

# RFscan™

## I. INTENDED USE

The **RFscan™** Latex Test is intended for the qualitative or quantitative determination of circulating rheumatoid factors.

## II. SUMMARY AND EXPLANATION

Rheumatoid factors are antibodies of various immunoglobulin classes, which are directed against specificities in the Fc portion of IgG molecules. Serum rheumatoid factor (RF) appears most commonly, and in the highest titer, in patients with the chronic systemic disease rheumatoid arthritis.<sup>1</sup> These autoantibodies are detected in approximately 70 percent of adult rheumatoid arthritis patients.<sup>2</sup> Therapeutic measures can suppress the inflammatory manifestations of the disorder and aid in minimizing musculoskeletal dysfunction. Hence, serological tests for the early detection of RF have significant clinical applications.

Many disease states other than rheumatoid arthritis have been shown on occasion to give seropositive RF results. Some RF assays have returned positive results in patients with disseminated sarcoidosis, syphilis, essential hypertension and hepatitis.<sup>1,3</sup> Furthermore, a small number of healthy people, especially the elderly, may also have RF.<sup>4</sup> As a rule these individuals display very low titers of RF and can usually be distinguished from rheumatoid arthritis patients by this means.

Using sheep erythrocytes sensitized with rabbit gamma-globulin, Waaler in 1940<sup>5</sup> and Rose et al, in 1948<sup>6</sup> related hemagglutination by human serum to the disease rheumatoid arthritis. In 1958, Singer and Plotz<sup>7</sup> eliminated many of the variables associated with sheep erythrocytes by replacing the erythrocytes with biologically inert latex particles coated with gamma-globulin. This method serves as the basis for the **RFscan™** Latex Test. Refinements in the preparation and stabilization of sensitized particles have led to an improved sensitivity and specificity of the test.

## III. PRINCIPLES OF THE PROCEDURE

The **RFscan™** Latex assay is based on the agglutination of IgG-coated uniform latex particles by patient serum containing rheumatoid factor. In the presence of agglutinating antibody (RF), the particles aggregate and form macroscopic clumps on the surface of the test card. In the absence of RF, no agglutination occurs, and the latex suspension remains homogeneous.

#### IV. REAGENTS

**Reagent A,**                    **RFscan™** Latex Antigen, vinyltoluene t-butylstyrene copolymer particles coated with heat-aggregated, purified human IgG; in glycine-buffered saline, with 0.01% thimerosal (preservative).

**Reagent B,**                    **RFscan™** Sample Diluent, glycine-buffered saline, with 0.01% thimerosal (preservative).

**Control +,**                    **RFscan™** Positive Control (Titerable), human serum containing rheumatoid factor in phosphate-buffered saline, with 0.01% thimerosal (preservative). See Assay Value Card for titer and International Unit (IU/mL) information.

**Control -,**                    **RFscan™** Negative Control, diluted human serum in phosphate-buffered saline, with 0.01% thimerosal (preservative).

**Precautions:**                For *in vitro* Diagnostic Use

**Reagents:** Do not use beyond the expiration date. Upon removal from refrigeration, allow reagents to warm to room temperature (15 to 30°C) before use. Do NOT mix reagents from different kit lot numbers.

The **RFscan™** Latex Antigen may require vigorous shaking to thoroughly re-suspend. Keep the vial tightly closed when not in use to prevent evaporation of the suspension medium. The suspension should be milky and homogeneous. Do not use the reagent if it shows signs of flocculation.

To assure proper drop delivery hold dispensing bottles vertically, dispensing one free-falling drop at a time.

**Controls:** Do not use the kit if positive and negative controls do not yield appropriate results.

The serum controls are derived from human blood tested by an FDA (U.S. Food and Drug Administration)-approved method for the presence of the antibody to HIV (human immunodeficiency virus) and HBsAG (hepatitis B surface antigen) and found to be nonreactive.

**WARNING:** Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, **SPECIMENS AND THESE REAGENTS SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING AN INFECTIOUS DISEASE.** The Food and Drug Administration recommends such material be handled at a Biosafety Level 2. BSL2 is referenced in the Centers for Disease Control/National Institutes of Health (CDC/NIH) manual, *Biosafety in Microbiological and Biomedical Laboratories*.

**Test Cards:** Cards must be flat for proper reactions. If necessary, flatten cards by bowing back in a direction opposite to that of the curl. Care should be taken not to finger-mark the test areas, since this may result in an oily deposit and improper test results. Use each card once and discard. Store cards in the original package in a dry area at room temperature.

**Reading of Test Results:** Results should be read promptly under a high intensity incandescent lamp. Fluorescent lighting is generally insufficient to distinguish minimally reactive results. The use of magnification in reading test results is not recommended.

**Storage of Reagents:** Refrigerate at 2 to 8°C. **DO NOT FREEZE.** Reagents should be recapped and returned to refrigerator when not in use.

