

IMMUNODOT™

RICKETTSIA TYPHI

 For In Vitro Diagnostic Use



INTENDED USE

The GenBio *R. typhi* (murine typhus) test is a semi-quantitative enzyme immunoassay for the detection of IgG and IgM antibodies to *Rickettsia typhi* as an aid in the presumptive diagnosis of murine typhus from serum, heparinized plasma or heparinized whole blood. This test is to be performed by trained medical personnel only.

SUMMARY AND EXPLANATION (1) (2) (3) (4) (5) (6)

Rickettsia typhi (*mooseri*), the etiologic agent of murine or endemic typhus, is distributed worldwide. It is transmitted primarily, but not solely, by rat fleas (*Xenopsylla cheopis*), which bite humans. Predominant manifestations of murine typhus include the sudden onset of fever, headache, and malaise and maculopapular rash. The triad of fever, severe headache, and rash is reported in 50% of the cases. Fever in adults ranges from 103 to 104°F. Most patients also experience pronounced muscular weakness. The incubation period, from actual infection to acute onset of symptoms, ranges from 8 to 16 days with an average of 11 days. Complications are rare and the mortality rate is lower than in epidemic typhus. Symptoms may last 9 to 14 days. Epidemiologic factors, clinical findings, exposure in endemic regions, and other laboratory results should be considered in diagnosing acute disease. Acute disease diagnosis will also include a positive laboratory confirmation in many cases. Antibody response, however, may either be delayed or eliminated in patients treated with antibiotics.

ASSAY PRINCIPLE

The GenBio ImmunODOT assay utilizes an enzyme-linked immunoassay (EIA) dot technique for the detection of both IgG and IgM antibodies. The antigen is 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 antigen to bind to the strip's solid support membrane. In the second stage, the reaction is enhanced by the 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 enzyme substrate reagent, which reacts with bound alkaline phosphatase to produce an easily seen, distinct spot.

REAGENTS

Assay Strip: Includes a positive human IgG control, negative control and four dilutions of *R. typhi* antigen

Diluent (#1): Consists of buffer salts with <0.1% NaN₃ (pH 6.2-7.6)

Enhancer (#2): Consists of sodium chloride with <0.1% NaN₃

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% NaN₃

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% NaN₃

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 (7). 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 (8). Follow recommended Universal Precautions for bloodborne pathogens as defined by OSHA (9), Biosafety Level 2 guidelines from the current CDC/NIH Biosafety

in Microbiological and Biomedical Laboratories (10), WHO Laboratory Biosafety Manual (11), and/or local, regional and national regulations.

STORAGE

Test kits and components should be at 2-8°C before use. Assuming good laboratory practices are used, opened reagents remain stable as indicated by the expiration date.

SPECIMEN COLLECTION AND HANDLING

The ImmunoDOT *Rickettsia typhi* assay can be performed on serum, heparinized plasma, or heparinized whole blood. The test requires approximately 10 µL serum or plasma or 20 µL whole blood. Serum, heparinized plasma and heparinized whole blood should be collected according to standard practices. Finger sticks 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 run at the same time. It is recommended that a convalescent specimen be collected from patients showing either an initially nonreactive result 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 15 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).

- 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.

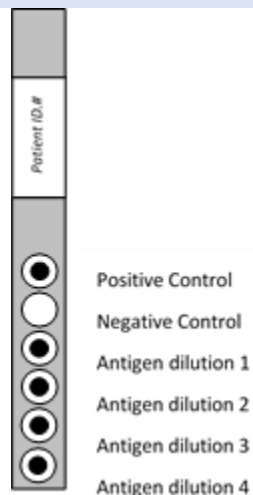
QUALITY CONTROL

The top two membrane windows of the Assay Strip are reagent controls. The top window is a positive reagent control (human IgG, as a control of proper test procedure) and must be positive for further interpretation. The next window is the reagent negative control (diluent and non-reactive proteins) and must be negative 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 must be 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 either be darker or lighter than the positive control depending on the antibody titer.

GenBio quality assures that its products perform as described. In addition, a positive control serum for *R. typhi* is separately available from GenBio. The performance of each kit may be confirmed upon receipt by running a determination using the positive control 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



The relative *R. typhi* antibody level is indicated by the number of reactive dots. The window closest to the negative control indicates the weakest antibody reaction while the bottom reactive window furthest from the negative control indicates the strongest reaction.

