SUMMARY AND EXPLANATION OF THE TEST

Rubella virus, the etiological agent of German measles, generally causes a mild viral disease which sometimes resembles common measles, but with none of the serious consequences often seen in young measles patients. When contracted in the first trimester of pregnancy, however, rubella may infect the fetus through the placenta causing deafness, cataracts, microcephaly and/or cardiac abnormalities in addition to hepatitis and/or chorioretinitis, thrombocytopenic purpura, anemia, and low birth weight. These multiple abnormalities are commonly referred to as a congenital rubella syndrome. Other consequences of rubella infection during pregnancy may include spontaneous abortion, miscarriage, and stillbirth.14,15,16

The availability of an attenuated rubella virus vaccine has greatly reduced the natural incidence of rubella infection. Nevertheless, it is recommended that all women of childbearing age be tested for the presence of rubella antibodies to assure that non-immune individuals are detected and subsequently vaccinated. Patient immune status to the rubella virus has been determined for many years using various serological methods. One widely accepted method is the hemagglutination inhibition test (HAI).17 Latex agglutination, in comparison with HAI, is quicker and easier to perform.

PRINCIPLE OF THE PROCEDURE

The ASI Rubella Test reagent is a suspension of polystyrene latex particles of uniform size coated with soluble rubella virus antigen from disrupted virus. Latex particles allow visual observation of the antigen-antibody reaction. When a patient’s serum containing antibodies to rubella virus agglutinates latex particles coated with soluble antigen, the mixture appears opalescent. A true negative result (no prozone) using undiluted samples indicates the absence of antibodies to the rubella virus (<1:128 titer). A negative result using diluted samples indicates that antibodies to rubella virus are absent or not at a dilution >1:20 (no agglutination). The diagnosis of primary or recent rubella infection is made by comparing antibody titers in paired sera. The timing of sample collection in paired sera is critical. The first sample (acute sera) should be collected as soon after rash onset as possible or at the time of exposure. The second sample (convalescent sera) should be obtained 10-21 days after the onset of rash or, at least 30 days after exposure, if no clinical symptoms appear. Acute and convalescent phase sera should be quantitatively analyzed simultaneously for antibodies to rubella along with reactivity and nonreactive controls. A four-fold or greater titer rise in acute and convalescent sera is indicative of a primary or recent rubella infection. In unresolved cases testing for the presence of rubella virus antigen is recommended as an additional indicator of infection.18

PERFORMANCE CHARACTERISTICS

QUALITATIVE TEST

A clinical study of the ASI Rubella Test was conducted using 282 sera diluted 1:10. There was a 98.5% overall agreement between the two methods. 162 of the 282 were also examined with ASI Rubella Test using the undiluted procedure and the results compared to those obtained with HAI. There was a 100% overall agreement between the two methods.

When the results obtained using ASI Rubella Test on a 1:10 dilution of these same 162 clinical specimens were compared with a second commercially available rubella latex test (also 1:10 dilution), a sensitivity and specificity of 100% was obtained.

A separate clinical study was conducted on 143 clinical serum samples comparing the results obtained with ASI Rubella Test (using a 1:10 serum dilution) to those obtained with HAI. Combining the two studies, a total of 425 sera were tested with a 98.4% overall agreement between the two methods.

MISQUANTITATIVE TEST

Clinical studies performed at two medical centers on a total of 100 sera compared titers obtained with ASI Rubella Test to those obtained using HAI. 75% of sera were within ±1 dilution interval; 96% of sera were within ±2 dilution intervals and 100% of sera were within ±3 dilution intervals.

Day to day reproducibility studies were conducted at two different medical centers. A panel of 6 sera with titers ranging from non-reactive to 1:160 by HAI was assayed with one lot of ASI Rubella Test using the quantitative procedure during three consecutive days. Results were 100% reproducible within one dilution. ASI Rubella Test results were confirmed using sera from 10 patients with rubella infection and sera from 10 patients who had no evidence of rubella. All 10 sera pools showed a four-fold or greater rise in titer. Four additional patients who received rubella vaccine were studied. Serocconversion was detected in all patients.

WARRANTY

This product is warranted to perform as described in its labeling and ARLINGTON SCIENTIFIC, INC. literature. ARLINGTON SCIENTIFIC, INC. disclaims any implied warranty of merchantability or fitness for a particular purpose and in no event shall ARLINGTON SCIENTIFIC, INC. be liable for consequential damages.
WARNINGS AND PRECAUTIONS

For in vitro Diagnostic Use

1. ASI Rubella TEST and CONTROLS contain sodium azide. Azides in contact with lead and copper plumbing may react to form highly explosive metal azides. When disposing of reagents containing azide, flush down the drain with large quantities of water to prevent azide buildup.

2. ASI Rubella TESTS CONTROLS contain human serum or plasma which has been treated at the donor level for HBsAg and for HIV-1, HIV-2 and HCV antibodies and found to be nonreactive. As no known test offers complete assurance that infectious agents are absent, the controls should be considered potentially infectious and universal precautions should be used. The CDC/NHAH Manual "Biosafety in Microbiological and Biomedical Laboratories" describes how these materials should be handled in accordance with Good Laboratory Practice.

