CE

ENTEROSCREEN-WB™

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

DEVICE

INTENDED USE

ENTEROSCREEN-WB™ is a rapid, self performing, qualitative, sandwich immunoassay for the detection and differentiation of IgM and IgG antibodies to *S. typhi* in human serum/plasma or whole blood specimen.

SUMMARY

Typhoid fever is a systemic prolonged febrile illness caused by a bacteria *Salmonella typhi*. The disease is transmitted through ingestion of food or water contaminated with faeces or urine of infected persons.

Acute typhoid fever is characterized by prolonged fever, disturbances of bowel functions (constipation or diarrhoea), headache, malaise and anorexia. Cough is common in the early stage of illness. Chronic carrier is determined when excretion of *S.typhi* in stools or urine lasts for longer than one year after onset of acute typhoid fever. 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. Specific agglutinins appear during the course of most of the attacks during the second week of infection. Detectable levels of IgM antibodies against *S.typhi* appear and persists for four months, IgG antibodies are detected thereafter and remain in blood for two years. The detection of IgM reveals acute typhoid in the early phase of infection, while the detection of both IgG and IgM suggests acute typhoid in the middle phase of infection. In areas of high endemicity, where the rate of typhoid transmission is high, the detection of specific IgG increases.

In the conventional Widal test the interpretation of results is done against a baseline titre in the same geographical area since titres of diagnostic significance differ in endemic or non-endemic areas. A paired sera with a fourfold rise in titer is needed for a meaningful result.

The limitations of the traditional methods have prompted novel tests to be developed. **ENTEROSCREEN-WB**™ qualitatively detects and differentiates between IgM and IgG class of antibodies specific to *S. typhi* in human serum/plasma or whole blood specimens.

PRINCIPLE

ENTEROSCREEN-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. **ENTEROSCREEN-WB**™ is a dual test device assembly comprising of an IgM detection test assembly and an IgG detection test assembly. The conjugate pad of the IgM test assembly consists of two components, Agglutinating sera for Human IgM conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. Similarly the IgG test assembly consists of Agglutinating sera for Human IgG conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test specimen flows through the respective membrane test assemblies, the Agglutinating sera for Human IgM or the Agglutinating sera for Human IgG -colloidal gold conjugate complexes with the *S. typhi* specific IgM or IgG antibodies in the specimen and travels on the membrane due to capillary action along with the rabbit globulin-colloidal gold conjugate. This complex moves further to the test regions of the respective test assembly where the specimen is immobilized by the *S. typhi* specific Lipopolysaccharide (LPS) O antigen coated at the test regions of the IgM/ IgG device assembly leading to formation of a pink to pink- purple colored band at the test regions of the respective test devices which indicates a positive IgM or IgG test result. The absence of this colored band in either of the test regions indicates a negative test result.

In both the test membrane assemblies the unreacted conjugate and unbound complex, if any move further on the membranes and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated on the membranes at the control region (C), forming a pink to pink-purple colored band. This control band acts as a procedural control and serves to validate the results.

REAGENTS AND MATERIALS SUPPLIED

ENTEROSCREEN-WB™ kit contains:

A. Individual pouches, each containing -

1. Dual test device :

   IgM Test Assembly: DEVICE — Membrane assembly pre-dispensed with Agglutinating sera for Human IgM-colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, *S. typhi* specific LPS O antigen and Agglutinating sera for rabbit globulin coated at the Test region 'T' and Control region 'C' respectively and

   IgG Test Assembly: DEVICE — Membrane assembly pre-dispensed with Agglutinating sera for Human IgG-colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, *S. typhi* specific LPS O antigen and Agglutinating sera for rabbit globulin coated at the Test region 'T' and Control region 'C' respectively.

2. Desiccant pouch.

B. PIPETTE Disposable Plastic Sample Applicator.

C. BUF Sample Running Buffer in a dropper bottle.

D. Package Insert.

REF	501030010	501030025
	10	25

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

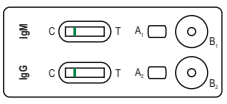
1. Read the instructions carefully before performing the test.
2. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
3. Do not use the kit beyond expiry date and do not re-use the test device.
4. Do not intermix reagents from different lots.
5. Contact with the contents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

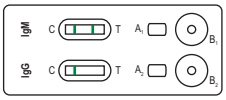
TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the kit components of **ENTEROSCREEN-WB**™ device to room temperature before testing.
2. Open a foil pouch by tearing along the "notch".
3. Remove the testing device and desiccant pouch. Check the color of the desiccant. It should be blue, if it has turned colorless or pink, discard the device and use another device. ***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 either whole blood / serum / plasma into the specimen port 'A1' and 5µl of same specimen into the specimen port 'A2' of the test device using a micropipette OR using the 5µl sample applicator provided in the kit, dip the sample applicator in the sample container and blot the sample in the sample port 'A1' and then using the same sample applicator, dip into the sample container again and blot the sample in the sample port 'A2'.
7. Add **four drops** each of sample running buffer into the buffer port 'B1'& 'B2' .
8. At the end of **15 minutes,** read results as follows:



Negative Result

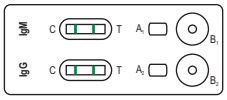
If only one colored band appears in the Control region 'C' of both the test windows. It indicates absence of antibodies to *S. typhi* in the specimen or that the amount of antibodies is below detection limit of the test.



