ImmunoDOT™

TORCH TEST

For *In Vitro* Diagnostic Use

INTENDED USE

The ImmunoDOT TORCH Test is an enzyme immunoassay (EIA) procedure intended for determining the presence of antibodies to *Toxoplasma gondii*, rubella virus, cytomegalovirus and herpes simplex virus in serum and heparinized whole blood to indicate previous or current infection by these agents. This product is intended for use in physician offices and clinical laboratories. This product is not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors.

SUMMARY AND EXPLANATION

When primary toxoplasma infection is acquired during pregnancy, there is a significant risk of infection to the fetus.[1,2] Toxoplasmosis is also a serious complication in the immunocompromised host,[3] and is considered in the differential diagnosis of infectious mononucleosis syndrome.

Rubella virus infection in a healthy individual typically presents few problems. On the other hand, infection of pregnant women during the first trimester often leads to serious sequelae in the infant.

Cytomegalovirus (CMV) is a common infectious agent in man. Primary infections in normal individuals are most often asymptomatic, although various syndromes may occur including CMV mononucleosis, hepatitis or pneumonitis.[4] In the United States, approximately 1 - 2% of all fetuses are born with congenital CMV infection acquired when the mother undergoes primary infection or reactivation of latent infection during pregnancy. Approximately 5 - 10% of infants become infected with CMV at delivery or shortly thereafter.[5,6] CMV infection is often a serious complication in patients undergoing immunosuppressive therapy.[4]

Two variants of herpes simplex virus (HSV) are recognized, type 1 and type 2. The two types of virus share many cross-reacting antigens and a majority of antibodies produced in response to an initial infection are reactive with both virus types.[7] This test detects antibodies to both virus types and does not differentiate between them.

Serological procedures can be very useful in ruling out a particular diagnosis. The absence of antibody to a given infectious agent can exclude that agent from consideration as a cause of the illness while a positive finding indicates past or present infection.[8] A variety of methods have been employed for the detection of antibodies including indirect immunofluorescence, complement fixation, passive hemagglutination, neutralization and enzyme-linked immunosorbent assays (ELISA) [9,10]. These procedures each require a serum sample and, in many cases, equipment such as a fluorescent microscope or spectrophotometer is necessary.

ASSAY PRINCIPLE

The ImmunoDOT TORCH Test utilizes an EIA dot technique for the detection of antibodies. The antigens are dispensed as discrete dots onto a solid membrane. After adding specimen to a reaction vessel, 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 phosphatase to produce an easily seen, distinct dot.

REAGENTS

Assay Strip positive control human IgG, *T. gondii* (RH strain), rubella virus (HPV - 77 strain), CMV (AD - 169 strain), HSV (MacIntyre strain) and negative control.

Diluent (#1). buffered diluent (pH 6.2-7.6),protein stabilizers with <0.1% NaN_3.

Enhancer (#2). sodium chloride with <0.1% NaN_3.

Conjugate(#3). alkaline phosphatase conjugated goat anti-human antibodies in buffered diluent (pH 6.2-8.5) with <0.1% NaN_3.

Developer(#4). 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 TORCH 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 deionized or distilled water must be used as Clarifier. 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.

Some assay components contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Warning - Potential Biohazardous Material. The antigens on the assay strip have been rendered non-infectious; nevertheless, caution should be exercised. Human sera used in the preparation of this product were tested and found nonreactive for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, HTLV-1 and hepatitis C virus. Because no test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting disease.[11]

Storage

Store reagents and assay strips at 2-8°C. Reagents must be at room temperature (15 - 30 °C) before use. Reagents must be used within one hour of placement in the heated workstation. Avoid contamination of reagents which may produce invalid results.

SPECIMEN COLLECTION AND HANDLING

The TORCH Test can be performed on either serum or heparinized whole blood. The test requires a volume of approximately 10 µL of serum or approximately 20 µL of whole blood.

Serum and heparinized whole blood are collected according to standard practices. Finger stick samples are stable at ambient temperature for one day. Serum and heparinized whole blood may be stored at 2-8°C for up to five days. Serum may be frozen below -20°C for extended periods. Freezing whole blood samples is not advised.

