Add 1 drop of the applicable Epiclone™ Anti-M or Anti-N phenotyping reagent to an genotype NN. Anti-M and Anti-N monoclonal IgG phenotyping reagents have been tested against red cell samples positive for the corresponding antigen. In Epiclone™ Anti-M and/or Epiclone™ Anti-N phenotyping reagents have been tested against red cell samples positive for the appropriate antigen.

**METHOD SUMMARY**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Tube</th>
<th>Anti-M, Anti-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validated Methods</td>
<td>Yes</td>
<td></td>
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</tbody>
</table>

**Tube Method**

1. Prepare a 3-5% suspension of test red cells in unbuffered isotonic saline or Celpresol™.
2. Add 1 drop of the suspension of test red cells.
3. Mix well and incubate at room temperature for 5 minutes.
4. Centrifuge at high speed (10000g) for 20 seconds.
5. Gently agitate the tube to dislodge the red cells and examine for agglutination. Record results.

**Cell Preparation**

Prepare a 3-5% suspension of test red cells in unbuffered isotonic saline or Celpresol™. Red cells not stored in Celpresol™ should be washed three times in Celpresol™ or unbuffered isotonic saline before use. Cells already suspended in Celpresol™ are suitable for use without further washing. Epiclone™ Anti-N is optimally reactive at 4° to 22°C; it rarely reacts by IAT. Both M and N antigens 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. Further anti-M or anti-N blood component.

**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 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 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 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.

**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 postpolyptide components of the sialoglycoprotein, glycophorin B. An allele, 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.

**STORAGE CONDITIONS**

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

**SPECIMEN COLLECTION AND PREPARATION**

Blood samples should be withdrawn aseptically and without the addition of anticoagulants. Tubes 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 Citrate may be tested up to 42 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. Samples may also be stored in Calpree™ at 2° to 8°C for up to 62 days. As Epiclone™ Anti-M and Epiclone™ Anti-N reagents are strictly pH dependant, red cells not stored in Calpree™ should be washed three times in Calpree™ as unbuffered isotonic saline before use. Cells already suspended in Calpree™ are suitable for use without further washing. 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**

1. Prepare a 3-5% suspension of test red cells in unbuffered isotonic saline or Calpree™.
2. Add 1 drop of the suspension of test red cells.
3. Mix well and incubate at room temperature for 5 minutes.
4. Centrifuge at high speed (10000g) for 20 seconds.
5. Gently agitate the tube to dislodge the red cells and examine for agglutination. Record results.

**Note:** ° C refrigeration at a speed and time appropriate for the centrifuge in use. Tests should not be read with any form of magnification.
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 glycophorin 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. Red cells that have a positive Direct Antiglobulin Test (DAT).
5. Other deviations from the recommended test methods.
6. Incorrect concentrations of red cells or expired reagents.
7. Incorrect reading of results.
8. Incorrect red cell suspension medium.

PRECAUTIONS

1. For in vitro diagnostic use only.
2. The product is intended for use with human blood.
3. Use of aged blood samples, reagents or supplementary materials.
4. Use of normal blood samples, reagents or supplementary materials.
5. Red cells that have a positive Direct Antiglobulin Test (DAT).
6. Other deviations 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.

REFERENCES

7. Scientific Subcommitteee of the Australian and New Zealand Society of Blood Transfusion Ltd Guidelines for Pretransfusion Laboratory Practice. 3rd Ed. 2007 (or current edition).