

Lupus Erythematosus Cell Test Magath And Winkle Method: A Diagnostic Test for Systemic Lupus Erythematosus

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ABSTRACT

Lupus Erythematosus (LE) cells were first discovered in 1948 by American clinical hematologists, Malcolm Hargraves and Robert Morton and a laboratory technician, Helen Richmond. Named LE cells because they are typically found in patients with Systemic Lupus Erythematosus (SLE) disease and are one of the antinuclear autoantibodies found in autoimmune diseases. LE cells are easily recognized under a microscope in the form of polymorphonuclear leukocytes (PMNs), usually neutrophils containing a homogeneous mass (LE Body) that is spherical in shape and reddish-purple in color, the nuclear lobes are pushed to one side and thinned, appearing trapped around the LE body. The LE cell test is carried out by destroying leukocyte cells so that they release their nucleoprotein and react with Immunoglobulin (Ig) G and this complex is phagocytized by normal PMN cells that are still present. The LE cell test was first performed to diagnose SLE, but is now rarely used because it has been replaced by a better test, namely the antinuclear antibody test (ANA) to diagnose SLE. However, LE cells are still considered the most important cells by some rheumatologists in the medical world. The LE cell test can be done in several ways, namely: the Magath and Winkle method (a modification of Zimmer and Hargraves), the Zinkham and Conley method, and the Mudrick method. Samples that can be used in the LE Cell test are: Heparinized venous blood, Oxalated venous blood, Defibrinated venous blood, and Clotted venous blood

Keywords: lupus erythematosus cells; systemic lupus erythematosus; antinuclear autoantibodies; magath and winkle method

INTRODUCTION

Lupus Erythematosus (LE) cells were first discovered in 1948 by American clinical hematologists, Malcolm Hargraves and Robert Morton and a laboratory technician, Helen Richmond. They have observed two unusual phenomena in several bone marrow preparations of patients with Systemic Lupus Erythematosus (SLE) disease, which they referred to as "Tart Cells" and "LE Cells" (LE Cell Phenomenon). Named Tart Cells because they look like tarts, and are called LE Cells because they are typically found in patients with SLE. Haserick, Lewis, and Bortz in 1950 demonstrated that L.E cells could be produced by incubating bone marrow with gamma globulin from LE patients. Mischer and Fauconnect in 1954 argued that it was gamma globulins that represented antibodies to components of the cell nucleus. [1-3]

LE cells are one of the antinuclear autoantibodies found in autoimmune diseases or after the administration of certain drugs. LE cells are easily recognizable under a microscope,

they are polymorphonuclear leukocytes (PMNs), usually neutrophils which contain a homogeneous mass (LE Body) in the cytoplasm that is spherical in shape and reddish-purple in color, the nuclear lobes are pushed to one side and thinned, appearing trapped around LE Body.[4,5,6] These inclusions represent the nuclei of damaged leukocytes and become homogeneous masses of reddish-purple in their cytoplasm and are phagocytized by normal polymorphonuclear leukocytes (PMN).[5] These cells are considered as LE cells that have not been fully formed or Pre-LE Cells.[7] LE cells need to be distinguished from Tart Cells (Pseudo LE Cells), which are monocytes that phagocytose other cells or other cell nuclei, especially lymphocyte nuclei with pyknotic nuclei, which are smaller than LE cells and lighter in color. LE cells are formed in vitro only because they require the presence of damaged leukocyte cells. [5,7,8] The LE cell test is carried out by destroying leukocytes so that they release their nucleoprotein and react with immunoglobulin (Ig) G and

this complex is phagocytized by normal and available PMN cells. [8,9]

