MEDICAL DIRECTOR APPROVAL: _____

SUPERVISOR APPROVAL: _____

SCOPE:

This procedure applies to testing performed in the laboratory section of HHRMC. The testing procedure, equipment, and data generated are subject to all policies and procedures of the Laboratory Quality Plan and Laboratory General Procedure Manual as well as facility generated Policies and Procedures.

PURPOSE:

The term rheumatoid factor evolved from the observation that serum from a high proportion of patients with rheumatoid arthritis agglutinated sheep erythrocytes sensitized with specific rabbit antibodies. Rheumatoid factor is defined as a group of antibodies specific to antigenic determinants on the Fc fragments of human and animal immunoglobulins. A positive latex test, indicating the presence of rheumatoid factor, may be decisive for the diagnosis of rheumatoid arthritis in patients with inflammatory arthritis.

PRINCIPLE:

The reagent is a suspension of polystyrene latex particles of uniform size coated with human immunoglobulin. Latex particles allow visual observation of the antigen-antibody reaction. If rheumatoid factor is present in the serum, the latex suspension changes its uniform appearance and a clear agglutination becomes evident. This change occurs because RF present in the serum reacts with the IgG coated to latex particles, starting the formation of a web between them.

When serum containing more than 10 IU/mL of RF is mixed with the latex reagent, a clear agglutination will appear.

Results are expressed in International Units per mL (IU/mL) based on the WHO International Reference Preparation of Rheumatoid Arthritis Serum. Our laboratory will result the corresponding titer result.

SPECIMEN COLLECTION AND HANDLING:

Conditions for patient preparation:

No patient preparation is necessary.

Specimen type:

Venous blood collection:
Collect venous samples via syringe or collection tube with no anticoagulant- SST or red top tube. Follow proper collection for each tube type.
Serum- centrifuge tube and perform testing on serum either the same day or up to 8 days after refrigeration at 2 to 8°C.

Specimen labeling:
All specimens must be properly labeled with patient's first and last name, patient date of birth, the date of collection, the collector's initials and the time the blood was drawn.

Specimen rejection:
EDTA sample tubes; tubes with incorrect or missing label information; grossly contaminated or leaking tubes; tubes that are QNS for testing. For unacceptable specimens, the technologist will notify the physician or their representative of the cancellation and cancel the test in the LIS documenting who was notified. Results that do not correlate with the patient (abnormal results inconsistent with patient condition) should be recollected. The technologist should notify the physician or their representative of the need to recollect the specimen, cancel the test in the LIS, and footnote the notification in the cancellation field.

EQUIPMENT AND MATERIALS:

Materials:

Sure-Vue™ RF kit which includes:
1 x 1.4 mL Latex reagent (suspension of polystyrene latex particle coated human IgG in a buffer)
1-mL positive control (Diluted positive human serum)
1-mL negative control (non-reactive diluted human serum)
All reagents contain sodium azide 0.1%
9 disposable slides with 6 sections each.

Materials not provided in kit:
Pipettes and tips capable of delivering 50 µl- - Class A **not** required.
Disposable stirrers
Mechanical rotator (80 – 100 rpm)
Timer
Normal saline (0.9% NaCl, only for semiquantitative technique)
Biokit RF Titer Control

QUALITY CONTROL:

Both positive and negative controls will be included in each batch of tests to ensure proper functioning of the system. Positive results must include titration of an RF positive control. Follow directions for the qualitative technique in evaluating controls. The reaction between the positive control and the latex 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 kit discarded if there is no positive reaction. **Never result patient tests without obtaining acceptable control results FIRST.**

Whenever a new kit lot number is put into use, crossover testing using the positive and negative controls from the kit previously in use must be performed. The latex reagent from the new kit must be tested against the positive and negative control from both the previous kit and its provided QC material.

