IMMUNODOT™
SALMONELLA TYPHI

IVD For In Vitro Diagnostic Use

INTENDED USE

The GenBio *Salmonella typhi* ImmunoDOT test is an enzyme immunoassay for the detection of antibodies to *Salmonella* (typhoid fever) for the serological confirmation of infections in serum, plasma or heparinized whole blood. This test is intended to be performed by trained laboratory personnel only.

SUMMARY AND EXPLANATION

Diagnosis of enteric fever is usually performed by the use of stool and urine cultures as well as febrile agglutinins to specific lipopolysaccharides and to somatic antigens. In the case of *Salmonella typhi* and *S. paratyphi*, the primary antigens tested for are the "O" antigens, the "H" flagellar antigens, and the Vi antigens. The febrile agglutinin test commonly referred to as the Widal test is widely used because of its simplicity. A four-fold rise in agglutinin titer is considered significant and diagnostic. Generally the antibodies are slow to develop, rise to a peak, and then recede. If the patient has been previously infected the antibody response is more rapid. The ImmunoDOT dots for the detection of typhoid antibodies are set to represent a 1:160 tube agglutination titer. The antigens include of the primary antigens listed above. Recent reports suggest that a titer of 1:160 or greater for "O" and "H" antigens has specificity of 97%, sensitivity of 30%, and an accuracy of 83.1% in the diagnosis of typhoid fever (1) (2).

ASSAY PRINCIPLE

The GenBio *Salmonella typhi* ImmunoDOT assay utilizes an enzyme-linked immunoassay (EIA) dot technique for the detection of both IgG and IgM antibodies. The antigens are dispensed as discrete dots onto a solid membrane. After adding specimen to a reaction cuvette, an assay strip is inserted, allowing patient antibodies reactive with the test antigens to bind to the strip's solid support membrane. In the second stage, the reaction is enhanced by removal of non-specifically bound materials. During the third stage, alkaline phosphatase-conjugated anti-human antibodies are allowed to react with bound patient antibodies. Finally, the strip is transferred to an enzyme substrate reagent, which reacts with bound alkaline phosphatase to produce an easily seen, distinct spot.

REAGENTS

Assay Strips: Includes a positive human control, negative control and *Salmonella typhi* antigens ("O," "H," and Vi)
Diluent (#1): Consists of buffer salts with <0.1% NaN_3 (pH 6.2-7.6)
Enhancer (#2): Consists of sodium chloride with <0.1% NaN_3
Conjugate (#3): Consists of alkaline phosphatase conjugated goat anti-human IgG and IgM antibodies in buffered diluent (pH 6.2-7.6) with <0.1% NaN_3
Developer (#4): Consists of 5-bromo-4-chloro-3-indolyl phosphate and p-nitro blue tetrazolium chloride in buffered diluent (pH 9.0-11.0) with <0.1% NaN_3

WARNINGS AND PRECAUTIONS

For In-Vitro Diagnostic Use. ImmunoDOT reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoDOT Assay System reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use any kits beyond the stated expiration date. Analytic quality water must be used. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since these may result in poor assay performance.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. It may be harmful if enough is ingested (more than supplied in kit). On disposal of liquids, flush with a large volume of water to prevent azide build-up (3). This dilution is not subject to GHS, US HCS and EU Regulation 2008/1272/EC labeling requirements.
The safety data sheet (SDS) is available at support.genbio.com or upon request.

Human source material. Material used in the preparation of this product has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV), and antibodies to human immunodeficiency virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease (4). Follow recommended Universal Precautions for bloodborne pathogens as defined by OSHA (5), Biosafety Level 2 guidelines from the current CDC/NIH Biosafety

in Microbiological and Biomedical Laboratories (6), WHO Laboratory Biosafety Manual (7), and/or local, regional and national regulations.

STORAGE

Store at 2-8°C. Reagents must be at room temperature (15-30° C) before use. Assuming good laboratory practices are used, opened reagents remain stable as indicated by the expiration date.

SPECIMEN COLLECTION AND HANDLING

GenBio *Salmonella typhi* ImmunoDOT assay can be performed with serum heparinized plasma or heparinized whole blood. The test requires approximately 10 µl of serum or plasma or 20 µl of whole blood. Serum, heparinized plasma, and heparinized whole blood should be collected according to standard practices. Finger stick samples are stable at ambient temperatures for one day. Serum, plasma or heparinized whole blood may be stored at 2-8°C for up to five days. Serum and plasma may be frozen below -20° C for extended periods. Freezing whole blood samples is not advised.
Single specimens are used to assess exposure; two specimens collected at different times from the same individual are used to show seroconversion. Paired specimens should be tested at the same time. It is recommended that a convalescent specimen be collected from patients showing either an initially nonreactive or weakly reactive result.

