Bromelin

For Specific Serological Studies

Qualitative procedure for use in antibody detection.

IVD

PACKAGING

Ref.: 101-0536	Cont.: 5 mL

Store at 2-8º C

PRINCIPLE OF THE METHOD

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.

CLINICAL SIGNIFICANCE

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. Bromelin destroys certain blood group antigens, notably M, N, S, Fya, Fyb and Xga, a property that may be useful for identification and separation of mixed antibodies.

REAGENTS

Chronolab Bromelin reagent is a ready to use liquid preparation of stabilised Bromelin. The reagent is standardised by serological methods for use in blood group antibody investigations. The reagent is supplied at optimal dilution for use with all 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 - 8º C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity.

SAMPLE COLLECTION AND PREPARATION

Blood samples should be drawn aseptically into EDTA and tested as soon as possible. If EDTA is unavailable, samples drawn into ACD, CPD or CPDA-1 are acceptable and may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS before being tested.

PRECAUTIONS

1. If a reagent vial is cracked or leaking, discard the contents immediately.
2. Do not use the reagent past the expiration date (see **Vial Label**).
3. Do not use the reagent if a precipitate is present.
4. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
5. 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.

CONTROLS AND ADVICE

1. It is recommended Chronolab Precise Weak Anti-D and appropriate red cells (ideally R1r 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 37º C.
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. In the **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
6. Use of reagent and interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagent is in use. The user must the determine suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22ºC ± 1ºC
- Positive control (R1r) and negative (rr) control red cells.
- Test tube centrifuge.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.
- Weak anti-D.

RECOMMENDED TECHNIQUES

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

1. Wash packed test red cells twice with PBS.
2. Place in a labelled test tube: 1 volume of Chronolab reagent and 1 volume of washed packed test red cells.
3. Mix thoroughly and incubate at 37º C for 15 minutes.
4. Wash cells once with PBS and then resuspend to 2-3% in PBS.
5. Place in a labelled test tube: 1 volume of test serum and 1 volume Bromelin treated test red cell suspension.
6. Mix thoroughly and incubate at 37º C 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

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

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume of Chronolab reagent and 2 volumes of test red cell suspension.
3. Mix thoroughly and incubate at 37º C for 15 minutes.
4. Wash cells three times with PBS and then resuspend to 2-3% in PBS.
5. Place in a labelled test tube: 1 volume of test serum and 1 volume Bromelin treated test red cell suspension.
6. Mix thoroughly and incubate at 37º C 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 the test red cells constitutes a positive test result and within accepted limitations of test procedure.
2. **Negative:** No agglutination of the test red cells constitutes a negative result and within the accepted limitations.

STABILITY OF THE REACTIONS

1. Tests should be read immediately after centrifugation.
2. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

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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 Bromelin: 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 Bromelain 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:
   - Contamination of test materials
   - Improper storage, cell concentration, incubation time or temperature
   - Improper or excessive centrifugation
   - Deviation from the recommended techniques

SPECIFIC PERFORMANCE CHARACTERISTICS

1. The reagent has been characterised by all the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Chronolab Bromelin reagent is tested by the **Recommended Techniques** against a panel of cells to ensure suitable reactivity.

BIBLIOGRAPHY

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