LORNE LABORATORIES LTD.
GREAT BRITAIN

SEROLOGICAL ENHANCEMENT MEDIUM
DIRECTIONS FOR USE

LISS-ADD: For Potentiating Serological reactions.

SUMMARY

Reducing the ionic strength of a test system increases the rate of red blood cell antigen-antibody binding. Low and Messeter in 1974 showed that the use of a low ionic strength solution enhances the rate of antibody uptake in first stage of agglutination, allowing incubation times to be shortened.

INTENDED PURPOSE

LISS-ADD is a 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 used by the recommended techniques, the solution will reduce the ionic-strength of a test system, increase the rate of red blood cell antigen-antibody binding and permits a substantial reduction in incubation time and an increases in the test sensitivity with many antibody specificities (see **Limitations**).

REAGENT

Lorne LISS-ADD is a low ionic strength solution containing glycine, sodium chloride, phosphate buffer and bovine albumin. 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 the 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 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. Contact with LISS together with bleach causes accelerated corrosion of base metals such as copper and iron. This should be borne in mind when considering the use of bleach for decontaminating pluming or apparatus with metal parts, which have also been in contact with LISS
9. 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. Lorne Precise Weak Anti-D and appropriate red cells (ideally R_1r and rr) shall 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 LISS 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. In the **Recommended Techniques** one drop is approximately 50 µl when using the vial dropper provided
5. 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.
6. The end user must determine the suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

- Anti-human globulin i.e. Lorne AHG Elite (Cat # 435010 or 415010) or anti-human IgG i.e. Lorne Anti-Human IgG (Cat # 401010 or 402010).
- 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 LISS-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. LISS-ADD cannot be used with enzyme treated red cells.
3. LISS-ADD cannot be used as a red cell suspending medium.
4. Weakly reactive Anti-A or Anti-B may not be detected using potentiating solutions.
5. Some IgM antibodies requiring room temperature incubation may not be reactive under the conditions of the recommended test procedure.
6. Deviation from the recommended ration of serum, cells and LISS-ADD may decrease the sensitivity of the test procedure.
7. Use of saline-diluted serum, or of eluates made into substrates other than fresh human serum, will result in increased ionicity and will therefore affect the sensitivity of the test.
8. False positive and false negative results may occur due to improper technique or contaminated test materials.
9. Not all antigen-antibody reactions are enhanced by LISS-ADD.

SPECIFIC PERFORMANCE CHARACTERISTICS

1. Prior to release, each lot of Lorne LISS-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 end 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[10].

BIBLIOGRAPHY

1. Low B., Messeter L. Antiglobulin test in low ionic strength salt solution for rapid antibody screening and crossmatching. Vox. Sang. 1974; **26**. 53-61.
2. Moore C., Mollison P.L. Use of low ionic strength saline medium in manual tests for antibody detection. Transfusion 1976; **16**. 291-296.
3. Wicker B., Wallas C.H. A comparison of low ionic strength saline medium with routine methods for antibody detection. Transfusion 1976; **16**. 469-472.
4. Voak D., Downie D.M., Darnborough J., Haigh T.J., Fairham S.A. Low ionic strength media for rapid antibody detection: optimum conditions and quality control. Med. Lab. Sci. 1980; **37**. 107-118.
5. Hiagh T.J., Fairham S.A. Advantages of low ionic strength saline (LISS) techniques in blood bank management. Med. Lab. Sci. 1980; **37**. 119-125.
6. Dynan P.K. Evaluation of commercially available low ionic strength salt (LISS) solutions. Med. Lab. Sci. 1981; **38**. 13-20.
7. Voak D., Downie M., Haigh T.J., Cook N. Improved antiglobulin tests to detect difficult antibodies: detection of Anti-Kell by LISS. Med. Lab. Sci. 1982; **39**. 363-370.
8. Phillips P.K., Bebbington C. The pH, conductivity and osmolality of low ionic strength solutions used within the U.K. for the antiglobulin test. Transfusion Medicine 1991; **1**. 155-158.
9. Guidelines for the Blood Transfusion Service in the United Kingdom, 6th Edition 2002. The Stationary Office.
10. 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	480010	100

TABLE OF SYMBOLS

Symbol	Definition	Symbol	Definition
	Manufacturer		Catalogue number
	Temperature limitation		Use by YYYY-MM-DD
	In vitro diagnostic medical device		Consult instructions for use.
	Authorised Representative		Lot number
	CE symbol with verification by a Notified Body		

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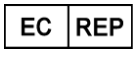

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