Cells	Results
A_1	Will show weak or no agglutination
A_2	Will show weak to +1 agglutination
B	Will show +1 to +2 agglutination
O	Will show strong +3 to +4 agglutination

TUBE METHOD (SECRETOR STATUS)

Agglutination of the red blood cells in tube '1' indicates that the Anti-H has not been neutralised and the patient is a non secretor. Inhibition of the agglutination of the red blood cells in tube '1' indicates that the soluble antigens in the saliva neutralised the Anti-H indicating that the patient is a secretor. The above interpretation is valid only if the tube '2' shows agglutination.

QUALITY CONTROL

Each batch of Anti-H Lectin is subjected to stringent internal quality control with positive control (ABO group O red cells) and negative / weak control (A_1 red cells) regarding titre, avidity and intensity to ensure constant quality of reagent.

Specificity: It is checked to ensure whether the reagent is specific for the purpose or not with Bombay group.

PROBLEMS IN BLOOD TYPING/SECRETOR STATUS

1. Improper identification of specimen.
2. Improper techniques like
   - Cell to reagent ratio.
   - Failure to identify haemolysis
   - Improper storage of Reagents.
   - Fibrin clots
   - Over incubation of cells and reagents.
3. Improper centrifuge calibration resulting in over/under centrifugation.
4. Improper centrifugation of saliva resulting in turbid supernatant.

REFERENCES

- Race RR, Sanger R. Blood Groups in Man, 6th edn. Oxford: Blackwell Scientific Publications 1975.
- Technical Manual American Association of Blood Banks, 9th edn. 1985.
- Daniels g. Human Blood Groups, 1st edn. Blackwell Science Ltd. 1995

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, in as much as that the test

in vitro diagnostic Reagent, not for medicinal use

J. Mitra & Co. Pvt. Ltd.
A-180-181, Okhla Ind. Area, Phase - 1, New Delhi - 110 020, India
Phone: +91-11-47130300, 47130500
E-mail: jmitra@jmitra.co.in **Internet:** www.jmitra.co.in

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Anti-H Lectin

ULEX EUROPAEUS LECTIN FOR SLIDE AND TUBE TESTS

The Anti-H Lectin is a purified extract from seeds of Ulex europaeus. The reagent contains a phytohaemagglutinin which is virtually specific for the H antigen on human red blood cells.

Anti-H Lectin is used to demonstrate the presence of H antigen on human red blood cells and in assessing the H secretor status of group 'O' individuals.

PRINCIPLE

Major percentage of the population (80%) have soluble ABO blood group antigens present in their body fluids. These patients are known as ABH secretors while remaining 20% are non secretors. Secretors contains the following ABH antigens in their body fluids:

O Secretors	:	Only H	A Secretors	:	A & H
B Secretors	:	B & H	AB Secretors	:	A, B & H
Non Secretors	:	None			

Human red blood cells possessing H antigen will agglutinate in the presence of seed extract of Ulex europaeus containing phytoagglutinin specifically directed towards it. Saliva also contains water soluble 'H' antigen which gets neutralised by Anti-H Lectin. Agglutination of red cells/neutralisation of Anti-H Lectin by saliva is a positive test result and indicates the presence of H substance on/in the red cells/saliva respectively. No agglutination of red cells/neutralisation of Anti-H Lectin by saliva is a negative test result and indicates the absence of H substance on/in the red cells/saliva respectively

STORAGE AND PACK SIZE:

The Anti-H Lectin is packed into 5 ml & 10 ml glass vials.

The reagent is stable at 2-8ºC for 24 months. Sodium azide is added to the reagent at 0.1 % concentration as preservative.

SAMPLE COLLECTION:

Blood : Blood sample should be collected with a suitable anticoagulant in a sterile stoppered container & should be tested immediately. If testing is delayed, blood should be stored at 2-8ºC & must be examined not later than 48 hours. Haemolysed & clotted samples should not be used for testing.

Saliva : For determination of secretor status. Collect 1ml of fresh saliva in a glass tube and boil for 10 minutes in a water bath. Centrifuge at 3500 rpm. for 10 minutes. Use clear supernatant immediately for testing. Samples may be frozen, if testing is proposed at a later date.

DESCRIPTION OF SYMBOLS USED

The following are graphical sysmbols used in or found on J. Mitra diagnostic products and packing. These sysbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2021.


Manufactured By

Catalogue Number

Lot Number
Batch Number


Manufacturing Date


Expiry Date


Do not use if package is damaged


Keep Dry

In vitro diagnostic medical device


Instruction for use

Temperature
Limitation


Caution
See instruction for use


Keep away from sunlight

Contains biological Material of Animal Origin

Country of Manufacture

WARNING FOR USERS

CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for professional use only and should be run by competent person only.
5. Do not pipette by mouth.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. Test shall be performed at room temperature (20ºC to 30ºC).
8. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed of in accordance with established safety procedures.
9. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
10. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
11. The reagent contain Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Center for Disease Control, Atlanta, Georgia, April 30, 1976.)
12. Only hh (Bombay) red cells will give a true negative reactive with this reagent.
13. The reagent occasionally shows slight turbidity after storage at 2-8ºC due to aggregation of solids and may appear light brown. However, this do not affect the quality of the reagent.
14. This reagent must not be diluted as further dilution will damage its ability to agglutinate the red blood cells.

ADDITIONAL MATERIALS REQUIRED

Glass slides, Test tubes (10X75mm), Pasteur Pipettes,Normal saline, Beakers, Centrifuge, Timer & Mixing sticks.

GROUPING TECHNIQUE

Separate the RBCs from the serum or plasma by centrifuging at 5000 rpm for 5 min.

(a) **Slide Method:** Place one drop each of Anti-H Lectin and negative control (normal saline) on a slide and add one drop of whole blood sample to both of them. Mix the cells & reagent with clean mixing stick & spread the mixture over an area of 2 cm. Rock the slide gently from side to side & observe for the agglutination within two minutes.

(b) **Tube Method (Blood):** Prepare a 5% suspension of red cells washed in isotonic saline solution. Place one drop of Anti-H Lectin and add one drop of 5% cells suspension in a tube. Shake to homogenise reagent and red cells suspension, then centrifuge for one minute at 1000 rpm. The reaction is read macroscopically by shaking gently the tube so as to loosen the cells pellet . If the red cells separate in one or more clumps, the reaction is positive. If the red cells return to a homogneous suspension, the reaction is negative.

(c) **Tube Method (Secretors Status):** Add two drops each of Anti-H Lectin in two different test tubes marked as '1' & '2'. Add 100 µl of saliva in tube '1' and 100µl normal saline in tube '2' and mix well. Incubate at room temperature for 10 mins. Add 50µl of 5% cell suspension of known 'O' red cells to both the tubes. Gently shake to mix the reagents and cells. Incubate at room temperature for 5 mins. Centrifuge for 1 min. at 1000 rpm. Results are read as in tube method (blood).

INTERPRETATION

SLIDE AND TUBE METHOD (BLOOD)

Agglutination of the red blood cells with the reagent is a positive test indicating the presence of H antigen on blood cells & absence of agglutination indicates that the cells do not possess H antigen and the cells are of Bombay phenotype (Oh). The results observed with different groups are as follows: