Instruction Manual No. M-990-510-HTG

Typhoid Vi IgM

ELISA KIT Cat. # 990-510-HTG

For Qualitative or Quantitative Determination of Human IgM antibodies against S. enterica serovar Typhi (Typhoid) polysaccharide (Vi) in Serum or Plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Human IgG antibodies against S. enterica serovar Typhi (Typhoid) polysaccharide (Vi) IgM ELISA # 990-510-HTM (96 tests)

Kit Components (96 tests)	Cat #
S. Typhi Vi antigen coated strip plate, (8x12 or 96 wells) # 990501	1 plate
Typhi Vi IgG Calibrator A (1.8 U/ml) #990502A (1 mL)	1 vial
Typhi Vi IgG Calibrator B (3.6 U/ml) #990502B (1 mL)	1 vial
Typhi Vi IgG Calibrator C (22 U/ml) #990502C (1 mL)	1 vial
Typhi Vi IgG Calibrator D (200 U/ml) #990502D (1 mL)	1 vial
All controls contain 0.02 % BND as preservative	
Anti-Human IgG-HRP Conjugate,100X (0.15 ml) # H-HuG-612	1 bottle
Sample Diluent, 20X, 10 ml #SD-20T	1 bottle
Wash buffer (100X) 10 ml # WB-100	1 bottle
HRP Substrate TMB Substrate Solution, 12 ml #80091	1 bottle
Stop Solution, 12 ml # 80101	1 bottle
Complete Instruction Manual	M-990-510 - HTG

Intended Use

ADI S. enterica serovar Typhi (Typhoid) polysaccharide (Vi) IgG ELISA is intended for the detection and the measurement of S. Typhi Vi-specific IgM antibodies in human serum and plasma. This ELISA is particularly designed to assess the antibody levels in humans vaccinated with new generation S. Typhi Vi based vaccines (Typhim and Typherix). It is intended for research use only (RUO).

Introduction

Typhoid fever or typhoid is a common worldwide bacterial disease transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium Salmonella enterica enterica, serovar Typhi. The disease has received various names, such as gastric fever, abdominal typhus, infantile remittant fever, slow fever, nervous fever and pathogenic fever. The name typhoid means "resembling typhus" and comes from the neuropsychiatric symptoms common to typhoid and typhus. The term enteric fever is a collective term that refers to severe typhoid and paratyphoid. The bacterium that causes typhoid fever may be spread through poor hygiene habits and public sanitation conditions, and sometimes also by flying insects feeding on feces. An estimated 16–33 million cases of typhoid fever occur annually. Its incidence is highest in children and young adults between 5 and 19 years old. These cases as of 2010 caused about 190,000 deaths up from 137,000 in 1990. Historically, in the pre-antibiotic era, the case fatality rate of typhoid fever was 10-20%. Today, with prompt treatment, it is less than 1%.

S. Typhi expresses a number of immunogenic structures on the surface, some of which provide a basis for serology identification. These include O (lipopolysaccharide), H (flagella) and the somewhat less immunogenic Vi capsule. S. Typhi exhibiting variation in these antigens are uncommon, with notable exceptions. S. Typhi found in Indonesia express variant H antigens including H:j and H:z66. Vi-negative S. Typhi isolates have been reported in Pakistan but are rare. Therefore, S. Typhi expressing O (O9, O12), Vi and H:d are ubiquitous in most endemic areas. Seroprevalence studies have been performed in endemic regions to determine antibody titers to O, H and Vi in the general population. Many individuals in endemic areas have cross-reactive antibodies even though they have

Twenty four (24) random human serum samples were analyzed at 1:100 dilution for antibodies to Typhi Vi-IgG and IgM using ADI ELISA. The data presented above suggest that many highly positive samples tend to be higher for both Vi IgG and IgM. However, some samples showed elevated IgG or IgM values. Significance of elevated Vi-IgG or IgM values with S. Typhi exposure or vaccine status is not established.

N	% +Ve for Vi IgG	% +Ve for Vi IgM
24	5/24 (20%)	12/24 (50%)

Given the higher % of positivity, researchers must establish their own +ve sample and suggest to test at 1:200 dilution. The data presented here should only be used as a guide and not as strict –ve and +ve values. ADI is in the process of conducting a large clinical trial to establish better sample values.

Inter-assay and Intra-assay variations

Intra-assay ~6-8% Inter-assay 8-12%

Sensitivity of Vi ELISA

ELISA sensitivity is optimized by antigen coating, antibody conjugate and sample dilution and other assay conditions (incubation time and temperature). Given the optimal ELISA assay testing, this test detects <2-5 ng/ml IgG or IgM. Sensitivity is established by sample values that can be clearly distinguished from blank values. At A450 values of 1.00, the sensitivity is about 20-30 ng/ml.

