



ANTI-H LECTIN

RECOGNITION OF H ANTIGEN IN HUMAN RED BLOOD CELLS AND SALIVA READ THE PACK INSERT CAREFULLY BEFORE PERFORMING THE TEST

CATALOGUE No. : Anti - HL

INTENDED USE : Anti-H Lectin is used for the recognition of H antigen on the human red blood cells. It is especially for assessing the H secretor status of group 'O' individuals and also in differential grouping of Aint subgroup along with the Anti-A1 Lectin.

INTRODUCTION : The H antigen is a basic blood group antigen present in human beings. There is considerable variation in the H antigen content in different individuals of the same ABO group but the general pattern indicates their strength as O>A2>A2B>B>A1>A1B. Water soluble H substance can also be demonstrated in saliva or body fluids of individuals who are secretors. Human red blood cells that do not agglutinate with Anti-H Lectin are classified as Bombay phenotype (Oh). The Bombay Phenotype is more common in India than other parts of the world and the estimated gene frequency of Oh phenotype in Bombay is 0.0066%. Anti-H Lectin is a ready to use purified extract of Ulex europaeus seeds. It contains a phytohaemagglutinin, which is virtually specific for the H antigen on human red blood cells.

PRINCIPLE: Human red blood cells possessing the H antigen will agglutinate in the presence of seed extracts (Lectins) containing phytohaemagglutinin specifically directed towards it. Water soluble H substance present in saliva neutralizes Anti-H Lectin. Agglutination of red blood cells or Neutralization of Anti-H Lectin by saliva is a positive test result and indicates the presence of H substance on or in the red cell or saliva respectively. No agglutination or Neutralization of Anti-H Lectin is a negative test result and indicates the absence of H substance on or in the red cell or saliva respectively.

STORAGE AND STABILITY: Store the reagents at 2-8°C. Do not use the reagent beyond the expiry date mentioned on it. Before performing the test bring the reagents to room temperature. Replace the reagents to 2-8°C soon after performing the test. DO NOT FREEZE THE REAGENTS.

1. The unopened kit is stable for 2 years from the date of manufacturing as indicated on the package.
2. The opened kit is stable for 6 months from the date of opening.
3. Repeated freeze thaw of reagents from 2-8°C to room temperature several times will reduce the stability of the kit.

PACK SIZE: Available in packs of 2ml/5ml/10ml.

TITRE: 1:256

AVIDITY: 2-7 Seconds

CONTENTS OF THE KIT : Anti-H Lectin = 2ml/5ml/10ml

MATERIALS REQUIRED BUT NOT PROVIDED:

1. Slide or Tube
2. Lancet
3. Applicator sticks
4. Saliva
5. Isotonic saline
6. Red blood cells positive for H antigen
7. Red blood cells negative for H antigen
8. Saliva positive for H antigen
9. Saliva negative for H antigen

SAMPLE COLLECTION AND PREPARATION:

For recognition of H antigen on human red blood cells

1. No special preparation of the patient is required prior to sample collection by approved techniques.
2. Samples should be stored at 2-8°C, if not tested immediately. Do not use haemolysed samples.
3. Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period.

EDTA or Heparin : 2 days

Sodium citrate or Sodium Oxalate : 14 days

ACD or CPD : 28 days

Clotted whole blood should be tested within 14 days.

For assessing secretor status in human saliva

1. Collect about 2ml of fresh saliva in a glass tube and incubate in a boiling water bath for 10 minutes.
2. Centrifuge at 3400 rpm (1000g) for 10 minutes.
3. Freeze the clear supernatant at -20°C immediately for further use.

TEST PROCEDURE:

Bring all reagents and samples to room temperature before testing.

A. SLIDE TEST:

1. Place one drop of Anti-H Lectin on a clean glass slide.
2. To the reagent drop, add one drop of whole blood and mix well.
3. Rock the slide gently back and forth.
4. Observe for agglutination at the end of two minutes. Peripheral drying should not be misinterpreted as a positive result.

B. TUBE TEST:

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of Anti-H Lectin into a test tube.
3. Add one drop of test red cell suspension into the test tube and mix well.
4. Centrifuge for one minute at 1000 rpm (125g) or 20 seconds at 3400 rpm (1000g).
5. Gently resuspend the cell button, observing for agglutination macroscopically.

C. TUBE TEST : (Secretor Status):

1. Add two drops of Anti-H Lectin into two glass tubes.
2. Add two drops of saliva into the tubes and mix well.
3. Incubate at room temperature for 10 minutes.
4. Add one drop of Positive and negative cell suspensions into the respective tubes, mix well and incubate at room temperature for 5 minutes.
5. Mix well and centrifuge for one minute at 1000 rpm (125g) or 20 seconds at 3400 rpm (1000g).
6. Gently resuspend the cell button and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS :

SLIDE AND TUBE TEST:

1. Agglutination is a positive test result and indicates the presence of H antigen.
2. No agglutination is a negative test result and indicates the absence of H antigen and the red cells being of Bombay phenotype (Oh).

TUBE TEST (Secretor Status):

1. Agglutination of the red cells indicates that the Anti-H Lectin has not been neutralized and the patient is a non-secretor.
2. No agglutination of the red cells indicates that the Anti-H Lectin has been neutralized and the patient is a secretor.

TROUBLE SHOOTING	
FALSE POSITIVE	
Cause/Error	Remedy
1. Contaminated blood specimen or reagents 2. Drying in slide test 3. Clotting of blood	<ul style="list-style-type: none"> • Make sure that there is no contamination of blood Specimen or reagent. • Use clean slide or tube for testing. • Do not read result after two minutes. • Test the sample immediately if anti-coagulant is not Added to the sample.

FALSE NEGATIVE	
Cause/Error	Remedy
1. Contaminated blood specimen or reagent	Make sure that there is no contamination of blood specimen or reagent Use clean slide or tube for testing Do not read the result after two human test.

WEAKLY DELAYED REACTION	
Cause/Error	Remedy
1. Prolonged storage of red blood cells. 2. Expired reagent	Store the blood sample with anticoagulant at 2-8°C for less than 30days check the expiry date on the reagent bottle

LIMITATIONS OF THE TEST:

1. The blood drop on the slide should not be allowed to dry; partial drying of the blood could be misinterpreted as agglutination.
2. Centrifugation should be perfect. Over-centrifugation or under-centrifugation may result in false negative interpretation.
3. Dislodgement of sedimented red cells in the tube test should be done as gently as possible, rough dislodgement may disrupt small or weak agglutinates and hence may lead to false negative interpretation.
4. The entire procedure should be carried out at room temperature. Warm or cold antibodies in the tested blood can cause agglutination and may lead to wrong interpretation.
5. Hemolysed blood samples should not be used.
6. It is recommended that known positive and negative cells must be included as controls with each test series.

REFERENCE:

1. Race R. & Sanger R., Blood Groups in man., 6th Ed., Blackwell Science, Oxford 1975.
2. Technical Manual of the American Association of Blood Banks, 9th Ed., 1985. 127-153.
3. Lee H.H., Rouger P., Germain C., Muller A. & Salmon C. (1983).



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