Stanbio RaPET® s-LE
Procedure No. 1160

Latex Agglutination Slide Test for the Detection and Quantitation of serum antinucleoprotein factors associated with systemic Lupus Erythematosis in Human Serum

Summary and Principle[1-7]

The demonstration of antinuclear antibodies by laboratory methods include immunifluorescence, LE cell test, and agglutination of coated particles. The antibodies that are believed to be most characteristic of s-LE are the ones directed against deoxyribonucleoprotein (DNP). These are the ones that are believed to cause the formation of the LE cell in vitro; this unusual happening occurs in 75-80% of those patients diagnosed as having s-LE. It is not necessary to have a positive LE cell test for the diagnosis of s-LE as this is test has been found negative in certain individuals having symptoms suggestive of s-LE. In these individuals, antinuclear antibodies may be demonstrated by methods other than the LE cell test.

Anti-DNP antibodies are demonstrated by a number of laboratory procedures which include the LE cell test, immunofluorescence, and agglutination of coated latex particles. The principle of the Stanbio RaPET® s-LE test is that when latex particles coated with DNP are brought into contact with a serum which contains anti-nuclear antibodies, there occurs agglutination which indicates a positive reaction. The reaction time for this occurence is within one minute.

Reagents

RaPET sLE Latex Reagent (white cap), Ref. No. 1161
Suspension of polystyrene latex particles coated with DNP extracted from fetal calf thymus in a buffer with sodium azide 0.1%.
sLE Positive Control (red cap), Ref. No. 1162
Stabilized s-LE positive human serum.
Negative Control (green cap), Ref. No. 1192
Stabilized human serum, negative for s-LE.

Precautions: *For In Vitro Diagnostic Use.*
sLE control sera have been tested and found nonreactive for Hepatitis B Surface Antigen (HBsAg), HIV 1/2, and HCV; however, all human serum products and patient specimens should be considered potentially hazardous and handled in the same manner as an infectious agent. Reagents contain sodium azide. This agent is known to react with copper and lead in sink drains to form explosive azides. Disposed materials should be flushed with large quantities of water to prevent azide accumulation.

Reagent Storage and Stability: Reagents are stable at 2-8°C until the expiration date shown on the label. DO NOT FREEZE!

Materials Provided

Plastic slide
Disposable pipette/mixer

Materials Required But Not Provided

Timer
Test tubes (Titration only)
Serological Pipettes (Titration only)
Physiological saline (0.9% NaCl) (Titration only)

Specimen Collection and Preparation[9-11]

It is recommended that serum only be used. Do not heat inactivate test sera or controls. Avoid repeated freeze-thawing of specimens. Do not use visibly hemolyzed specimens, as these have been known to produce false-positive results.

Sample Stability: Serum is stable for 48 hours when stored at 2-8°C.

Interfering Substances: Use only a clean, dry slide washed in mild detergent and rinsed with distilled water.

Patients with Rheumatoid Arthritis, Sjogren Syndrome, Mixed Connective Tissue Disease, Progressive Systemic Sclerosis and Discoid L.E. may show reactivity when using this test. Many widely used drugs may, in fact, induce a systemic lupus erythematosis syndrome. Hydralazine, isoniazid, procainamide and a number of anti-convulsant drugs fall into this category.

Latex reagent **must** be shaken vigorously for 30 seconds before use in order to avoid aggregation of latex particles.

Procedure (Screening)

1. Bring all reagents to room temperature and shake latex vigorously for 30 seconds prior to use. Do not use a vortex mixer.
2. Place in separate divisions (cells) of the same slide:

Serum	1 drop
Positive Control (red cap)	1 drop
Negative Control (green cap)	1 drop

3. Add one drop of sLE latex reagent to each cell.
4. Mix with flat end of pipette/mixer and spread fluid evenly over each cell.
5. Tilt the slide back and forth slowly (or use rotator) for 1 minute while observing for agglutination. Observe for agglutination not longer than 1 minute,

Procedure (Semi-quantitative)

Prepare dilutions of the specimens as shown below:
1. For each test serum to be titrated label 6 test tubes.
2. To each tube add 0.2 mL physiological saline.
3. To tube No. 1 add 0.2 mL of undiluted test serum.
4. Serially make two-fold dilutions by mixing contents of tube No.1 with a pipette and transferring 0.2 mL to tube No. 2. Repeat serial transfers for each tube. For the 6 tubes, the dilutions range from 1:2 to 1:64. If required, additional serum dilutions can be added.
5. Proceed with step 5 as in screening method above. The serum s-LE antibody titer is the highest dilution of serum showing agglu tination of the latex reagent within one minute after mixing.

Quality Control: Positive and Negative controls should be run with each series and results compared with patient specimens.

Results

Any degree of agglutination visible within one (1) minute is to be interpreted as positive. Test is considered s-LE negative when no difference in agglutination is observed between specimen and negative control.

Performance Characteristics[8]

A study was conducted on 155 subjects which included 29 patients with active sLE, 23 with clinically inactive sLE, 8 having connective tissue diseases,a nd the remainder (95) were controls. The test was compared with a standard LE cell preparation test and a fluorescent ANA test.

For the serum from the 29 active s-LE patients, the latex showed 82% positive, the LE cell prep showed 86% positive, and tge ANA test showed 82% positive. On the serum from the 23 clinically inactive sLE patients, the latex gave 19% positive results, the LE cell prep gave 19%, and the ANA test 71%.

Those patients having connective tissue disease showed no positive reactions with the latex test, but the LE cell prep gave a 17% positive reaction while the ANA procedure gave a 50% positive reaction.

The remaining controls which were made up of normal people and from patients who had diseases which included anemia, infectious mononucleosis and rheumatic heart disease, showed a 1% positive result with both the latex test and the LE cell prep., while the ANA gave 6% positive results.

Additional published studies have confirmed the sensitivity and specificity of the s-LE latex test.

References

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