The LE cell test was first performed to diagnose SLE, but is now rarely used because it has been replaced by a better test, namely the antinuclear antibody test (ANA) to diagnose SLE. However, LE cells are still considered the most important cells by some rheumatologists in the medical world. The phenomenon of LE cells in the course of SLE disease indicates the involvement of autoantibodies and complements in phagocytosis of the cell nucleus. The LE cell test is positive in 50%-75% of individuals with acute disseminated lupus, as well as in other autoimmune diseases such as rheumatoid arthritis, chronic (lupoid) hepatitis, scleroderma, dermatomyositis, polyarteritis nodosa, acquired hemolytic anemia, and Hodgkin's disease, as well as in adults taking certain medications such as phenylbutazone and hydralazine. The presence of LE cells is evidence of the presence of autoantibodies or LE factors, but the absence of LE cells does not mean that the individual does not have SLE disease. [6-9-11]

Formation of LE Cells (LE Cell Phenomenon) requires [4,5]:

- (1) Damaged leukocyte nucleus.
- (2) Leukocyte cells that are damaged will release their nucleoproteins.
- (3) LE factor presents in the patient's serum.
- (4) Immunoglobulin G and antibodies against Deoxyribonucleic Acid (DNA), nucleoproteins, or histones.
- (5) Viable PMN leukocytes.

Nucleoprotein released from damaged leukocyte cells will react with LE factor in serum, in the form of immunoglobulin (Ig) G. Depolymerization of the nucleus occurs, the chromatic structure is lost, a homogeneous mass of complement (LE Body) is formed and this complex attracts PMN (chemotaxis), then is phagocytized by normal viable PMN leukocytes (neutrophils), resulting in the formation of LE cells.

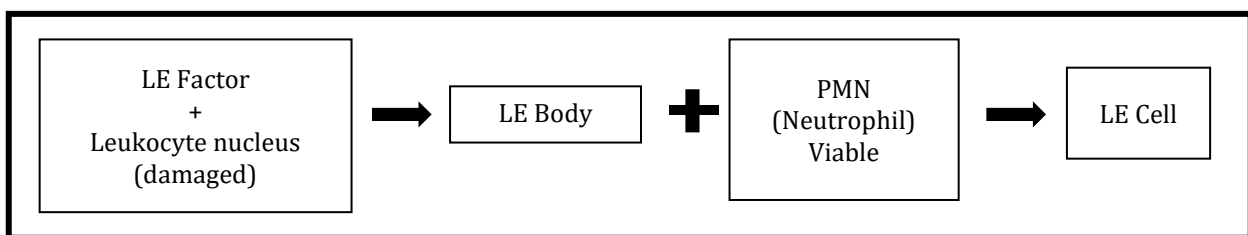


FIGURE 1: LE Cell Formation [5]

