The OnSite TORCH Panel Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection and differentiation of antibodies (IgG and IgM) to Toxoplasma gondii (T. gondii), rubella virus, Cytomegalovirus (CMV), herpes simplex virus 1 (HSV-1), and herpes simplex virus 2 (HSV-2) in human serum, plasma, or whole blood. It is intended to be used by professionals as a screening test and provides a preliminary test result to aid in the diagnosis of infection with T. gondii, rubella virus, CMV, HSV-1, and HSV-2.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

T. gondii is an obligate intracellular protozoan parasite with a worldwide distribution (Source Toxo – 1,2). Serological data indicates that approximately 30% of the population of most industrialized nations is chronically infected with the organism (Source Toxo – 3). Women infected with T. gondii during pregnancy possess a risk of transmission to their unborn child. Seronegative women should avoid risk factors for T. gondii transmission including owning cats, eating raw and undercooked meats, and gardening (Toxo Review).

Rubella virus infection most often occurs during childhood and manifests with mild symptoms. However, if a rubella virus infection occurs during pregnancy, a group of birth defects collectively known as congenital rubella syndrome (CRS) may develop, including congenital eye defects, deafness, congenital heart diseases, and mental retardation (Source Rubella – 1). The presence of anti-rubella virus IgM or high titers of anti-rubella virus IgG (>200 IU/mL) (Source Rubella – 2) are suggestive of acute rubella infection. Lower titers of anti-rubella virus IgG (10–15 IU/mL) (Source Rubella – 3, 4) are suggestive of convulsions and immunosuppressive illness. A patient without protective titers of anti-rubella virus IgG (<10 – 15 IU/mL) is considered to be at risk of acquiring a rubella virus infection during pregnancy (Source Rubella – 3, 4).

Cytomegalovirus (CMV) infections are widespread and usually asymptomatic; however, the virus may persist as a latent or chronic infection (Source CMV – 1). The majority of individuals that contract CMV infections remain asymptomatic (Source CMV – 2). Congenital transmission of CMV can cause hearing loss, mental retardation, or central nervous system motor disorders in infected infants (SOURCE CMV – 5, 6). The presence of anti-CMV IgM is suggestive of primary infection (Source CMV – 8, 9). Differentiation of anti-CMV IgG and IgM can help discriminate between primary and recurrent infections since anti-CMV IgM is rarely found in recurrent infections (Source CMV – 8).

HSV-1 and HSV-2 are neurotropic DNA viruses that are responsible for recurrent and primary genital and orofacial herpetic infections. The HSV-1 genotype accounts for approximately 75% of HSV-1 infections, while the HSV-2 genotype accounts for approximately 25% of HSV-2 infections (Source HSV – 1, 2). HSV-1 is generally acquired during childhood via nonsexual contact and affects mainly the orofacial area (Source HSV-1). HSV-2 is almost always sexually transmitted and is the main cause of genital herpes. HSV-1 and HSV-2 can infect both the genital and orofacial areas (Source HSV-2 – 1). HSV-1 and HSV-2 infections have different prognoses. Type-specific serological diagnosis is beneficial, which can be achieved by using glycoprotein G1 and glycoprotein G2 as recommended by the CDC (Source HSV-1 – 7). The OnSite TORCH Panel Rapid Test uses both HSV-1 glycoprotein G1 and HSV-2 glycoprotein G2 to differentiate antibodies from the two viruses.

The TORCH Panel Rapid Test detects and differentiates IgG and IgM antibodies present in serum, plasma or whole blood that are generated in response to each of the TORCH Panel diseases (T. gondii, rubella virus, CMV, HSV-1, and HSV-2). The test can be performed within 10 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE

The OnSite TORCH Panel Rapid Test is a lateral flow chromatographic immunoassay consisting of 5 panel strips assembled in one cassette.

The Toxo IgG/IgM Rapid Test consists of: 1) a burgundy colored conjugate pad containing T. gondii antigens conjugated with colloidal gold (Toxo conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with mouse anti-human IgG for detection of IgG anti-T. gondii, the M line is pre-coated with mouse anti-human IgM for detection of IgM anti-T. gondii, and the C line is pre-coated with a control line antibody.

The Rubella IgG/IgM Rapid Test consists of: 1) a burgundy colored conjugate pad containing rubella virus antigens conjugated with colloidal gold (rubella conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing three test lines (G1, G2 and M lines) and a control line (C line). The G1 and G2 lines are pre-coated with mouse anti-human IgG for detection of IgG anti-rubella virus, the M line is pre-coated with mouse anti-human IgM for detection of IgM anti-rubella virus, and the C line is pre-coated with a control line antibody.

The CMV IgG/IgM Rapid Test consists of: 1) a burgundy colored conjugate pad containing CMV antigens conjugated with colloidal gold (CMV conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with mouse anti-human IgG for detection of IgG anti-CMV, the M line is pre-coated with mouse anti-human IgM for detection of IgM anti-CMV, and the C line is pre-coated with a control line antibody.