Initially nonreactive: Samples interpreted as nonreactive (no reactive dots) indicate antibodies are not present in the sample. Since antibodies may not be present during early disease, confirmation 2-3 weeks later is indicated for laboratory diagnosis. At this later time, patients showing weak reactions (1 or 2 dots) should be further tested by alternate methods or retested 2-3 weeks later. A convalescent serum with a significant reaction (3 or 4 dots) indicates the formation of specific antibody against *R. typhi*. An initially negative result followed by a positive result implies seroconversion.

Initially weakly reactive: Weakly reactive specimens (1 or 2 dots) should be cautiously interpreted. In normal populations, weakly reactive samples are infrequent but possible. Confirmation using a sample collected 2-3 weeks later (paired acute and convalescent sera) is recommended. Three and four positive dots in the second sample confirm the presence of recent specific antibody. **[Caution: If patient has been treated with antibiotics, the convalescent serum sample may not show a higher antibody level than the acute sample].** If the sample remains 1 or 2 dots, a second methodology should be considered or sample interpreted as taken beyond rising titer (titer declining). See TABLE 1 for interpretations.

Initially reactive: Samples interpreted as strongly reactive (3 or 4 dots) 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 murine typhus should not be made based on results of GenBio *R. typhi* ImmunoDOT test alone, but in conjunction with other clinical signs and symptoms and other laboratory findings. Interpret serology results with caution in patients who have received drug therapy, since antibody response may diminish after treatment.
- Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.
- Known cross reactions between rickettsial antigens must be considered during interpretation, since some epitopes are known to react with other rickettsial antibodies. Because of the presence of common antigens within the same rickettsial group, and among different rickettsial groups, the GenBio *R. typhi* ImmunoDOT test may react with other rickettsial species, e.g. *R. rickettsii* and *R. prowazekii*.
- Since serological assay methods may yield different results for weakly reactive samples, a second serological method (i.e., an alternative method that tests specifically for IgM or IgG separately) is recommended.

• 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 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.

PERFORMANCE CHARACTERISTICS

COMPARATIVE STUDIES WITH PRESUMED NEGATIVE AND POSITIVE SERA

Normal population sera (presumptive negative): Ninety-five (95) presumptive normal samples were tested to demonstrate type specificity. Sixty-five (65) were normal serum samples received at a reference laboratory. These samples were lipemic, hemolyzed, or icteric samples used to assure suitable assay performance on such specimens. An additional thirty (30) asymptomatic, normal subjects were also tested.

Presumptive positive sera: Like many tests, positives may be defined as clinical cases or assay positives. For rickettsial diseases, positives are described based on the assay result since clinical definitions are difficult to assign. An assay-positive sample is not necessarily from a patient with murine (endemic) typhus. The following are presumptive positive samples collected from clinically suspect patients and tested to have at least 1:16 IFA titer.

Thirty-three (33) presumptive positive samples were tested. All samples were obtained from a U.S. medical reference laboratory for rickettsial diseases. The samples tested were from clinical cases in foreign countries diagnosed as having murine typhus.

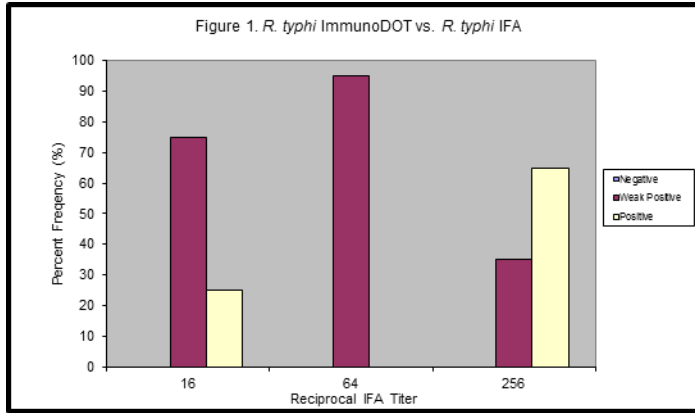
Comparison assays: All 128 samples were screened using latex agglutination and IFA assays. Thirty-three (33) clinical samples were also screened by ELISA (12) (13; 14). Discrepancies between the ImmunoDOT *R. typhi* and Latex assay were resolved using the results of an IFA assay. An IFA or Latex assay result of 1:16 or greater was used to identify positive samples.

