LORNE LABORATORIES LTD.
GREAT BRITAIN

SEROLOGICAL ENHANCEMENT MEDIUM
DIRECTIONS FOR USE

PEG-ADD: For Potentiating Indirect Antiglobulin Techniques.

SUMMARY

It is well established that reducing the ionic strength of a test system increases the rate of antigen-antibody binding in indirect antiglobulin tests. The sensitivity of indirect antiglobulin techniques can also be increased by the use of polyethylene glycol (PEG) as a potentiating medium.

INTENDED PURPOSE

PEG-ADD is a high molecular weight polymer containing low ionic strength solution that is intended for use in blood grouping for cross matching and antibody screening procedures when used in accordance with the recommended techniques stated in this IFU.

PRINCIPLE

When added to a solution of immuno-reactants, high molecular weight polymers displace other molecules, thereby increasing the amount of contact between antigen and antibody. When PEG is dissolved in a low ionic strength solution, the enhancement properties of both are combined and a smaller volume can be added to each test than if PEG is dissolved in normal ionic strength saline.

REAGENT

Lorne PEG-ADD is a low ionic strength solution contains glycine, a phosphate buffer and polyethylene glycol. 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 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 reagent, 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. No known tests can guarantee products derived from 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 Lorne Precise Weak Anti-D and appropriate red cells (ideally R_1r and rr) be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show the expected results.
2. The antiglobulin technique can only be considered valid if all negative tests react positively with IgG sensitised red cells.
3. The PEG-ADD solution, red cell suspensions and test sera should be at room temperature prior to use to avoid encountering unwanted positive reactions due to “cold” antibodies.
4. The use of polyspecific anti-human globulin with Lorne PEG-ADD may result in non-specific reactions.
5. In the **Recommended Techniques** one drop is approximately 50 µl when using the vial dropper provided
6. 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.
7. The user must determine the suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

- Anti-human IgG i.e. Lorne Anti-Human IgG (Cat # 402010 or 401010).
- Coombs cells washer.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- IgG sensitised red cells i.e. Lorne Coombs Control Cells (Cat # 970010).
- Lorne Precise Weak Anti-D (Cat # 209005).
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive (ideally R_1r) and negative (rr) control red cells.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.

RECOMMENDED TECHNIQUE

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 2 volumes test serum, 1 volume red cell suspension and 2 volumes Lorne PEG-ADD.
3. Mix thoroughly and incubate at 37ºC for 15 minutes.
4. Wash red cells 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.
5. Add 2 volumes of anti-human globulin to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
7. Gently resuspend the cells and read for agglutination.

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of red cells constitutes a positive test result.
2. **Negative:** No agglutination of the red cells constitutes a negative result.

STABILITY OF THE REACTION

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. Red cells that have a positive DAT due to a coating of IgG cannot be typed by the indirect antiglobulin technique.
2. Lorne PEG-ADD should not be used as a red cell suspending medium.
3. Not all antigen-antibody reactions are enhanced by the use of Lorne PEG-ADD in the indirect antiglobulin technique.
4. Red cells tend to aggregate in the presence of linear polymers therefore Lorne PEG-ADD may only be used in the indirect antiglobulin test.
5. IgM antibodies may not be detected by the indirect antiglobulin technique.
6. False positive or false negative results may also occur due to:
   - Contamination of test materials
   - Improper cell concentration
   - Improper incubation time or temperature
   - Improper or excessive centrifugation
   - Improper storage of test materials or omission of reagent
   - Deviation from the recommended techniques

SPECIFIC PERFORMANCE CHARACTERISTICS

1. Prior to release, each lot of Lorne PEG-ADD has been shown to enhance many antigen-antibody reactions when used by the **Recommended Technique**.
2. The solution 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[5].

BIBLIOGRAPHY

1. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6
2. B.Wenz, J.Apuzzo and D.P.Shah, Evaluation of the polyethylene glycol-potentiated indirect antiglobulin test, Transfusion 1990-Vol.30, No.4
3. R.S.Shirey, J.S.Boyd and P.M.Ness, Polyethylene glycol versus low-ionic-strength solution in pretransfusion testing: a blinded comparison study, Transfusion 1994-Vol.34, No.5
4. Guidelines for the Blood Transfusion Service in the United Kingdom, 6th Edition 2002. The Stationary Office.
5. 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
10 ml	485010	100
1000 ml	485000*	10,000

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

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