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Anti-D Monoclonal (IgG)

Diagnostic reagent for the in-vitro qualitative detection of Blood groups antigenic determinant D in Human Red Blood Cells manually.

REF: BS.1/AD01.010.0010 REF: BS.1/AD03.010.0030

CLINICAL SIGNIFICANCE

Levine and Stetson discovered the Rh blood group system in 1940. The D antigen determinant is the most clinically significant non-ABO red blood cell antigen and the corresponding antibodies have been implicated in causing both Transfusion Reactions and Haemolytic Disease of the Newborn. Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells or are derived from a human B cell line through EBV transformation.
Approximately 85 % of the Caucasian population is Rh positive. The collective term Du is widely used to describe red cells which have a weaker expression of the D antigen determinant than normal.
BioScien reagent will detect most examples of partial and weak D red cells by direct agglutination in slide test and in tube technique.

METHOD PRINCIPLE

The test principle is direct agglutination for human red blood cells possessing D antigen determinant. No agglutination generally indicates the absence of the D antigen determinant. All negative test results should be further tested for D^u (weak/partial D´s) by performing the Du test procedure, as described later.

REAGENT COMPOSITION

Reagents:	Composition
Anti-D Monoclonal	Monoclonal of IgG antibodies diluted in: - Phosphate buffer - bovine albumin - Sodium azide 0.1%

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagents contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Blood Group reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien Anti-D reagent is ready-to-use and is stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).

Deterioration
The ***BioScien*** Anti-D reagent can be damaged due to microbial contamination or on exposure to extreme temperatures which is indicated by reagents turbidity. It is recommended that such reagent should be discarded.

SPECIMEN COLLECTION AND PRESERVATION

Clean and dry glassware free from detergents must be used for sample collection; blood samples drawn with anticoagulant are used. Samples should be stored at 2-8 °C if not tested immediately. Do not use hemolyzed samples.
Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period:

- EDTA or heparin : 2 days
- Sodium citrate or Sodium oxalate : 14 days
- ACD or CPD : 28 days

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes (12x75 mm)
- Glass slides (50x75 mm)
- Centrifuge
- Stop watch
- Mixing sticks
- Pipettes (drop volume 40 to 50 µl)
- Isotonic saline solution
- Anti-Human Globulin reagent

ASSAY PROCEDURE

Allow the reagents and samples to reach room temperature.

Tube Test

1. Prepare a 3-5% unwashed red blood cell suspension in Isotonic saline solution.
2. Place one drop reagent Anti-D IgG in an appropriately labeled test tubes.
3. Pipette into each of the test tubes, one drop (50µl) of the test red cell suspension and mix well.
4. Incubate at 37 C for 15 minutes.
5. Wash the red blood cells twice with isotonic saline solution and discard the remaining liquid from the last wash.
6. Add one drop of Anti-human globulin Anti-IgG to the red blood cell pellet.
7. Shake to mix, then centrifuge for 15 seconds at 1000 rpm.
8. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutination.
9. Read and record the reaction immediately.

STABILITY OF THE REACTIONS

1. Read all tube tests straight after centrifugation.
2. Complete washing steps without interruption and centrifuge and read tests immediately after reagent addition because delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.
3. Slide tests should be interpreted within two minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
4. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

READING AND INTERPRETATION

	Tube Technique
Positive	Agglutination as indication for the presence of corresponding D antigen determinant.
Negative	No agglutination as indication for the absence of corresponding D antigen determinant.

QUALITY CONTROL

The reactivity of all Anti-D reagents should be confirmed by testing with known positive and negative red blood cells to monitor the performance of the reagent. Good physiological saline may be used as a negative control, if the expected results have not been observed; the reagent should not be used. For more information please contact ***BioScien*** technical support.

LIMITATIONS OF PROCEDURE

Some conditions that may cause false positive results are:

- Contamination of sample or reagents
- Autoantibodies
- Improper storage or preparation of red blood cells
- Antibodies to antibiotics or other reagents
- Cold Antibodies

REFERENCES

1. Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity, Nature, 256, 495-497.
2. Lee H.H., Rouger P, Germain C., Muller A. & Salmon C. (1983), The production and standardisation of monoclonal antibodies as AB blood group typing reagents. Symposium of International Association of Biological Standardisation on Monoclonal antibodies.
3. Human Blood Groups by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995.
4. HMSO, Guidelines for the Blood Transfusion Services, 2nd Ed., 1994.
5. AABB Technical manual, 13th Ed., 1999.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		