

**NATIONAL INSTITUTE OF BIOLOGICALS
NOIDA
BLOOD REAGENT LABORATORY**

Monographs for Blood Grouping Lectins

Anti- H

Source

Anti- H (Lectin) is an extract of (*Ulex europaeus*) seeds prepared in a saline diluent.

The H antigen is present in variable quantity on human red blood cells, depending on the ABO blood group of the individual. The greatest amount is present on red blood cells of group O, intermediate amounts occur on red blood cells of groups A₂ and certain other A-subgroups, A₂B and B, least amounts are present on red cells of A₁ and A₁B. Bombay group (O_h) does not contain H antigen and does not react with Anti-H lectin.

Principle of the Test

For resolving problems related to ABO subgroups by direct agglutination tests, wherein agglutination of the test red blood cells indicates the presence of the H antigen, and variability in the strength of the reaction may be a reflection of variable expression of the antigen. No agglutination indicates that the H antigen is absent, or possibly that its expression on the test red blood cells is too weak to be detected.

Anti- H reagents are tested for their Physical Appearance, Color, Potency, Intensity, Specificity, Rouleaux, and Haemolysis and Prozone parameters and have values at least equal to the appropriate International Standards or suitable reference preparation used.

Physical Appearance and Color:

Anti-H reagent should be clear with no turbidity, precipitate, particles or gel formation by visual inspection. Anti-H (Lectin) blood grouping Reagents are colorless to mild amber color liquid.

Potency

It is defined as the reciprocal of the greatest reagent dilution for which the reaction is graded at 1+. Anti-H (Lectin) reagent should have an average potency value at least equal to that of the reference preparation.

Type of Reagent	Test Red Blood Cells	Titer
Anti-H (Lectin)	O (2 cells) A ₂ (2 cells) A ₁ * (2 cells)	1:8 to 1:16 (1+) 1:4 to 1:8 (1+) 1:1 – 1:2 (1+) *In rare cases negative reaction is observed

Intensity

It is defined by the appearance of agglutination obtained after mixing undiluted Anti-H (Lectin) reagent with washed red blood cell suspension. For this 1 volume of the reagent is added to 1 volume of 40% red cell suspension on a slide and mixed for 3 minutes. At the end of 3 minutes, reaction grade is compared with the strength of reaction obtained with the reference preparation.

Type of Reagent	Test Red Blood Cells	Intensity
Anti-H (Lectin)	O (2 cells) A ₂ (2 cells) A ₁ (2 cells)	Strong 2 + to 4+ (within 3 minutes) 2+ (within 3 minutes) <2+ (within 3minutes)

Specificity

Reagent is tested with red cell suspension which exhibits the antigen corresponding to the reagent antibody and yields positive reaction and red cell suspension which lacks the antigen corresponding to reagent antibody yields negative reaction.

For this 1 volume of Anti-H (Lectin) reagent is mixed in a test tube with 1 volume of 2-3% red cell suspension which exhibits the antigen corresponding to the reagent antibody and red cell suspension which lacks the antigen corresponding to reagent antibody. Centrifuge the tube at 1000rpm for 1 minute. Tube with corresponding antigen gives positive reaction and the tube which lacks the antigen gives negative reaction.

Type of Reagent	Test Red Blood Cells	Specificity
Anti-H (Lectin)	O (2 cells) A ₂ (2 cells) A ₁ (2 cells) Oh (2 cells)	Positive Positive Positive Negative

Reactivity

Haemolysis

It is defined as the release of hemoglobin from the red cells as a result either of osmotic effect or of the breaking up of the red blood cells. No immune hemolysis should be observed with the red blood cells used for specificity.

Type of Reagent	Test Red Blood Cells	Haemolysis
Anti-H (Lectin)	O (2 cells)	Absent
	A ₂ (2 cells)	Absent
	A ₁ (2 cells)	Absent
	Oh (2 cells)	Absent

Rouleaux

It is defined as a form of pseudo agglutination in which the red blood cells look like pile of coins. The red blood cells lacking the antigen corresponding to the reagent antibody should not show rouleaux formation. For this 1 volume of Anti-A₁ (Lectin) is mixed in a test tube with 1 volume of 2-3% red cell suspension which lacks the antigen corresponding to reagent antibody and the tube is centrifuged at 1000rpm for 1 minute and observed microscopically for rouleaux formation.

Type of Reagent	Test Red Blood Cells	Rouleaux
Anti-H (Lectin)	Oh (2 cells)	Absent

Prozone

It is defined as a phenomenon in which negative reactions are obtained with low dilutions of an antibody, while a positive reaction is obtained with higher dilutions. For this 1 volume of Anti-A₁ (Lectin) is mixed with 1 volume of 2-3% red cell suspension in test tubes which exhibit the antigen corresponding to the reagent antibody for “15 minutes”, “30 minutes”, and “60 minutes”. If the reaction grades are the same or increase as the incubation time increases, no prozone is present and if the reaction grades decrease as the incubation time increases, prozone is present.

Type of Reagent	Test Red Blood Cells	Specificity
Anti-H (Lectin)	O (2 cells)	Absent
	A ₂ (2 cells)	Absent
	A ₁ (2 cells)	

Expiration date. The expiration date for Anti-H (Lectin) reagent is not less than 1 year at 2° to 8°C.

Storage. Store at a temperature between 2° to 8°C.

