**METHOD SUMMARY**

**Tube** | **Reagent** | **Cat**
---|---|---
| | Anti-M, Anti-N | Anti-M
| Validated Methods | Yes | Yes
| Reagent Volume | 1 | 1
| Cell Volume | 3-5% | 0.8% / 3%
| Incubation Time | 5 mins | Immediate Spin
| Temperature | Room Temp | Room Temp
| Spin (Speed/Time) | High for 20 seconds |


**REAGENT DESCRIPTION**

Epiclone™ Anti-M and Epiclone™ Anti-N monoclonal phenotyping reagents are prepared from murine monoclonal IgG antibodies. When used by the recommended methods these reagents will cause agglutination of the red cells carrying the specific M and/or N antigens. Epiclone™ Anti-M and Anti-N phenotyping reagents have been tested against red cell samples positive for the appropriate antigen, to ensure adequate potency, and against a panel of cells that are negative for the appropriate antigen to ensure specificity. The panel includes red cells positive for low incidence antigens, particularly those associated with the MNNS system, whenever suitable cells are available. The reagents contain Bovine Albumin, macromolecular potentiators (Epiclone™ Anti-M only) and Sodium Azide as a preservative. Each reagent has been optimised for use without further dilutions or additions. The clones used to produce these reagents are: Epiclone™ Anti-M is LM110/140 and Epiclone™ Anti-N is HM1.

**STORAGE CONDITIONS**

Store at 2° to 8°C (Refrigerate. Do Not Freeze).

**PRINCIPLE OF THE TEST**

The agglutination of red cells by a specific reagent indicates the presence of the corresponding antigen on those cells, whilst a negative reaction signifies the absence of the corresponding antigen. Red cells expressing the M and N antigens will agglutinate in the presence of the corresponding specific antibody in Epiclone™ Anti-M and/or Epiclone™ Anti-N.

**BACKGROUND**

**Blood Group System**

In 1927, Landsteiner and Levine first reported the M and N antigens on human red cells. For most serological purposes the M and N antigens act as products of allelic genes and three phenotypes MM, MN and NN are detectable using Anti-M and Anti-N phenotyping reagents.

Anti-M is not considered clinically significant and is not implicated in transfusion reactions or Haemolytic Disease of the Foetus and Newborn (HDN), except in extremely rare cases. Anti-N is also not considered clinically significant. Some authors recommend that when anti-M or anti-N antibodies are active at 37°C by Indirect Antiglobulin Test (IAT) are encountered, a crossmatch of compatible blood should be provided.

**Antigen/Antibody Characteristics**

M and N antigens are determined by amino-acid polymorphism in the polypeptide chain of the major sialoglycoprotein, glycophorin A. Linked to M and N are two further antigens, S and s, which are determined by polypeptide components of the sialoglycoprotein, glycophorin B. An N-like antigen, known as 'N' is also carried on glycophorin B, irrespective of the MN status of the glycophorin A. Both M and N antigens are expressed on cord cells.

Anti-M and anti-N antibodies in human sera often show marked dosage effects and weak examples may only react with red cells homozygous for the corresponding antigen. Anti-M is a relatively common antibody in human sera; it can be either IgG or IgM in form and usually reacts best in saline tests at 4° to 22°C, although examples may be seen that react at 37°C and by IAT. The activity of anti-M is often enhanced by acidification of the serum to pH 6.5, but reactions may become non-specific at a pH of less than 6.0. The addition of Bovine Albumin to a final concentration of 10-12% w/v may also aid in the detection of weak anti-M antibodies at 22°C. Anti-N is less commonly found, but has been reported as either an alloantibody or as an autoantibody in patients on haemodialysis. Anti-N is usually IgM in form and is optimally reactive at 4° to 22°C; it rarely reacts by IAT. Both M and N antibodies are destroyed when red cells are treated by certain enzymes, such as Papain and ficin, and this is useful in the identification and confirmation of anti-M and anti-N antibodies. Neither anti-M nor anti-N bind complement.

**Gene Frequency**

The expected results and frequencies of the phenotypes in the Australian blood donor population are given below:

<table>
<thead>
<tr>
<th>Reactions obtained with:</th>
<th>Presumed Genotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-M</td>
<td>Anti-N</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>MM</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>MN</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>NN</td>
</tr>
</tbody>
</table>

**SPECIMEN COLLECTION AND PREPARATION**

Blood samples should be withdrawn aseptically with or without the addition of anticoagulants. Tests should be performed as soon as possible after collection of the sample. If testing the blood samples is delayed, samples should be stored between 2° to 8°C. Samples collected into EDTA or Heparin may be tested up to 7 days from the date of withdrawal provided storage has been at 2° to 8°C. Clotted samples may be tested up to 14 days from the date of withdrawal provided storage has been at 2° to 8°C. Samples collected in Citrate may be tested up to 42 days from the date of withdrawal provided storage has been at 2° to 8°C. Cells may also be stored in Celpresol™ at 2° to 8°C for up to 42 days.