Specificity and Species Cross reactivity

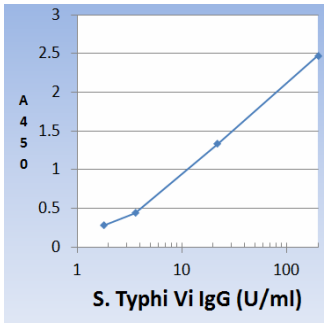
Human Anti-S. Typhi Vi IgG ELISA only detect IgG with no detection of IgM or IgA. Similarly Vi IgM ELISA only detects IgM and not IgG or IgA isotypes. This kit is designed to detect Vi-IgG or IgM in human samples. It cannot be used for testing samples other than human (mouse, rat, rabbit etc). ADI has other species (mouse, rabbit etc) ELISA kits for S. Typhi Vi-IgG.

Other important information

Rabbits immunized with S. Typhi (whole killed bacterium) and two commercially available S. typhi Vi vaccines (purified Vi polysaccharides) and also Vi-rEP experimental vaccines and tested in ELISA. Highest titer antibodies were produced by Vi-rEP vaccine but all vaccines produced significant antibody titer after 1 month and 2 month post immunization. Therefore, ADI's Vi-IgG and IgM ELISA may be useful to detect and measure antibodies to Vi-polysaccharides in vaccinated samples.

References: Szu SC (2013) Vaccine 31, 1970-1974; FeryL (2004) Clin Exp Immunol;136:297–303;Staats HF (2010) Clin Vaccine Immunol17:412–9 Ivanoff B (1994) Bull WHO 72, 957-971; .Jesudason M (2002) Ind. J. Med. 116, 70-72; Gopalakrishna V (2002) Med J; 43: 354; Olsen SJ (2004) J. Clin. Microbiol. 42, 1885-1889; Shanta D (2006) Infect. Dis. 56, 359-365.

Calculation of Results



Read A450/630nm values of the wells.

1. Subtract blank (diluent) values from all values including calibrators and samples.
2. Calculated the mean values for Calibrators A-D and samples.

Interpretation of Results (typical example)

	Mean A450 (minus blanks)
Calibrator A (-ve)	0.270
Calibrator B (cut-off)	0.660
Calibrator B (positive)	2.00

ADI has set cut-off values at ~0.660. All values below the cut-off are either negative of equivocal.

All samples above the cut-off values may be considered positive for Vi-IgG antibodies,

Important note: ADI negative, cut-off and positive values were assigned from limited human samples. We strongly recommend that users established their own negative, cut-offs, and positive values depending upon the demographics and vaccine status or exposure to other diseases, ADI calibrators are meant to be used as quality control for the kit and not for establishing diseases or vaccine status of samples.

Vi-IgG and IgM Values in normal population (N=Negative; P=Positive)

Samples	Vi-IgG Values	Inter-pretation	Vi-IgM Values	Interpretation
1.	0.304	N	0.315	N
2.	0.532	N	0.859	P
3.	0.666	N	1.349	P
4.	0.545	N	1.730	P
5.	0.271	N	0.092	N
6.	0.885	P	1.747	P
7.	0.280	N	1.313	P
8.	1.299	P	0.907	P
9.	0.363	N	0.850	P
10.	1.903	P	0.110	N
11.	2.807	P	0.373	N
12.	1.478	P	0.900	P
13.	1.014	P	0.574	N
14.	0494	N	0.863	P
15.	0.464	N	0.470	N
16.	0.279	N	0.435	N
17.	0.204	N	0.486	N
18.	0.272	N	0.467	N
19.	0.520	N	1.746	P
20.	0.553	N	0.901	P
21.	0.176	N	0.881	N
22.	0.357	N	0.596	N
23.	0.270	N	0.907	P
24.	0.46	N	0.583	N

no clinical record of typhoid. Additionally, such raised antibody levels frequently cannot be detected in patients with culture confirmed typhoid. Problems have also been encountered during the testing of commercial serological tests, including Typhidot and Tubex.

Diagnosis is made by any blood, bone marrow or stool cultures and with the Widal test (demonstration of salmonella antibodies against antigens O-somatic and H-flagellar). The **Widal test** is time-consuming, and often, when a diagnosis is reached, it is too late to start an antibiotic regimen. Typhidot-M is a dot enzyme immunoassay for the detection of specific IgG/IgM antibody to Salmonella typhi OMP antigen Salmonella typhi.

