

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 health problem. The organisms are non-capsulated, non sporulating, facultative anaerobic bacilli. The outer membrane proteins (OMP) 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 anti-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 British and European Standard EN ISO 15223-1:2016.

 Manufactured By	 <i>In vitro</i> diagnostic medical device
 No. of tests	 See Instruction for use
 Lot Number Batch Number	 Temperature Limitation
 Manufacturing Date	 Caution, see instruction for use
 Expiry Date	 Catalogue Number
 Do not use if package is damaged	 Keep away from sunlight
 Single Time use only	

KIT PRESENTATION

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. Do not open the foil pouch to remove the product until it attains room temperature and you are ready to perform the test.
10. **Optimal test performance requires strict adherence to the test procedure described in the instruction manual.**

PRECAUTION

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

KIT STORAGE & STABILITY

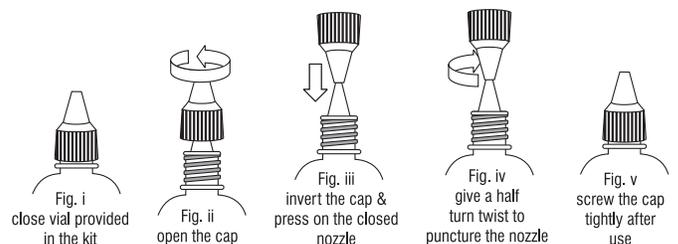
The kit should be stored at 2-30°C in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. Advantage Typhi IgM & IgG Card should not be frozen and must be protected from exposure to humidity.

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:



TEST PROCEDURE

1. Bring the complete kit & sample to be tested to room temperature prior to testing. Once the test kit 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 identification number.
3. Add 1 drop (35 μ l) of serum / plasma sample using sample dropper into the sample well of the Test Card.
4. Add 2 drops of Assay Buffer to the sample well of the Test Card and screw cap the vial after use.
5. Allow reaction to occur during the next 20 minutes.
6. 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.

R.T.
20-30°



Important Note: Do not read result after 20 minutes.

7. 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



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

IgG REACTIVE



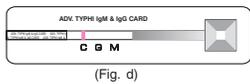
As shown in (Fig. b) appearance of red coloured line in the control region 'C' and Test region; IgG region 'G' indicates that the sample is reactive for S. typhi IgG antibodies.

IgM REACTIVE



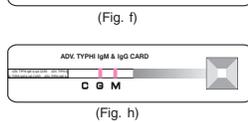
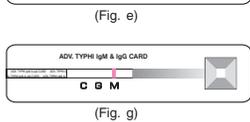
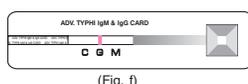
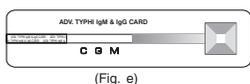
As shown in (Fig. c), appearance of red coloured line in the control region 'C' and Test region; IgM region 'M' indicates that the sample is reactive for S. typhi IgM antibodies.

NON-REACTIVE



As shown in (Fig. d), 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



The test is invalid if no control line appear after completion of test either with clear background or pinkish background as shown in (Fig. e, f, g & h). 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 device and test sample.

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 typhoid 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 Rheumatoid arthritis antibodies, SLE antibodies may show cross-reactivity.
5. As with all diagnostic tests, all results must be correlated 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 typhoid 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 typhoid 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 licensed commercially available test. The samples included cross-reacting samples; Rheumatoid factor, anti-nuclear antibodies etc. 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	500	500	499
S. typhi IgM Positive	85	85	82
S. typhi IgG Positive	55	55	54

Adv. Typhi IgM antibodies :

Sensitivity : 93.18% Specificity : 99.6%

Adv. Typhi IgG antibodies :

Sensitivity : 96.4% Specificity : 99.6%

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

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 thereof.

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 endemicity. *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.

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



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