LORNE LABORATORIES LTD.
GREAT BRITAIN

STABILISED PAPAIN REAGENT
DIRECTIONS FOR USE

Papenzyme-Plus: For Specific Serological Studies

SUMMARY

Enzymes are particularly useful in detecting antibodies of the Rh system and offer a valuable addition to the range of serological techniques used for antibody identification, especially where it is suspected that there is a mixture of antibodies. Papain destroys certain blood group antigens, notably M, N, S, Fy^a, Fy^b and Xg^a, a property that may be useful for identification and separation of mixed antibodies.

INTENDED PURPOSE

The reagent contains an enzyme that is capable of enhancing blood group agglutination reactions in the detection of anti-erythrocytic antibodies when tested in accordance with the recommended techniques stated in this IFU.

PRINCIPLE

Enzymes may potentiate agglutination in at least two different ways: by reducing surface charge of red cells and by removing structures, which sterically interfere with the access of antibody molecules.

REAGENT

Lorne Papenzyme-Plus reagent is a ready to use liquid preparation of stabilised papain. The reagent is standardised by serological methods for use in blood group antibody investigations. 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 optimal dilution for use with all the recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see **Vail 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, 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.

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.

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_1r 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. One stage mixed techniques, in which enzyme, serum and red cells are mixed without purposeful delay and incubated together, are not recommended for use in the screening of patients' sera for atypical antibodies or in compatibility testing of patients' sera with donors' red cells.
3. An auto-control is recommended because enzymes can considerably enhance the reactions of cold agglutinins and so many normal sera react with enzyme-treated cells at room temperature and in some cases at 37ºC.
4. Deviation from the recommended methods of use may result in false positive or false negative results. This includes very slight changes in buffers or in solutions, which may result in sub-optimal pH for enzyme treatment.
5. 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.
6. In the **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
7. 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.
8. The user must determine the suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED BUT NOT SUPPLIED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive control (R_1r) and negative (rr) control red cells.
- Test tube centrifuge.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.
- Weak anti-D i.e. Lorne Precise Weak Anti-D (Cat # 209005).

RECOMMENDED TECHNIQUES

A. Two-Stage Technique (using packed red cells)

1. Wash packed red cells twice with PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume Lorne Papenzyme-Plus and 1 volume washed packed red cells.
3. Mix thoroughly and incubate at 37ºC for 15 minutes.
4. Wash cells once with PBS or Isotonic saline and then resuspend to 2-3% in PBS or Isotonic saline.
5. Place in a labelled test tube: 1 volume of test serum and 1 volume Papenzyme-Plus treated red cell suspension.
6. Mix thoroughly and incubated at 37ºC for 15 minutes.
7. Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
8. Gently resuspend each cell button and read macroscopically for agglutination.

Two-Stage Technique (using 2-3% red cells)

1. Prepare a 2-3% suspension of washed red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume Lorne Papenzyme-Plus and 2 volumes of red cell suspension.
3. Mix thoroughly and incubate at 37ºC for 15 minutes.
4. Wash cells three times with PBS or Isotonic saline and then resuspend to 2-3% in PBS or Isotonic saline.
5. Place in a labelled test tube: 1 volume of test serum and 1 volume Papenzyme-Plus treated red cell suspension.
6. Mix thoroughly and incubated at 37ºC for 15 minutes.
7. Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
8. Gently resuspend each cell button and read macroscopically for agglutination.

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of red cells constitutes positive test result within accepted limitations of the test procedure.
2. **Negative:** No agglutination of the red cells constitutes negative test result within accepted limitations.

STABILITY OF THE REACTIONS

1. Read all tubes tests immediately after centrifugation.
2. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those **recommended**.

LIMITATIONS

1. All enzyme preparations are subject to some loss of potency in storage. It may therefore be necessary to increase the recommended treatment time towards the expiry date of the preparation in order to ensure maximum sensitivity.
2. Improper ratios of Papenzyme-Plus: cell suspension may result in excessive haemolysis.
3. The standard one stage technique is a convenient method for used with potent blood grouping reagents, but it is relatively insensitive for antibody detection or compatibility testing. This is due to the presence of protease inhibitors in serum and also the ability of papain to cleave Ig molecules.
4. Care should be taken to maintain the sterility of the enzyme preparation since they readily become contaminated with microorganisms that can result in false negative or false positive reactions.
5. Enzyme tests do not detect all antibodies of probable clinical significance.
6. Extended incubation may cause weakened positive or false negative reactions due to enzyme degradation of Ig molecules.
7. False positive or false negative results may also occur due to:

- Improper cell concentration
- Improper incubation time or temperature
- Improper or excessive centrifugation
- Improper storage of test materials or omission of reagent
- Inadequate technique e.g. standard one-stage enzyme technique is less sensitive than two stage technique.

SPECIFIC PERFORMANCE CHARACTERISTICS

1. Prior to release, each lot of Reagent was tested using the recommended test methods listed in this IFU. The tests complied with the test requirements as stated in the current version/issue of the "Guidelines for the Blood Transfusion Services in the United Kingdom".

DISCLAIMER

1. The user is responsible for the performance of the reagent by any methods other than those mentioned in the **Recommended Techniques**.
2. Any deviations from the **Recommended Techniques** should be validated prior to use[5].

BIBLIOGRAPHY

1. Mollison PL. Blood Transfusion in Clinical Medicine, 8th Edition. Blackwell Scientific, Oxford 1987; Chapter 7
2. Boorman and Dodd, Blood Group Serology, 5th ed. Churchill Livingstone (1977) 67, Technique 8.6.B.
3. Phillips PK, Farr AD (Ed). Quality assurance and control in clinical laboratories. Med Lab Sci 1984; 32.
4. Waters AH et al, Guidelines for compatability testing in hospital blood banks. J Clin Lab Haemat 1987; **9**: 333-341.
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	Tests per vial
10 ml	441010	200
1000 ml	441000*	20,000

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

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