There are few vaccines licensed for use for the prevention of typhoid: **the live, oral Ty21a vaccine** (sold as Vivotif by Crucell) and the injectable **Typhoid polysaccharide vaccine** (sold as Typhim Vi by Sanofi Pasteur and Typherix by GlaxoSmithKline). Both are 50% to 80% protective and are recommended for travellers to areas where typhoid is endemic. There exists an **older, killed-whole-cell vaccine** that is still used in countries where the newer preparations are not available, but this vaccine is no longer recommended for use because it has a higher rate of side effects (mainly pain and inflammation at the site of the injection). A new vaccine based upon Vi-rEPA (recombinant Pseudomonas aeruginosa exoprotein A) has been shown to confer 90% protection for 4 years in 2-5 yrs old children.

PRINCIPLE OF THE TEST

Alpha Diagnostic's Typhoid Vi IgG antibody test kit is based on the principle of indirect ELISA). Vi antigen is bound on the surface of the microtiter strips. Diluted sample or standards are pipetted into the wells of the microtiter plate. Vi IgG antibodies bind to the immobilized Vi antigen. After incubation at room temperature, the plate is rinsed with wash buffer and plates are incubated with anti-human-IgG peroxidase conjugate. After another washing step, the substrate (TMB) solution is added that produces blue color in the antibody positive wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The color is measured using an ELISA reader at 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color. Results can also be expressed as –ve or +ve in comparison to the cut-off standards.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Distilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

PRECAUTIONS

Do not ingest or swallow and reagents. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken. No reagents from different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for testing. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

Prepare 1:10 initial stock of all samples by diluting in sample/conjugate diluent (10 ul sample in 90 ul diluent). The presence of dye does not interfere with the ELISA. Antibodies are stable in this diluent and can be kept at 4oC for weeks. It also avoids freezing and thawing of the original samples. All subsequent test dilutions (1:100 or more) of samples should be made fresh on the day of the test. For example, samples that will be tested at 1:100 will be diluted another 1:10 fold from the 1:10 sample stocks. High antibody samples should be diluted more (e.g. for 1:500 dilution, dilute initial 1:10 stock another 50 fold or 10 ul in 490 ul of 1x diluent).

Users may choose to test their samples at a dilution different than 1:100 recommended in this manual (e.g. 1:50 or 1:200 etc) and compare the control groups with experimental group or vaccinated vs non-vaccinated. A difference in the A450 values of one group Vs another should be indicated of the Vi antibody.

REAGENTS PREPARATION

Dilute Wash buffer 1:100 with water. Dilute 10 ml stock in 1-L distilled water. Store diluted buffer at 4oC for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.

Dilute Sample Diluent 1:20 with water (1 ml stock in 19 ml water)**.** Keep at 4oC. Prepare as necessary. 1x Sample diluent is used for sample dilution and also for the antibody conjugate (10 ml per plate).

Dilute antibody-HRP conjugate 1:100 with 1X sample diluent**. Prepare conjugate only in required amounts**. Prepare 1 ml for every strip (10 ul stock in 1 ml of 1X sample diluent or prepare 10 ml for full plate)**. Do not store diluted conjugate beyond the assay date.**

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{O}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8^{O}C or stored frozen in small aliquots and should be stable for 3 months.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* Read instructions carefully before the assay.

Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4oC. Do not touch the bottom of the wells.

ELISA Test Procedure

1. Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. Prepare 1x Wash buffer, 1x sample diluent, and 1x antibody conjugate. **All samples should be diluted 1:100 (see sample preparation)**. **Do not dilute the calibrators**. We recommend preparing a replica of samples on small ELISA tubes or blank ELISA plates for a quick transfer of samples to the coated plate using multi-pipette.
2. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
3. Add **100 ul of diluted anti-IgG-HRP conjugate** to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp (25-28oC).
4. Wash the wells 3 times as in step 3.
5. Add **100 ul TMB substrate solution**. This time also the substrate blank is pipetted. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp. Blue color develops in positive controls and samples.
6. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).
7. Measure the **absorbance at 450 nm** and a reference filter 630nm using an ELISA reader within 60 min.

WORKSHEET OF A TYPICAL ASSAY

Wells	Calibrators /Samples	A450	Net A450	Results
A1/A2	Sample diluent Blanks	0.1000	-	
B1/B2	Calibrator A (-ve)	0.370	0.270	
C1/C2	Calibrator B (cut-off)	0.760	0.660	
D1/D2	Calibrator C (positive)	2.100	2.00	
D1/D2	Calibrator C (positive)	2.100	2.00	
E1/E2	Sample 1	0.410	0.310	-ve
F1/F2	Sample 2	0.910	0.900	+ve