## V. **SPECIMEN COLLECTION AND PREPARATION**

Serological specimens should be collected under aseptic conditions. Allow whole blood to clot; then centrifuge to remove particulate matter from the serum. Decant promptly. Samples may be stored refrigerated for several days or frozen at -20°C for extended storage. Repeated freezing and thawing of serum is not recommended. The use of hemolyzed, markedly lipemic, or contaminated samples may cause difficulty in the interpretation of test results. No special preparation of the patient is required prior to specimen collection.

## VI. **PROCEDURES**

Review "Precautions" and "Specimen Collection and Preparation" prior to performing procedures. The testing area, reagents, test specimens and test components should be at room temperature (20-25°C) when used.

**Materials provided:**

Catalog No. 265001	(100 Tests)
<b>Reagent A,</b> RFscan™ Latex Antigen,	5.5 mL
<b>Reagent B,</b> RFscan™ Sample Diluent,	2 x 60.0 mL
<b>Control +,</b> RFscan™ Reactive Control (human serum)	1.5 mL
<b>Control -,</b> RFscan™ Nonreactive Control (human serum)	1.5 mL
<b>Test Cards</b>	8
<b>Assay Value Information,</b>	1
<b>Dispenstirs™,</b> Dropper/Spreader	100

**Materials Required But Not Provided:** Micropipettor with disposable tips, 50 and 100 µL delivery, serological pipets (1 or 2 mL) or variable micropipet (1,000 µL), 12 x 75 mm test tubes and rack.

Also required are the necessary equipment and labware used for preparation, storage and handling of serologic specimens.

**Procedural Overview:**

The RFscan™ Latex Test may be used for either a qualitative or quantitative estimation of rheumatoid factor in patient sera. The screening test uses undiluted patient serum. If agglutination is evident, dilute the sample 1:20 with the RFscan™ Sample Diluent provided with the kit, and repeat the test. *Only those patient samples, which remain positive at 1:20, are considered positive for rheumatoid factor.* These sera may be further analyzed by the card titration method described in "Quantitation (Card Titration Method)."

**VII. PERFORMANCE OF TESTS**

Screening (Qualitative):

1. Mark the card to identify the controls and all samples being tested.
2. Place one drop each of the **Control +** and **Control -** onto the appropriate test circle.
3. Deliver one drop of serum onto a vacant circle on the test card, using a separate **Dispenstirs™** device for each patient serum.
4. Re-suspend **Reagent A** by shaking vigorously. Deliver one drop **Reagent A** to each serum (patients and controls) on the card. Mix each serum circle with the flat end of the corresponding **Dispenstirs™** device; spread the contents within the entire test circle area. Avoid cross contamination of test areas in adjacent circles.

5. Rock the card with a gentle rotary motion for 60 sec, while observing each test area for agglutination by tilting the test card so that the flow of latex across each test area can easily be seen.

## VIII. INTERPRETATION OF TEST RESULTS

**Qualitative Test:** The positive control should show agglutination, while the negative control should show no agglutination.

**NOTE:** UNDILUTED SAMPLES EXHIBITING AGGLUTINATION MUST BE DILUTED 1:20 AND REASSAYED. ONLY THOSE SAMPLES THAT REMAIN POSITIVE AT 1:20 ARE CONSIDERED POSITIVE FOR RHEUMATOID FACTOR.

**Quantitation (Card Titration Method):** Follow Steps 1 through 11 for each serum (or control) to be tested. Use a different pipet or pipet tip for each patient. The large test card is recommended for use with the card titration method.

1. Label a test tube for each patient (or control) to be tested.
2. Deliver 1.9 mL of **Reagent B** into each tube.
3. Deliver 100  $\mu\text{L}$  of patient serum (or control) into the corresponding tube. Mix the contents well. Each tube now contains a 1:20 dilution of the patient serum (or control).
4. Pipet 50  $\mu\text{L}$  of **Reagent B** in test circles 2 through 6.
5. Pipet 50  $\mu\text{L}$  of the 1:20 patient serum (or control) dilution into test circle 1.
6. Pipet 50  $\mu\text{L}$  of the 1:20 patient serum (or control) dilution into test circle 2. Mix the contents of the test circle by several aspiration/delivery cycles from the pipet.
7. Using the same pipet tip, aspirate 50  $\mu\text{L}$  of the mixture from test circle 2 and deliver it into test circle 3. Mix as before and transfer 50  $\mu\text{L}$  of the mixture into test circle 4.
8. Continue the mix and transfer procedure through test circle 6.
9. Discard 50  $\mu\text{L}$  from test circle 6.

Circle No.	Dilution	Circle No.	Dilution
------------	----------	------------	----------

1	1:20	4	1:160
2	1:40	5	1:320
3	1:80	6	1:640

10. Re-suspend the **Reagent A** by shaking vigorously. Deliver one drop of **Reagent A** to each circle. Mix and distribute the contents evenly within the entire circle using the flat end of a clean **Dispenstirs™** device.
11. Rock the card with a gentle rotary motion for 60 sec while observing for agglutination by tilting the test card so that the flow of latex across each circle can easily be seen. The last circle exhibiting agglutination determines the titer.

