INSTRUCTIONS FOR USE

L.E.S. LATEX

LATEX AGGLUTINATION SLIDE TEST FOR THE DETERMINATION OF ANTI-N-DNA ANTIBODIES ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS (S.L.E.)

1 – CLINICAL SIGNIFICANCE AND INTENDED USE

For ***in Vitro*** diagnostic use only

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of unknown cause that affects multiple organ systems (articulations, skin, kidneys, central nervous system, heart, lungs). Immunologic abnormalities, especially the production of a number of antinuclear antibodies (ANA), are another prominent feature of this disease. The clinical course is marked by spontaneous remissions and relapses. Its multisystemic manifestations and the complications from the use of immunosuppressive agents make the diagnosis and management of this entity challenging. The detection of ANA antibodies by laboratory methods include immunofluorescence, LE Cells test and agglutination of coated latex particles. These antibodies anti-DNP are believed to cause the formation of the LE cell in vitro, with this unusual event occurring in 75-80% of those patients diagnosed as having SLE. Some patients having symptoms suggestive for SLE had been found negative with LE Cells Test. In these individuals, ANA antibodies may be demonstrated by methods other than the LE cell test, as latex agglutination or immunofluorescence.

L.E.S. LATEX is a rapid agglutination procedure, developed for the direct detection and the semi-quantitation on a slide of antideoxyribonucleoprotein antibodies (anti-DNP) in human serum.

2 - PRINCIPLE OF THE METHOD

The assay is performed by testing a suspension of latex particles coated with DNP against unknown serums. The presence or absence of a visible agglutination indicates the presence or absence of anti-DNP antibodies in the samples tested.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
L.E.S. LATEX CND: W0102100116 EDMA: 12.10.01.16; RDM: 1555421/R	Latex agglutination test	**UB80800** (62 tests)	1 glass bottle containing latex for L.E.S., suspension of polystyrene latex particles coated with DNP (calf thymus) in a buffered solution. Contain Sodium azide < 0.1% (3,1 mL = 62 tests) 1 glass bottle containing Positive Control: human serum with anti-DNP activity. Contain Sodium azide < 0.1% (0.5 mL) 1 glass bottle containing Negative Control: stabilized liquid control, contain Sodium azide < 0.1% (0,5 mL) Slide, 6 test areas: plastic waterproof sheets for reaction (11 items) Sticks (1x25): plastic sticks for mixing (3 items) Secondary packaging: cardboard box.
L.E.S. CONTROLLI CND: W0102100116 EDMA: 12.50.01.13; RDM: 1555441/R	Controls for latex agglutination test	**UD80802** (2x0,5 mL)	1 glass bottle containing Positive Control: human serum with anti-DNP activity. Contain Sodium azide <0.1% (0.5 mL) 1 glass bottle containing Negative Control: stabilized liquid control, contain Sodium azide <0.1% (0,5 mL) Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Mechanical rotator with adjustable speed at 80-100 r.p.m. Timer or clock. Pipettes. Saline solution (9 g/L NaCl, only for semi-quantitation procedure).

5 - PRECAUTIONS AND WARNINGS

- L.E.S. LATEX is a kit for in vitro diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- Components of human origin have been tested and found to be negative for the presence of antibodies anti-HIV 1+2 and anti-HCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious.
- The sensitivity of the test may be reduced at low temperatures. Allow the reagents and samples to reach room temperature (15-30ºC/59-86ºF) before use to have best results.
- Do not use after expiration date or if the packaging is damaged. The quality of the reagent cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.
- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it

6 - STORAGE CONDITIONS AND SHELF LIFE

All the kit components will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze: frozen reagents could change the functionality of the test.

Mix reagents gently before use.

Reagents deterioration: Presence of particles and turbidity in controls; don't use it. Bacterial contamination of reagents or specimens may cause false positive results.

7 – SPECIMENS COLLECTION

Fresh, clear serum. Stable 7 days at 2-8ºC or 3 months at –20ºC. Do not use highly hemolysed or lipemic samples.

8 - TEST PROCEDURE

Allow the components of the kit to reach to room temperature (15-30ºC/59-86ºF) prior to testing.

