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 15223-1:2021.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 30°C	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
	Keep Dry	2	Single Time use only
BIO	Contains biological Material of Animal Origin	IN	Country of Manufacture

KIT PRESENTATION

5 Test pack | 10 Test pack | 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. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
6. Do not pipette by mouth.
7. All materials used in the test and samples should be disposed off in accordance with established safety procedures/ guidelines.
8. 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.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
10. 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).
11. **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:

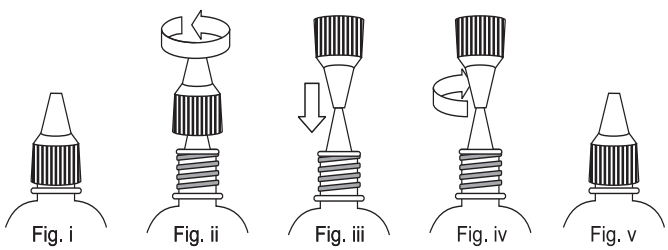


Fig. i Fig. ii Fig. iii Fig. iv Fig. v

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.
3. Label the test card with patient's name or identification number.
4. Add 1 drop (35 μl) of human serum/ plasma sample into the well using the dropper provided as shown in fig. (a). (Use separate dropper/ microtip for each specimen).

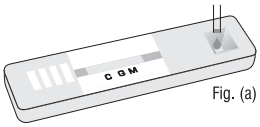


Fig. (a)

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.

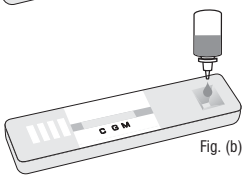


Fig. (b)

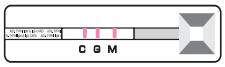
6. Allow reaction to occur during the next 20 minutes.
7. Read results at 20 minutes. Positive results may appear as early as 5-10 minutes. However, negative results must be confirmed only at 20 minutes.

Important Note: **Do not read result after 30 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 purplish pink colour line in Test region; IgM region 'M' and IgG region 'G' indicates that the sample is reactive for both S. typhi IgM & IgG antibodies.

IgM REACTIVE

(Fig. d)

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

IgG REACTIVE

(Fig. e)

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

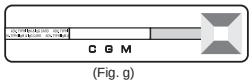
NON-REACTIVE

(Fig. f)

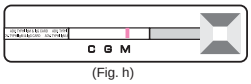
As shown in (Fig. f), appearance of one distinct red coloured line in the control region 'C' only (with no line in the IgM region 'M' & IgG region 'G') indicates that the sample is non-reactive for S. typhi antibodies.

INVALID

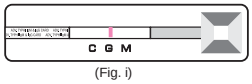
The test is invalid if no control line appear after completion of test either with clear background or pinkish background as shown in (Fig. g, h, i & j). The test should be treated as Invalid which may be because of following reasons:



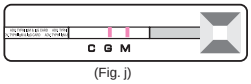
(Fig. g)



(Fig. h)



(Fig. i)



(Fig. j)

(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 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 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 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 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
S. typhi Antibody Negative	200	200	200
S. typhi Antibody Positive	60	60	60
Cross-reactive Sample	27	27	27

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 10%.

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

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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.
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VER-02 R-07

in vitro diagnostic Reagent, not for medicinal use

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