Cromatest

LINEAR

RF-Waaler CE

CONTENTS			
REF	2375005	RF-Waaler	50 Tests
	2375010	RF-Waaler	100 Tests
For *in vitro* diagnostic use only			

RF-Waaler

Determination of rheumatoid factors

SLIDE TEST

PRINCIPLE

RF-Waaler Test is a rapid slide agglutination procedure, based on a modification of the Rose-Waaler technique[1-3] developed for the direct detection and semi-quantitation of rheumatoid factors (RF) in serum.
The assay is performed by testing a suspension of stabilized sheep red cells sensitized with rabbit gamma globulin, against the unknown serums. The presence or absence of a visible agglutination, indicates the presence or absence of RF in the samples tested.

REAGENT COMPOSITION

R — **RF-Waaler Reagent**. Suspension of stabilized sheep red cells (SRC) in a buffered saline solution, sensitized with rabbit IgG anti-sheep erythrocyte. Contains 0.95 g/L sodium azide.

CONTROL + — Human serum with an activity equivalent to appr. 25 IU/mL. Contains 0.95 g/L of sodium azide.

CONTROL - — Animal serum with an activity < 5 IU/mL. 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 contact with skin or mucous membranes.

PACKAGING CONTENTS

REF 2375005, kit 50 tests.
1 vial RF-Waaler Reagent, 1x1 mL Positive control, 1x1 mL Negative control, 3 Test cards and 1x50 disposable stirrers.

REF 2375010, kit 100 tests.
2 vials RF-Waaler Reagent, 1x1 mL Positive control, 1x1 mL Negative control, 3 Test cards and 2x50 disposable stirrers.

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.
After the clear serum has been separated it may be stored at 2-8ºC up to one week or for longer periods at –20ºC.

MATERIAL REQUIRED

- Automatic pipettes.
- Saline solution (0.9% NaCl, only for semi-quantitation procedure).

PROCEDURE

I. Qualitative Test

1. Bring the test reagents and samples to room temperature (Note 1).
2. Resuspend the Reagent vial gently. Aspirate dropper several times to obtain a thorough mixing.
3. Place 1 drop (50 µL) of the serum under test into one of the circles on the card. Dispense 1 drop of positive control serum and 1 drop of negative control into two additional circles.
4. Add 1 drop of RF-Waaler Reagent to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Leave the card undisturbed on a flat surface for **2 minutes**. Immediately after that time tilt the card *very carefully* once to about 30º from horizontal and rest slide flat again for 1 additional minute (Note 2).
7. 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.

II. Semi-quantitative Test

1. For each specimen to be tested place with an automatic pipette 50 µL of 0.9% saline solution into each of the circles of a card. Do not spread diluent.
2. To circle one add 50 µL of specimen to the saline solution and, using the same tip, mix the saline solution with the sample by repeated aspiration and expulsion of the fluid and transfer 50 µL of the mixture to the saline solution in the second circle.
3. Continue with the 2-fold serial dilutions in a similar manner up to the sixth circle, and discard 50 µL from this circle. Final sample dilutions will be: 1:2, 1:4, 1:8, 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 (Note 3).
If the highest dilution tested is reactive repeat the test starting with a preliminary 1:16 dilution. Use a 1:50 dilution of negative control serum in 0.9% saline solution to the replace the 0.9% saline solution in the new 2-fold dilution series.
The approximate RF level (IU/mL) present in the sample may be obtained multiplying the titer of the last positive dilution by the minimum detectable unit (analytical sensitivity).

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 antigen.
The positive control should produce clear agglutination. If the expected result is not obtained, do not use the kit.

EXPECTED VALUES[4,5]

Of those patients with a clinical diagnosis of rheumatoid arthritis approximately 70-80% are seropositive for rheumatoid factor. Positive results were shown for nearly all patients with variants of rheumatoid arthritis such as Felty's or Sjogren's syndrome.
A positive result can be expected in less than 5% of healthy individuals, while in the population aged 60 years and older as many as 30% may be seropositive using agglutination tests for the detection of rheumatoid factor.

CLINICAL SIGNIFICANCE[6-8]

Rheumatoid factors found in the sera of most patients with rheumatoid arthritis as well as in a variety of other diseases, are a group of antibodies most belonging to the IgM class directed against determinants on the Fc fragment of the patients'IgG immunoglobulin.
Rheumatoid factors testing has a high diagnostic value on a tentative diagnosis made on the basis of case history and clinical findings.

PERFORMANCE CHARACTERISTICS

- The minimum detectable unit (analytical sensitivity) is of approximately 8 IU/mL (6-16 IU/mL), tested against a RF standard traceable to WHO Reference Material 64/1.
- Diagnostic specificity: 93.6%.
- Prozone effect: No prozone effect was detected up to 600 IU/mL.
- Results obtained with this reagent did not show significant differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Hemoglobin (<10 g/L), bilirubin (<20 mg/dL) and lipemia (<10 g/L) do not interfere. Other substances may interfere[9].

LIMITATIONS OF THE PROCEDURE

- Positive reactions do occur in conditions other than rheumatoid arthritis such as mononucleosis, hepatitis, syphilis, various other infections and in elderly patients. When tested by the quantitative test, however, most of these specimens give very low results.
- False negative results may be given by patients in the early or in sub-clinical chronic phases of the disease.

NOTES

1. The sensitivity of the test may be reduced at low temperatures. The best results are achieved at 15-25ºC.
2. The delays in reading the results may generate in over-estimation of the antibody present.
3. Titers obtained with sensitized sheep cells do not compare with titers obtained with the latex test. Differences in titer do not reflect a difference between methods in the ability to detect rheumatoid factors.

SOURCES OF ERROR

- Bacterial contamination of controls and specimens as well as freezing and thawing of the 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 all reactants are removed and then with distilled water. Allow to air dry, avoiding the use of organic solvents as they may impair the special finish on the slide.
- The RF-Waaler Reagent must not be used beyond its expiry date because a prolonged storage can affect the sensitivity of the suspension.

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