Monoclonal Blood Grouping Antibodies for Slide and Tube Tests

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ANTI-A
ANTI-B
ANTI-A,B

SUMMARY

Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells.
Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity.
Human red blood cell antigens can be divided into four groups A, B, A,B and O depending on the presence or absence of the corresponding antigens on the red blood cells. Approximately 41% of the Caucasian population have the A Antigen, 9% have the B Antigen, 4% have both A and B antigens, while the remaining have neither the A nor the B antigen.

REAGENT

Anti-A, Anti-B, and Anti-A,B are ready to use reagents prepared from the supernatants of mouse hybridoma cell cultures.
These antibodies of immunoglobulin class IgM are mixture of several monoclonal antibodies of the same specificity but having the capacity of recognising different epitopes of the human red blood cell antigens A and B.
Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8°C . DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE

Human red blood cells possessing A and/or B antigen will agglutinate in the presence of antibody directed towards the antigen.
Agglutination of red blood cells with Anti-A, Anti-B, Anti-A,B reagents is a positive test result and indicates the presence of the corresponding antigen.
Absence of agglutination of red blood cells with Anti-A, Anti-B, and Anti-A,B reagents is a negative test result and indicates the absence of the corresponding antigen.

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NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded.
4. Reagents are not from human source, hence contamination due to HBsAg, HIV and HCV is practically excluded.

SAMPLE COLLECTION AND PREPARATION

No special preparation of the patient is required prior to sample collection by approved techniques.
Samples should be stored at 2-8°C if not tested immediately. Do not use haemolysed 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

ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS

Glass slides (60 x 85 mm), Test tubes (12 x 75 mm), Pasteur pipettes, Isotonic saline, Centrifuge, Timer, Mixing sticks.

TEST PROCEDURE

Bring reagents and samples to room temperature before testing.

SlideTest

1. Place one drop or 50µl Anti-A or Anti-B or Anti-A,B reagents using the reagent dropper separately on a clean glass slide.
2. To each reagent drop, add one drop or 25 µl of whole blood.
3. Mix well with a mixing stick uniformly over an area of approximately 2.5 cm^2.
4. Rock the slide gently, back and forth.
5. Observe for agglutination macroscopically at three minutes.

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	Test RBC	Specification	Results
Specificity and sensitivity Anti A (IgM)	A1	Positive	Positive
	A2	Positive	Positive
	A2B	Positive	Positive
	B	Negative	Negative
	0	Negative	Negative

	Test RBC	Specification
Specificity and sensitivity Anti B (IgM)	B	Positive
	A1B	Positive
	A1	Negative
	0	Negative

Tube Test

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of Anti-A, Anti-B, or Anti-A,B reagents using the reagent dropper into corresponding labeled test tubes.
3. Pipette into each of test tubes, one drop or 50 µl red cell suspension and mix well.
4. Centrifuge for one minute at 1000 RPM (125 g) or 20 sec. at 3400 RPM(1000g)
5. Gently resuspend the cell button, observing for agglutination macroscopically.

INTERPRETATION OF RESULTS

Slide and tube tests

Agglutination is a positive test result and indicates the presence of A and/or B antigen. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative test result and indicates the absence of A and/or B antigen.

NOTES

1. (a) Anti-A, Anti-B and Anti-A,B reagents do not show a reaction with crypt antigens (T, Tn, Tk activatedcells).
(b) Anti-B is truly negative reacting with acquired B characteristics.
2. In the tube test procedure, it is recommended that tubes with negative reactions should be recentrifuged and results read again after 5 minutes so that weak antigens are not overlooked.

Monoclonal Blood Grouping Antibodies for Slide and Tube Tests

3. As under centrifugation or over centrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and determine the time required for achieving the desired results.
4. Results of forward grouping obtained by using Anti-A, Anti-B and Anti-A,B reagents should always be reconfirmed by performing reverse grouping with known red cells.
5. It is strongly recommended that red cells with known ABO characteristics should be occasionally run, preferably on a daily basis so as to control reagent performance and validate the test results.
6. After usage the reagents should be immediately recapped and replaced to 2-8°C storage.
7. The label minimum titre claim is based on, A group cells for Anti-A reagent, B group cells for Anti-B reagent, AB group cells for Anti-A,B reagents. This is based on titration procedure as recommended by the manufacturer. Any deviation in test procedure could result in variable results.

