SUMMARY
Reducing the ionic strength of a test system increases 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).

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 - 8ºC 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 2-8ºC. Specimens collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal.

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 biological 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 metal bases 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 Rr 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 22ºC ± 1ºC.
- Positive (ideally Rr) and negative (rr) control red cells.
- Volumetric pipettes.

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 37ºC 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.

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.


**AVAILABLE REAGENT SIZES**

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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

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