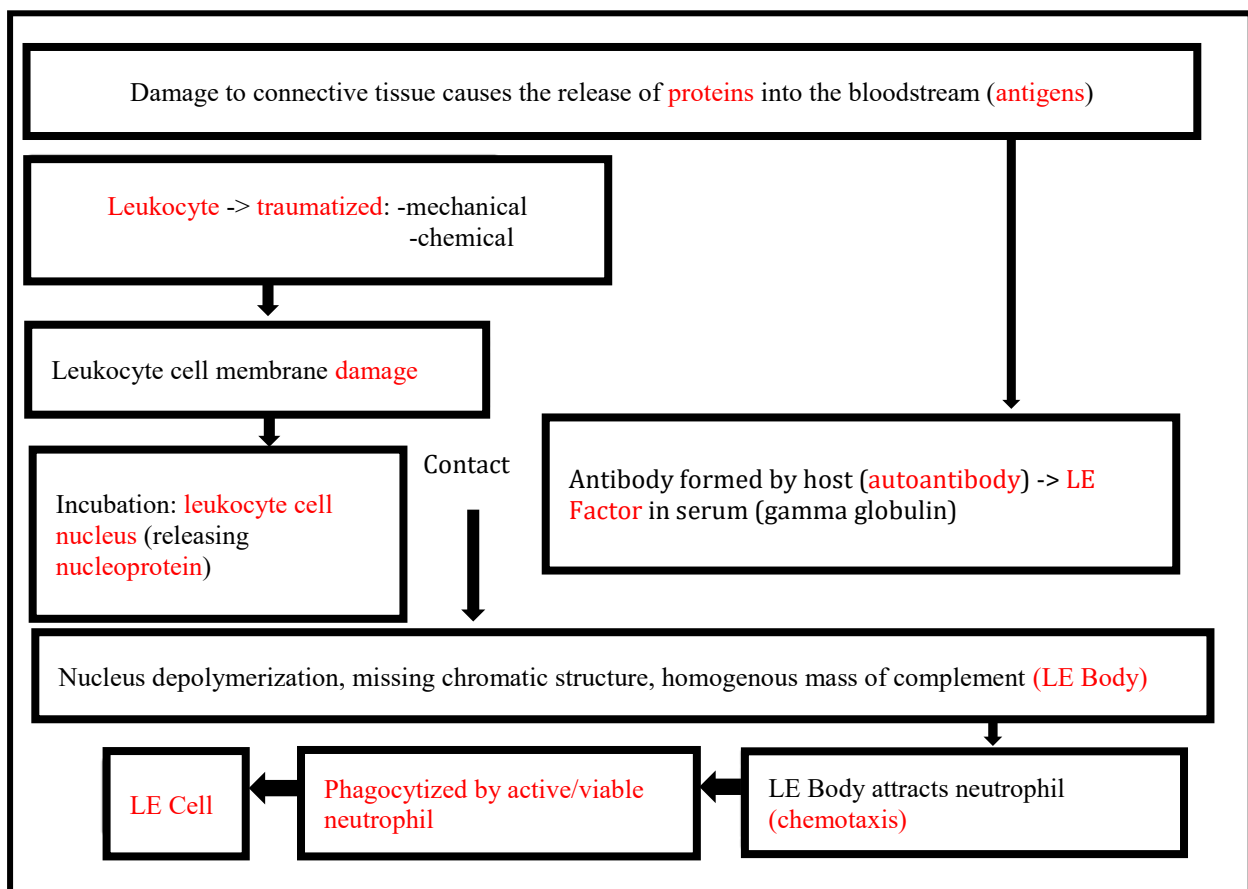


FIGURE 2: LE Cell Formation [4]

