



## 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.

### 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 increase 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 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

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.

### 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. Specimens collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. Other samples should be typed within 48 hours. 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 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. 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 plumbing 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. It is recommended Lorne Precise Weak Anti-D and appropriate red cells (ideally R<sub>1</sub>r 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 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 40 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 user must determine the suitability of the reagent for use in other techniques.

### REAGENTS AND MATERIALS REQUIRED

- Anti-human globulin i.e. Lorne Polyspecific AHG Elite (Cat # 435010) or anti-IgG i.e. Lorne Monospecific Anti-IgG (Cat # 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).
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22°C ± 1°C
- Positive (ideally R<sub>1</sub>r) and negative (rr) control red cells.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37°C ± 2°C.

### RECOMMENDED TECHNIQUE

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 2 volumes test serum, 1 volume test red cell suspension and 2 volumes Lorne LISS-ADD.
3. Mix thoroughly and incubate at 37°C for 15 minutes.
4. Wash test red cells 4 times with PBS, 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 test red cells constitutes a positive test result.
2. **Negative:** No agglutination of the test 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 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<sup>10</sup>.

## BIBLIOGRAPHY

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2. Moore C., Mollison P.L. Use of low ionic strength saline medium in manual tests for antibody detection. Transfusion 1976; **16**: 291-296.
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5. Haigh T.J., Fairham S.A. Advantages of low ionic strength saline (LISS) techniques in blood bank management. Med. Lab. Sci. 1980; **37**: 119-125.
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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. H.M.S.O. Current Edition.
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
10 ml	480010
1000 ml	480000

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

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