MATERIALS PROVIDED

Assay Strips	Conjugate (#3)
Diluent (#1)	Developer (#4)
Enhancer (#2)	Reaction Vessels

MATERIALS REQUIRED BUT NOT PROVIDED

Workstation	Timer
Pipets	Specimen collection apparatus (e.g., finger sticking device, venipuncture equipment)
Positive Control serum	Absorbent toweling to blot dry assay strips
	Analytic quality water

SET-UP

1. Turn on Workstation and adjust to proper temperature if necessary. Refer to Workstation Instructions.
2. Remove 4 Reaction Vessels (per test) from the product box and insert into appropriate slots in Workstation. For the large Workstation, add water up to the fill line of the provided rinse container. For the small Workstation, use an appropriate container and sufficient water to cover all reactive windows of the assay strip.
3. Place 2 mL Diluent (#1) in Reaction Vessel #1; 2 mL Enhancer (#2) in Reaction Vessel #2; 2 mL Conjugate (#3) in Reaction Vessel #3; and 2 mL Developer (#4) in Reaction Vessel #4.
4. Wait ten minutes before beginning “Assay Procedure”. During this time, specimen(s) may be added (step #5), Assay Strips labeled (step #6), and inserted into the Strip Holder (step #7).
5. Add patient specimen (approximately 10 µL serum or plasma or 20 µL of whole blood) to Reaction Vessel #1.
6. Appropriately label the Assay Strips.
7. If the large Workstation is used, insert the label end of the Assay Strip into the Strip Holder, one per groove, taking care not to touch the assay windows.

ASSAY PROCEDURE

1. Prewet Assay Strip by immersing in water for 30-60 seconds.
2. Using several (5 - 10) quick up and down motions with the Assay Strip, mix reagent and specimen thoroughly in Reaction Vessel #1. Let stand for 10 minutes.
3. Remove Assay Strip from Reaction Vessel and swish in the water. Use a swift back and forth motion for 5-10 seconds allowing for optimal washing of the Assay Strip’s membrane windows.
4. Place Assay Strip into Reaction Vessel #2. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 5 minutes.
5. Remove Assay Strip from Reaction Vessel #2 and swish in water as described (step #3).
6. Place Assay Strip into Reaction Vessel #3. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 15 minutes.
7. Remove Assay Strip from Reaction Vessel #3 and swish in water as described (step #3). DO NOT remove the Assay Strip from the water.
8. Allow the Assay Strip to stand in the water for 5 minutes.
9. Remove Assay Strip from water and place into Reaction Vessel #4. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 5 minutes.
10. Remove Assay Strip from Reaction Vessel #4 and swish in water as described (step #3).

11. Blot and allow Assay Strip to dry. It is imperative that tests of borderline specimens be interpreted after the Assay Strip has been allowed to dry.

READING THE ASSAY STRIP

Positive	A dot with an EASILY SEEN, distinct border is visible in the center of the window. The outer perimeter of the window must be white to pale gray.
Negative	If no dot is seen or a dot is difficult to see, interpret it as negative.

QUALITY CONTROL

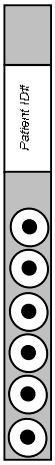
The top membrane window of the Assay Strip contains reagent controls: the dot in the top window is a positive reagent control and must be positive for further interpretation. The area around the positive control dot is the reagent negative control (diluent and non-reactive proteins) and must be negative (white to pale-gray) for further interpretation. Reagent controls assure that reagents are active and that the test has been performed properly. If either reagent control is invalid, the test results should not be reported, and the test repeated. The intensity of the positive control dot must not be used as a calibrator. Positive reactions in the other antigen windows of the strip may be either darker or lighter than the positive control depending on the antibody titer.

GenBio quality assures that its products perform as described. The performance of each kit may be confirmed upon receipt by performing a determination using a well characterized positive serum and obtaining a positive result.

The assay's reagent temperature is between 42-48°C. Due to heat transfer loss, the Workstation temperature is set higher. The appropriate Workstation temperature setting is listed in the Workstation's package insert. (Contact Technical Services for additional guidance if an alternate heat source is used).

