specimen. the results is reactive for Anti-HCV.
3. **Invalid Result:** If the control band "C" is not visible in the result window after performing the test, the result is considered invalid. The specimen must be tested using a new test device.

### Performance Characteristics:

ErbaQik HCV test has been tested using in-house panel of positive and negative clinical samples confirmed by leading commercial anti HCV ELISA and Lateral Flow test and the correlation between these two systems was found to be 100%.

| Status | Positive | Negative | Total |
|---|---|---|---|
| Positive | 135 | 0 | 135 |
| Negative | 0 | 627 | 627 |
| **Total** | | | 762 |

**Sensitivity- 100%** **Specificity- 100%**

### Limitations and Interferences

1. This is only screening test to detect the presence of antibodies against HCV. All specimens detected reactive must be cross checked by using other techniques like ELISA, PCR, Western Blot
2. A definitive clinical diagnosis should not be based on the single test but should only be made by the physician after all clinical and laboratory



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findings have been evaluated.

3. The test is designed for use with serum or plasma or whole blood samples only. Use of other body fluids, including urine or saliva has not been established.
4. A negative result can occur if the quantity of the analyte of interest present in the specimen is below the detection limits of the assay or the analyte of interest that are detected are not present during the stage of disease in which a sample is collected.

## Warning

1. For *In-vitro* diagnostic use only. Do not re-use the test device.
2. The instruction must be followed exactly to get accurate results. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.
3. Do not eat or smoke while handling specimens.
4. Wear protective gloves while handling specimens. Wash hands afterwards.
5. Avoid splashing or aerosol formation.
6. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials in a biohazard container.
7. Clean up spills thoroughly using an appropriate disinfection.

## Limited Express Warranty Disclaimer

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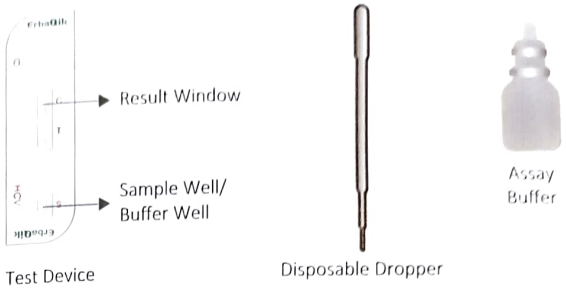
## *References;*


1. Grakoui, A. R. A. S. H., et al. "Expression and identification of hepatitis C virus polyprotein cleavage products." Journal of virology 67.3 (1993): 1385-1395
2. Cho, Young Gyu, et al. "Cloning and over expression of the highly immunogenic region of HCV genome from Korean patients." Mol Cells 3 (1993): 407-416.
3. Neville, J. A., et al. "Antigenic variation of core, NS3, and NS5 proteins among genotypes of hepatitis C virus." Journal of clinical microbiology 35.12 (1997): 3062-3070.
4. Gordon, Julian, and Charles SG Pugh. "Lateral flowcromatographic binding assay device." U.S. Patent No.4,956,302. 11 Sep. 1990.
5. Yoshikawa, A., et al. "Serodiagnosis of hepatitis C virus infection by ELISA for antibodies against the putative core protein (p20c) expressed in Escherichia coli." Journal of immunological methods 148.1-2 (1992): 143-150.


## Description of Material provided




Test Device

Disposable Dropper

## Test Procedure

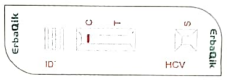

Add 10 µL (serum/plasma) or
20 µL whole blood specimen to the sample well (S)

Add 3 drops of assay buffer
to well (S)

**00:20:00** Read the results at 20 Minutes

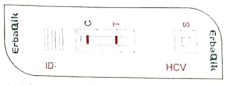
## Interpretation of the test

**Negative**



Only Control (C) band is developed

**Positive**



Both Control(C) and HCV test (T) bands are developed

**Invalid**

No control band in the result window

## Kit Presentation

Contents:

- 10/25/50/100 Test devices packed individually, disposable dropper, Assay buffer sufficient for the provided number of tests.
- 1 Instruction for Use

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Issue : 01, 06/05/2019