Positive Result

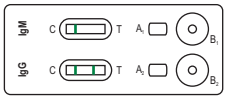
IgM positive

In addition to a colored band appearing in the Control region 'C' in both the IgM and IgG Test windows, a colored band appears in the Test region 'T' of the IgM test window. The presence of only IgM antibodies is indicative of current acute typhoid infection. The intensity of the test band may be more or less than the Control band, depending upon the concentration of antibodies in specimen.



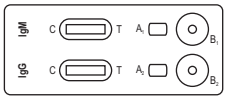
IgM and IgG positive

In addition to a colored band appearing in the Control region 'C' in both the IgM and IgG Test windows, a colored band is observed in the Test region 'T' of both IgM and IgG Test windows. The presence of both IgM and IgG antibodies indicates acute typhoid fever (in the middle stage of *Salmonella typhi* infection). The intensity of the test band may be more or less than the Control band, depending upon the concentration of IgM and IgG antibodies in specimen.



IgG positive

In addition to a colored band appearing in the Control region 'C' in both the IgM and IgG Test windows, a colored band appears in the Test region 'T' of the IgG test window. The presence of only IgG antibodies is indicative of previous *Salmonella typhi* infection (in which case current fever may not be due to typhoid) or relapse or re-infection. The intensity of the test band may be more or less than the Control band, depending upon the concentration of antibodies in specimen.



Invalid Result

The test is invalid if the Control band in both or either one of the devices is not visible at fifteen minutes. Verify the test procedure and repeat the test with a new device.

PERFORMANCE CHARACTERISTICS

Internal Evaluation

In an in-house study, the performance of **ENTEROSCREEN-WB**™ was evaluated using a panel of seventy specimens of WIDAL-positive (of varying reactivity) and WIDAL-negative sera. The results of the evaluation are as follows:

SPECIMEN DATA	WIDAL	ENTEROSCREEN-WB™	COMMERCIAL RAPID TEST
No. of specimens tested	70	70	70
No. of positive specimens	16	16	16
No. of negative specimens	54	54	54

Based on this evaluation:
Sensitivity of **ENTEROSCREEN-WB**™ : 100%
Specificity of **ENTEROSCREEN-WB**™ : 100%

External Evaluation-I

Fifty samples that were Widal positive/Widal negative sera were evaluated with **ENTEROSCREEN-WB**™ in a reputed hospital in Amritsar, India.The results of the evaluation are as follows:

SPECIMEN DATA	WIDAL	ENTEROSCREEN-WB™
No. of specimens tested	50	50
No. of positive specimens	5	5
No. of negative specimens	45	45

Based on this evaluation:
Sensitivity of **ENTEROSCREEN-WB**™ :100%
Specificity of **ENTEROSCREEN-WB**™ :100%

LIMITATIONS OF THE TEST

1. 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 **ENTEROSCREEN-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. **ENTEROSCREEN-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. High titer Rheumatoid factor may result in a false positive reaction.
10. A low extent of cross reactivity may be observed with *S. paratyphi* infection.

WARRANTY

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

BIBLIOGRAPHY

1. Hatta M et al., Simple dipstick assay for the detection of Salmonella typhi-specific IgM antibodies and the evolution of the immune response in patients with typhoid fever. Am. J. Trop. Med. Hyg., 66(4), 2002, pp. 416-421.
2. Gopalkrishna V., Sekhar W. Y., Soo E. H., Vinsent R. A. & Devi S., Singapore Med J. 2002 Vol. 43(7) 354-358.
3. House Deborah, Wain John, Ho Vo A., Diep To S., Chinh Nguyen, Bay Phan V., Vinh Ha, Due Minh, Parry Christopher M., Dougan Gordon, White Nicholas J., Hien Tran Tinh & Farrar Jeremy J. J. of Clin. Microbiol. 2001 Vol. 39 (3) 1002-1007.
4. Bhutta ZA et al., Rapid Serologic Diagnosis Of Pediatric Typhoid Fever In An Endemic Area: A Prospective Comparative Evaluation Of Two Dot-Enzyme Immunoassays And The Widal Test. Am. J. Trop. Med. Hyg. 61(4), 1999, pp. 654-657.
5. Agarwal PK et al., Typhoid Fever. JIACM 2004; 5(1):60-4.
6. Data on file: Viola Diagnostic Systems.

SYMBOL KEYS

Temperature Limitation	Manufacturer	DEVICE Device	Xn NaN., R22. S23-46-61 Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions.
Use by	Consult Instructions for use	PIPETTE Disposable Plastic Sample Applicator	
Date of Manufacture	REF Catalogue Number	BUF Sample Running Buffer	
LOT Batch Number / Lot Number	IVD *In vitro* Diagnostic Medical Device	This side up	EC REP Authorised Representative in the European Community
Σ Contains sufficient for <n> tests	Do not reuse	Do not use if package is damaged	

Manufactured by:

Viola Diagnostic Systems

A Division of Tulip Diagnostics (P) Ltd.

Plot No. 33, Sector-3, I.I.E. SIDCUL, Pantnagar, U. S. Nagar, Uttarakhand - 263 153, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

Website: www.tulipgroup.com Email: sales@tulipgroup.com

EC REP

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

0923/VER-04