INTERPRETATION

Results are interpreted in terms of staining intensity for each antigen well on a scale from 0 (negative reaction) through 4. An equivocal reaction (one in which the intensity of the dot is greater than 0 but less than +1) is recorded as ± or 0+. Intermediate reactions may be denoted with a "+". For example, a test that stains with intensity between 3 and 4 is scored a 3+.



IgG/IgM Positive Control (Negative Control is area around dots)

Salmonella H, type a

Salmonella H, type b

Salmonella H, type d

Salmonella O, type Vi

Salmonella O, type D

Initially nonreactive: Samples interpreted as nonreactive (no reactive dots) indicate antibody not present in the sample. Since antibodies may not be present during early disease, confirmation 3-5 weeks later is indicated for laboratory diagnosis. At this later time, patients showing weak reactions should be further tested by alternate methods or retested 10-14 days later. A convalescent serum with a significant reaction indicates the formation of specific antibody against the indicated organism. An initially negative result followed by a positive result implies seroconversion.

Initially weakly reactive: Weakly reactive specimens should be cautiously interpreted. In normal populations, weakly reactive samples are infrequent but possible. Confirmation using a sample collected 3-5 weeks later (paired acute and convalescent sera) is recommended. A stronger reaction (based on an increase in the strength of color development) in the convalescent sera confirms the presence of recent, specific antibody. [Caution: If this is a cross reactive antibody, the convalescent serum sample may not show a higher antibody level than the acute sample]. If sample reading remains weakly reactive, a second methodology (IgM) should be considered or the sample may be interpreted as taken beyond rising titer (titers declining).

Initially reactive: Samples interpreted as strongly reactive may indicate the presence of specific antibody. Antibody presence alone cannot be used for diagnosis of acute infection, however, because antibodies from prior exposure may circulate for a prolonged period of time.

LIMITATIONS

- Treatment is often indicated prior to completion of serologic diagnosis, which requires at least two weeks. Diagnosis of these diseases should not be made based on results of GenBio *Salmonella typhi* ImmunoDOT assay alone, but in conjunction with other clinical signs and symptoms and other laboratory findings.
- Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.
- Since serological assay methods may yield different results for weakly reactive samples, a second serological method (i.e. an alternate method that tests specifically for IgM or IgG separately) is recommended (see INTERPRETATION section above).
- This ImmunoDOT assay is designed to be used for screening for the presence of Salmonella antibodies. If one or more dots are positive and a titer is indicated, another methodology such as the febrile tube test should be performed.
- There are many known antigenic similarities and cross-reactions.
- Paired samples should be tested at the same time to minimize variability in testing. A known positive control and known negative sera should be tested to monitor test performance.
- GenBio ImmunoDOT assays should not be used as a substitute for culture.
- The positive control dot is not a reading guide and should not be used to compare antibody strength.
- Performance characteristics for GenBio *Salmonella typhi* ImmunoDOT assay are still being defined.
- The IgG results suggests that the samples tested by IgM and found to be weakly reactive in the IgM test and normal by culture may have had a recent Salmonella infection as demonstrated by the IgG response.
- Users of the Salmonella ImmunoDOT test should establish their own cut-off values based on seroprevalence studies.

EXPECTED RESULTS

The number of antibody positive subjects in a population depends on two factors: disease prevalence and clinical criteria used to select the tested population. Because very few positives should be seen in a randomly screened population in a non-endemic area, most serology tests are not specific enough to screen non-endemic populations. Even in an endemic region, serology screening often yields many false positives if used to randomly screen patients. Serology tests are most useful to test patients in an endemic region with signs and symptoms consistent with the disease.

Antibody levels are generally low or absent during early infection. Symptomatic patients may have no antibody during the first 1-2 weeks after exposure and the antibody titer will rise with time. Secondary infections with the same antigen usually give rise to higher titers consistent with a specific anamnestic response.

Non-specific anamnestic responses may occur with heterologous species or closely related serovariants.

This table, adapted from the DIFCO package insert, presents data that will be helpful in interpreting serological tests with the Salmonella antigens. The values tabulated will vary somewhat in certain cases.

BACTO-ANTIGEN	PATHOLOGY	TIME OF MAXIMUM TITER	SIGNIFICANT TITER
Salmonella H Antigen d (Typhoid H)	Typhoid Fever	4-5 weeks	1:80
Salmonella O Antigen Group D (Typhoid O)	Typhoid Fever	3-5 weeks	1:80* over 1:160 indicative
Salmonella H Antigen a (Para A)	Paratyphoid Fever	3-5 weeks	1:80*
Salmonella H Antigen b (Para B)	Paratyphoid Fever	3-5 weeks	1:80*

*Significant in non-vaccinated individuals.