As Epiclone™ Anti-M and Epiclone™ Anti-N reagents are strictly pH dependant, red cells not stored in Celpresol™ should be washed three times in Cepresol™ or unbuffered isotonic saline before use. Cells already suspended in Cepresol™ are suitable for use without further washing.

The unbuffered isotonic saline used for washing the red cells should have a pH of not less than 6.0. Low pH saline solutions may result in weak reactions being obtained with the Epiclone™ Anti-M and Epiclone™ Anti-N reagents.

**RECOMMENDED METHODS**

**Tube Method**

1. Prepare a 3-5% suspension of test red cells in unbuffered isotonic saline or Cepresol™.
2. Add 1 drop of the applicable Epiclone™ Anti-M or Epiclone™ Anti-N phenotyping reagent to an appropriately labelled, clean glass test tube (10x75mm or 12x75mm).
3. Add 1 drop of the suspension of test red cells.
4. Mix well and incubate at room temperature for 5 minutes.
5. Centrifuge at high speed (1000rpm) for 20 seconds*.
6. Gently agitate the tube to dislodge the red cells and examine for agglutination. Record results.

* Or centrifuge at a speed and time appropriate for the centrifuge in use.

Tests should not be read with any form of magnification.
Column Agglutination Technology (CAT) 0.8% Method (BioVue™ and BioRad™/DiaMed™) for Anti-M – Immediate Spin (IS)
1. Prepare a 0.8% suspension of test red cells in Celpresol™ LISS.
2. Label a BioVue™ Neutral card or a BioRad™/DiaMed™ ID-Card NaCl, Enzyme Test and Cold Agglutinins card.
4. Add 50μL of the suspension of 0.8% test red cells (to be phenotyped).
5. Centrifuge according to the manufacturer’s instructions.
6. Read according to the manufacturer’s instructions.

Column Agglutination Technology (CAT) 3% Method (BioVue™ only) for Anti-M – Immediate Spin (IS)
1. Prepare a 3% suspension of test red cells in Celpresol™.
2. Label a BioVue™ Neutral card.
3. Add 40μL of Epiclone™ Anti-M phenotyping reagent.
4. Add 10μL of the suspension of 3% test red cells (to be phenotyped).
5. Add 40μL of Celpresol™.
6. Centrifuge according to the manufacturer’s instructions.
7. Read according to the manufacturer’s instructions.

INTERPRETATION OF RESULTS
Agglutination of the test red cells constitutes a positive result and indicates the presence of the appropriate antigen. No agglutination of the test red cells indicates the absence of the relevant antigen.

A positive reaction with Epiclone™ Anti-M reagent denotes the presence of the M antigen on the test red cells. A positive reaction will also occur with the very rare Mg+ M- N- red cells. A negative reaction denotes the absence of the M antigen.

A positive reaction with Epiclone™ Anti-N reagent denotes the presence of the N antigen on the test red cells. The reagent does not react with the ‘N’ antigen on glycoporphin B. A negative reaction denotes the absence of the N antigen.

CONTROLS
The use of controls is essential in the performance of all blood grouping tests. Control samples should be tested in parallel with the test sample.

Positive Control – red cells known to be heterozygous for the antigen as appropriate for the phenotyping reagent in use.

Negative Control – red cells known to lack the antigen as appropriate for the phenotyping reagent in use.

LIMITATIONS OF PROCEDURE
False results may occur due to:
1. Incorrect technique.
2. Presence of gross rouleaux.
3. Use of aged blood samples, reagents or supplementary materials.
4. Contaminated blood samples, reagents or supplementary materials.
5. Red cells that have a positive Direct Antiglobulin Test (DAT).
6. Other deviation from the recommended test methods.
7. Incorrect concentrations of red cells or expired reagents.
8. Incorrect reading of results.
9. Incorrect red cell suspension medium.

PRECAUTIONS
1. For in vitro diagnostic use only.
2. The material from which this product was derived is from non-human sources, there is no risk of HIV or HBsAg infection. However, good laboratory practice requires safe handling procedures are used.
3. Sodium Azide 0.1% w/v is added as a preservative. Users should be aware of the toxicity and cumulative explosive nature of Sodium Azide and take appropriate precautions when handling and discarding this reagent.
4. This product should be clear; turbidity may indicate bacterial contamination. The reagent should not be used if a precipitate or particles are present.
5. As Anti-M and Anti-N reactions are strictly pH dependent, care should be taken to ensure the red cell suspension medium prepared is unbuffered isotonic saline with a pH of not less than 6.0 and the level of cell washing performed is appropriate.
6. The bovine material used is from an approved source free of Bovine Spongiform Encephalopathy (BSE).

REFERENCES

Consult instructions for use
In vitro diagnostic medical device
Catalogue number
Temperature limitation
Manufacturer

bioCSL, Celpresol and Epiclone are Trademarks of CSL Limited.