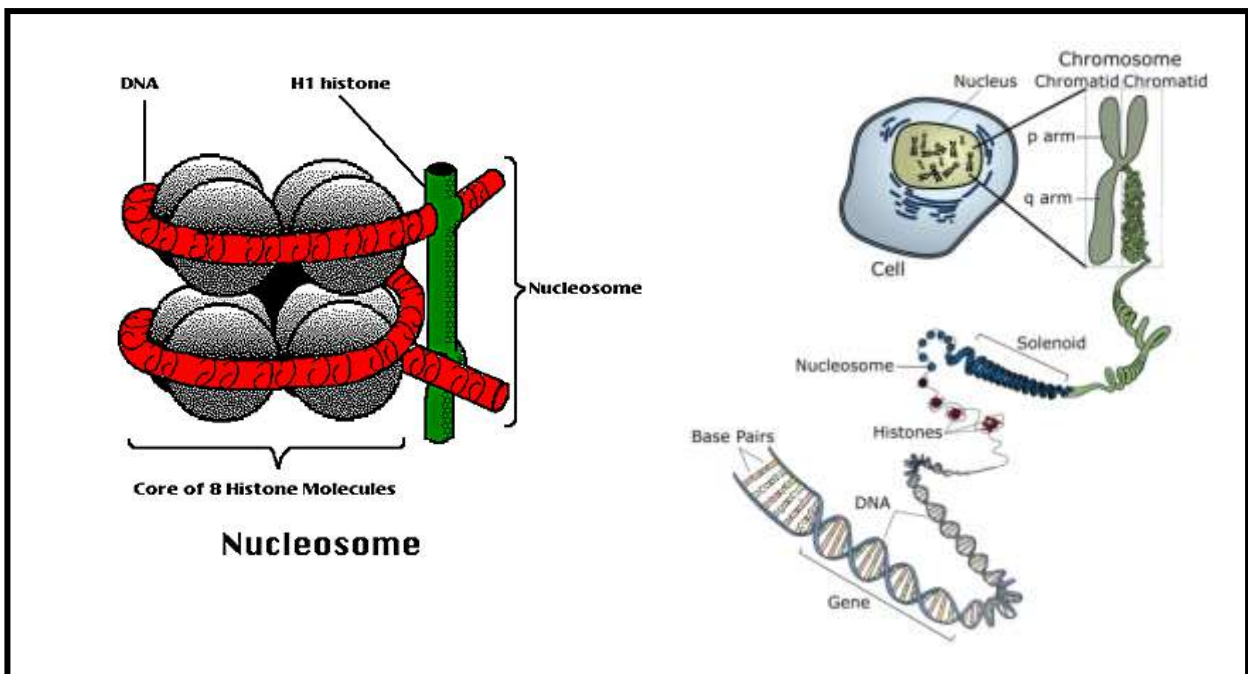


FIGURE 3: Nuclear Depolymerization [4]

The LE cell test can be done in several ways, namely: the Magath and Winkle method (a modification of Zimmer and Hargraves), the Zinkham and Conley method, and the Mudrick method. Samples that can be used in the LE Cell test are Heparinized venous blood, Oxalated venous blood, Defibrinated venous blood, and Clotted venous blood.[4,6,7] In this paper, we will discuss the LE cell test carried out at the Central Laboratory of dr. Wahidin Sudirohusodo Central General Hospital, Makassar, South Sulawesi, Indonesia, according to the Magath and Winkle method (modification of Zimmer and Hargraves) using clotted venous blood samples.

OBJECTIVE

The purpose of this test is to find out the method and principle of the LE cell test to support the diagnosis of SLE.

METHOD [4,7,8,12]

A. Pre Analysis

(1) Patient preparation

- Preferably before the LE cell examination is performed, the patient does not receive corticosteroid treatment for at least a week before the examination.
- The history of medication needs to be considered.

(2) Sample preparation

Clotted venous blood.

(3) Instruments and materials

Instruments:

- | | |
|---------------------|---|
| 1. Test tube | 6. Microscope |
| 2. Win Trobe tube | 7. Copper wire filter 30 wires per inch |
| 3. Pasteur pipette | 8. Object glass |
| 4. Centrifuge | 9. 37°C incubator |
| 5. Mortar & stamper | |

Materials:

1. Clotted venous blood 8-10 ml
2. Methanol
3. pH 6,8 buffer solution prepared from 26,5 cc of Na₂HPO₄·2H₂O + KH₂PO₄ to 100 cc
4. Ready to use Giemsa stain, composition: azure, eosin, methylene blue.



FIGURE 4: Instruments

B. Analytic**(1) Test principle**

The blood sample was heated in a 37°C water bath. The clotted sample was ground in a wire sieve. The filtrate is accommodated in a centrifuge tube/ Win Trobe tube. After centrifugation, a layer of leukocytes will be obtained at the top of the red blood cell layer that has been compressed. The leukocyte layer was taken to be removed, stained with Giemsa stain, and then viewed with a microscope.

(2) Method

The Magath and Winkle Method (modification of Zimmer and Hargraves):

1. Take 8-10 ml of venous blood and allow the blood to clot in a clean, dry tube.
2. Leave the tube for 2 hours at 27°C or 30 minutes in an incubator or water bath at 37°C.
3. Separate the clot from the serum, then the clot is filtered and crushed using a stamper through a copper wire sieve on a mortar.
4. The filtrate was filtered on a mortar, put into a Win Trobe tube, and centrifuged at 2000 rpm for 10 minutes.
5. Discard the serum, take the topmost cell layer (mostly "buffy coat") using a Pasteur pipette, and make a smear preparation.
6. Smear preparation:
 - With the right hand, place the leveling glass to the left of the drop of blood.
 - Move the leveling glass to the right until it touches the drop of blood.
 - Let the blood stick and spread evenly on the edge of the flat glass.
 - Immediately slide the glass to the left at an angle of 30-45°. Do not press the leveling glass down.

