LIMITATIONS OF THE PROCEDURE

1. Test results obtained with **ASI Rubella Test** must be evaluated by the physician in light of the clinical symptoms shown by the patient.

2. **ASI Rubella Test** has been tested for the detection of rubella antibodies in serum. Performance with plasma has not been established.

3. To verify that the procedure works properly the use of reactive, weak reactive and nonreactive controls is recommended.

4. Acute and convalescent sera must be tested simultaneously. The absence of a four-fold titer rise does not exclude the possibility of exposure and infection.

5. Reaction times longer than specified may cause false positive results due to a drying effect.

6. Temperature of the reagents and samples is crucial to test outcome: it should be between 20 and 30° C.

EXPECTED VALUES

A positive qualitative test on either undiluted samples (≥1-2 IU/ml) or samples diluted 1:10 (≥10-20 IU/ml and equivalent to the HAI test at 1:8) indicates previous infection with rubella virus. Each individual laboratory must determine the antibody level which it considers clinical protection against future rubella infection.

A true negative result (no prozone) using undiluted samples indicates the absence of antibodies to the rubella virus (<1-2 IU/ml). A negative result using samples diluted 1:10 indicates that antibodies to rubella virus are absent or at a level <10-20 IU/ml.

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, while 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 reactive and nonreactive controls. A four-fold or greater titer rise between acute and convalescent sera is indicative of a primary or recent rubella infection. In unresolved cases testing for the presence of rubella IgM is recommended as an additional indicator of infection.[1,6]

PERFORMANCE CHARACTERISTICS

QUALITATIVE TEST

A clinical study of the **ASI Rubella Test** was conducted using 282 sera diluted 1:10. There was a 99.5% overall agreement between the two methods. 162 of the 282 were also assayed 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 to those obtained using another commercially available rubella 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 99.4% overall agreement between the two methods.

SEMIQUANTITATIVE 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 dilutions intervals.

Day to day reproducibility studies were conducted at two different medical centers. A panel of 6 sera with titers ranging from nonreactive 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 studied using paired sera from 10 naturally occurring infections. All 10 serum pairs showed a four-fold or greater rise in titer. Four additional patients who received rubella vaccine were studied. Seroconversion was detected in all pairs of sera.

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.

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ASI
RUBELLA TEST

For *in vitro* diagnostic use

Catalog Number	Kit Size
600030	30 Tests
600100	100 Tests
600500	500 Tests

CPT Code: 86762

INTENDED USE: The **ASI Rubella Test** is a rapid latex particle agglutination test for the qualitative and semiquantitative determination of rubella virus antibodies in serum. The test aids in the diagnosis of recent or active rubella infection and in the determination of immune status. These materials are intended to be acquired, possessed, and used only by health professionals.

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 hepatosplenomegaly, icterus, 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.[1,2,3,4]

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).[4,5] Latex agglutination, in comparison with HAI, is quicker and easier to perform.[6]

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 serum containing antibodies against rubella virus is mixed with the latex reagent, the uniform appearance of the latex suspension will convert to a visible agglutination.

The sensitivity of the **ASI Rubella Test** kit is 1-2 IU/ml when performed with undiluted serum. This is greater than the sensitivity of the HAI at a 1:8 dilution. The sensitivity of the **ASI Rubella Test** kit is 10-20 IU/ml when run with serum diluted 1:10. This is approximately equal to the sensitivity of the HAI at 1:8. The **ASI Rubella Test** has a specificity of >99%.

REAGENTS

RUBELLA LATEX REAGENT - Suspension of polystyrene latex particles coated with soluble virus antigen from disrupted virus in a buffer, with 0.1% sodium azide as preservative.

REACTIVE CONTROL - Human serum diluted to a titer of 1:160, with 0.1% sodium azide as preservative.

WEAK REACTIVE CONTROL - Human serum diluted to a titer of 1:10, with 0.1% sodium azide as preservative.

NONREACTIVE CONTROL - Diluted nonreactive human serum, with 0.1% sodium azide as preservative.

DILUTION BUFFER - Phosphate buffered saline pH 7.2, containing bovine serum albumin, with 0.1% sodium azide as preservative.

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 tested 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/NIH Health Manual "Biosafety in Microbiological and Biomedical Laboratories" describes how these materials should be handled in accordance with Good Laboratory Practice.[7]

3. Reagents must be well mixed before use.

4. The vaccine virus strain used in the preparation of **ASI Rubella Test** latex reagent has been previously disrupted. Bioassay 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. Any cuts, abrasions or other skin lesions should be suitably protected.

Handling and Procedural Notes

1. In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for reagents or samples.

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

3. Do not interchange components of one kit with those of another kit.

4. To maintain optimal sensitivity, the **ASI Rubella Test** kit should be removed from the refrigerator approximately 5 minutes prior to use. Warm the latex reagent by rolling the vial between hands. Replace in 2-8° C immediately after use.

5. When suspending latex, do not shake. Invert the vial several times until the latex reagent is uniformly suspended without visible clumping.

6. Although the reagents and controls contain preservatives, they remain sensitive to contamination. Handle with the necessary precaution. Discard latex reagent, buffer or controls if they become contaminated.

