

Subject/Title: ASI RF DIRECT SLIDE TEST		Doc#: 6004-700DC CLSI
Effective Date: 04/18	Supersedes Revision/Date: 09/08	Revision: 04/18
Prepared by: ASI	QA Approval by:	Copy/Dept.:

FOR IN VITRO DIAGNOSTIC USE

Cat. No.	700025	25	Tests
	700100	100	Tests
	7001000	1000	Tests

CPT Code: 86430

- 1 **INTENDED USE:** The **ASI RF Direct Slide Test** is a slide agglutination assay for the qualitative and semiquantitative determination of rheumatoid factor (RF) in human serum. No initial dilution of patient samples is required for this test. These materials are intended to be acquired, possessed and used only by health professionals.
- 2 **SUMMARY AND EXPLANATION OF THE TEST:** It has been determined that sera from a high percentage of patients suffering from rheumatoid arthritis contain RF.^{1,2} Rheumatoid factors are immunoglobulins of any isotype with antibody activity directed against antigenic sites on the Fc region of human or animal immunoglobulin G (IgG). Waaler indicated that sensitized sheep erythrocytes could be used as a reagent to detect RF. Singer and Plotz described a method of detecting RF using a suspension of fine plastic granules coated with human gamma globulins that agglutinated in the presence of RF.^{3,4}
- 3 **PRINCIPLE OF THE PROCEDURE:** The **ASI RF Direct Slide Test** reagent contains latex particles coated with purified human gamma globulins. The sensitivity of the latex reagent is 8.0 IU/ml with no predilution of samples required. Calibration is established against WHO.⁵ The specificity of the latex reagent is >95%.
- 4 **REAGENTS**
 - 4.1 RF DIRECT LATEX REAGENT - Inert latex particles coated with gamma globulins, with 0.1% sodium azide as preservative.
 - 4.2 GLYCINE-SALINE SOLUTION - Glycine buffer in saline, with 0.1% sodium azide as preservative.
 - 4.3 CONTROLS (REACTIVE, NONREACTIVE) - Human serum or defibrinated plasma (liquid), with 0.1% sodium azide as preservative.
- 5 **WARNINGS AND PRECAUTIONS**
For *In Vitro* Diagnostic Use.
 - 5.1 ASI RF DIRECT LATEX REAGENT, GLYCINE-SALINE SOLUTION and CONTROLS contain sodium azide. Azides in contact with lead and copper plumbing may react to form highly explosive metal azides. When disposing of reagents containing azide, flush down the drain with large quantities of water to prevent azide build-up.
 - 5.2 ASI RF DIRECT LATEX REAGENT and CONTROLS contain human serum or plasma which has been tested at the donor level for HBsAg and for HIV-1, HIV-2 and HCV antibodies and found to be nonreactive. As no known test offers complete assurance that infectious agents are absent, the LATEX REAGENT and CONTROLS should be considered potentially infectious and universal precautions should be used. The CDC/NIH Health Manual "Biosafety in Microbiological and Biomedical Laboratories" describes how these materials should be handled in accordance with Good Laboratory Practice.
 - 5.3 Do not pipet by mouth.
 - 5.4 Do not smoke, eat, drink or apply cosmetics in areas where plasma/serum samples are handled.
 - 5.5 Any cuts, abrasions or other skin lesions should be suitably protected.

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6 HANDLING AND PROCEDURAL NOTES

- 6.1 In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for reagents or samples.
- 6.2 Do not use past the expiration date indicated on the kit.
- 6.3 Do not interchange components of one kit with those of another kit.

7 STORAGE INSTRUCTIONS

- 7.1 Store all reagents at 2-8°C in an upright position when not in use. Do not freeze reagents. Pipets and slides do not require refrigeration.

8 INDICATIONS OF DETERIORATION

- 8.1 Turbidity or precipitation in controls is indicative of deterioration and the component should not be used.
- 8.2 Bacterial contamination of reagents or specimens may cause false positive results.

9 SPECIMEN COLLECTION AND STORAGE

- 9.1 Use only serum that is free from contamination. Test samples should not be heat-inactivated.
- 9.2 It is preferable to test samples on the day of their collection. If samples cannot be tested immediately, maintain them in their original tubes at 2-8°C and test within 48 hours.
- 9.3 Serum samples stored longer than 48 hours should be stored at -20°C or below until testing. Avoid repeated freezing and thawing of specimens.
- 9.4 If necessary before testing, centrifuge the specimens at a force sufficient to sediment cellular components.
- 9.5 Samples to be sent out for testing should be placed on ice packs and packaged like any other biohazardous material that could potentially transmit infection.

10 PERFORMANCE OF THE TEST

Materials Provided:

	25 Tests	100 Tests	1000 Tests
RF DIRECT LATEX REAGENT	1.0 ml	4.0 ml	10 x 4.0 ml
REACTIVE CONTROL	0.5 ml	1.0 ml	10 x 1.0 ml
NONREACTIVE CONTROL	0.5 ml	1.0 ml	10 x 1.0 ml
GLYCINE-SALINE SOLUTION	50 ml	100 ml	10 x 100 ml
0.05 ml Disposable Stirrer Pipets	25	100	1000
Disposable Test Cards (6-well)	5	17	170

11 ADDITIONAL MATERIALS REQUIRED

- 11.1 Timing device
- 11.2 13 x 75 mm test tubes
- 11.3 Volumetric pipet to deliver 0.25 ml
- 11.4 Mechanical rotator (optional)

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12 TEST PROCEDURE

12.1 PREPARATION FOR THE ASSAY

- 12.1.1 Allow all reagents and samples to warm to room temperature (20–30°C) before use. Remove reagents from foam holders. Do not heat reagents in a water bath.
- 12.1.2 All reagents are ready for use as supplied. Gently mix the reagents before use; avoid foaming.
- 12.1.3 Gently mix the LATEX REAGENT before each use to ensure homogeneity.