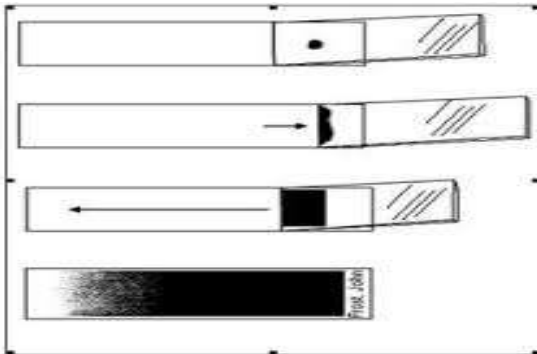


FIGURE 5: Creating smear preparation [12]

preparation by dripping methanol onto the preparation, so that the preparation is completely covered. Leave it for ± 2 minutes. Then remove excess methanol from the preparation.

8. Color the smear preparation with Giemsa's solution which has been diluted with buffer pH 6.8 (1:9), and leave on for 30 minutes. Then rinse the preparation under running water and place the preparation in an upright position and allow it to air dry.
9. Examine the smear under a 1000x magnification microscope, then look for the LE cells. Differentiate LE Cells with Tart Cells (Pseudo LE Cells) and Rosette Cells (Pre-LE Cells).
 - a. LE cells (Figures 3 and 4):
 - LE cells are neutrophil cells that phagocytize the nuclei of other leukocytes that have been damaged and form a homogeneous globular mass (LE Body) with reddish-purple color in the cytoplasm. Although the LE Body is basically a core, the core structure is completely invisible.
 - The nuclei of phagocytic neutrophils are pushed to one side, the nuclear lobes appear trapped around the LE Body.
 - The mass of the nucleus looks homogeneous.
 - b. Tart Cells (Pseudo LE Cells) (Figure 5):
 - Need to be distinguished from LE cells.
 - Tart cells are monocytes that phagocytize other monocyte cells/other cells, especially lymphocyte nuclei with pyknotic nuclei, which are smaller than LE cells and lighter in color.
 - The chromatin threads are still visible in the nucleus.
 - c. Rosette Cells (Pre-LE Cells) (Figure 6):
 - LE Body is surrounded by many PMN leukocytes.
 - This Rosette Cell form is not a diagnosis for LE phenomena. These cells are considered as Immature LE Cells or Pre-LE Cells.

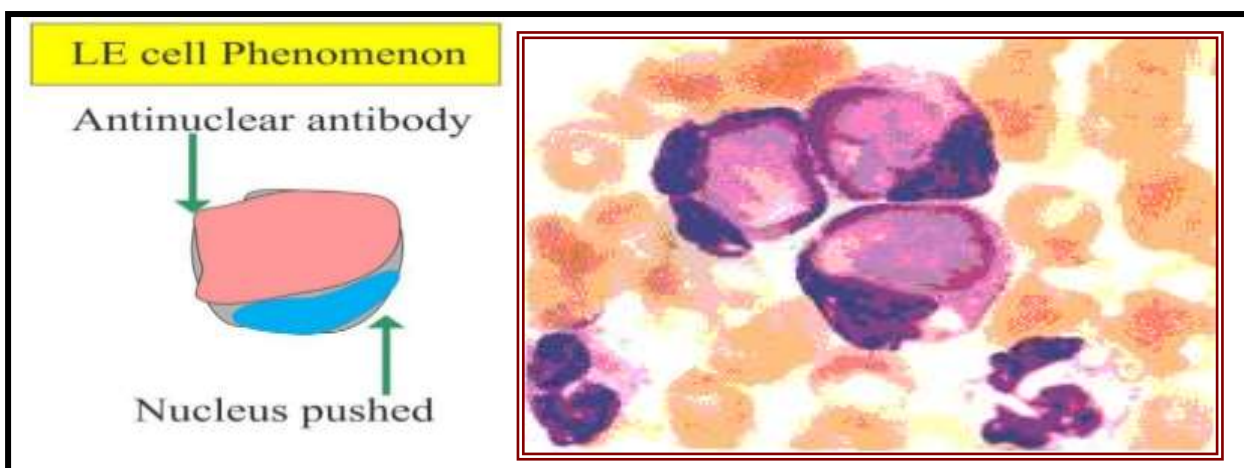


FIGURE 6: LE Cells [5,10]

