LORNE LABORATORIES LTD.
GREAT BRITAIN

ANTI-HUMAN GLOBULIN AND ENZYME CONTROL REAGENT
INSTRUCTIONS FOR USE

Precise Weak Anti-D: For Control of Antiglobulin and Enzyme Techniques.

SUMMARY
Careful control of both manual and automated techniques for detecting antibodies is essential. Testing of a weak IgG Anti-D with group O R_1r red cells in parallel with routine antiglobulin or enzyme tests will confirm the efficacy or otherwise of the chosen technique.

INTENDED PURPOSE
The reagent is intended as a sensitivity control for AHG and Enzyme techniques that are used in blood banking to detect clinically significant blood group antibodies.

PRINCIPLE
When used by the recommended techniques, the reagent will cause agglutination (clumping) of red cells carrying Rh D antigen. No agglutination (no clumping) when tested against Rh D positive red cells usually indicates a problem with the anti-human globulin or enzyme test (see **Limitations**).

REAGENT
Lorne Precise Weak Anti-D Control Reagent is prepared from pools of human serum containing low activity Anti-D. The pool is diluted in inert serum to give a final concentration of <0.10 IU/ml Anti-D. ABO antibodies are not absorbed. 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 in-use and 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 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 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 reagent 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.
10. Once the vial content is used, discard the empty vial in a yellow burn bin.

DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES
Manage waste according to local, state, and national regulations. For more information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

CONTROLS AND ADVICE
1. A a positive control (ideally OR_1r cells) and a negative control (Orr cells only) shall be tested in parallel with each batch of tests.
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 **Tube Technique** one volume is approximately 50µl when using the vial dropper provided.
4. The use of the reagents and the interpretation of results must be carried out by trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
5. End user must determine suitability of the reagents for use in other techniques.
6. The reagent is used in a manual test procedure. It is the responsibility of the end user to determine the suitability of the device in other techniques and/or test systems.
7. Where applicable, the use of calibrated or verified equipment is required.
8. Plasma being a complex biological material, can sometimes lead to the formation of fibrin threads. These threads can physically affect the clarity of the antibody solution, resulting in the observed precipitation.
9. If a serious incident has occurred (as defined in relevant regulations) which can be attributed to Lorne's reagent than the end user of the reagent must immediately report this to the manufacturer and the Competent Authority of the country where the incident took place.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED
- Anti-human globulin i.e., Lorne AHG Elite (Cat # 435010 or 415010) or anti-IgG i.e., Lorne Anti-Human IgG (Cat # 401010 or 402010).
- IgG sensitised red cells i.e., Lorne Coombs Control Cells (Cat # 970010).
- Enzyme reagent i.e., Lorne Papenzyme-Plus (Cat # 441010) or Lorne Bromelite (Cat # 443010).
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Test tube centrifuge.
- Coombs cell washer.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.
- Volumetric pipettes.

RECOMMENDED TECHNIQUE
Lorne Precise Anti-D should be tested in parallel (as a test sample) with all indirect antiglobulin and enzyme techniques using the techniques stated in the **Recommended Techniques** for the reagent being controlled.

INTERPRETATION OF TEST RESULTS
1. **Positive:** Agglutination of the OR_1r test red cells constitutes a positive test result and within the accepted limitations of the test procedure, indicates that there was no problem with the antiglobulin or enzyme test.
2. **Negative:** No agglutination of the OR_1r test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates that there is a problem with the antiglobulin or enzyme test. This could be due to one or more of the following factors:
   - Inadequate serum: cells ratio
   - Inadequate incubation of tests
   - Inadequate cell washing
   - Deterioration or omission of AHG reagent
   - Improper centrifugation
   - Excessive agitation, at the reading stage
   - Inactivity of enzyme preparation
   - Inadequate technique e.g., standard one-stage enzyme techniques are less sensitive than two stage or phased one-stage techniques

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. Lorne Precise Anti-D is for use with group O cells only because the ABO antibodies have not been absorbed.
2. This control may contain low levels of Anti-C or Anti-E.
3. The reagent must not be used for Rh D typing or for preparing sensitised cells for assuring Anti-IgG activity in negative Anti-Human Globulin tests.
4. In case of ambiguous results, it is recommended to wash red blood cells at least 2 times.
5. Stored blood may give weaker reactions than fresh blood
6. False positive or false negative results may also occur due to:
   - Contamination of test materials
   - Improper cell concentration, incubation time or temperature
   - Improper or excessive centrifugation
   - Introduction of human serum/gamma globulins into test
   - Improper storage of test materials or omission of reagent
   - Deviation from the recommended techniques

SPECIFIC PERFORMANCE CHARACTERISTICS
1. Prior to release, each lot of this reagent is tested using the recommended test methods listed in this IFU. The tests complied with the test requirements as stated in the in-house specifications.
2. The Quality Control of the reagent 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.

3. The specificity of the reagent is confirmed by screening with a panel of group O Rh D negative red cells which bear antigens with an incidence of 1% or greater in the random population.
4. Antibodies to Xg^a, Do^a, Yt^a, Co^b, Wr^a, Bg^a and V^w may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cell. This can also be said for Yt^b, M^g and V^w and other low frequency antigens which may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cells
5. The reagents comply 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 Techniques**. Any deviations from the **Recommended Techniques** should be validated prior to use[7].

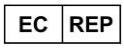
BIBLIOGRAPHY

1. Engelfriet CP, Voak D. International reference polyspecific anti-human globulin reagents. Vox Sanguinis 1987; **53**, 241-247
2. Voak D, Downie DM, Moore BPL, Ford DS, Engelfriet DP, Case J. Replicate tests for detection and correction of errors in anti-human globulin (AHG) tests: use of optimum conditions and quality control. Haematologia 1988; **21**, 3-16
3. Marion E.Reid & Christine Lomas-Francis, Blood Group Antigens & Antibodies, SBB Books, New York 2007; Page 190.
4. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 3.
5. AABB Technical Manual, 16th edition, AABB 2008.
6. Guidelines for the Blood Transfusion Service in the United Kingdom, 6th Edition 2002. The Stationary Office.
7. 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
Precise Weak Anti-D	5 ml	209005	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
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