12.2 ASSAY PROTOCOL- QUALITATIVE

- 12.2.1 Using the stirrer pipets, deliver one free-falling drop (0.05 ml) of each serum sample onto a separate circle on the test card. Use a fresh stirrer pipet for each sample. When using the stirrer pipet, keep it in a vertical position to ensure accurate delivery. Repeat by adding one free-falling drop of REACTIVE or NONREACTIVE CONTROL from the dropper vials supplied. Note the location of each sample by using the numbers located below and to the right of each circle.
- 12.2.2 Expel the contents of the LATEX REAGENT dropper and refill. Add one drop of the reagent to each serum specimen and to each control.
- 12.2.3 Using the flat end of the stirrer pipets, mix each specimen and control serum with the LATEX REAGENT, in a circular manner, over the entire area in the circles of the card.
- 12.2.4 Gently tilt and rotate the card for two (2) minutes and observe for agglutination. All test results should be compared to both REACTIVE and NONREACTIVE CONTROLS.

12.3 ASSAY PROTOCOL- SEMIQUANTITATIVE

- 12.3.1 Prepare serial dilutions of patient serum in GLYCINE-SALINE SOLUTION in test tubes as follows:

<u>Tube</u>	<u>Dilution</u>	<u>Composition</u>
1	1:2	0.25 ml of serum + 0.25 ml GLYCINE-SALINE SOLUTION. Mix.
2	1:4	0.25 ml from tube 1 + 0.25 ml GLYCINE-SALINE SOLUTION. Mix.
3	1:8	0.25 ml from tube 2 + 0.25 ml GLYCINE-SALINE SOLUTION. Mix.
4	1:16	0.25 ml from tube 3 + 0.25 ml GLYCINE-SALINE SOLUTION. Mix.
5	1:32	0.25 ml from tube 4 + 0.25 ml GLYCINE-SALINE SOLUTION. Mix.

Testing on additional dilutions should be performed as needed.

- 12.3.2 Using each dilution as a separate test specimen, apply the samples to the card as described in step 1 of the Qualitative Assay Protocol and proceed with steps 2 through 4 of the Qualitative Assay Protocol. Include undiluted sample if not tested previously on that day with the same lot of LATEX REAGENT.

13 QUALITY CONTROL

- 13.1 REACTIVE and NONREACTIVE CONTROLS should be included in each test run to confirm optimal reactivity of the LATEX REAGENT. If control samples do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not elicit the expected results for the control samples, discontinue use of the test and contact ASI Technical Support at (800)654-0146.

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14 INTERPRETATION OF RESULTS

14.1 INTERPRETATION OF RESULTS- QUALITATIVE

Agglutination indicates an RF concentration of greater than or equal to 8 IU/ml in the serum sample. Sera that elicit a positive result should be retested and titered using the Semiquantitative Assay Protocol.

14.2 INTERPRETATION OF RESULTS- SEMIQUANTITATIVE

The highest dilution in which visible agglutination occurs is considered the endpoint titer. The corresponding RF concentration (in IU/ml) is calculated as the product of the endpoint dilution factor and the assay cutoff values as shown in the following table.

<u>Dilution</u>	<u>RF IU/ml</u>
NEAT*	8
1:2	16
1:4	32
1:8	64
1:16	128
1:32	256

*NEAT = undiluted

For example, if the endpoint dilution is 1:32, the corresponding RF serum concentration would be 32 x 8, or 256 IU/ml.

15 LIMITATIONS OF THE PROCEDURE

- 15.1 False positive tests may occur using samples from patients with systemic lupus erythematosus, hepatitis, scleroderma, lymphomas, cirrhosis of the liver and other infections. The frequency of false positive results is not high under these conditions but the possibility does exist.
- 15.2 Contaminated, lipemic, or grossly hemolyzed sera should not be used because of the possibility of nonspecific results.
- 15.3 Plasma samples should not be used because of the possibility of nonspecific results.
- 15.4 Temperature of the reagents and samples is crucial to test outcome. It should be between 20° and 30° C.
- 15.5 Reaction times longer than specified might cause false positive results due to a drying effect.
- 15.6 In accord with all diagnostic methods, a final diagnosis should not be made on the result of a single test, but should be based on a correlation of test results with other clinical findings.

16 EXPECTED VALUES

- 16.1 The clinical significance of the detection of rheumatoid factor lies in differentiating between rheumatoid arthritis and rheumatic fever; in the latter, RF is never present.⁶ Patients diagnosed with rheumatoid arthritis have rheumatoid factor in their serum 70% - 80% of the time.⁷ A high percentage (90%) of patients with rheumatoid arthritis variants such as Felty's and Sjögren's syndromes are positive for rheumatoid factor.⁸

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17 REFERENCES

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TECHNICAL INFORMATION: (801) 489-8911 or (800) 654-0146