Ruba-Latex

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For *in vitro* diagnostic use only			

Ruba-Latex

Determination of anti-rubella antibodies

SLIDE TEST

PRINCIPLE

Ruba-Latex Test is a rapid slide agglutination procedure, developed for the direct detection of anti-rubella antibodies in human serum.
The assay is performed by testing a suspension of latex particles coated with soluble rubella inactivated virus against unknown samples. The presence or absence of a visible agglutination, indicates the presence or absence of anti-rubella antibodies in the sample tested.

REAGENT COMPOSITION

R1	**Ruba-Latex Reagent**. Suspension of polystyrene latex particles coated with soluble rubella inactivated virus. Contains 0.95 g/L of sodium azide.
R2	**Diluent:** Phosphate buffer, BSA. Contains 0.95 g/L of sodium azide.
CONTROL +	Human serum with an anti-rubella concentration ≥ 10 IU/mL. Contains 0.95 g/L of sodium azide.
CONTROL -	Animal serum. Contains 0.95 g/L of sodium azide.

Precautions: Components of different human origin have been tested and found to be negative for the presence of antibodies anti-HIV 1+2 and anti-HCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious.
Warning: The reagents in this kit contain sodium azide. Do not allow to contact with skin or mucous membranes.

PACKAGING CONTENTS

REF 2730005 kit 100 tests.
1x 1.6 mL Ruba-Latex Reagent, 1x50 mL Diluent, 1x 1 mL Positive control, 1x 1 mL Negative control, 17 Test cards and 2 x 50 disposable pipettes.

STORAGE AND STABILITY

Store at 2-8ºC. Do not freeze. Frozen reagents could change the functionality of the test.
Reagent and Controls are stable until the expiry date stated on the label.

REAGENT PREPARATION

Reagent and Controls are ready to use.

SAMPLES

Fresh, clear serum (Note 1).
Samples should be stored at 2-8ºC up to one week or for longer periods at –20ºC.

MATERIAL REQUIRED

- Automatic pipettes.
- Mechanical rotator, adjustable at 100 r.p.m.
- Laboratory alarm clock.

PROCEDURE

I. Qualitative Test

1. Bring the test reagents and samples to room temperature (Note 2).
2. Dilute the sample with Diluent according the sensitivity of the latex reagent* which is printed on the outside of the box.

Cut off 10 IU/mL	
Latex sensitivity*	Dilution
2.5 UI/mL	1/4 (50 µL sample + 150 µL Diluent)
2.0 UI/mL	1/5 (50 µL sample + 200 µL Diluent)
1.5 UI/mL	1/6.7 (50 µL sample + 200 µL Diluent)

3. Resuspend the antigen vial gently. Aspirate dropper several times to obtain a thorough mixing.
4. Place 1 drop (25 µL) of the diluted sample under test into one of the circles on the card. Dispense 1 drop of positive control and 1 drop of negative control into two additional circles.
5. Add 1 drop of Ruba-Latex Reagent to each circle next to the sample to be tested.
6. Mix the contents of each circle with a disposable pipettes while spreading over the entire area enclosed by the ring. Use separate pipettes for each mixture.
7. Rotate the slide slowly by means of a mechanical rotator (100 r.p.m.) for a period of **8 minutes** (Note 3).
   Observe immediately under a suitable light source for any degree of agglutination.

Reading

Nonreactive: Smooth suspension with no visible agglutination, as shown by negative control.

Reactive: Any degree of agglutination visible macroscopically (Note 4).

II. Semi-quantitative Test

1. For each specimen to be tested place 25 µL of Diluent into each of the circles of a card. Do not spread diluent.
2. To circle one add 25 µL of diluted specimen used in the qualitative test to the Diluent and, using the same tip, mix the Diluent with the sample by repeated aspiration and expulsion of the fluid and transfer 25 µL of the mixture to the saline Diluent in the second circle.
3. Continue with the 2-fold serial dilutions in a similar manner up to the sixth circle, and discard 25 µL from this circle . Final sample dilutions of the diluted sample will be: 1:2, 1:4, 1:16, 1:32, 1:64.
4. Test each dilution as described in steps 4-7 for the Qualitative Test.

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Reading

Same as in Qualitative Test. The titer of the specimen is reported as the highest dilution that shows reactivity. The next higher dilution should be negative.
When expressing the titer in IU/mL, multiply the sensitivity of the latex reagent (which is expressed in the outside of the box) by the reciprocal of the last dilution giving positive result.

e.g. Latex reagent sensitivity: 1.5 IU/mL
Last positive dilution: 1/8
IU/mL = 1.5 x 8 = 12 IU/mL

The diagnosis of acute rubella infection should be confirmed by the presence of IgM antibodies or by comparing the antibody titers between two samples obtained at the beginning of the rash and 1-3 weeks later. A four-fold or greater rise in the titer between these two samples is considered diagnostic of acute infection.

QUALITY CONTROL

Positive and negative controls should be run daily following the steps outlined in the Qualitative Test, in order to check the optimal reactivity of the reagent.
The positive control should produce clear agglutination. If the expected result is not obtained, do not use the kit.

EXPECTED VALUES

Young adults present a antibody prevalence against natural infection or vaccination about 80%. Generally, adults are not affected by the disease although women of childbearing age are susceptible to be infected.

CLINICAL SIGNIFICANCE[1-5]

Rubella is a viral disease caused by the rubella virus, and children and adults may be affected. When pregnant women are affected, specially in the first 3 months of the pregnancy, the virus may affect to the fetus causing several disturbances as spontaneous abortion and stillbirth. The application of the rubella vaccine to the pregnant women reduce considerably the incidence of the disease, nevertheless, it is recommended that all women of childbearing age should be tested for the presence of antibodies to detect the immunity state and to proceed with the vaccination.

ANALYTICAL PERFORMANCES

- The sensitivity of the latex reagent has been adjusted to detect 10 IU/mL of the 1st International Standard 1996 Rubi-I-94 from WHO (NIBS).
- Precision: The reproducibility of the measurements of 10 samples in 3 consecutives days is 100% (± one dilution).
- The analytical sensitivity referred to a EIA reagent is 100%.
- The diagnostic specificity referred to the same reagent is 98.1%.
- Results obtained with this reagent did not show significant differences when compared with reference reagents. Details of the comparison experiments are available on request.

LIMITATIONS OF THE PROCEDURE

- Both samples, acute and convalescent sera, should be tested at the same time. The absence of a four-fold rise in the titer does not exclude the possibility of exposure and infection.
- False negative results due to the prozon effect, should be retested with a 1/20 dilution if there is any suspicion that the sample comes from an infected patient.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory

NOTES

1. The diagnostic of an acute infection requires two samples. The first sample should be collected as soon as possible after the rush unset and the second 1-3 weeks later.
2. The sensitivity of the test may be reduced at low temperatures. The best results are achieved at 15-25ºC.
3. Delays in reading the results may generate in over-estimation of the antibody present.
4. It is recommended to repeat the assay in pregnant women one month later after the first assay in order to detect a possible seroconversion.

SOURCES OF ERROR

- Bacterial contamination of controls and specimens as well as freezing and thawing of the Ruba-Latex Reagent may lead to false positive results.
- Traces of detergent in the test cards may give false positive results. Wash used cards first under tap water until reactants are removed and then with distilled water. Allow to dry in air, avoiding the use of organic solvents as they may impair the special finish on the slide.
- The Ruba-Latex Reagent must not be used beyond its expiry date because a prolonged storage can affect the sensitivity of the suspension.

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