**Procedural Notes:** Extended incubation of more than 1 min may produce false positive results due to evaporation of the test mixture on the card.

#### IX. DETERMINATION OF RESULTS

Results are obtained by establishing whether agglutination has occurred in the test circles. Observation of clearly visible macroscopic clumping of latex particles as the test card is tilted back and forth constitutes a positive result. **ONLY THOSE SAMPLES WHICH REMAIN POSITIVE AT 1:20 ARE CONSIDERED POSITIVE FOR RHEUMATOID FACTOR.** A milky homogenous mixture with no visible agglutination constitutes a negative result. Agglutination noted on undiluted serum but not observed on a 1:20 dilution also constitutes a negative result. With the card titration method, the titer is determined to be the greatest dilution giving a positive reading.

The **RFscan™** Positive Control is traceable to the WHO International Reference Preparation. Using the **RFscan™** Positive Control as a secondary standard, results may be expressed in IU/ml following the formula:

$$\text{Sample IU/mL} = \frac{\text{IU/mL Positive Control} \times \text{titer of sample}}{\text{Titer of control}}$$

For positive control IU/mL information, refer to enclosed Assay Value Card.

**Quality Control:** Positive and negative controls are provided in each **RFscan™** kit. The positive control should show agglutination, while the negative control should show no

agglutination. If the Card Titration Method is used, the positive control should exhibit agglutination through the titer given in the Assay Value Information.

#### X. **LIMITATIONS OF THE PROCEDURES**

The clinical significance of any test result depends upon its relationship to other medical data. Disease diagnosis and management should be based on an evaluation of all relevant patient information.

#### XI. **EXPECTED VALUES**

Rheumatoid factor is present in the sera of most patients with classic rheumatoid arthritis. Although a positive RF test result is highly suggestive of rheumatoid arthritis, it is not diagnostic for this condition since RF also appears in a small percentage of healthy individuals and in some patients with other disorders.

#### XII. **PERFORMANCE CHARACTERISTICS**

Comparison of **RFscan**<sup>TM</sup> Latex Test versus a commercially available sheep RBC agglutination procedure and another commercially available latex determination was performed on patients with clinically diagnosed rheumatoid arthritis, and on patients with autoimmune or connective tissue diseases including systemic lupus erythematosus, osteoarthritis, systemic sclerosis, fibromyositis, vasculitis and polymyalgia. Of 121 negative results by the **RFscan**<sup>TM</sup> latex agglutination assay, all were negative by a leading commercial latex agglutination assay; one sample was borderline positive (i.e., lowest possible titer) by hemagglutination. Likewise, of 25 positive results by the **RFscan**<sup>TM</sup> assay, all were positive by both hemagglutination and another latex agglutination procedure.

#### XIII. **AVAILABILITY**

**RFscan**<sup>TM</sup> 100 Test Kit (Qualitative), Cat. No. 265001.  
**Dispenstirs**<sup>TM</sup>, 0.05 mL, Box of 500, Cat. No. 272905.

#### XIV. **REFERENCES**

1. Howell, D.S., J.M. Malcolm, R. Pike, and B. Broome. 1960. The F II agglutinating factors in serums of patients with non-rheumatic diseases. *Am. J. Med.* 29:662-671.
2. Rothfield, N.F. 1969. Serologic tests in rheumatic diseases. *Postgrad. Med.* 45:116-121.

3. Bartfield, H. 1960. Incidence and significance of seropositive tests for rheumatoid factor in non-rheumatoid diseases. *Ann. Intern. Med.* 52:1059-1066.
4. Froehlich, C.J., and R.C. Williams, Jr. 1980. Tests for detection of rheumatoid factors. *In* N.R. Rose, and H.F. Friedman (ed.) *Manual of clinical immunology*, 2nd ed. American Society for Microbiology, Washington, D.C.
5. Waaler, E. 1940. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. *Acta Pathol. Microbiol. Scand.* 17:172-179.
6. Rose, H.M., C. Ragan, E. Pearce, and M.O. Lipmann, 1948. Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proc. Soc. Exp. Biol. Med.* 68:1-6.
7. Singer, J.M., and C.M. Plotz. 1956. The latex fixation test: Application to the serologic diagnosis of rheumatoid arthritis. *Am. J. Med.* 21:888-892.

**TECHNICAL INFORMATION:** In the United States, telephone BD Diagnostic Systems Technical Services, toll free (800) 638-8663.

Approved by:

\_\_\_\_\_  
Supervisor Date

\_\_\_\_\_  
Director Date

Reviewed:

PI Rev. 6/95  
Rev. 4/97  
Rev.11/25/02