Evaluation of presumptive positives is more complex, since true clinical cases are difficult to identify. The IFA result (screen at 1:16) or confirmation using paired sera was used to resolve discrepancies between the latex assay and ImmunoDOT *R. typhi*.

ImmunoDOT vs. IFA: Out of 33 presumptive positive samples (including paired sera), both *R. typhi* ImmunoDOT and IFA tests provided positive results in 29 samples and negative results in 4 samples. There were no discrepant samples.

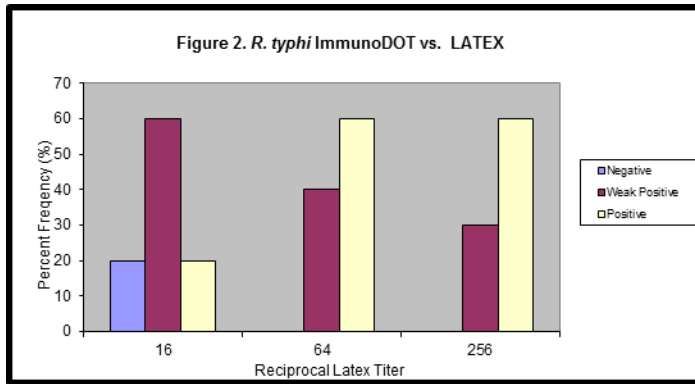
Figure 1 shows results of ImmunoDOT tests with 33 clinical samples compared with IFA titer. The frequency is the percentage of samples at a given IFA titer that gave the indicated ImmunoDOT reaction.

Figure 1



ImmunoDOT vs. latex: A total of 95 normal specimens was tested. The latex assay and *R. typhi* ImmunoDOT yielded similar results with normal specimens. Latex incorrectly identified two samples as weakly positives. The *R. typhi* ImmunoDOT test was in agreement with IFA in identifying these samples. Of the normal sera tested, the *R. typhi* ImmunoDOT did not indicate any false positives. A comparison between the *R. typhi* ImmunoDOT and latex titer is illustrated in Figure 2. Thirty-three sera from presumed typhus patients were tested by both Latex (serial dilutions at 1:16, 1:64, and 1:256) and ImmunoDOT. The frequency is the percentage of samples at a given Latex titer that gave the indicated ImmunoDOT reaction. Of the 33 presumptive positive samples tested, three results differed between the latex assay and *R. typhi* ImmunoDOT (samples 92074, 92079, and 92095). Each discrepancy was resolved using either IFA and/or documented antibody rise in titer (as in the case of sample 92079). In two instances, the samples were weakly positive with *R. typhi* ImmunoDOT test and negative by the latex test. All of those samples were positive by IFA. One sample was reactive by *R. typhi* ImmunoDOT and negative by the latex test. This sample was positive by IFA.

Figure 2



Correlation data for all test comparisons are presented in Table 1 and Table 2.

Comparative study with paired sera: Ten acute and convalescent serum pairs were tested by IgG ELISA, IFA, and latex agglutination. Not all of these sera showed a rise in titer by any test method (Table 1).

Table 1: R. typhi ImmunoDOT CLINICAL TRIALS

Pairs	ImmunoDOT	ELISA IgG	ELISA IgM	IFA Titer	Latex Titer
A1	3	2.91		256	64
A2	3	1.49		256	64
B1	3	0.08	1.66	256	64
B2	3	2.48		256	256
C1	0	0.05		0	0
C2	2	0.64		16	64
D1	3	1.32		256	256
D2	3	0.96		256	256
E1	1	0.32	1.37	16	16
E2	3	2.53		256	256
F1	3	-		16	256
F2	0	0.17		0	16
G1	1-2	1.99		256	256
G2	3	2.79		256	256
H1	2	1.28	0.05	256	16
H2	3	0.77		256	256
I1	2	1.28		256	16
I2	2	1.46		256	256
J1	3	0.69		256	64
J2	2	1.27		256	0

*ImmunoDOT were graded by the number of positive dots: (0) = negative, (+1-4) = positive; ELISA is positive at > 0.5; IFA and latex are positive at 1:16.

Note: IFA performed with polyvalent FITC conjugate.

