Anti-A Lectin from Dolichos biflorus for the tube, slide, plate tests and gel technique

INTENDED USE AND PRINCIPLE
Anti-A, Lectin from Dolichos biflorus is intended for the A, blood group determination with conventional techniques or with the gel technique. Anti-A, Lectin agglutinates adult human erythrocytes of blood group A and AB belonging to the subgroup A, B, or AB. The classification of group A into A, A, or weaker variants is especially used in blood banking (choice of donors) or in genetic studies.

When having a concentration of 4 µg/ml, this Phytolectin shows strong reactions with A, erythrocytes, by binding itself to N-acetylgalactosamine, which provokes the agglutination of A, cells. Very weak unspecific reactions may be observed with A, erythrocytes. Only when having a minimum concentration of 400 µg/ml, Dolichos biflorus Lectin agglutinates A, as well as A, erythrocytes.

For in vitro Diagnostic Use.

REAGENT
Anti-A, Lectin reagent consists of a purified and stable plant haemagglutinin (Phytemagglutinin) from Dolichos biflorus in a diluent containing 0.1% (w/v) sodium azide as a preservative. Titer: > 1.8 with A, erythrocytes using tube technique. The assigned titer was obtained at manufacture according to the manufacturer’s quality control procedure.

Warning: This product contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded lead and copper plumbing to form highly explosive metal azides. If discarded in the presence of a strong oxidizing agent, a violent reaction can occur with explosive results.

STABILITY
Do not expose to extreme temperatures. Store at 2-8 °C when not in use. Turbidity may indicate reagent deterioration or microbial contamination; discard if present. The clear brownish appearance of the liquid is due to the method of extraction during the manufacturing process and has no impact on the quality of the reagent. Do not use beyond expiration date. If stored appropriately at 2-8 °C the product is stable after the first opening until the indicated expiration date.

SPECIMEN COLLECTION AND PREPARATION
Collect blood specimens using acceptable phlebotomy techniques. Specimens may be collected in commonly used anticoagulants (e.g. in EDTA, Cilstrate) or without anticoagulant if tube, slide or plate techniques are used. The testing should be performed as soon as possible after the blood draw to minimise the risk of false positive or false negative results through possible contamination and inappropriate storage. Therefore fresh cells are preferred for testing; blood specimens not used immediately or requiring shipment should be stored at 2-8 °C. Blood specimens exhibiting gross haemolysis or contamination should not be used. Reagent red blood cells should be used according to the manufacturer’s instructions.

PROCEDURE
Reagent provided
Anti-A, Lectin, 1x5 ml, cat. no. 213437

Materials required but not provided
Tube, slide and plate tests
- Test tubes 1x75 mm, e.g. cat. no. 401855
- Physiologic saline, e.g. Immusol Compact, cat. no. 213580
- Pipette (drop size ~ 50 µl)
- Glass slides
- Plates
- Calibrated centrifuge, e.g. Immucent®, III, cat. no. 213597
- Timer

Gel Technique
- DG Gel Neutral (Diagnostic Grifols, SA)
- Centrifuge, e.g. DG Spin for DG Gel Cards (Diagnostic Grifols, SA)
- DG Gel Sol (Diagnostic Grifols, SA)

The reagents as well as the samples to be tested must be brought to room temperature (18-25 °C) prior to testing.

Tube Test
1. Prepare a 2-3% red blood cell suspension in physiologic saline of the once washed erythrocytes.
2. Place 1-2 drop(s) of Anti-A, Lectin in a properly labeled tube.
3. To each tube add 1 drop of a freshly prepared 2-3% red blood cell suspension in physiologic saline.
4. Shake the tubes to mix reagents.
5. Incubate for 1 min. at room temperature.
6. Centrifuge for 20 seconds at 750 rcf* or use a time and speed appropriate to the calibration of the centrifuge.
7. Gently resuspend cells completely and examine immediately macroscopically for agglutination. Grade and record results.

Plate Test
1. Place 1-2 drop(s) of Anti-A, Lectin onto a clean, labeled glass slide.
2. Add 1 drop of whole blood (35-45% cell suspension).
3. Mix well over an area approximately 20x40 mm in diameter by gently and continuously rocking the slide.
4. Tilt or rotate the slide at room temperature and examine macroscopically for agglutination over a period not to exceed 2 minutes. Do not confuse any drying of the mixture with agglutination. Grade and record results.

Card Method
Anti-A, Lectin can be used both in a manual method and with automatic instruments. For the automatic system, see the instrument user manual.
1. Prepare a 1 % suspension in DG Gel Sol of red blood cells to be tested.
2. Pipette 50 µl of this red blood cell suspension and 50 µl of Anti-A, Lectin in the incubation chamber of a microtube of the DG Gel Neutral Card.
3. Incubate for 10 min at room temperature.
4. Centrifuge the DG Gel Card in a card centrifuge.
5. Grade and record results.

QUALITY CONTROL
Positive and negative controls should be tested in parallel each day the reagent is in use. To differentiate between specific and non specific reactions (rouleaux formation, cold agglutinins) an autocontrol can be run in parallel, in which the Anti-A, Lectin is replaced by the patient’s serum. Tests must be considered invalid if controls do not show the expected reactions. To ensure proper centrifugation, each individual centrifuge should be calibrated for the specific test procedure being performed.

RESULTS
Interpretation
Agglutination = positive test result, A, antigen present;
No agglutination = negative test result, A, antigen not present.

The reading and interpretation of gel cards has to be done according to the manufacturer’s instructions.

LIMITATIONS OF PROCEDURE
1. Perform the subgroup A determination only if the blood group A or AB has been previously established.
2. The reading of the tube tests must be proceeded immediately after centrifugation.
3. Plate test results: weakly reacting A, samples or doubtful test results have to be retested using the tube technique.
4. When using the slide technique weaker reactivity may be observed.
5. The reaction strength of positive results depends on the age of the used blood sample.
6. Newborns do not possess fully expressed A subgroups.
7. False positive or false negative results can occur due to microbial or chemical contamination of test materials and sample, improper incubation temperature and/or time, improper storage of materials, improper centrifugation, omission of test reagents, incorrect red cell concentration and certain disease states.
8. The erythrocytes tested with Anti-A Lectin may get of a darker red color, which does not have any influence on the test result.
9. Any modifications of the test procedures described in this instructions for use require validation by the user.
10. Take under consideration all limitation statements in the instructions for use of the used gel cards and in the user manual of the automatic instruments.

SPECIFIC PERFORMANCE CHARACTERISTICS
Anti-A Lectin fulfills the requirements of the IVD-D 98/79/EC.

WARRANTY
This product is warranted to perform as described in its labeling and in the product literature and Medion Grifols Diagnostics AG disclaims any implied warranty of merchantability or fitness for any other purpose and in no event shall Medion Grifols Diagnostics AG be liable for any consequential damages arising out of the aforesaid express warranty.

BIBLIOGRAPHY