IMMUNODOT™
RICKETTSIA CONORII

IVD For In Vitro Diagnostic Use

INTENDED USE

The GenBio ImmunoDOT *Rickettsia conorii* (Mediterranean Spotted Fever) test is a semi-quantitative enzyme immunoassay for the detection of total IgG and IgM antibodies to *Rickettsia conorii* for the serological confirmation from serum, plasma or heparinized whole blood. This test is intended to be performed by trained medical technologists only.

SUMMARY AND EXPLANATION (1) (2) (3)

Rickettsia conorii, the etiologic agent of Mediterranean Spotted Fever (MSF), also referred to as Boutonneuse fever, is endemic in the Mediterranean regions of Africa and Europe as well as India. It is transmitted by ticks, which also use dogs and rodents as hosts.

Predominant manifestations of MSF include fever, headache, myalgia, rash, and rash on palms. The triad of fever, headache, and rash is reported in 50% of the cases. Fever, in adults, ranges from 103 to 105°F. Maculopapular rash and petechiae first appear on the extremities then progress to the trunk. The incubation period, from actual infection to acute onset of symptoms, ranges from 5 to 7 days. Complications are rare and the mortality rate is low, even in untreated cases. Symptoms may last 10 to 20 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 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 phosphate to produce an easily seen, distinct spot.

REAGENTS

Assay Strip: Includes a positive human IgG control, negative control and four dilutions of *R. conorii* antigen
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 (4). 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 (5). Follow recommended Universal

Precautions for bloodborne pathogens as defined by OSHA (6), Biosafety Level 2 guidelines from the current CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (7), WHO Laboratory Biosafety Manual (8), 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 ImmunoDOT *R. conorii* assay can be performed on serum, heparinized plasma or heparinized whole blood. The test requires approximately 10µl of serum or plasma or 20µl 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 run 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 water 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 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).

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.

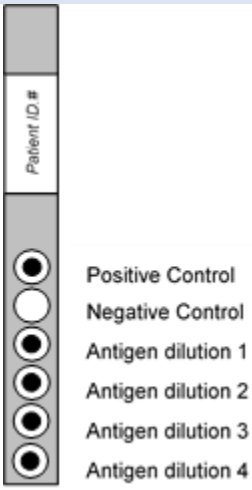
QUALITY CONTROL

The top 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. 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 be either 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, moderately positive (1:32-1:64) for *R. conorii*, 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. conorii* antibody level is indicated by the number of reactive dots; the more reactive dots, the higher the antibody level.

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 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. conorii*. 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 or four positive dots in the second sample confirms the presence of recent, specific antibody. [Caution: If the patient has been treated with antibiotics, the convalescent serum sample may not show a higher antibody level than the acute sample]. If sample remains 1 or 2 dots, a second methodology should be considered or sample interpreted as taken beyond rising titer (titers declining).

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 MSF should not be made based on results of GenBio ImmunoDOT R. conorii 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 groups, and among different rickettsial groups, the GenBio ImmunoDOT R. conorii test may react with other rickettsial species, e.g., R. rickettsii and *R. typhi*.
- 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.

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.

PERFORMANCE CHARACTERISTICS (9)

During a *R. conorii* epidemic in southern Africa, more than 30% of exposed subjects were symptomatic. Samples from 169 exposed subjects were testing using commercial IFA and ImmunoDOT kits. Table 1 illustrates performance using symptomatology as a disease criterion. Table 2 shows the relative performance compared to IFA.

Table 1: Performance using Symptomatology Criteria

Condition	ImmunoDOT Reactive	
	Acute	Convalescent
Asymptomatic	49% (18/37)	51% (49/97)
Possible Case	25% (2/8)	66% (6/9)
Probable Case	62% (16/26)	93% (25/27)

Table 2: Relative Performance

Sera	Sensitivity	Specificity
Acute	100%	48%
Convalescent	100%	33%

BIBLIOGRAPHY

1. **Elisberg, B L and Bozeman, F M.** The Rickettsiae. [book auth.] E H Lennette and N J Schmidt. *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections.* 1979.
2. **Fiset, P.** Clinical Laboratory Diagnosis of Rickettsial Disease of Man. [book auth.] G D Hsuing and R H Green. *Handbook of Clinical Laboratory Science Vol. 1, Part 2.* West Palm Beach : CRC Press, 1978, p. 361.
3. **Centers for Disease Control.** *Rickettsial Disease Surveillance Report No. 2.* Washington DC : U.S. Department of Health and Human Services, 1979.
4. **US Centers for Disease Control.** *Manual Guide – Safety Management No. CDC–22 Decontamination of Laboratory Sink Drains to Remove Azide Salts.* Atlanta : Centers for Disease Control, 1976.
5. —. *HHS Publication No. (CDC) 93-8395, 3rd ed: Biosafety in Microbiological and Biomedical Laboratories.* Washington DC : US Government Printing Office, 1993.
6. **US Department of Labor, Occupational Safety and Health Administration.** *29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.*
7. **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.
8. **World Health Organization.** *Laboratory Biosafety Manual 3rd ed.* Geneva : World Health Organization, 1991.

9. **Broadhurst, L E, et al.** Laboratory Evaluation of a Dot Blot enzyme Immunoassay for Serologic Confirmation of Illness due to Rickettsia conorii. *Am J Trop Med.* 1998, Vol. 58, 6, p. 786.

QUICK REFERENCE PROCEDURE

IMMUNODOT R.CONORII

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.

GenBio

15222-A Avenue of Science
San Diego, CA 92128

EC REP

MedEnvoy Global B.V.
Prinses Margrietplantsoen 33 - Suite 123
2595 AM The Hague
The Netherlands

ITALY CONTACT

Meridian Healthcare srl
68, via G. Guglielmino
Tremestieri Etneo, CT 95030
Phone: +39 095 725 68 69
FAX: +39 095 725 44 54