3. Reagents must be well mixed before use.

4. The virus vaccine strain used in the preparation of ASI Rubella Test latex reagent has been previously disrupted. Bioassays procedures demonstrate that disrupted virus is inactivated. However it is recommended that users follow the same safety regulations in effect for the handling of other types of potentially infectious material.

5. Do not pipet by mouth.

6. Do not smoke, eat, drink or apply cosmetics in areas where plasma/serum samples are handled.

7. All tests, whether or not the results are positive, should be handled with the same safety regulations in effect for the handling of other types of potentially infectious material.

8. Do not use past the expiration date indicated on the kit.

9. Do not mix different lot numbers of test or control samples.

10. Do not use plasma.

ASSAY PROTOCOL - QUALITATIVE

When testing undiluted specimens, the weak reactive control and nonreactive control should be used undiluted by following the procedure outlined in the steps below. The weak reactive control should show agglutination different from the uniform appearance of the nonreactive control. If no agglutination takes place the test should be repeated, and if there is no positive reaction the kit should be discarded.

When testing specimens at a 1:10 dilution, the weak reactive control should be used diluted 1:10 by following the procedure outlined in the steps below. It is unnecessary to dilute the nonreactive control for testing. The weak reactive control should show agglutination different from the uniform appearance of the nonreactive control. If no agglutination takes place, the test should be repeated. At a 1:5 dilution of the weak reactive control previously prepared. If there is no visible reaction, continue testing specimens as the weak reactive control is formulated to produce agglutination at a titer of 1:1/5 one dilution. If there is no positive reaction the kit should be discarded.

For undiluted specimens:

1. Place 25 µl of the sample (or drop of control) onto one of the circles of the disposable card.
2. Using a new plastic stirrer for each circle, spread the sample over the entire surface of the circle.
3. Dispose one drop of the latex reagent onto each circle containing the sample.
4. Place the card on an automatic rotator and cover to maintain humidity. Rotate at 100 rpm for 8 minutes.
5. Immediately following the 8 minute rotation, read for the presence or absence of agglutination.

For a 1:10 slope specimen:

1. Prepare a 1:5 dilution of the sample (or weak reactive control) on the disposable card by pipetting 100 µl of the dilution buffer and 25 µl of the sample (or a drop of control) in the square section of the card and mix several times with the same pipet (it is unnecessary to dilute the nonreactive control before testing).
2. Transfer 25 µl of the dilution buffer on the circle beside the square section.
3. Transfer 25 µl of the 1:5 dilution from the square section into the dilution buffer and mix several times with the same pipet. Discard 25 µl from the circle.
4. Using a new plastic stirrer for each circle, spread the sample over the entire surface of the circle.
5. Dispose one drop of the latex reagent onto each circle containing a sample.
6. Place the card on an automatic rotator and cover to maintain humidity. Rotate at 100 rpm for 8 minutes.
7. Immediately following the 8 minute rotation, read for the presence or absence of agglutination.

ASSAY PROTOCOL - SEMIQUANTITATIVE

The reactive and weak reactive controls should be treated as if they were samples by following steps 1-7 outlined below. Substitute one drop of control for the 25 µl of patient specimen. The nonreactive control should be tested undiluted.

1. Prepare a 1:5 dilution of the sample (or control) on a square section of the disposable card by pipetting 100 µl of the dilution buffer and 25 µl of the sample (or a drop of control) and mix several times with the same pipet.
2. Place 25 µl of the dilution buffer on the circles marked 1:10 to 1:160 of the disposable card.
3. Transfer 25 µl of the 1:5 dilution from the square section directly into the dilution buffer in the circle marked "Laboratory". Repeat this procedure until the contents of all circles are spread.
4. Dispose one drop of the latex reagent onto each of the different circles containing the serial dilutions.
5. Place the card on an automatic rotator and cover to maintain humidity. Rotate at 100 rpm for 8 minutes.
6. Immediately following the 8 minute rotation, read for the presence or absence of agglutination.

QUALITY CONTROL

Controls with graded reactivity should be included in each test run to confirm optimal reactivity of the latex reagent. If control samples do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not exhibit the expected results for the control samples, discontinue use of the test and contact ASI Technical Support at 800.654.0146.

INTERPRETATION OF RESULTS - QUALITATIVE

The presence of any visible agglutination, significantly different from the nonreactive control, indicates the presence of anti-rubella antibodies. The reactive control should show agglutination at a titer of 1:160 or greater. The weak reactive control should show agglutination different from the nonreactive control at a titer of 1:8. The data collected will correlate with that obtained using the HAI test.

INTERPRETATION OF RESULTS - SEMIQUANTITATIVE

The approximate rubella titer will correspond to the highest serum dilution that still presents a clearly visible agglutination. The nonreactive control should show no agglutination. When the semi-quantitative test is performed with a patient specimen, the presence of antibodies against rubella virus in the serum sample indicates recent exposure to the rubella virus. A qualitative test performed on a single sample serum can be used to estimate the immune status of the individual.

The approximate rubella titer will correspond to the highest serum dilution of the patient specimens that still presents a clearly visible agglutination. The reactive control should show agglutination at a titer of 1:1-10. The data collected will correlate with that obtained using hemagglutination inhibition assays. This protocol will fail to detect low levels of antibodies found in patient samples that are positive undiluted.