BIBLIOGRAPHY

1.Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity. Nature, 256, 495-497.
2.Human Blood Groups, by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995.

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IVD

Monoclonal Blood Grouping Antibodies for Slide and Tube Tests

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ANTI-D IgM/IgG

SUMMARY

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. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity. Human red Blood cells are classified as Rho (D) positive or Rho (D) negative depending on the presence or absence of Rho (D) antigen on them. Approximately 85 % of the Caucasian population is Rho (D) positive. The Du phenotype is a traditional definition to describe the weak / partial D´s that can be detected with Meditest anti-D Rho (IgM & IgG). About 60 % of the Dus(weak / partial D´s) may react with Meditest Anti-D (Rho) reagent in slide test and about 90 % may be detected by the tube technique.

REAGENT

Meditest Anti-D (Rho) is ready to use reagent prepared from supernatants of cell cultures with antibody producing B lymphocytes obtained through EBV transformation and is a blend of antibodies of immunoglobulin class IgM and IgG. These antibodies are a mixture of several antibodies of the same specificity but having the capability of recognizing different epitopes of the human red blood cell antigen D (Rho). Meditest Anti-D (Rho) reagent is a blend of IgM and IgG class of Anti-D (Rho), a characteristic which accords versatility to the reagent. It gives an avid saline reacting slide / tube test reagent the capability of detecting Du (weak / partial D´s) in the Anti-human globulin phase. Each batch reagent undergoes quality control at various stages of manufacture for its specificity, avidity and performance.

REAGENT STORAGE AND STABILITY

1.Store the reagent at 2-8°C.DO NOT FREEZE.
2.The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

Monoclonal Blood Grouping Antibodies for Slide and Tube Tests

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PRINCIPLE

Human red blood cells possessing D antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with Meditest Anti-D (Rho) reagent is a positive test result and indicates the presence of D (Rho) antigen. No agglutination with Meditest Anti-D (Rho) reagent is a negative test result and indicates the absence of the D (Rho) antigen.

SAMPLE COLLECTION AND PREPARATION

No special preparation of the patient is required prior to sample collection by approved techniques. Samples should be stored at 2-8°C if not tested immediately. Do not use haemolysed samples. Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period:

EDTA or Heparin : 2 days Sodium citrate or sodium Oxalat : 14 days

ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS

Glass slides (60 x 85 mm), Test tubes (12x 75 mm), Test tube rack, Pasteur pipettes, Isotonic saline, Centrifuge, Timer, Mixing sticks, Anti Human Globulin (Coombs) reagent.

TEST PROCEDURE

Bring reagents and samples to room temperature before testing.

Slide Test

1. Place one drop or 50µl Anti D (Rho reagent using the reagent dropper on a clean glass slide.
2. To the reagent drop add a drop of whole blood
3. Mix well the reagent and blood sample with a mixing stick uniformly over an area of approximately 2.5 cm^2
4. Rock the slide gently, back and forth.
5. Observe for agglutination macroscopically at the end of three minutes.

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Tube Test

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of Meditest Anti-D (Rho) into the labelled test tubes.
3. Pipette into each of the test tubes, one drop of the 5% red cell suspension and mix well.
4. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Gently resuspend.

INTERPRETATION OF RESULTS

Slide and Tube Tests

a) Agglutination with the Anti D (Rho) is a positive test result and indicates the presence of D antigen. Do not interpret peripheral drying or fibrin strands as agglutination.
b) No agglutination with Anti D (Rho) is a negative test result and indicates the absence of D antigen.

NOTES

1. As under centrifugation and over centrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required of achieving the results.
2. It is strongly recommended that as a routine quality control measure with known D positive and D negative red cells be occasionally run, preferably on a daily basis to validate reagent performance.
3. After usage, the reagents should be immediately recapped and replaced to 2-8°C storage.
4. Cord Cells heavily sensitized with Anti D (Rho) may give false negative result in immediate spin test.
5. False positive reactions may occur if the test subject has cold agglutinins.

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(1) Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity. Nature, 256, 495-497.
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