Qualitative test

1. Gently shake the suspension for homogenization of the latex particles.

2. Always use positive and negative controls as references.
3. Place **30 µL of the serum** under test into one of the circles on the slide. Dispense **1 drop of each Positive and Negative control** into two additional circles.
4. Add **1 drop or 40 µL of Latex reagent** to each circle next to the sample to be tested (serum, positive and negative control).
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide, either manually or with a mechanical stirrer 80 to 100 rpm for **1 minute***.
7. Observe the presence or absence of visible agglutination immediately.

**Samples giving indeterminate results may be retested increasing the rotation period to 2 minutes. Reaction times longer that 2 minutes might cause false positive results.*

Semiquantitative test

For each specimen to be tested place with a pipette 30 µl of saline solution (NaCl 9 g/L) into each of the 6 circles of a slide.
To circle one add 30 µl of specimen to the saline solution and, using the same tip, mix the saline solution with the sample by repeated aspiration and expulsion of the fluid and transfer 30 µl of the mixture to the saline solution in the second circle.
Continue with the 2-fold serial dilutions in a similar manner up to the sixth circle, and discard 30 µl from this circle. Final sample dilutions will be: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64.
Test each dilution as described in steps 4-7 for the Qualitative Test.

9 – READING, INTERPRETATION AND CALCULATION

Qualitative test: Nonreactive: smooth suspension with no visible agglutination, as shown by negative control.
Reactive: any degree of agglutination visible macroscopically.

Semiquantitative tests: same as Qualitative test. The titer is defined as the highest dilution showing reactivity. The next higher dilution should be negative.

10 – QUALITY CONTROL

Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation. All result different from the negative control result, will be considered as a positive.

11- EXPECTED VALUES

A positive result indicates the level of anti-deoxyribonucleoprotein antibodies (DNP) is in the range commonly found in systemic lupus erythematosus.

12 – CHARACTERISTICS

Analytical performance. Serum samples were tested with L.E.S. LATEX : 29 had active SLE, 23 had clinically inactive SLE, 8 had connective tissue diseases and the remaining 95 were clinically normal or had some nonrelated diseases (anemia, infectious mononucleosis and rheumatic diseases). Results were compared with a standard LE Cell preparation assay and a fluorescent ANA method.

	Found	L.E.S. TEST Mascia Brunelli	LE Cell Preparation	F-ANA Test	Total
Active SLE	Positive	24 83%	25 86%	24 83%	29
Inactive SLE	Positive	4 17.4%	4 17.4%	16 70%	23
Connective tissue diseases	Positive	0 0%	1 12.5%	4 50%	8
Clinically normal/non related diseases	Positive	1 1%	1 1%	6 6%	95

13 – LIMITATIONS OF THE METHOD

- Serum from patients with scleroma, rheumatoid arthritis, dermatomyositis and a variety of connective tissue diseases may elicit agglutination in the L.E.S. Test.
- As high levels of antibodies might affect the degree of agglutination, positive samples should be re-assayed using semi-quantitative procedure.
- Plasma samples should not be used because of the possibility of non-specific results.
- Bacterial contamination of controls and specimens as well as freezing and thawing of the L.E.S. TEST reagent may lead to false positive results.
- Drugs such as hydralazine, isoniazid, procainamide and a number of anticonvulsant drugs can induce an SLE syndrome.
- As with all diagnostic tests, a final diagnosis cannot rely on the outcome of a single test and must be supported by other clinical and laboratory data.
- The components of this I.v.D. were always tested together without compatibility with components from other manufacturers. While not excluding the possibility that these components can be used with components of the same formulation but produced by other companies, there is no experimental evidence of such compatibility.

14 – REFERENCES

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4. Holman HR, Kunkel HG, Science 1957; 126: 263
5. Miescher PA, Strassel R. Vox Sang 1957; 2: 283-287
6. Miescher PA, Rothfield N, Miesher A. Lupus Erythematosus 1966; EL Dubois, Ed., Blakiston Co., New York
7. Rothfield NF, Thythyon JJ, McEwan C, Miescher P. Arth Rheuma 1961; 4: 223-229.
8. Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4th Edition. AACC Press (1995).

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (DXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 3	Updated layout and content	2022/10

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

Mascia Brunelli S.p.A., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@masciabrunelli.it; export@masciabrunelli.it; web: www.masciabrunelli.it