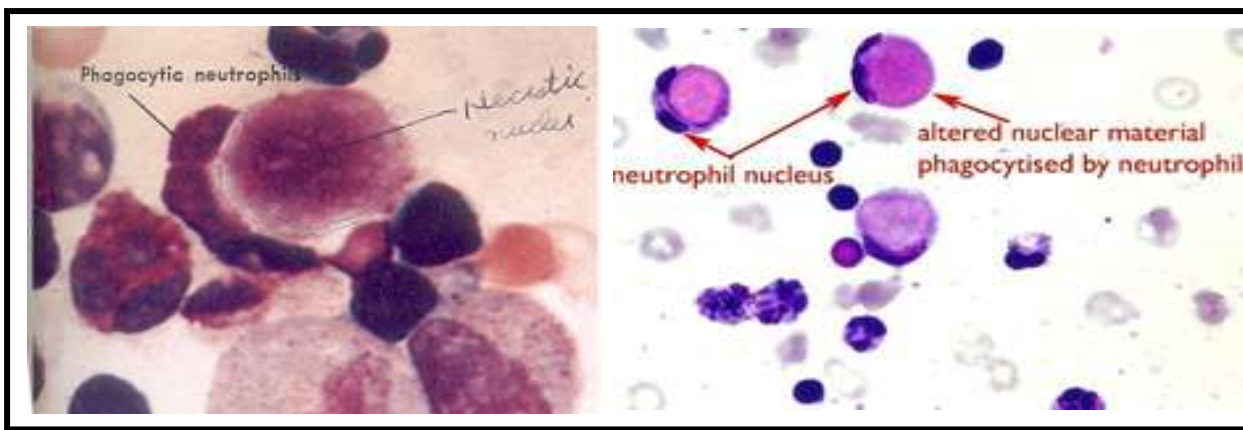


FIGURE 7: LE Cells [5,12]



FIGURE 8: Tart Cell (Pseudo LE Cell) [5,10]

C. Post analytic [4,6,8,9,12]

(1) Interpretation

a. Positive result (+): if obtained at least 2 LE cells.

Positive LE cell results can support the diagnosis of SLE, especially if it is obtained with the typical clinical symptoms of SLE. We recommend that you continue with the ANA test, anti-Deoxyribonucleic Acid (Anti DNA), or anti-Smith (Anti-Sm). If a large number of LE cells is found, it should be reported per 1000 neutrophils. False-positive results in: rheumatoid arthritis, chronic (lupoid) hepatitis, scleroderma, dermatomyositis, polyarteritis nodosa, acquired hemolytic anemia, Hodgkin's disease, and other collagen diseases, as well as reactions to certain drugs such as phenylbutazone and hydralazine.

b. Negative result (-): if there are less than 2 LE cells.

False-negative results in: SLE patients receiving corticosteroid treatment (associated with the effect of lowering/suppressing plasma levels of Immunoglobulin G) and in severe leukopenia.

(2) Sensitivity and Specificity

The LE cell test is positive in 75% of patients with SLE. It has a sensitivity of 85% and a specificity of 93%.

(3) Weaknesses and Strengths

The disadvantage of the LE cell test is that it has a lower sensitivity and specificity than the ANA test with a sensitivity of 91% and a specificity of 98%, it takes a long time to carry out the examination, and depends on the skill of the operator. However, the LE cell test is still used or considered to be carried out in the regions and the price of the examination is cheaper than the price of the ANA test.

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