PROCEDURE:

A. Qualitative Technique:

1. Allow reagents and samples to reach room temperature (20 to 30°C).
2. Mark positions on the test slide for specimens and, as needed, for positive and negative controls.
3. Gently shake the latex reagent vial to disperse and suspend the latex particles in the buffer solution. Vigorous shaking should be avoided.
4. Dispense 50 µl undiluted patient sample or 1 drop of control solution onto prelabeled section of the disposable slide.
5. Place one drop of latex reagent next to the drop of sample or control.
6. Mix both drops with a stirrer covering the whole surface of the slide section.
7. Manually, or using a mechanical rotator, rotate the slide gently for 2 minutes.
8. Observe and record the presence (Positive) or Absence (Negative) of agglutination.

Note: The test should be read after 2 minutes incubation under the conditions described above. If test reading is delayed beyond 2 minutes, the latex suspension may dry out **giving a false agglutination pattern. If this is suspected, the specimen must be retested.**

RESULTS:

POSITIVE REACTIONS:
3+ Large clumping with clear background
2+ Moderate clumping with fluid slightly opaque in background
1+ Small clumping with opaque fluid background

NEGATIVE REACTIONS:
No visible clumping, uniform suspension.

B. Semiquantitative Technique

1. Label slide sections 1-6 for each positive patient and the positive control (Biokit RF titer control).
2. Place 50 µL of normal saline in sections 2-6 for each sample being tested.
3. Place 50 µL of serum or control onto section 1. Place 50 µL of serum or control onto section 2.
4. Using the same pipette take in and release several times the mixture made on section 2 and transfer 50 µL of the mixture to section 3.
5. Repeat this series through section 6, discarding 50 µL from section 6.
6. Gently shake the latex reagent and add one drop of reagent to each section.
7. Mix both drops with a stirrer covering the whole surface of the slide section.
8. Manually, or using a mechanical rotator, rotate the slide gently for 2 minutes.
9. Observe and record the presence (Positive) or Absence (Negative) of agglutination.
10. Positive results at a titer of greater than 1:256 will be reported as >1:256.

Results from both patient testing and controls must be recorded on the RA Quality Control and Patient Testing Log located in the Kits Testing Binder in the Hematology area. The approximate titer will correspond to the highest serum dilution that still presents a clearly visible agglutination. (See chart).
Results of crossover testing, when performed, must also be documented.

Results should be entered into the LIS database under workstation 800 under the patient accession number.

Section	1	2	3	4	5	6
Saline µL	-	50	50	50	50	50
Serum µL	50	50	50	50	50	50
Mix and transfer µL		50	50	50	50	50-
Dilution	1:1	1:2	1:4	1:8	1:16	1:32
IU/mL	10	20	40	80	160	320

EXPECTED RESULTS:

Establishing "normal values" for rheumatoid factor is difficult since its presence has little diagnostic value if it is not accompanied by signs that suggest the existence of rheumatoid arthritis.
In at least 70-80% of patients with RA the existence of RF can be proven through significant titers. The highest titers are usually related to the most serious cases of RA. The remaining patients are considered seronegative having RF titers falling in the normal range or not detected by conventional tests.
In approximately 3-5% of the normal population, RF may be found at low titers. The proportion increases with the age of the population being studied.

LIMITATIONS OF PROCEDURE:

As with all diagnostic assays, the results of this test kit should be interpreted in light of the clinical symptoms shown by the patient.
Reading results after more than 2 minutes may result in a false positive reaction.
The strength of agglutination is not necessarily indicative of relative rheumatoid factor concentration.
Since increased levels of RF may accompany certain acute immune responses such as infectious mononucleosis, certain diseases such as sarcoidosis, systemic lupus erythrematosis, and Sjögren's syndrome and may also be found in a considerable percentage of elderly individuals, the interpretation of the clinical significance of a positive test result must be made with caution. Less commonly, a positive test may result in situations where chronic inflammatory disease is suspected such as bacterial endocarditis, tuberculosis, leprosy, etc.
Certain patients with RA may show negative results for RF.

REFERENCES:

.
Sure-Vue™ RF package insert, Biokit, S. A. Barcelona, Spain. 12/99 I-03/05.