



LECTIN BLOOD GROUPING REAGENTS
DIRECTIONS FOR USE

Anti-N (*Vicia Graminae*): For Tube and DiaMed-ID Techniques.

SUMMARY

The N antigen is part of the MNSs system and was reported in 1927. Anti-N generally reacts at room temperature and so is rarely implicated in Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

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 N antigen, after centrifugation. No agglutination generally indicates the absence of the N antigen (see **Limitations**).

REAGENT

Lorne Anti-N Lectin blood grouping reagent is prepared from an extract of *Vicia graminae* 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

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. Reagent will remain stable for up to 7 days when subjected to temperatures not exceeding 30°C.

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

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 a positive control (ideally heterozygous cells) and a negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. In the **Recommended Techniques** one volume is approximately 40µl when using the vial dropper provided.
3. 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-CellStab.
- DiaMed ID-Incubator equilibrated at 37°C ± 2°C.
- 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 (M+M+) control red cells.
- Test tube centrifuge.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37°C ± 2°C.

RECOMMENDED TECHNIQUES

A. Tube Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume of Lorne reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and then incubate at 37°C for 15 minutes.
4. Centrifuge all the tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination

B. DiaMed-ID Micro Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in an ID-Diluent.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50 µl of test red cell suspension and 25µl of Lorne reagent.
4. Incubate the ID-Card(s) for 15 minutes at 37°C.
5. Centrifuge ID-Card(s) for 10 minutes at 90 rcf or for a suitable alternative time and force.
6. Read macroscopically for agglutination.

INTERPRETATION OF TEST RESULTS

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

STABILITY OF THE REACTIONS

1. Tube tests must be read immediately after centrifugation. Delays may cause 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. Stored blood may give weaker reactions than fresh blood.
2. 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-N Lectin reagent is tested by the **Recommended Technique** against a panel of antigen-positive red cells to ensure suitable reactivity.
3. The Quality Control of the reagent was performed using red cells that had been washed twice with PBS prior to use.
4. 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 Techniques**.
2. Any deviations from the **Recommended Techniques** should be validated prior to use⁶.

BIBLIOGRAPHY

1. Widman FK. Technical Manual, 9th Edition. American Association of Blood Banks, Arlington, VA, 1985; Chapter 8
2. Race RR, Sanger R. Blood Groups in Man, 6th Edition. Blackwell Scientific, Oxford 1975; Chapter 2
3. Mollison PL. Blood Transfusion in Clinical Medicine, 8th Edition. Blackwell Scientific, Oxford 1987; Chapter 7
4. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6
5. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
6. British Committee for Standards in Haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and cross matching. Transfusion Medicine, 1995, 5, 145-150.








AVAILABLE REAGENT SIZES

Vial Size	Catalogue Number
2 ml	312002
1000 ml	312000

For the availability of other sizes, please contact:

Lorne Laboratories Limited
Unit 1 Danehill
Cutbush Park Industrial Estate
Lower Earley, Reading,
Berkshire, RG6 4UT
England
Tel: +44 (0) 118 921 2264
Fax: +44 (0) 118 986 4518
E-mail: info@lornelabs.com

TABLE OF SYMBOLS

	Batch Number		<i>In-vitro Diagnostic</i>
	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		