Storage Instructions

Store all reagents at 2-8° C in an upright position when not in use. Do not freeze. Pipets and cards do not require refrigeration. The reagents can be damaged, or the latex sensitivity can be altered, by improper storage and/or handling.

SPECIMEN COLLECTION AND STORAGE

1. Use fresh serum collected by centrifuging clotted blood.

2. If the test cannot be carried out on the same day, the serum must be stored between 2-8° C for no longer than 8 days after collection. For longer periods the samples must be frozen (-20° C).

3. It is not necessary to inactivate the serum.

4. As in all serological tests, hemolytic or contaminated serum must not be used.

5. Do not use plasma.

6. For diagnosis of rubella infection, paired sera (acute and convalescent) should be obtained. The acute sera should be collected as soon after rash onset as possible or at the time of exposure and the 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 sera should be tested simultaneously for antibodies to rubella using the semiquantitative procedure.[1]

PERFORMANCE OF THE TEST

Materials Provided:

	30 Tests	100 Tests	500 Tests
RUBELLA LATEX REAGENT	0.5 ml	1.6 ml	5 x 1.6 ml
REACTIVE CONTROL	0.5 ml	1.0 ml	2 x 1.0 ml
WEAK REACTIVE CONTROL	0.5 ml	1.0 ml	2 x 1.0 ml
NONREACTIVE CONTROL	0.5 ml	1.0 ml	2 x 1.0 ml
DILUTION BUFFER	5 ml	35 ml	2 x 35 ml
DISPOSABLE STIRRER	33	120	600
TEST CARDS (9 WELL)	4	14	70

Additional Materials Required:
- Mechanical rotator set at 100±5 rpm with humidity cover
- Timing device, minute and second capability
- Automatic pipets

TEST PROCEDURE
PREPARATION FOR THE ASSAY

1. Remove reagents from refrigeration approximately 5 minutes prior to use; warm the latex reagent to room temperature (20-30° C) by rolling vial between hands.

2. Mark the test card to identify each sample or control.

3. Invert the latex vial several times to disperse and suspend the latex particles. Vigorous shaking should be avoided.

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 with the 1:5 dilution of the weak reactive control previously prepared. If there is positive reaction, continue testing specimens as the weak reactive control is formulated to produce agglutination at a titer of 1:10± one dilution. If there is no positive reaction the kit should be discarded.

For undiluted specimens:

1. Place 25 µl of the sample (or a 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. Dispense 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±5 rpm for 8 minutes.

5. Immediately following the 8 minute rotation, read for the presence or absence of agglutination.

For a 1:10 specimen dilution:

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. Dispense 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±5 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 to the circle marked 1:5.

4. Using the same pipet, transfer 25 µl of the 1:5 dilution from the square section directly into the buffer in the circle marked 1:10 and mix several times with the same pipet. The serum in this circle is now a 1:10 dilution.

5. With the same pipet, transfer 25 µl of the 1:10 dilution into the buffer in the circle marked 1:20, and mix.

6. Repeat step 5 in succession through the circle marked 1:160.

7. Discard 25 µl from the circle marked 1:160.

8. Using a new plastic stirrer for each sample and control, spread the serum or control dilutions over the entire surface of the circle starting at the highest dilution. Using the same stirrer proceed to the next lower dilution and spread the serum dilution in a similar way. Repeat this procedure until the contents of all circles are spread.

9. Dispense one drop of the latex reagent onto each of the different circles containing the serum dilutions.

10. Place the card on an automatic rotator and cover to maintain humidity. Rotate at 100±5 rpm for 8 minutes.

11. 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 elicit 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 antibodies against rubella virus in the serum sample. This indicates previous exposure to the rubella virus. A qualitative test performed on a single serum sample can be used to estimate the immune status of the individual.

When a negative result is obtained on undiluted serum, the sample should be retested at 1:10, as occasionally a decrease in the degree of agglutination has been reported with high titered specimens.[8,9] High titered specimens, when tested undiluted, may cause the migration of agglutinated particles to the periphery of the circle.

When the **ASI Rubella Test** is initially performed on samples which have been diluted 1:10, the sensitivity obtained is approximately equal to that obtained with the HAI test at 1:8. The data collected will correlate with that obtained using hemagglutination inhibition assays. This protocol will fail to detect low levels of antibodies found in samples that are positive undiluted.[10]

INTERPRETATION OF RESULTS - SEMIQUANTITATIVE

The approximate rubella titer will correspond to the highest serum dilution that still presents a clearly visible agglutination. The reactive control should show agglutination at a titer of 1:160 or greater. The weak reactive control should show agglutination at a titer of 1:10± one dilution. The nonreactive control should show no agglutination.

When the semiquantitative test is performed with an acute and convalescent serum from the same patient, a four-fold or greater rise in antibody titer or seroconversion is indicative of a primary or recent rubella infection. Also a seroconversion may be seen after a vaccination procedure. Some persons previously exposed to rubella may demonstrate a rise in antibody titer. This is thought to represent reinfection and these patients rarely develop symptoms.[11]