CE

ENTEROCHECK® - WB

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

DEVICE

INTENDED USE

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

SUMMARY

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

ENTEROCHECK®-WB qualitatively detects the presence of IgM class of Lypopolysaccharide (LPS) specific to *S. typhi* in human serum/plasma or whole blood specimens.

PRINCIPLE

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

The unreacted conjugate and unbound complex, if any along with the rabbit globulin - colloidal gold conjugate move further on the membrane and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated on the membrane at the control region (C), forming a pink to pink-purple coloured band. This control band acts as a procedural control and serves to validate the results.

REAGENTS AND MATERIALS SUPPLIED

ENTEROCHECK®-WB kit contains:

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

B. PIPETTE : Disposable Plastic Sample Applicator.

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

D. 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.

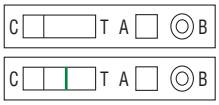
5. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
6. Handle all specimens as if potentially infectious. Follow standard biosafety guidelines for handling and disposal 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 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 whole blood / serum / plasma into the specimen port 'A' 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 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** If IgM antibodies to *S.typhi* are not present, only one coloured band appears in the Control Window (C).
	**Positive Result** 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** 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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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.
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6. Data on file: Zephyr Biomedicals.

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	Do not use if package is damaged
Contains sufficient for <n> tests	LOT — Batch Number / Lot Number	PIPETTE — Disposable Plastic Sample Applicator	
EC REP — Authorised Representative in the European Community	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.		

Manufactured by:

Zephyr Biomedicals

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EC REP

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