The HSV-1 IgG/IgM Rapid Test consists of: 1) a burgundy colored conjugate pad containing HSV-2 type specific glycoprotein G1 antigens conjugated with colloidal gold (HSV-1 conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with mouse anti-human IgG for detection of IgG anti-HSV-1, the M line is pre-coated with mouse anti-human IgM for detection of IgM anti-HSV-1, and the C line is pre-coated with a control line antibody.

The HSV-2 IgG/IgM Rapid Test consists of: 1) a burgundy colored conjugate pad containing HSV-2 type specific glycoprotein G1 antigens conjugated with colloidal gold (HSV-2 conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with mouse anti-human IgG for detection of IgG anti-HSV-2, the M line is pre-coated with mouse anti-human IgM for detection of IgM anti-HSV-2, and the C line is pre-coated with a control line antibody.

The Toxo IgG/IgM Rapid Test consists of: 1) a burgundy colored conjugate pad containing T. gondii antigens conjugated with colloidal gold (Toxo conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with mouse anti-human IgG for detection of IgG anti-T. gondii, the M line is pre-coated with mouse anti-human IgM for detection of IgM anti-T. gondii, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen, will bind to the antigen conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgG forming a burgundy colored M line, indicating an IgM positive result for that particular disease.

IgG antibodies to the target, if present in the specimen, will bind to the antigen conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgG forming a burgundy colored G line, indicating an IgG positive result for that particular disease. In the case of rubella, an IgG anti-rubella virus titer ≥15 IU/mL produces a burgundy colored G1 test line. An IgG anti-rubella virus titer ≥2250 IU/mL produces burgundy colored G1 and G2 test lines.

Absence of any test lines (M, G, G1, or G2) suggests a negative result for that particular test strip. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control antibodies regardless of color development on any of the test lines. If the C line does not develop, the test result for that test strip is invalid, and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One dosicant
2. 10 μL capillary tubes
3. Sample Diluent (REF SB-R0253C, 5 mL/bottle)
4. One package insert (instruction for use)

MATERIALS MAY BE REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. Lancing device for whole blood test

WARNINGS AND PRECAUTIONS

For in Vitro Diagnostic Use

This package insert must be read completely before performing the test. Failure to follow the insert may lead to inaccurate test results.

Do not open the sealed pouch unless ready to conduct the assay.

Do not use expired devices.

4. Bring all reagents to room temperature (15-30°C) before use.

Do not use different types of M as a substitute for the components in this kit.

Do not use hemolyzed blood specimens for testing.

Wear protective clothing and disposable gloves while handling the kit reagents and clinical samples. Wash hands thoroughly after performing the test.

Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.

Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.

Dispose of all specimens and materials used to perform the test as biohazardous waste.

Handle the negative and positive controls in the same manner as the patient specimens.

The test result should be read 10 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside 10-15 minutes should be considered invalid and must be repeated.

Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2-20°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before testing. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle using standard bio-safety procedures.

Plasma
Step 1: Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by venipuncture.
Step 2: Separate the plasma by centrifugation.
Step 3: Carefully withdraw the plasma into a new pre-labeled tube.

Serum
Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
Step 2: Allow the blood to clot.
Step 3: Separate the serum by centrifugation.
Step 4: Carefully withdraw the serum into a new pre-labeled tube.
Test specimens as soon as possible after collecting. If not tested immediately, store specimens at 2-8°C for up to 5 days. For longer storage, specimens should be frozen at -20°C.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross hemolysis, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Whole Blood
Drops of whole blood can be obtained by different methods, such as venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer® ). Do not use hemolyzed blood for testing.

The whole blood specimens must be tested within 24 hours of collection. The specimens should be stored in refrigeration (2-8°C), if not tested immediately.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen.

Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen ID number.

Step 4: Fill the capillary tube with specimen not exceeding the specimen line as shown in the images below. The volume of specimen is approximately 10 µL. For better precision, transfer specimen using a pipette capable of delivering a 10 µL volume.

Hold the capillary tube vertically, dispense the entire specimen into the center of the sample well making sure that there are no air bubbles.

Immediately add 2 drops (about 60-80 µL) of Sample Diluent to the sample well with bottle positioned vertically.

Step 5: Set up the timer.

Step 6: Read results at 10-15 minutes. Positive results may be visible in as short as 1 minute. If the C line does not develop, perform the test with a new device.

QUALITY CONTROL

1. Internal Control: This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.

2. External Control: Good Laboratory Practice recommends using external positive and negative controls to ensure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature during storage of the kit falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. NEGATIVE RESULT:
If only the C line develops, the test indicates that antibodies to the target are not detected in the specimen. The result is negative or non-reactive.

2. POSITIVE RESULT:
2.1 In addition to the presence of the C line, if only the M line develops, the test result indicates the presence of IgG antibodies to the target. The result is IgG target positive or reactive.

2.2 In addition to the presence of the C line, if only the M line develops, the test indicates the presence of IgM antibodies to the target. The result is IgM target positive or reactive.

