ImmunoDOT™

TORCH IgM TEST

For *In Vitro Diagnostic* Use

INTENDED USE

The ImmunoDOT TORCH IgM Test is an enzyme immunoassay (EIA) procedure intended for determining the presence of IgM antibodies to *Toxoplasma gondii*, rubella virus, cytomegalovirus and herpes simplex virus in serum to indicate previous or current infection by these agents.

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). 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 IgM 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 IgM, *T. gondii*, 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 IgM 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 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 for Day 2 procedure must be at room temperature (15-30°C) before use. Avoid contamination of reagents.

SPECIMEN COLLECTION AND HANDLING

ImmunoDOT Test is performed on serum. The test requires 10 µL of serum. Lipemic or hemolyzed serum has not been shown an acceptable specimen.

Store samples at room temperature for no longer than eight hours. If the assay will not be completed within eight hours, refrigerate the sample at 2-10°C. If the assay or shipment of the samples will not be completed within 48 hours, freeze at -20°C.

PROCEDURE

Materials Provided

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

Materials Required But Not Provided

Reaction vessel rack

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 dry assay strip

Positive control serum

Negative control serum

Assay Procedure

Day 1

1. Remove one Reaction Vessel per test from the product box and insert into first row of slots in reaction vessel rack.
2. Appropriately label the Assay Strips.
3. If Strip Holder 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.
4. Fill Clarifier Vessel or an appropriate container with sufficient water to cover **all** reactive windows of the assay strip.
5. Place 2 mL Diluent (#1) in Reaction Vessel #1.
6. Add 10 µL patient specimen or control to Reaction Vessel #1.

7. Prewet Assay Strip by immersing in Clarifier for 30-60 seconds.
8. Using several (5-10) quick up and down motions with the Assay Strip, mix reagent and specimen thoroughly in Reaction Vessel #1. Let stand overnight **in the refrigerator (2-10°C).**

Day 2

1. Remove Reaction Vessel #1 from refrigerator.
2. Allow all reagents to reach room temperature.
3. Prepare fresh Clarifier vessel (refer to Day 1, step 4).
4. Remove three Reaction Vessels per test and insert in the remaining slots in the reaction vessel rack.
5. Place 2 mL Enhancer (#2) in Reaction Vessel #2, Conjugate (#3) in Reaction Vessel #3 and 2 mL Developer (#4) in Reaction Vessel #4.
6. Remove Assay Strip from Reaction Vessel #1 and swish in fresh Clarifier. Use a swift back and forth motion for 5-10 seconds allowing for optimal washing of the Assay Strip's membrane windows.
7. Place Assay Strip into Reaction Vessel #2. Mix thoroughly with several (5-10) quick up and down motions. Let stand at room temperature for **10 minutes.**
8. Remove Assay Strip from Reaction Vessel #2 and swish in Clarifier as described (step 6).
9. Place Assay Strip into Reaction Vessel #3. Mix thoroughly with several (5-10) quick up and down motions. **Let stand for 30 minutes.**
10. Remove Assay Strip from Reaction Vessel #3 and swish in Clarifier as described (step 6). **Do not** remove the Assay Strip from the Clarifier.
11. Allow the Assay Strip to stand in the Clarifier for **5 minutes.**
12. Determine the incubation time for step 13.

    Note: To determine the incubation time required for this step, the actual room temperature is measured and the required incubation time is used. Table 1

Table 1: Developer Incubation Times

Room Temperature (°C)	Reaction Vessel #4 Incubation Time (min)
18	40
20	35
22	25
24	20
26	15
28	10

13. Remove Assay Strip from Clarifier and place into Reaction Vessel #4. Mix thoroughly with several (5-10) quick up and down motions. Let stand the appropriate amount of time.
14. Remove Assay Strip from Reaction Vessel #4 and swish in Clarifier as described (step 6).
15. 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.

INTERPRETATION

Negative:	A negative result for each antigen demonstrates little or no antibody presence.
Positive:	A positive result for each antigen demonstrates the presence of that antibody. In the case of toxoplasma and rubella infections the presence of IgM may indicate current or recent infection, but should not be considered relevant unless the specific IgG antibodies are also present. CMV and HSV IgM serology interpretation is more complex since recurrent infections may also cause specific IgM reactivity.

LIMITATIONS

If testing of a sample occurs less than five days following primary infection, detectable specific antibody may not yet be present. Specific IgG reactivity must be present to interpret a positive IgM reactivity.

This test is a qualitative, screening procedure and cannot be used to detect rises in antibody titer.

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

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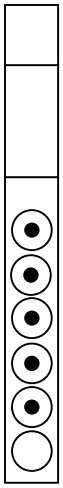

QUICK REFERENCE PROCEDURE

ImmunoDOT TORCH IgM

- Add 10 µL serum to Vessel #1.
- **Prewet** assay strip in Clarifier for 30 - 60 seconds.
- Place strip in Vessel #1, mix, **let stand overnight in the refrigerator.**
- Bring Reagents #2, #3 and #4 to room temp.
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #2, mix, **let stand 10 min.**
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #3, mix, **let stand 30 minutes.**
- Remove strip, place in Clarifier, **let stand 5 min**.
- Place strip in Vessel #4, mix, **let stand according to Table 1.**
- 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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