CLINICAL FINDINGS

STUDY 1: Fifty-six human blood donors were screened using the GenBio Salmonella ImmunoDOT test. The donors were from Baltimore County and City, MD, USA. The only known parameter was that these individuals were clinically well and had normal chemistries as defined by a panel > 18 analytes. Forty-five of the 56 donors were completely negative for IgM antibodies to spotted *Salmonella* antigens. Eleven of the donors had one or more antigens positive at a 1:160 to 1:320, but did not have more than three antigens positive.

STUDY 2: Samples were obtained from Jakarta, Indonesia and the Philippines with defined clinical histories and cultures. These samples were tested using conjugate containing only antihuman IgM and an IgG removal device, proSorb G™.

SALMONELLA IGM RESULTS: INDONESIA AND PHILIPPINES

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
H-a	1++	2	2	3+	1++	-	-	-	1+	+/-	4	2++	4	+/-	+/-
H-b	1++	2++	3	3+	2	-	-	1++	1	1++	3++	2++	3	1+	1++
H-d	3++	3	4	4	2++	-	-	+/-	3	3	4	1	3++	+/-	-
0-vi	1	+/-	+/-	2++	1++	-	-	-	+/-	-	1+	+/-	+/-	-	-
0-d	2++	2++	4	4	2+	-	-	-	2++	3	2++	-	2+	-	-
OX	ST	ST	ST	ST	ST	EC	KP	EC	PS	EA	NG	NG	SP	SP	SP
RC	NF	NF	ST	NF	NF	NF	NF	NF	NF	NF	NF	SP	NF	NF	NF

KEY

SP = *Salmonella paratyphi* A	KP = *K. pneumoniae*
ST = *Salmonella typhi*	EA = *E. aerogenes*
NF = Normal flora	PS = *Pseudomonas*
NG = Normal growth	OX = Oxgall culture
EC = *E. cloaca*	RC = Rectal Swab culture

The same samples were retested using the total Ig conjugate to assess past exposure to *Salmonella*.

SALMONELLA IGG RESULTS: INDONESIA AND PHILIPPINES

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
H-a	4	4	+/-	3++	4	-	-	3++	3+	3++	4+	3++		1++	2+
H-b	4	4	1+	4	4	1	2	3++	2	3++	3+	3		3+	3++
H-d	4	4	+/-	4	4	-	1	3+	3	4	3+	1++		2++	+/-
0-vi	1+	2++	2++	3+	2++	+/-	+/-	1	+/-	-	2+	2++		1	2++
0-d	4	2++	-	3++	3++	-	-	3+	2++	3++	2	+/-		2++	-
OX	ST	ST	ST	ST	ST	EC	KP	EC	PS	EA	NG	NG	SP	SP	SP
RC	NF	NF	ST	NF	NF	NF	NF	NF	NF	NF	NF	SP	NF	NF	NF

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6. **US Department of Health and Human Services.** *HHS Publication No. (CDC) 21-11: Biosafety in Microbiological and Biomedical Laboratories. 5th ed.* Washington DC : US Government Printing Office, 2009.
7. **World Health Organization.** *Laboratory Biosafety Manual 3rd ed.* Geneva : World Health Organization, 1991.

QUICK REFERENCE PROCEDURE

IMMUNODOT S.TYPHI

Set-Up

- Make sure Workstation is at temperature.
- Place reaction Vessels into slots in Workstation and add water to the rinse container.
- Place 2 mL Diluent (1) in Vessel #1; 2 mL Enhancer (2) in Vessel #2; 2 mL Conjugate (3) in Vessel #3; and 2 mL Developer (4) in Vessel #4.
- Wait 10 minutes

Procedure

- Add 10 µL serum to Vessel #1.
- Prewet assay strip in water for 30 - 60 seconds.
- Place strip in Vessel #1, mix, let stand 10 min.
- Remove strip, place in water, swish 5-10 sec.
- Place strip in Vessel #2, mix, let stand 5 min.
- Remove strip, place in water, swish 5-10 sec.
- Place strip in Vessel #3, mix, let stand 15 min.
- Remove strip, place in water, let stand 5 min.
- Place strip in Vessel #4, mix, let stand 5 min.
- Remove strip, place in water, swish, blot, dry, and read.

To place an order for ImmunoDOT products, contact your local distributor, or call GenBio directly for the distributor nearest you and for additional product information.
For assistance, please call toll-free 800-288-4368.

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