IMMUNODOT™
COXIELLA BURNETII (Q FEVER)

IVD For In Vitro Diagnostic Use

INTENDED USE

The ImmunoDOT test is an enzyme immunoassay for the qualitative detection of antibodies to *Coxiella burnetii* in serum specimens and is used as an aid in the diagnosis of *C. burnetii* (Q fever) infection. The test is used as an aid for diagnosis of *C. burnetii* (Q fever) infection in subjects suspected with consistent signs and symptoms to suspect the infection.

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

Q fever is a zoonosis caused by *Coxiella burnetii*, an obligate gram-negative intracellular bacterium. *C. burnetii* infects various hosts, including humans, ruminants (cattle, sheep, goats), and pets and, in rare cases, reptiles, birds, and ticks. This bacterium is excreted in urine, milk, feces, and birth products. These products, especially the latter, contain large numbers of bacteria that become aerosolized after drying. *C. burnetii* is highly infectious, and only a few organisms can cause disease.

Because of its spore like life cycle, *C. burnetii* can remain viable and virulent for months. Infection can be acquired via inhalation or skin contact, and direct exposure to a ruminant is not necessary for infection. Rare human-to-human transmissions involving exposure to the placenta of an infected woman and blood transfusions have been reported. Sexual transmission is also possible.

C. burnetii infection in livestock often goes unnoticed. In humans, acute *C. burnetii* infection is often asymptomatic or mistaken for an influenza like illness or atypical pneumonia. In rare cases, *C. burnetii* infection becomes chronic, with devastating results, especially in patients with preexisting valvular heart disease. Because of its highly infectious nature and has an inhalational route of transmission, *C. burnetii* is recognized as a potential agent of bioterrorism. The Centers for Disease Control and Prevention (CDC) classifies Q fever as a Category B agent.

ASSAY PRINCIPLE

The ImmunoDOT assay utilizes an enzyme-linked immunoassay (EIA) dot technique for the detection of IgG and IgM antibodies to *C. burnetii*. 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 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 positive human antibody and negative control and four dilutions of *C. burnetii* antigens, three Phase II dots and one Phase I dot

Diluent (#1): Consists of buffer salts with <0.1% NaN3 (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 (5). 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 (6). Follow recommended Universal Precautions for bloodborne pathogens as defined by OSHA (7), Biosafety Level 2 guidelines from the current CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (8), WHO Laboratory Biosafety Manual (9), and/or local, regional and national regulations.

STORAGE

Store at 2-8°C. Bring reagents to room temperature (15-30°C) before use. Once reagents are opened assure storage at 2-8°C and avoid contamination, especially microbial contamination. Assuming good laboratory practices are used, opened reagents remain stable as indicated by the expiration date.

SPECIMEN COLLECTION AND HANDLING

ImmunoDOT test is performed using 10 µl of serum, which should be collected according to standard practices. Serum may be stored at 2-8°C for up to five days and frozen below -20°C for extended periods.
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 a 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 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”.
5. Add patient specimen (approximately 10 µL) 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. A false positive dot may be identified if the assay strip is not dry when interpreted.

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.

Equivocal reactivity must be cautiously interpreted. If in doubt, interpret as nonreactive.

QUALITY CONTROL

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.)
Quality control procedures should follow applicable guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Unless otherwise required, it is recommended that control sera be tested upon receipt of a kit. If the control is not reactive, results should not be reported and GenBio Technical Service should be contacted before the kit is used again.
The kit uses reagent controls to assure performance each time a test is performed. The Positive Control window (well #1) contains human serum and tests reagent reactivity. It must be reactive but the intensity must not be used as a calibrator. As a negative reagent control check, the backgrounds around dots and the bottom window (#6) must be white. If the positive is not reactive or the negative is reactive, do not interpret the assay strip.

INTERPRETATION

Single Specimen, Interpretation: If Phase 1 dot is reactive, and if all three Phase 2 dots are reactive, report as positive. Otherwise, report as negative.
Equivocal: If only Phase 1 or Phase 2 is positive, the result should be cautiously interpreted. Report as Equivocal or Phase 1 positive associated with consistent symptomatology may indicate chronic infection.
Initially weakly reactive: Weakly reactive specimens (1 or 2 dots) should be cautiously interpreted. Collection of convalescent specimen is recommended.
Seroconversion: Seroconversion occurs when the first serum pair (acute) reports negative and the convalescent specimen result is positive.

Equivocal reactivity must be cautiously interpreted. If in doubt, interpret as negative. Additional information such as symptomatology and/or IFA may be required to distinguish acute versus chronic disease.