2.3 In addition to the presence of the C line, if both the M and S lines develop, the test indicates the presence of IgG antibodies and IgM antibodies to the target. The result is IgG and IgM target positive or reactive.

Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made.

3. INVALID:
If no C line develops on any test strip, the assay is invalid regardless of any color development on the test lines (G and M) as indicated below. Only the test strip without C line development is invalid. All other results are valid. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. Clinical Performance for IgM Test:
Samples were collected from susceptible subjects and tested with the OnSite TORCH Panel Rapid Test and with a commercial rapid test or ELISA on the market. Sensitivities and specificities are shown in the following table:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Overall Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxo</td>
<td>91.6%</td>
<td>98.5%</td>
<td>97.8%</td>
</tr>
<tr>
<td>Rubella</td>
<td>98.3%</td>
<td>95.0%</td>
<td>97.7%</td>
</tr>
<tr>
<td>CMV</td>
<td>98.2%</td>
<td>99.1%</td>
<td>98.6%</td>
</tr>
<tr>
<td>HSV-1</td>
<td>90.6%</td>
<td>91.4%</td>
<td>90.7%</td>
</tr>
<tr>
<td>HSV-2</td>
<td>93.8%</td>
<td>96.0%</td>
<td>95.3%</td>
</tr>
</tbody>
</table>

2. Clinical Performance for IgG Test:
Samples were collected from susceptible subjects and tested with the OnSite TORCH Panel Rapid Test and with a commercial rapid test or ELISA on the market. Sensitivities and specificities are shown in the following table:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Overall Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxo</td>
<td>100.0%</td>
<td>99.0%</td>
<td>99.0%</td>
</tr>
<tr>
<td>Rubella</td>
<td>99.0%</td>
<td>100.0%</td>
<td>99.6%</td>
</tr>
<tr>
<td>CMV</td>
<td>97.6%</td>
<td>99.1%</td>
<td>98.8%</td>
</tr>
<tr>
<td>HSV-1</td>
<td>80.0%</td>
<td>85.6%</td>
<td>85.0%</td>
</tr>
<tr>
<td>HSV-2</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

3. Cross-Reactivity:
No false positive test results were observed on 4-10 specimens from the following disease states or special conditions:

<table>
<thead>
<tr>
<th>Disease State</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Overall Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAV</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>HBV</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>HCV</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>HEV</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>HIV</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>NCG</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Dengue</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>H. pylori</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>TB</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>T. pallidum</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Typhoid</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>ANA</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>HAMA</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>RF (up to 2,500 IU/mL)</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

4. Interference:
Common substances (such as pain and fever medication and blood components) may affect the performance of the OnSite TORCH Panel Rapid Test. This was studied by spiking these substances into three levels of IgG and IgM standard controls (negative, weak, positive and strong positive). The results demonstrate, at the concentrations tested, the substances studied do not affect the performance of the OnSite TORCH Panel Rapid Test.

List of potentially interfering substances and concentrations tested:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Albumin</td>
<td>60-200 mg/L</td>
</tr>
<tr>
<td>2. Bilirubin</td>
<td>20-150 mg/dL</td>
</tr>
<tr>
<td>3. Creatinine</td>
<td>442-1,500 µM</td>
</tr>
<tr>
<td>4. EDTA</td>
<td>1-40 µM</td>
</tr>
<tr>
<td>5. Glucose</td>
<td>5.0-20.0 µM</td>
</tr>
<tr>
<td>6. Human IgG</td>
<td>50-150 mg/dL</td>
</tr>
<tr>
<td>7. Hemoglobin</td>
<td>2-10 g/dL</td>
</tr>
<tr>
<td>8. Heparin</td>
<td>1,000-3,000 IU/L</td>
</tr>
<tr>
<td>9. Salicylic acid</td>
<td>4.34 mmol/L</td>
</tr>
<tr>
<td>10. Sodium citrate</td>
<td>3.8%</td>
</tr>
</tbody>
</table>

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to T. gondii, rubella virus, CMV, HSV-1, and HSV-2 in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate test results.

2. The OnSite TORCH Panel Rapid Test is limited to the qualitative detection of antibodies to T. gondii, rubella virus, CMV, HSV-1, and HSV-2 in serum, plasma or whole blood. The intensities of the test lines do not have linear correlation with the antibody titers in the specimen.

3. A negative or non-reactive result for an individual subject indicates absence of detectable T. gondii, rubella virus, CMV, HSV-1, and HSV-2 antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with T. gondii, rubella virus, CMV, HSV-1, or HSV-2.

4. A negative or non-reactive result can occur if the quantity of the anti-T. gondii, rubella virus, CMV, HSV-1, and HSV-2 antibodies present in the specimen is below the detection limits of the assay or the antibodies that are detected are not present during the stage of the disease in which a sample is collected.

5. Infection may progress rapidly. If the symptom persists, while the result from OnSite TORCH Panel Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
6. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor (>2,500 IU/mL) may affect expected results.

7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES