
ACCUTEX SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) LATEX TEST

A Qualitative and Semiquantitative Serological Test For Detection Of Antinuclear Antibodies in Serum or Plasma as an Aid in the Diagnosis of Systemic Lupus Erythematosus.

SUMMARY

Serum from patients with systemic lupus erythematosus (SLE) almost always contains autoantibodies to one or more nuclear antigens. ^(1,2) These antinuclear antibodies (ANA) may be either IgG, IgM or IgA. ^(2,4) They are present in about 95% of active untreated SLE patients, but are not completely specific for the disease. ^(1,2) Patients with other autoimmune diseases, such as rheumatoid or juvenile arthritis, scleroderma, chronic discoid lupus, polyarteritis nodosa, dermatomyositis, chronic liver disease and drug induced lupus, also may have detectable levels of antinuclear antibodies, although these are usually present in substantially lower titers than in systemic lupus erythematosus. ^(5,7) Circulating ANA levels may or may not correlate with disease activity in SLE patients.

Assays for antinuclear antibodies are the most widely used serological tests for the detection of systemic lupus erythematosus. Methods of ANA determination include the LE cell test, immunofluorescence, immunoperoxidase, complement fixation and particle agglutination tests. ^(1-3,6,9,10) The chief advantage of the particle agglutination test is its great speed relative to other tests.

Antibodies to deoxyribonucleoprotein (DNP) are the most common ANA encountered in SLE, being found in over 90% of patients at some time during the course of the disease. ^(6,11) Anti-DNP appears to be responsible for the characteristic LE cell phenomenon and gives rise to homogeneous patterns of nuclear staining in immunofluorescent/ immunoperoxidase ANA tests. ^(1,11)

PRINCIPLE

The Accutex Systemic Lupus Erythematosus Latex Test provides a suspension of polystyrene latex particles which have been coated with deoxyribonucleoprotein. The antinuclear antibodies produced in systemic lupus erythematosus bind to the DNP coating the latex particles. This binding is evident by rapid agglutination of the latex.

STABILITY AND STORAGE

Indication of deterioration: lack of clear agglutination with the Positive Control Serum, agglutination with the Negative Control Serum or extreme turbidity in either control serum. The reagents in this kit are stable until their expiration date when stored as directed. However, as with most reagents, they can be damaged by improper handling, especially temperature extremes. Use of the Positive and Negative Control Sera provided will permit detection of reagent deterioration.

Store reagents at 2-8°C when not in use. All other kit components may be stored at room temperature if desired. Do not freeze reagents. Discard any unused control serum when the SLE Latex Reagent is depleted.

SPECIMEN COLLECTION AND HANDLING

Use fresh serum or plasma free from contamination. Collect blood in a clean dry tube and allow to clot at room temperature for at least 10 minutes before removing serum. If not tested immediately, specimens may be stored at 2-8°C for a maximum of 72 hours. If longer storage is required, the sample may be frozen and tested at a later time. Repeated freezing and thawing should be avoided.

Specimens must be clear and free of particulate matter before testing. Contaminated or grossly hemolyzed specimens should not be used.

PROCEDURE

REAGENTS

For in vitro diagnostic use only.

- 1 SLE Latex Reagent: a suspension of polystyrene latex particles coated with calf thymus deoxyribonucleoproteins in a stabilization buffer.
- 1 SLE Positive Control Serum: a human serum pool known to be positive for ANA.
- 1 SLE Negative Control Serum: a human serum pool known to have a negative reaction with the Accutex SLE Latex Reagent.

All three reagents contain 0.1% sodium azide as a preservative.

- Test Slide
- Disposable Pipet/Stir Sticks

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Mechanical slide rotator (optional)
- Physiologic saline (0.85% or 0.9% sodium chloride)
- Serological pipets or safety pipetting device with disposable tips.
- Disposable test tubes (12 x 75mm, 10 x 75mm or 13 x 100mm)
- Test tube rack.

Warning: Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. If discarded into sink, flush with a large volume of water to prevent azide buildup.

Warning: Human sourced material. Treat as potentially infectious. Each donor unit used in the preparation of this product has been tested by an FDA-approved method and found non-reactive for the presence of HBsAg and antibody to HIV Virus. Because no known test method can offer complete assurance that hepatitis B virus, HIV Virus, or other infectious agents are absent, all human blood based products should be handled in accordance with good laboratory practices using appropriate precautions as detailed in Centers for Disease Control/National Institute of Health Manual "Biosafety in Microbiological and Biomedical Laboratories, 1984."

PROCEDURE OUTLINE

A. QUALITATIVE TEST

1. Bring reagents and specimens to room temperature before use.
2. Place one drop (50 ul) of the SLE Positive Control on the first field of the reaction slide. Place one drop (50 ul) of the SLE Negative Control on the second field. The remaining fields are used for test specimens. Using pipets provided, place one drop of each specimen on successive fields. Retain the Pipet/Stir Sticks for mixing step.
3. Gently resuspend the SLE Latex reagent and add one drop to each test field. Use Pipet/Stir Stick to spread reaction mixture over entire test field.
4. Rotate slide for 3 minutes and read immediately under direct light.

