LORNE LABORATORIES LTD.
GREAT BRITAIN

ANTI-HUMAN GLOBULIN AND ENZYME CONTROL REAGENT
DIRECTIONS 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.

PRINCIPLE

When used by the recommended techniques, the reagent will cause agglutination (clumping) of red cells carrying Rh D antigen. No agglutination 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 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 37°C and –25°C 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 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. 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.

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 that a positive control (ideally R_1r cells) and a negative control (rr cells only) be tested in parallel with each batch of tests.
2. In the **Tube Technique** one volume is approximately 50µl when using the vial dropper provided.
3. The use of the reagents 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.
4. User must determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- 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).
- Coombs cell 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).
- 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)
- Positive (ideally R_1r) and negative (rr) control red cells.
- Test tube centrifuge.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.

RECOMMENDED TECHNIQUE

Lorne Precise Anti-D should be tested in parallel 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 R_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 R_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 due to the fact that 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. 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
   - 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. The reagent has been characterised by the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Lorne Precise Weak Anti-D is tested by the **Recommended Techniques** against a panel of antigen-positive red cells to ensure suitable reactivity.
3. The Quality Control of this reagent was performed using red cells that had been washed twice with PBS or Isotonic saline prior to use.
4. 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.
5. 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
6. The reagents comply 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 Techniques**.

2. Any deviations from the **Recommended Techniques** should be validated prior to use[6].

BIBLIOGRAPHY

1. BCSH. Guidelines for compatibility testing in hospital blood banks. Standard Haematology Practice 1991; **14**, 150-163
2. Engelfriet CP, Voak D. International reference polyspecific anti-human globulin reagents. Vox Sanguinis 1987; **53**, 241-247
3. UKBTS/NIBSC. Guidelines for Blood Transfusion Service in the United Kingdom, Section 3 Guidelines for reagents for Blood Group Serology and HLA typing. 1996.
4. 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
5. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
6. 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
5 ml	209005
1000 ml	209000*

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

For the availability of other sizes, please contact:

Lorne Laboratories Limited
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Tel: +44 (0) 118 921 2264
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TABLE OF SYMBOLS

LOT	Batch Number	IVD	*In-vitro* Diagnostic
REF	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		