LENTIN BLOOD GROUPING REAGENTS
DIRECTIONS FOR USE

**Anti-H Lectin: For Tube Techniques.**

**SUMMARY**

The H antigen is part of the Hh system and is found on all red cells except those of O, (Hh) Bombay phenotype, which is extremely rare.

<table>
<thead>
<tr>
<th>Anti-H</th>
<th>Phenotype</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A+</td>
<td>99.9%</td>
</tr>
<tr>
<td>0</td>
<td>H-</td>
<td>Very rare</td>
</tr>
</tbody>
</table>

H is the precursor of A and B and so group A and B people have less H than O people. The order of reactivity of Anti-H with red cells of various ABO groups is:

<table>
<thead>
<tr>
<th>Strong ——— Weak</th>
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<tbody>
<tr>
<td>O</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>A,B</td>
</tr>
<tr>
<td>A, B</td>
</tr>
</tbody>
</table>

**PRINCIPLE**

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

**REAGENT**

Lorne Anti-H Lectin blood grouping reagent is prepared from an extract of *Ulex europaeus* seeds, diluted with 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**

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. This reagent has undergone transportation stability studies at 37°C and -25°C as described in document EN/13640:2002.

**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 2-6°C. EDTA and citrate samples should be typed within 7 days of collection. 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 or isotonic saline before being tested.

**PRECAUTIONS**

1. The reagent is intended for *in vitro* diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagent past the expiration date (see Vial Label).
4. Do not use the reagent if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. 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.
7. 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.
8. 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.

**DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES**

For information on disposal of the reagent and decontamination of a spillage site see Material Safety Data Sheets, available on request.

**CONTROLS AND ADVICE**

1. It is recommended known A₂ and A₁ control red cells be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. In the Tube Technique one volume is approximately 50μl when using the vial dropper provided.
3. Use of the reagent and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagent is in use.
4. User must determine suitability of the reagent for use in other techniques.

**REAGENTS AND MATERIALS REQUIRED**

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Known group A, and A₁ control red cells.
- PBS solution (pH 6.8-7.2) or Isotonic saline solution (pH 6.5-7.5).
- Test tube centrifuge.

**Volumetric pipettes.**

**RECOMMENDED TECHNIQUE**

A. Tube Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS or isotonic saline.
2. Place in a labelled test tube: 1 volume Lorne Anti-H reagent and 1 volume test red cell suspension.
3. Mix thoroughly and incubate at room temperature for 5 minutes.
4. Centrifuge all tubes for 30 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination

**INTERPRETATION OF TEST RESULTS**

1. Positive: Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the H antigen on the test red cells.
2. 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 H antigen on the test red cells.

**STABILITY OF THE REACTIONS**

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

**LIMITATIONS**

1. Lorne Anti-H Lectin may react with test red cells that are Tn-polyagglutinable or Cad-positive.
2. Stored blood may give weaker reactions than fresh blood.
3. 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**

1. The reagent has been characterised by all the procedures mentioned in the Recommended Technique.
2. Prior to release, each lot of Lorne Anti-H Lectin reagent is tested by the Recommended Technique against a panel of antigen-positive red cells to ensure suitable reactivity. 
3. Lorne Anti-H has been formulated to be non-reactive with most A₁ and A₂B red cells and reactive with A₂ red cells.
4. The Quality Control of the reagent was performed using red cells that had been washed twice with PBS or isotonic saline prior to use.
5. The reagent complies with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

**DISCLAIMER**

1. The user is responsible for the performance of the reagent by any method other than those mentioned in the Recommended Technique.
2. Any deviations from the Recommended Technique should be validated prior to use.

**BIBLIOGRAPHY**

AVAILABLE REAGENT SIZES

<table>
<thead>
<tr>
<th>Vial Size</th>
<th>Catalogue Number</th>
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<tbody>
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<td>2 ml</td>
<td>115002</td>
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<tr>
<td>1000 ml</td>
<td>115000*</td>
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</tbody>
</table>

*This size is For Further Manufacturing Use (FFMU) only and is therefore not CE marked.

For the availability of other sizes, please contact:

Lorne Laboratories Limited
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Fax: +44 (0) 118 986 4518
E-mail: info@lornelabs.com

TABLE OF SYMBOLS

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<th>In-vitro Diagnostic</th>
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