

Atlas Anti-M

HUMAN BLOOD GROUPING REAGENTS

For Tube and DiaMed-ID Techniques.

SUMMARY

M antigen is part of the MNS system and was first reported in 1927. M antigen expression on red cells can show dosage. Anti-M has rarely been implicated in Haemolytic Disease of the Newborn or in Haemolytic Transfusion Reactions.

Anti-M	Anti-N	Phenotype	Caucasians %	Afro-Americans %
+	0	M+N-	28	26
+	+	M+N+	50	44
0	+	M-N+	22	30

PRINCIPLE

The reagent will cause agglutination (clumping) of test red cells, that carry the M antigen, after centrifugation. No agglutination generally indicates the absence of the M antigen (see Limitations).

REAGENT

Atlas Human Anti-M blood grouping reagent is prepared from human serum diluted in a sodium chloride solution containing bovine albumin. The reagent is supplied at optimal dilution for use with all recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see **Vial Label**.

STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8°C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity

SAMPLE COLLECTION AND PREPARATION

Blood samples drawn with, or without anticoagulant may be used for antigen typing. If testing is delayed then store specimens at 2 - 8°C. EDTA and citrate samples should be typed within 48 hours. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS before being tested

PRECAUTIONS

- The reagent is intended for *in vitro* diagnostic use only.
- If a reagent vial is cracked or leaking, discard the contents immediately.
- Do not use the reagent past the expiration date (see Vial Label).
- Do not use the reagent if a precipitate is present.
- Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
- The reagent has been filtered through a 0.2 µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
- The reagent contains < 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
- Materials used to produce reagent were tested and found negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
- No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

CONTROLS AND ADVICE

- It is recommended a positive control (ideally MN cells) and a negative control (NN cells) be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
- Most proteolytic enzymes destroy M reactivity.
- In the Tube Technique one volume is approximately 40µl when using the vial dropper provided.
- Use of reagent and interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagent is in use. The user must determine suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- DiaMed ID-Cards (Neutral).
- DiaMed ID-Centrifuge.
- DiaMed ID-Diluent: e.g. ID-Cel[Stab.
- Glass test tubes (10 x 75, mm or 12 x 75 mm).
- Phosphate Buffered Saline (PBS): NaCl 0.9%• pH 7.0 ± 0.2 at 22°C ± 1°C
- Positive (ideally M+N+) and negative (N+N+) control red cells.
- Test tube centrifuge.
- Volumetric pipettes.

RECOMMENDED TECHNIQUES

A. Tube Technique

- Prepare a 2-3% suspension of washed test red cells in PBS.
- Place in a labelled test tube: 1 volume of Atlas Anti-M reagent and 1 volume of test red cell suspension.
- Mix thoroughly and incubate at 4°C ± 2°C for 15 minutes.
- Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
- Gently resuspend red cell button and read macroscopically for agglutination

B. DiaMed-ID Micro Typing Technique

- Prepare a 0.8% suspension of washed test red cells in an ID-Diluent.
- Remove aluminium foil from as many microtubes as needed.
- Place in appropriate microtube: 50µl of test red cell suspension and 25µl of Atlas Anti-M reagent.
- Incubate the ID-Card(s) for 15 minutes at 4°C ± 2°C.
- Centrifuge ID-Card(s) for 10 minutes at 90 rcf or for a suitable alternative time and force.
- Read macroscopically for agglutination.

INTERPRETATION OF TEST RESULTS

- Positive: Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the appropriate antigen on the test red cells.
- Negative: No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of the appropriate antigen on the test red cells.

STABILITY OF THE REACTIONS

- Tests should be read immediately after centrifugation. Delays may result in dissociation of antigen-antibody complexes leading to false negative, or *weak* positive reactions.
- Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

- Antibodies directed at low frequency antigens may occur as unsuspected contaminants in blood grouping antisera. In addition, certain antigens (eg. Bg, Sd^a) can be present in an exalted state on red blood cells. These phenomena may be the source of rare false positive reactions, which *may* occur with more than one lot of a given specificity.
- It is not possible to claim the absence of all contaminating antibodies, as red cells carrying antigens of low frequency or exalted antigens are not always available for testing.
- Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative reactions and so caution should always be exercised when assigning genotypes on the basis of test results.
- False positive or false negative results may also occur due to:
 - Contamination of test materials
 - Improper storage, Cell concentration, incubation time or temperature
 - Improper or excessive centrifugation
 - Deviation from the recommended techniques

SPECIFIC PERFORMANCE CHARACTERISTICS

- The reagent has been characterised by all the procedures mentioned in the Recommended Techniques.
- Prior to release, each lot of Atlas Anti-M reagent is tested by the Recommended Techniques against a panel of antigen-positive red cells to ensure suitable reactivity.
- The presence of contaminating antibodies to antigens with an incidence of 1% or greater within the random population has been excluded either in tests employing the appropriate antigen-negative red cells or in tests employing reagents previously absorbed to remove interfering specificities.
- Antibodies to Xg^a, Do^a, Yt^a Co^b, Wr^a, Bg^a and V^w may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cell. This can also be said for Yt^b, M⁹ and V^w and other low frequency antigens which may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cells
- The Quality Control of the reagent was performed using red cells that had been washed twice with PBS prior to use.

DISCLAIMER

- The user is responsible for the performance of the reagent by any method other than those mentioned in the Recommended Techniques.
- Any deviations from the Recommended Techniques should be validated prior to use⁶.

BIBLIOGRAPHY

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