PROCEDURE

Materials Provided

ImmunoDOT TORCH Assay Strips	Reaction Vessels
Diluent (#1)	Package Insert
Enhancer (#2)	
Conjugate (#3)	
Developer (#4)	

Materials Required But Not Provided

GenBio Workstation

Specimen collection apparatus (e.g., finger sticking device, venipuncture equipment)

Timer

Analytic quality distilled or deionized water to be used as Clarifier

Pipets

Absorbent toweling to blot assay strip dry

Positive control serum

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 Clarifier vessel provided. 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, Assay Strips labeled and inserted into the Strip Holder.
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 Clarifier 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 5 minutes.
3. Remove Assay Strip from Reaction Vessel and swish in the Clarifier. 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 Clarifier 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 Clarifier as described (step #3). DO NOT remove the Assay Strip from the Clarifier.
8. Allow the Assay Strip to stand in the Clarifier for 5 minutes.
9. Remove Assay Strip from Clarifier 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 Clarifier 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 window of the assay strip is a positive reagent control and must be positive for further interpretation. The bottom window is the reagent negative control and must be negative for further interpretation. Reagent controls serve the purpose to assure the user that the individual reagents used are active at the time of use. If either reagent control is invalid, 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 each kit lot performs as described. In addition, a positive control serum (Product No. 2215), moderately positive for all antigens, is separately available. The performance of each kit lot may be confirmed upon receipt by running a determination using the positive control serum and obtaining a positive result for each of the four TORCH Test antigens.

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

Negative: A negative result for each antigen demonstrates little or no antibody presence, indicating the patient may be susceptible to primary infection.

Positive: A positive result for each antigen demonstrates the presence of that antibody.

T. gondii	Indicates previous or current infection and immunity to future primary infection.
Rubella	The presence of "any level of detectable antibody should be considered presumptive evidence of immunity." [12]
CMV	Indicates previous or current infection and presumed capability of transmitting the viral infection although such individuals are not necessarily currently contagious. Presence of antibody does not assure protection from disease.
HSV	Indicates previous or current infection with HSV, type 1 or type 2. This test does not differentiate HSV-1 and HSV-2 antibodies but detects both antibodies. Presence of antibody to HSV does not imply protection from disease. For example, previous oral HSV infection does not protect against genital infection.

LIMITATIONS

If testing of a sample occurs less than five days following primary infection, detectable specific antibody may not yet be present.

This test is a qualitative, screening procedure and cannot be used to detect rises in antibody titer or to diagnose active infection. The assay is not intended for final selection of CMV negative donors for blood transfusion or organ transplantation. The test may be used to screen potential recipients.

Antibody screening in the compromised host must be interpreted with caution. The antibody response of an immunosupressed individual may differ from that of the immunocompetent host.

Since maternal antibody will be detected in infants under one year of age, assessment of previous infections or immune status of infants is inappropriate using this test alone.

EXPECTED RESULTS

The prevalence of antibodies to *T. gondii*, rubella virus, CMV, and HSV in any given population has been shown to be dependent on age, socioeconomic status and geographic location. On the average, approximately 30% of adults have antibody to *T. gondii*, 60% to CMV, and 80% to HSV.[13,14,15,16,17] The primary factor affecting the prevalence of rubella antibody is the vaccination policy within the population. The U.S. vaccination policy was implemented during the 1970's, and has consistently lowered the number of antibody negative individuals.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

Serum and heparinized whole blood finger stick samples from overtly healthy adults and children were tested. Serum results are presented. The heparinized whole blood results did not differ significantly. Corresponding IFA screening for *T. gondii* (1:16), CMV (1:16), and HSV (1:10) were performed. For rubella, the corresponding screening methods at site A were latex agglutination (1:10) and EIA. At site B the methods were HAI and latex agglutination (1:10). For rubella, the one observed false negative sample (equivocal by EIA) was positive in the assay on retest. The false positive results were resolved to be antibody positive using a 1:1 (undiluted) latex agglutination test. Table 1 presents the serum results.

