

ANTI-H LECTIN
ULEX EUROPAEUS LECTIN FOR SLIDE AND TUBE TESTS

SUMMARY

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%.

REAGENT

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.

Anti-H lectin is used for recognition of the H antigen on human red blood cells. It is useful, especially for assessing the H secretor status of group 'O' individuals and also in differential grouping of A_{int} subgroup along with Anti-A₁ lectin.

REAGENT STORAGE AND STABILITY

- a) Store the reagent at 2-8°C. DO NOT FREEZE.
- b) The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE

Human red blood cells possessing the H antigen will agglutinate in the presence of seed extract (lectins) containing phytohaemagglutinin specifically directed towards it. Water soluble H substance present in saliva neutralises Anti-H lectin. Agglutination of red blood cells / Neutralization of Anti-H lectin by saliva is a positive test result and indicates the presence of H substance on/in the red cell / saliva respectively.

No agglutination / Neutralization of Anti-H lectin is a negative test result and indicates the absence of H substance on / in the red cell / saliva respectively.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. Extreme turbidity may indicate microbial contamination / reagent deterioration. Such reagents should be discarded.

SAMPLE COLLECTION AND PREPARATION

For recognition of H antigen on human red blood cells

No special preparation of the patient is required prior to sample collection by approved techniques.

Samples should be stored at 2-8°C, if not tested immediately. Do not use haemolysed samples.

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

- a) Collect about 2 ml of fresh saliva in a glass tube and incubate in a boiling water bath for 10 minutes.
- b) Centrifuge at 3400 rpm (1000 g) for 10 minutes.
- c) Use the clear supernatant immediately for the study or freeze immediately if to be tested later.

ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS

Glass slides (50 x 75 mm), Test tubes (10 x 75 mm), Pasteur pipettes, Isotonic saline, Centrifuge, Timer, Mixing sticks. Red blood cells positive for H antigen, Red blood cells negative for H antigen, Saliva positive for H antigen, Saliva negative for H antigen.

PROCEDURE

Bring all reagents and samples to room temperature before testing.

Slide Test

1. Place one drop of Anti-H lectin on a clean glass slide.
2. Add one drop of whole blood to be tested on the slide and mix well with a mixing stick uniformly over an area of approximately 2.5 cm².
3. Rock the slide gently, back and forth.
4. Observe for agglutination macroscopically at two minutes.

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. Pipette into the test tube, one drop of the test red cell suspension and mix well.
4. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Gently resuspend the cell button, observing for agglutination macroscopically.

Tube Test (Secretor Status)

1. Place two drops of Anti-H lectin into two clean glass tubes.
2. Pipette two drops of saliva into the tubes and mix well.
3. Incubate at room temperature for ten minutes.
4. Add one drop of negative and positive cell suspensions respectively into the tubes, mix well and incubate at room temperature for five minutes.
5. Mix well and centrifuge for one minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
6. Gently resuspend the cell button in each test tube observing for agglutination macroscopically.

INTERPRETATION OF RESULTS

Slide and Tube Tests

Agglutination is a positive test result and indicates the presence of H antigen.

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)

Agglutination of the red cells indicates that the Anti-H lectin has not been neutralised and the patient is a non-secretor.

No agglutination of the red cells indicates the Anti-H lectin has been neutralised and the patient is a secretor.

REMARKS

1. Do not interpret peripheral drying or fibrin strands as agglutination.
2. It is recommended that known negative and positive cells must be included as controls with each test series.
3. As undercentrifugation or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required for achieving the desired results.

WARRANTY

This product is designed to perform as described on the label and the package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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