Clinical interpretation of Table 1:

A1/A2:	Samples taken past peak serological titer; titers declining.
B1/B2:	Samples taken early in course where IgM antibody predominates as demonstrated by R. typhi Immuno-DOT, ELISA, and latex results.
C1/C2:	Possible exposure to rickettsial agent cross-reactive with R. typhi. Definitive serological diagnosis is not possible.
D1/D2:	Samples taken at peak of infection, beyond rising titer.
E1/E2:	4-fold rise confirms active recent infection.
F1/F2:	Declining titers suggest recent infection.
G1/G2:	Change in ELISA and R. typhi ImmunoDOT infection.
H1/H2:	Change in IFA, R. typhi ImmunoDOT, and Latex suggests active recent infection. ELISA result appears in error.
I1/I2:	Samples taken past peak infection, titers declining.
J1/J2:	Samples taken at or past peak infection, titers stable or declining.

Table 2: R. typhi ImmunoDOT Clinical Trials

Sample	Pairs	ImmunoDOT	ELISA IgG	ELISA IgM	IFA titer	LATEX titer
92004		4	2.84		256	256
92002		4	2.78		256	256
92030		3	2.71		256	64
92033		2	1.78		256	256
92034		1	1.28		64	64
92035		2	2.63		256	16
92047		4	1.97		256	256
92048	A2	3	1.49		256	64
92049	B1	3	0.08	1.66	256	64
92050	A1	3	2.91		256	64
92051	C2	2	0.64		16	64
92054		0	0.07		0	0
92056		2	1.56		256	16
92058	C1	0	0.05		0	0

Sample	Pairs	ImmunoDOT	ELISA IgG	ELISA IgM	IFA titer	LATEX titer
92061	B2	3	2.48		256	256
92062	D1	3	1.32		256	256
92063	D2	3	0.96		256	256
92064		2	2.25		64	64
92068	E2	3	2.53		256	256
92069	E1	1	0.32	1.37	16	16
92073	F1	3	1.00		16	256
92074	F2	0	0.17		0	16
92075	G1	1	1.99		256	256
92076	G2	3	2.79		256	256
92079	H1	2	0.18	0.50	16	0
92081	H2	3	0.77		256	256
92085		2	1.91		256	256
92088	I2	2	1.46		256	256
92090	I1	3	1.28		256	16
92093		1	1.45		256	256
92094	J1	3	0.69		256	64
92095	J2	2	1.27		256	0
S0002		0	0.03		0	0
Correlation			32/33		33/33	30/33

ImmunoDOT were graded by the number of positive dots: (0) = negative, (+1-4) = positive; ELISA is positive at > 0.5; IFA and latex are positive at 1:16.

Conclusion: Based on these data, the comparative sensitivity between Latex and *R. typhi* ImmunoDOT is 93% (27/29) and the specificity is 98% (93/95). After resolution of the results from discrepant samples, the final **relative and comparative sensitivity between ImmunoDOT and IFA is 100% with a specificity of 100%.**

COMPARATIVE STUDY WITH MATCHED SERA

A 59 year old male presented in a rickettsial unit with fever, headache, rash, myalgia, microscopic hematuria and heart murmur. The patient had been working with RMSF, typhus, and scrub typhus rickettsiae and was accidentally stuck with an *R. typhi*-contaminated needle on 04-27-92. The following serological studies were performed using currently available methodologies in the rickettsial laboratory (Table 3).

Table 3: Description of a recently acquired case of murine typhus

Serum Sample	ELISA (IgM)	ELISA (IgG)	IFA (IgM)	IFA (IgG)	ImmunoDOT
DM 92-4	64	< 64	64	< 64	0
DM 92-3	< 64	< 64	< 64	< 64	0
4-18-92					
DM 92-2	128	128	128	128	+2
6-22-92					
DM 92-3C	256	256	256	256	+3
6-22-92					
DM 92-6	256	1024	256	1024	+4
6-24-92					
DM 92-7	≥ 2048	≥ 2048	≥ 2048	≥ 2048	+4
7-2-92					
DM 92-8	≥ 2048	≥ 2048	≥ 2048	≥ 2048	+4
7-14-92					

ImmunoDOT were graded by the number of positive dots: (0) = negative, (+1-4) = positive; ELISA is positive at >0.5; IFA and latex are positive at 1:16. Serum DM 92-4 stored prior to study.

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QUICK REFERENCE PROCEDURE

IMMUNODOT R.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 15 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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