INTERPRETATION

Agglutination of the red blood cells is a positive test result and indicates the presence of the A1 antigen and absence of agglutination indicates that the cells does not possess A1 Antigen and are A2 subgroup.

QUALITY CONTROL: Each batch of Anti - A_1 Lectin is subjected to stringent internal quality control regarding specificity, avidity, intensity and titre to ensure constant quality of reagent.

Avidity: It is defined as the reactivity time (in seconds) taken by the antibodies to show the agglutination and is tested by slide method with 40% red cells suspension.

Intensity: It shows the strength of the reaction ie clumping. It is tested by slide method with 40% red cells suspension & is graded as :

+/- Doubtful for agglutination Repeat test
+1- Small clumps scattered in the test area
+2- Two or more clumps of equal size
+3- One big clump with some small clumps
+4- One big clump in the centre.

Titre: Titre is checked by tube method with 2-5% cell suspension. It is defined as the reciprocal of highest dilution of antibodies which gives agglutination. The reagent is diluted up to 256 dilution by two fold serial dilution.

Specificity: It is checked to ensure whether the reagent is specific for the purpose or not. For example specificity is checked with A2 & A2B cells.

LIMITATIONS OF PROCEDURE

1. Test using antisera of the ABO blood group system should be performed at room temperature (25ºC±5ºC) and never incubated at high temperatures.
2. The A1 antigen is not fully expressed on the red blood cells of new born infants and false negative results may occur.
3. Red cells upto 28 days old may be tested with this reagent; however positive reactions may be weaker than those obtained with fresh red cells.
4. Contaminated blood specimens and/or supplementary materials used in the procedures described in this protocol may interfere with the results.

SPECIFIC PERFORMANCE CHARACTERISTICS

When properly stored and used according to the procedures described under Direction for Use, this reagent will agglutinate red cells which have the A_1 antigens. The potency of this reagent is standardised by comparison to a previously approved lot. The reactivity of each lot is demonstrated in tests with

in vitro diagnostic Reagent, not for medicinal use

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J. Mitra & Co. Pvt. Ltd.

Anti A_1 Lectin
(Dolichos biflorus)

Qualitative Test for the Detection of A_1 Antigen on Human Red Blood Cells

INTRODUCTION

Erythrocytes possessing the A antigen can be subdivided into A_1 and A_2 cells. Group A red blood cells which are agglutinated with Anti A_1 Lectin are said to be sub group A_1. Those which are not agglutinated by Anti A_1 lectin fall into subgroups weaker than A_1, the majority being classified as A_2. Approximately 80% of the population of blood group A is A1 while the remaining 20% are A_2 or weaker subgroup.

PRINCIPLE

The procedures used with this reagent are based on the principle of agglutination described by Landsteiner. Normal human red blood cells posessing antigens will clump in the presence of antibody directed toward the antigens. Renkonen showed that some extracts of seeds (lectin) contain powerful agglutinins with human blood group specificity. The extract of the seeds from Dolichos biflorus is virtually specific for A1 antigen on red blood cells.

REAGENT

Anti A_1 lectin (Dolichos biflorus) is designed for use in agglutination tests for the detection of the A_1 antigen on human red cells. This reagent is a purified extract of the seeds of Dolichos biflorus, containing a phytohemagglutinin (lectin) which agglutinates red blood cells of the subroup A_1 or A_1B.

STORAGE AND PACK SIZE:

Anti-A_1 Lectin is packed into 5 ml & 10 ml dropper vial. Anti-A_1 Lectin is stable for 24 months at 2-8ºC from the date of manufacturing.

Do not use the kit beyond expiration date. Bring it to room temperature (25±5%) before use.

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	IVD	In vitro diagnostic medical device
REF	Catalogue Number		Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution
	Expiry Date		See instruction for use Keep away from sunlight
	Do not use if package is damaged	BIO	Contains biological Material of Animal Origin
	Keep Dry	IN	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 *in vitro* diagnostic 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. Extreme turbidity may indicate microbial contamination. Serologic testing is necessary to recognize reagent deterioration. It is recommended that the reagent should be tested with appropriate positive and negative controls according to the methods described in this protocol.

Positive Control: Red blood cells known to possess the antigen (s) towards which the antiserum is directed.

Negative Control: Red blood cells known to lack the antigen (s) towards which the antiserum is directed.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection. Blood collected with an anticoagulant (ACD, CPD, Sodium Citrate, Ammonium Oxalate, EDTA and Heparin) should be used in the test procedures. The blood sample should be tested as soon as possible following collection. If a delay in testing occur. The sample should be stored at 2-8ºC.

Red blood cells collected and stored in ACD or CPD have been shown to maintain reactivity for 28 days while RBCs collected and stored in sodium citrate or oxalate are reactive for atleast 14 days. If red blood cells are collected in EDTA or heparin they should be tested within 48 hours.

Blood obtained by finger puncture may be tested directly in the slide method.

However to avoid clotting, blood collected in this manner should be mixed quickly with the test serum.

ADDITIONAL MATERIALS REQUIRED

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

Required Supplementary materials

DIRECTION FOR USE

Tube Method

1. Prepare a 3-4% suspension of red blood cells in Isotonic Saline.
2. To a test tube add 1 drop of Anti-A_1 lectin.
3. Using a Pasteur pipette add 1 drop of the 3-4% red blood cell suspension to the above test tube.
4. Mix well and Centrifuge for 1 minute at 1000 rpm (100-125 rcf). The centrifugal force applied to cell/serum mixture should be the minimum required to produce a “button” of red cells and a clear supernatent. Overcentrifugation i.e. the application of forces in excess of the minimum required to produce a button causes the cells to adhere to the bottom of the test tube. Vigorous agitation is then necessary before they can be resuspended. During such agitation weak agglutination may be dispersed causing a positive reaction to be missed under centrifugation i.e. the failure to apply forces necessary to cause the cells to form a button and a clear supernatent may result in weak or negative reaction. Exact speed and time of centrifugation cannot be recommended which will cover the wide variety of centrifuges available. Therefore each laboratory must calibrate its own equipment and determine the time required at a given speed to achieve the desired result.
5. Resuspend the cells by gentle agitation and examine macroscopically for agglutination.

Slide Method

1. Take whole blood sample to be tested.
2. Using a Pasteur pipette, place 1 drop of the whole blood sample on the slide.
3. Place 1 drop of Anti A_1 lectin next to the whole blood sample on the slide.
4. Mix the whole blood sample and Anti A_1 lectin mixture well with an applicator stick.
5. Tilt the slide back and forth and observe for agglutination within 1 minute. Test that do not show agglutination within 1 minute are considered NEGATIVE. Do not interpret drying or fibrin strands as agglutination.