B. SEMIQUANTITATIVE TEST

1. Bring reagents and specimens to room temperature before use.
2. Using physiologic saline, dilute the specimens 1:2, 1:4, 1:8, 1:16, 1:32, or as needed.
3. Place one drop (50 ul) of each dilution on successive fields of the reaction slide.
4. Gently resuspend the SLE Latex Reagent and add one drop to each field on the reaction slide. Use Pipet/Stir Stick to spread reaction mixture over entire test field.
5. Rotate slide for 3 minutes and read immediately under direct light.

QUALITY CONTROL

SLE Positive and Negative Control Sera should be included in each test series. The Positive Control Serum should produce clear agglutination; the Negative Control Serum should produce no agglutination.

RESULTS

A. QUALITATIVE TEST

Agglutination (positive reaction) indicates the level of antinuclear antibody (specifically, anti-DNP) is in the range commonly found in systemic lupus erythematosus. Positive test specimens should be subjected to the Quantitative Test. The lack of agglutination (negative reaction) indicates that the level of anti-DNP is below that commonly encountered in SLE.

B. SEMIQUANTITATIVE TEST

The titer of antinuclear antibodies (anti-DNP) is the reciprocal of the highest dilution which exhibits a positive reaction. Titer changes in sequential samples from the same patient may or may not correlate with disease activity.

LIMITATIONS OF TEST PROCEDURE

Although the Accutex SLE Latex Reagent is highly sensitive and specific for antinuclear antibodies, a diagnosis of systemic lupus erythematosus or the presence of other autoimmune disease should not be made on the basis of a positive test result without the support of patient history and other clinical evidence. Similarly, a negative test result cannot completely rule out systemic lupus erythematosus. Incubation of the test for longer than the recommended time may cause false positive reactions.

About 10% of the normal healthy population below the age of 60 years have antinuclear antibodies.⁽⁷⁾ The titer of ANA in these individuals is usually too low to be detected by the Accutex SLE Latex Test. In apparently normal persons age 60 and above, the incidence of ANA increases.^(7,12) The significance of such ANA is uncertain since many persons in this group may have degenerative, subclinical and/or occult diseases. The presence of ANA in titers lower than those generally found in SLE have been associated with serum from some patients with other autoimmune diseases, such as rheumatoid or juvenile arthritis, scleroderma, chronic discoid lupus, polyarteritis nodosa and dermatomyositis, chronic liver disease, tuberculosis, atypical pneumonia, anaplastic carcinoma, lymphoma and drug-induced lupus.⁽⁵⁻⁷⁾

The Accutex SLE Latex Reagent is designed to agglutinate in the presence of levels of ANA commonly found in systemic lupus erythematosus. The titers of ANA found in most cases of other autoimmune diseases and in normal healthy individuals are generally too low to produce agglutination in this test.

SPECIFIC PERFORMANCE CHARACTERISTICS

To assure proper performance, Accutex SLE Latex Reagent has been tested by the procedures described against a panel of human sera of established reactivity levels.

No false positive reactions were observed with the Accutex SLE Latex Reagent in testing performance of 75 samples from apparently healthy individuals. Samples from 145 patients with ANA titers of 1:10 or greater by immunofluorescence (IF) were tested with the Accutex SLE Latex Test. The results of this testing are presented in the following table:

Accutex SLE Latex Test Results On Sera With Known ANA Immunofluorescence Patterns

IF/IP Titer	IMMUNOFLUORESCENCE PATTERNS		
	Homogeneous	Speckled	Nucleolar
1:10	2/8	4/60	—
1:20	1/3	3/22	—
1:40	6/6	4/10	—
1:80	2/2	4/8	—
>1:160	13/13	5/8	1/5

* Indicated in table as number positive/number tested.

Titers of ANA below 1:20 by immunofluorescence test are generally considered insignificant. Thus, the results of the Accutex SLE Latex Test on samples having titers of 1:20 and above are a good measure of the sensitivity of the test. With those sera that had titers of 1:20 or greater by immunofluorescence in this series, the Accutex SLE Latex Test gave positive reactions with 22 of 24 samples (92%) with homogeneous patterns, 16 of 48 samples (33%) with speckled patterns and 1 of 5 samples (20%) with nucleolar patterns.

In another series of tests on samples from patients with ANA titers > 1:160 by the indirect immunofluorescence technique, reactivity that is highly suggestive of a diagnosis of SLE, the Accutex SLE Latex Test gave positive reactions with 31 of the 36 samples (86%). The 4 samples that failed to react with the Accutex SLE Latex Test also were negative with another commercial SLE latex reagent tested.

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