BLOOD GROUPING REAGENTS IVD

ANTI-A, ANTI-B, ANTI-AB, ANTI-D(IgM+IgG)

FOR SLIDE AND TUBE TEST

PRODUCT CODE	DESCRIPTION
SB001	ANTI-A
SB002	ANTI-B
SB003	ANTI-AB
SB004	ANTI-D (IgM+IgG)
SB005	ABD Blood Grouping Kit
SB008	ABD, AB Blood Grouping Kit

INTENDED USE

These reagents are *in vitro* diagnostic medical device for professional use, intended for *in vitro* determination of ABO group and Rh typing of human blood cell antigens.

PRINCIPLE

The manual technique employed, on a plate or in a tube utilizes the principle of hemagglutination. Test red blood cells bearing an antigen agglutinate in the presence of the reagent containing the corresponding antibody.

REAGENT COMPOSITION

Anti-A

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatants of in vitro culture of hybridoma of murine origin. The cell line used is 9113 D10. The reagent is a clear blue solution.

Anti-B

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatant of *in vitro* culture of hybridoma of murine origin. The cell line used is 9621A8. The reagent is a clear yellow solution.

Anti-AB

The reagent is prepared from monoclonal antibody in a storage medium. The monoclonal antibody is produced from supernatants of in vitro culture of hybridoma of murine origin. The cell lines used is 152D12+9113D10. The reagent is a clear colorless solution.

Anti-D(IgM+IgG)

The reagent is prepared from a monoclonal blend of IgM+IgG antibodies in a storage medium. The monoclonal antibodies are produced from supernatants of *in vitro* culture of hybridoma of human origin. The cell lines used are P3X61+P3X21223B10+P3X35+P3X290. The reagent is a clear colorless solution.

All the above reagents contain Sodium azide (<0.1%) and Bovine albumin as preservative/stabilizer.

STORAGE AND STABILITY

The sealed reagent is stable up to expiry date stated on the vial label, when stored at 2-8^{0}C. Do not use if turbid. Do not dilute. Do not use the reagent beyond the expiry date mentioned on the vial. It is advisable to minimize its time outside the refrigerator and to avoid leaving it at room temperature between two uses. DO NOT FREEZE

SAMPLE

Blood collected in anticoagulant: EDTA, heparin or citrate.

Storage at 2-8°C below 48 hours. Hemolyzed blood should not be used.

PRECAUTIONS

It is advisable to wear gloves and safety goggles and handle test samples of human origin with caution. Do not use damaged or leaking reagents. This reagent contains <0.1% Sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded to sink flush with a large volume of water to prevent azide build up. As this reagent is of animal origin care must be taken during use and disposal as there is a potential infection risk.

PROCEDURE

PLATE TECHNIQUE AT ROOM TEMPERATURE

On a rigorously clean plate, using the vial dropper, apply one drop of each reagent. Take one drop of blood and apply next to each drop of the reagent. Take one drop of blood and apply next to each drop of the reagent, taking care not to create contact between the drops. Mix the blood and reagent using the end of the stirrer so as to create a regular lozenge of diameter 2 to 3 cm. Incubate the plate a room temperature and without stirring for 30 seconds. Hold the plate at room temperature and without stirring for 30 seconds. Hold the plate and give it a rolling movement for 3 minutes while macroscopically observing the possible appearance of agglutinates. Read the reaction immediately.

DIRECT METHOD IN A TUBE AT ROOM TEMPERATURE

Prepare a 5% suspension of red blood cells in isotonic saline solution. Using the vial dropper, place one drop of Anti-A, Anti –B, Anti-AB & Anti D reagent in to corresponding labeled test tubes. Add a drop of 5 % RBC suspension. Shake to homogenize the mixture, then centrifuge at 1000 RPM for 1 minute. Read macroscopically while gently shaking the tubes. Note the appearance of any agglutination.

INTERPRETATION

With both the above methods, if there is agglutination (the red blood cells form one or several clumps) the reaction is positive and the antigen or at least one of the antigens corresponding to the reagent used is present on the red blood cells tested

If there is no agglutination (red blood cells form a homogenous suspension), the reaction is negative and the antigen is not present on the red blood cells.

In case of Anti D agglutination, a positive test indicates presence of Rh antigen i.e. Rh Positive. No agglutination or a negative test indicated either presence of weaker variants of Rh antigens like 'Du' or absence of Rh antigen. This needs confirmation by carrying out indirect Coomb's test.

Drying at the periphery or fibrin strands should not be misinterpreted as agglutination.

NOTE:

1. The reactions must be read immediately after centrifuging and re-suspending.
2. Anti D (IgM+IgG) have the special feature of recognizing certain rare antigen motives of type RH33 (RoHar) and may thus yield discordant reactions with polyclonal reagents that recognize them little or not at all.
3. For specific identification of partial D antigen, the use of any 3rd party kit for this purpose is recommended.
4. A false positive reaction may occur if the subject tested has cold agglutinatinins.
5. Anti D (IgM+IgG) cannot ensure the recognition of all weak or variant subjects, due to the variability of antigen motifs.
6. Certain discordances (negative reaction for the direct hemagglutination method and positive reaction for the indirect antiglobulin method) may occur with Anti D (IgM+IgG). A weak and/or partial D antigen may be suspected.
7. A false –positive reaction may occur, when Anti D (IgM+IgG) is used in the indirect antiglobulin method (IAT), if the RBC from the test subject shows a positive reaction in the direct antiglobulin test (DAT)
8. A negative reaction obtained in an indirect antiglobulin test can be validated with IgG-sensitized red blood cells (not provided with this kit)

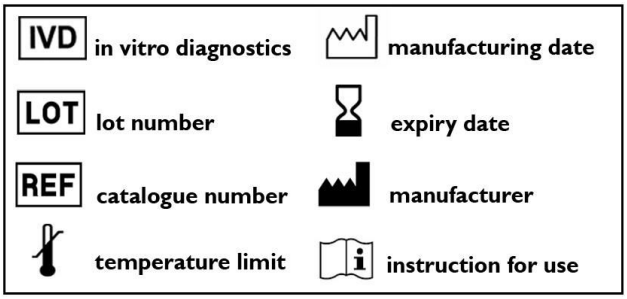
LIMITATIONS

1. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. All negative slide tests should be confirmed by tube testing to confirm absence of weak sub groups.
3. Stored blood may give weaker reactions than fresh blood.
4. Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.
5. False positive or false negative results can occur due to contamination of test material, improper reaction temperature, improper storage materials, omission of test reagents and certain disease states or deviation from recommended technique.

BIBLIOGRAPHY

1. Human blood groups, Geoff Daniels, 1st edition, Blackwell Science, Oxford 1995.
2. Common Technical Specifications (CTs) for the in vitro diagnosis medical devices of annex II, list A, of directive 98/79/EC are notified in the official journal of the European communities under document No.C (2202) 1344, with EEA relevance (2202/364/CE)
3. Mannessier .L Blood transfusion center Lille France. The use of monoclonal antibodies as blood grouping reagents: applications, advantages and problems. Congress of the Italian society for blood transfusion –Rome-June 1992.
4. Blood group serology, Boorman, Dodd and Lincoln, Churchill Livingstone, 6th edition.

SYMBOL ON LABELS



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