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Procedure No. \_\_\_\_\_

<b>Procedure</b>	<b>Rubagen - NCCLS</b>
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<b>Prepared by</b>	<b>Date Adopted</b>	<b>Supersedes Procedure #</b>

<b>Review Date</b>	<b>Revision Date</b>	<b>Signature</b>

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**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 uniform appearance of the latex suspension will convert to a visible agglutination.

**SPECIMEN:**

**Type:** Use fresh serum. **DO NOT USE PLASMA**

For diagnosis of rubella infection, paired sera (acute and convalescent) should be obtained. The acute sera should be collected as early as possible after exposure or as soon after rash onset and the convalescent sera should be obtained 10 to 21 days later 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.

For qualitative antibody assay a single sample is sufficient

**Handling Conditions:** If the test can not be performed on the same day of sample collection, the serum may be stored at 2-8° C for no longer than 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.

**EQUIPMENT AND MATERIALS****Materials Required:**

- Rotator
- Serological or automatic pipettes
- Stop watch or timer

**Materials Provided:**

- Latex reagent
- High positive control
- Low positive control
- Negative control
- Disposable slides
- Dilution buffer
- Stirrers

**Storage Requirements:** Reagents and controls should be stored at 2-8° C.

**DO NOT FREEZE.****PROCEDURE - QUALITATIVE (Step-wise)**

- Allow the reagents and controls to reach room temperature (20 to 30°C).
- Gently invert the reagent vial to disperse and suspend the latex particles. Vigorous shaking should be avoided.
- Label test card appropriately for each sample and control to be tested.
- Place 1 drop (25 uL) of low positive and negative control and each patient sample being tested in the appropriate circle.
- Spread all controls and samples evenly in the test circles using the stirrers supplied in the kits.
- Dispense one free falling drop of latex from the reagent vial directly to each control and patient sample in the circles.
- Place each card on rotator and rotate for eight minutes at 100 rpm under a moistened humidifying cover.
- After eight minutes, read for the absence or presence of agglutination.
- Use low positive and negative control for qualitative procedure.

*Note:* Before performing a series of determinations check the latex reagent with the low positive control and negative control included in the kit. When testing undiluted specimens, the low positive control should be used undiluted by following the procedure outlined above. The low positive control should show agglutination different from the uniform appearance of the negative control. If no agglutination takes place the test should be repeated, and if there is still no positive reaction the kit should be discarded.

**QUALITATIVE (with a 1:10 dilution):**

- Allow reagents and controls to reach room temperature (20° - 30° C).
- Gently invert the reagent vial to disperse and suspend the latex particles. Vigorous shaking should be avoided.
- Label test card appropriately for each sample and control to be tested.
- Place 100 uL of the dilution buffer in the appropriate labeled squares for each patient sample and control.

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- Place 25 uL of the dilution buffer in each circle for all controls and patient samples being used.
- Take 1 drop (25 uL) of the low positive control and/or patient sample and place in appropriate square and mix with the dilution buffer. This will be a 1:5 dilution. Do this for all patient samples and controls. (The negative control need not be diluted for testing.)
- Take 25 uL of the 1:5 low positive and/or patient sample dilution and place in appropriate circle and mix with dilution buffer. This will be a 1:10 dilution. Do this for all patient samples and controls.
- Spread all controls and samples evenly in the test circles using the stirrers supplied in the kits. Spread over the entire surface of the circle.
- Dispense one free falling drop of latex from the reagent vial directly to the sample in the circle.
- Place card on rotator and rotate for eight minutes at 100 rpm under a moistened humidifying cover.
- After eight minutes, read for the absence or presence of agglutination.

*Note:* Use low positive and negative control for qualitative procedure. The sensitivity obtained with this procedure is similar to the sensitivity obtained with HAI methods.

#### **PROCEDURE - QUANTITATIVE (Stepwise)**

1. Allow the 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.
3. Label test card appropriately for each control and patient.
4. Prepare a 1:5 dilution of the sample (or control) on a square section of the quantitative disposable slide by pipetting 100 uL of the dilution buffer and 25 uL of the sample (or drop of control) and mix several times with the same pipette.
5. Place 25 uL of the dilution buffer on the circles marked 1:10 to 1:160 of the quantitative pipette.
6. Transfer 25 uL of the 1:5 dilution from the square section to the circle marked 1:5.

7. Using the same pipette, transfer 25 uL of the 1:5 dilution from the square section directly into the buffer in circle marked 1:10 and mix several times with the same pipette. The serum in this circle is now a 1:10 dilution.
8. With the same pipette, transfer 25 uL of the 1:10 dilution into the buffer in circle marked 1:20 and mix.
9. Repeat step 8 in succession through circle marked 1:160.
10. Discard 25 uL from circle marked 1:160.
11. Using a new plastic stirrer for each sample dilution and control, start at the highest numbered circle and spread the sample dilution to fill the entire circle. Using a new stirrer for each new row, proceed to the next lower circle and spread the serum dilution in a similar manner. Repeat this procedure until the sample dilutions of all circles are spread.
12. Dispense one free falling drop of latex from the reagent vial directly to each control and sample dilutions in the circle.
13. Place carefully on rotator and rotate for eight minutes at 100 rpm under a moistened humidifying cover.
14. After eight minutes, read for the absence or presence of agglutination.

The following chart represents a quantitative test card showing serum dilutions and results obtained when testing for rubella.

<b>Section</b>	<b>Undiluted</b>	<b>1:5</b>	<b>1:10</b>	<b>1:20</b>	<b>1:40</b>	<b>1:80</b>	<b>1:160</b>
<b>Patient Sample</b>	NA	P	P	P	P	N	N
<b>High Positive Control</b>	NA	P	P	P	P	P	P
<b>Low Positive Control</b>	NA	P	P	N	N	N	N
<b>Negative Control</b>	N	NA	NA	NA	NA	NA	NA

Not Applicable = NA

Negative = N

Positive = P

*Example:* The patient has a 1:40 antibody titer.

### **REPORTING 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 re-tested 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 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.

**Quantitative:**

The approximate rubella titer will correspond to the highest serum dilution that still presents a clearly visible agglutination.

The high positive control should show at least the titer indicated on the label. The low positive control should show agglutination within one dilution of the titer indicated on the label. The negative control should show no agglutination.

When the quantitative test is performed with an acute and convalescent serum from the same patient, a four-fold or greater rise in antibody titer or sero-conversion is indicative of a primary or recent rubella infection. Also, a sero-conversion 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 re-infection and these patients rarely develop symptoms.

**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.

**Limitations:**

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- 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 recommended.
- 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.

**EXPECTED VALUES:**

A positive quantitative 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 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 as possible after rash onset 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 positive and negative 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.