LIMITATIONS

- Treatment is often indicated prior to completion of serologic diagnosis, which requires at least two weeks. Diagnosis of Q fever should not be made based on results of the GenBio Q Fever 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 among rickettsial antigens must be considered during interpretation. Because of the presence of common antigens within the same rickettsial groups, and among different rickettsial groups, the *C. burnetii* ImmunoDOT test may react with other rickettsial species, e.g., *Rickettsia rickettsii*.
- Serological assay methods may yield different results for weakly reactive samples. An alternate method is recommended for these specimen types. If the patient has been treated with antibiotics, the serum sample, including the convalescence serum pair, may not report as a clinical positive. An alternate methodology should be considered.
- Results obtained from immunocompromised individuals should be interpreted with caution.

EXPECTED RESULTS

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.
Phase 1 or 2 specific antibody may be present in a healthy population. As an example, forty-seven (47) sera collected from U.S. blood donors (presumed to be healthy adults) were tested using ImmunoDOT. Three (3) subjects reported Phase 1 positive and two (2) different subjects reported Phase 2 positive results. It is noted that all ImmunoDOT positive serum pairs (see Sensitivity)

reported both a Phase 1 and Phase 2 positive result. There are no clinically defined cases that the ImmunoDOT reported as only Phase 1 or Phase 2 positive; however IFA methods vary. Presumably this is due to the increased Phase 1 sensitivity of the enzyme immunoassay system.
Twelve (26%) of the sera reported one Phase 2 dot reactive and one (2%) serum reported two (2) Phase 2 dots reactive. These 13 (28%) sera are reported as negative.

PERFORMANCE CHARACTERISTICS

Study Population: Serum pairs collected from thirty-eight (38) patients clinically defined with *C. burnetii* are tested. These serum pairs are part of a *C. burnetii* reference serum panel maintained by the university laboratory. All patients exhibited symptomatology and laboratory results consistent with *C. burnetii* infection.
Relative Performance, compared to IFA: The indirect immunofluorescence assay (IFA) method is often used sero-diagnostic method to detect anti-*C. burnetii*. Each of the 76 specimens (38 serum pairs) are tested with four commercial IFA kits (Phase 1 and 2, IgG and IgM) and four university developed IFA test methods (Phase 1 and 2, IgG and IgM). These specimens are also tested with ImmunoDOT. All testing was performed by the university laboratory. IFA interpretation is made using the following criteria:

Table 1: Interpretation Criteria

Method	Positive Interpretation Criteria
Phase 1 IgG IFA	Antibody detected at screening dilution (titer). Commercial method screening titer is 1:16 and university method screening titer is 1:20.
Phase 2 IgG IFA	Four-fold dilution (titer) difference between serum pair specimens or either serum pair specimen reports antibody dilution (titer) greater than 1:1000
Phase 1 IgM IFA	Antibody detected at screening dilution (titer). Commercial method screening titer is 1:16 and university method screening titer is 1:10.
Phase 2 IgM IFA	Antibody detected at screening dilution (titer). Commercial method screening titer is 1:16 and university method screening titer is 1:10.

There is no significant difference (p=0.05, Chi-square, confidence limits based on Gart and Nam's score method with skewness correction) between assay methods.

Table 2: Sensitivity

Method	Phase 1	Phase 2	Combined
ImmunoDOT	67-91%	67-91%	67-91%
Commercial IFA	61-87%	67-91%	70-93%
University IFA	55-83%	55-83%	67-91%

Serology tests are most useful to test patients with signs and symptoms consistent with the disease and predictive values depend on incidence within the tested population. An example of an asymptomatic population, represented by forty-seven (47) healthy U.S. blood donors, is shown below.

Table 3: Specificity

	Specificity
Phase 1	96% (45/47)
Phase 2	94% (44/47)

PRECISION

Interpretation requires observation/reading of a blue-brown dot. Precision is 100% reproducible for strongly reactive ("positive") dots. Equivocal reactivity ("borderline dot color") exhibits high analyte and reader interpretation variability.

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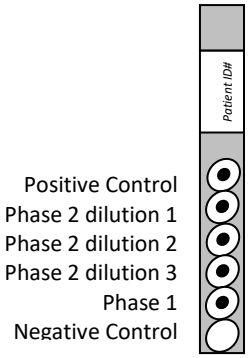

QUICK REFERENCE PROCEDURE

IMMUNODOT COXIELLA BURNETII (Q FEVER)

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 of 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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