Table 1: Sensitivity and Specificity of TORCH Test

Item	% Sensitivity	% Specificity
T. gondii	94% (68/72)	>99% (123/123)
Rubella virus	>99% (363/363)*	98% (39/40)*
CMV	95% (100/105)	>99% (43/43)
HSV	96% (103/107)	98% (47/48)

* Rubella studies were conducted in two outside facilities:

Site	Sensitivity		Specificity	
	Initial	Resolved	Initial	Resolved
A	177/178	180/180	18/20	18/18
B	180/180	183/183	21/25	21/22

Reproducibility

Reproducibility results (within test and between test) are presented in Table 2. The studies of *T. gondii*, CMV, and HSV were conducted by three outside medical assistants on fifty heparinized whole blood samples and serum samples. The study of rubella virus was conducted by three outside medical assistants and two outside laboratory technicians (sites A and B) on a negative, low positive (1:8 HAI), moderate, and high serum sample in replicates of eleven.

Table 2: Reproducibility of TORCH Test

Item	Negative Specimen*	Positive Specimen*
T. gondii	99% (172/174)	94% (118/126)
Rubella virus	>99% (55/55)	99% (163/165)
CMV	98% (118/120)	94% (169/180)
HSV	97% (122/126)	98% (171/174)

* (# correct replicates/total number replicates)

Cross-Reactivity

There is neither measurable cross-reactivity between HSV or CMV antigens nor to varicella-zoster virus antigen. For example, serum and blood samples which were negative for antibody to HSV in the TORCH Test were found to contain antibody to CMV and/or VZV when tested by IFA. Five clinical samples were evaluated for each potential cross-reaction.

It was also demonstrated that false positive reactions were not obtained for any of the TORCH Test antigens when at least one sample negative for the test antibody contained high titers of antinuclear antibodies.

Predictive Values

Positive (PPV) and negative (NPV) predictive values are dependent on the assay sensitivity and specificity and the prevalence of antibody positive cases in the population. Predictive values are used to assess the usefulness of a test for an intended use within a defined population. As an aid, Table 3 presents calculated predictive values for selected populations. Because these tests are intended to screen an overtly healthy population to assess antibody status, a high positive predictive value is preferred for the intended use.

Table 3: Predictive Values for TORCH Test

Item	Antibody Prevalence (%)	Positive* Predictive Value (%)	Negative* Predictive Value (%)
T. gondii [3]			
Mt. States	3	>99	>99
Northeast	20	>99	99
Test population	31	>99	98
Rubella			
Hypothetical	99	>99	>99
Hypothetical	95	>99	>99
Test population	90	>99	>99
CMV[13]			
4-10 year olds	30	94	98
Adults	60	98	94
Test population	68	99	92
HSV[17]			
21-25 year olds	55	99	95
Adults	80	>99	86
Test population	68	99	92

* PPV = True Positives/(True Positives + False Positives)

NPV = True Negatives/(True Negatives + False Negatives)

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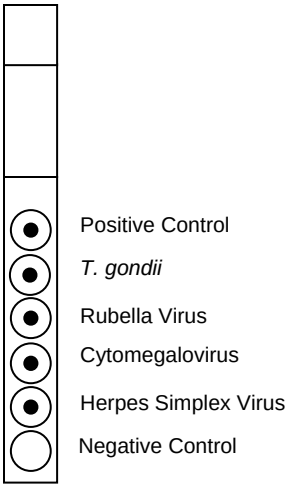

QUICK REFERENCE PROCEDURE

ImmunoDOT TORCH

Set-Up

- Make sure Workstation is at temperature.
- Place reaction Vessels into slots in Workstation and add water to the Clarifier Vessel.
- 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 Clarifier for 30 - 60 seconds.
- Place strip in Vessel #1, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #2, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #3, mix, **let stand 15 min.**
- Remove strip, place in Clarifier, **let stand 5 min**.
- Place strip in Vessel #4, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, 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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