LORNE LABORATORIES LTD.
GREAT BRITAIN

MONOCLONAL ANTI-COMPLEMENT REAGENT
DIRECTIONS FOR USE

Anti-C3d Monoclonal: For Direct Tube Techniques.

SUMMARY

Without complement, sensitisation and agglutination by an antibody would be incomplete and ineffectual. The complement system proteins make up a highly complex system involving as many as 24 chemically and biologically distinct entities.

INTENDED PURPOSE

This reagent is a blood grouping reagent intended to be used to qualitatively detect the presence or absence of complement factors C3d and C3b on human red cells when tested in accordance with the recommended techniques stated in this IFU.

PRINCIPLE

The reagent contains antibodies against C3 complement factors (C3d and C3b) on human red cells and will cause direct agglutination (clumping) of red cells that are sensitised with C3 complement factors (C3d and C3b). No agglutination generally indicates the absence of C3 complement factors (C3d and C3b on human red cells (See **Limitations**).

REAGENT

Lorne Monoclonal IgM Anti-C3d blood grouping reagent contains mouse monoclonal anti-C3d, Clone BRIC-8. The reagent does not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user. The reagent is supplied at optimal dilution, for use with all the 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 - 8ºC 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 37ºC and –25ºC as described in document BS EN ISO 23640:2015.

SAMPLE COLLECTION AND PREPARATION

Blood samples can be collected into EDTA, citrate, CPDA anticoagulants or as a clotted sample. The samples should be tested as soon as possible following collection. If a delay in testing should occur, store the samples at 2-8°C. Samples displaying gross haemolysis or microbial contamination should not be used for testing. Blood samples showing evidence of lysis may give unreliable results. It is preferable (but not essential) to wash all blood samples 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, but is not supplied sterile. 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. Materials used to produce the products were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
9. 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 (C3d and C3b coated 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. Before use, let the reagent warm up to room temperature. As soon as the reagent has been used, put the reagent back in storage at 2-8ºC.
3. In the **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
4. The use of the reagent and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
5. The user must determine the suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED BUT NOT SUPPLIED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive control (C3d and C3b coated) and negative control cells.
- Centrifuge capable of spinning at 1000 g for 20 seconds.
- Volumetric pipettes.

RECOMMENDED TECHNIQUE

A. Direct Antiglobulin Technique (DAT)

1. Wash 1 volume of red cells (2–3% suspension in PBS or Isotonic saline) 4 times with PBS or Isotonic saline, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
2. Add 2 volumes of Lorne Anti-C3d to each dry cell button.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of red cells constitutes a positive test result and within the accepted limitations of the test procedure, indicates the presence of complement (C3d and/or C3b) on the red cells.
2. **Negative:** No agglutination of the red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of complement (C3d and/or C3b) on the red cells.

STABILITY OF THE REACTIONS

1. Washing steps should be completed without interruption and tests centrifuged and read immediately after addition of the reagent. Delays may result in dissociation of antigen-antibody complexes, causing false negative or weak positive results.
2. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those **recommended**.

LIMITATIONS

1. Inadequate washing of red cells may result in neutralisation of the reagent.
2. Following completion of the wash phase excess residual saline may dilute the Anti-C3d reagent, reducing its potency.
3. Positive direct antiglobulin results due to complement sensitisation may not reflect *in vivo* complement fixation if test cells are from a previously refrigerated clotted blood specimen.
4. A negative direct antiglobulin test result does not necessarily preclude clinical diagnosis of ABO Haemolytic Disease of the Newborn or Auto Immune Haemolytic Anaemia. It also does not necessarily rule out HDN, especially if ABO incompatibility is suspected.
5. 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

SPECIFIC PERFORMANCE CHARACTERISTICS

1. Prior to release, each lot of these reagents was tested using the recommended test methods listed in this IFU. The tests complied with the test requirements as stated in the current version/issue of the “Guidelines for the Blood Transfusion Services in the United Kingdom”.
2. The anti-C3d potency has been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Controls (NIBSC):
   - Anti-AHG reference standard 96/666
3. Anti-C3d potency is demonstrated in tests employing cells coated with C3.
4. The presence of contaminating heterospecific agglutinins or antibodies to C4d has been excluded in tests employing red cells of all ABO groups and cells coated with C4d.
5. The Quality Control of the reagents was performed using red cells with phenotypes that were verified by a UK blood transfusion centre and had been washed with PBS or Isotonic saline prior to use.

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[6].

BIBLIOGRAPHY

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2. The Department of Health and Social Security. Health Services Management Antiglobulin Test. False negative results, HN (Hazard) (83) 625 Nov 1983.
3. Bruce M, Watt AH, Hare W, Blue A, Mitchell R. A serious source of error in antiglobulin testing. Transfusion 1986; **26**: 177-181.
4. Voak D, Downie DM, Moore BPL, Ford DS, Engelfreit CP, Case J. Replicate tests for the detection and correction of errors in AHG (AHG) tests: optimum conditions and quality control. Haematologia 1988; **21**(1): 3-16.
5. Guidelines for the Blood Transfusion Service in the United Kingdom, 6th Edition 2002. The Stationary Office.
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	Tests per vial
2 ml	427002	20
1000 ml	427000*	10,000

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

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