## TITLE: Rubella Testing

## PRINCIPLE:

The rubagen 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 rubella virus antibodies is mixed with the latex reagent, the latex suspension will lose its uniform appearance and a visible agglutination will become evident.

**CLINICAL SIGNIFICANCE:**

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 still birth.

The availability of an attenuated rubella virus vaccine has greatly reduced the natural incidence of rubella infection. Testing for the presence of rubella antibodies assure that nonimmune individuals are detected and subsequently vaccinated.

PERSONNEL

Medical Technologist

**MATERIALS REQUIRED**

Rotator

25 ul pipette

MATERIALS PROVIDED

Latex reagent

High positive control (human sera diluted to a titer of 1:160)

Low positive control (human sera diluted to a titer of 1:10)

Negative control (Diluted non-immune human sera)

Disposable slides

Dilution buffer

Stirrers

NOTE: Reagents and controls should be stored at 2-8 degrees C. DO NOT FREEZE.

SAMPLE COLLECTION

Use fresh serum. If the test cannot be performed on the same day of sample collection, the serum must be stored between 2-8 degrees C for up to 8 days after collection. For longer periods, the sample must be frozen (-20 C). It is not necessary to inactivate the serum. As in all serological tests, hemolytic or contaminated serum must not be used. DO NOT USE PLASMA.

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 together with a positive and negative control must be performed simultaneously using the quantitative procedure. Quantitative Rubella Testing is sent to Quest.

For qualitative antibody assay, a single sample is sufficient.

QUALITY CONTROL

1. The latex reagent should be tested with each of the controls, positive and negative, each day the test is performed.
2. All controls should be used following the steps of the Qualitative Technique.
3. The reaction between the positive control and the reagent should show a clear agglutination, different from the uniform appearance of the negative control. If no agglutination takes place, the test should be repeated, and the senior tech should be notified if a positive reaction is not detected.
4. Enter all QC results in the Quality Control Program in the LIS.

Refer to the LIS Procedure Manual for complete instructions.

### QUALITATIVE

With undiluted specimen

1. Allow reagents and controls to reach room temperature (20-30 C).
2. Gently invert the reagent vial to disperse and suspend the latex particles.

 Vigorous shaking should be avoided.

1. Label test card appropriately for each sample and control to be tested.
2. Place 25 ul of high positive, low positive and negative control and each patient sample being tested in the appropriate circle.
3. Spread all controls and samples evenly in the test circles, using the stirrers supplied in the kit.
4. Dispense one free falling drop of latex from the reagent vial directly to each control and sample in the circles.
5. Place each card on rotator and rotate for eight minutes at 100 rpm under a moistened humidifying cover.
6. After 8 minutes, read for the absence or presence of agglutination.

NOTE: All negative samples must be tested using the 1:10 dilution.

With a 1:10 specimen dilution:

1. Allow reagents and controls to reach room temperature (20-30 C).
2. Gently invert the reagent vial to disperse and suspend the latex particles.

 Vigorous shaking should be avoided.

1. Label test card appropriately for each sample to be tested.
2. Prepare a 1:5 dilution of the patient sample by pipetting 100 ul of the dilution buffer and 25 ul of the sample in the appropriate labeled squares for each patient sample.
3. Place 25 ul of dilution buffer in the circle beside the patient’s square section.
4. Transfer 25 ul of the 1:5 dilution in the square section and place in the 25ul buffer circle and mix. Do this for all patient samples.
5. This will be a 1:10 dilution. .
6. Spread all controls and samples evenly in the test circles using the stirrers supplied in the kit.
7. Dispense one free falling drop of latex from the reagent vial directly to the sample in the circle.
8. Place card on rotator and rotate for eight minutes at 100 rpm under a moistened humidifying cover.
9. After eight minutes, read for the absence or presence of agglutination.

### INTERPRETATION OF RESULTS

Qualitative - The presence of any visible agglutination, significantly different from the negative 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. High titered specimens, when tested undiluted, may cause the migration of agglutination particles to the periphery of the circle.

When the rubagen assay 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.

### EXPECTED VALUES

A positive test on either undiluted samples (1-2 IU/ml) or samples diluted 1:10 (10-20 IU/ml) is equivalent to the HAI test at 1:8 and 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 indicated the absence of antibodies to the rubella virus (l-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.

Positive Reactions:

3+ Large clumping with clear background

2+ Moderate clumping with fluid slightly opaque in background

1+ Small clumping with opaque fluid in background

Negative Reactions:

No visible clumping, uniform suspension.

### REPORTING RESULTS

Report all results through the Laboratory Information System. Refer to LIS Procedure Manual for complete instruction.

QUALITATIVE: Negative = None detected

 Positive = Detected

**LIMITATIONS**

-Test results obtained with Rubagen must be evaluated by the physician in light of the clinical symptoms shown by the patient.

-Rubagen has been tested for the detection of rubella antibodies in serum.

 Performance with plasma has not been established.

-To verify that the procedure works properly the use of positive and negative controls is required.

-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.

### INTERFREING SUBSTANCES

###### See Fisher Sure-Vue Rubella Package Insert for interfering substances

### REFERENCE

Sure-Vue Rubella Package Insert

06/15

I-09/15

Fisher Healthcare

Houston, Texas 77038 , 05/2008



