

Cells	Results
A <sub>1</sub>	Will show weak or no agglutination
A <sub>2</sub>	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<sub>1</sub> red cells) regarding 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 kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

*in vitro* diagnostic Reagent, not for medicinal use

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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 5ml glass vials. The reagent is stable at 2-8°C 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 2-8°C & 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 symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the British and European Standard EN ISO 15223-1:2016.

	Manufactured By		In vitro diagnostic medical device
	Catalogue Number		See Instruction for use
	Lot Number		Temperature Limitation
	Batch Number		Caution
	Manufacturing Date		See instruction for use
	Expiry Date		Keep away from sunlight
	Do not use if package is damaged		Keep Dry

### 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 *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. 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 121°C at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. 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.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. 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" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976.)
10. Only hh (Bombay) red cells will give a true negative reactive with this reagent.
11. The reagent occasionally shows slight turbidity after storage at 2-8°C due to aggregation of solids and may appear light brown. However, this do not affect the quality of the reagent.
12. 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 plasma by centrifuging blood at 5000 rpm for 5 mins.

- (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 homogeneous 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: