The use of papain in routine blood group serology was originally reported by Low in 1955. Papain is usually used in its two-stage technique, to agglutinate enzyme-treated red cells. This enhanced sensitivity is especially useful prior to the administration of RhD antibody. bioCSL Papain Solution may also destroy certain blood group antigens, thereby preventing agglutination by the corresponding antibody. A reduction in the number of antigen-positive cells or cells with high antigen expression and do not demonstrate a discernible pattern on an antibody identification panel. The use of papainised cells may cause a significant increase in reaction strength, allowing the antibody to demonstrate agglutination of all antigen-positive cells and therefore confirm the antibody identity. Some antibodies may be detectable only on enzyme treated cells and often appear to have Rh system specificity (usually anti-E). They are known as “enzyme only” antibodies, are generally naturally occurring and are considered to be of no clinical significance.

Antibody Detection

Enzyme techniques may be used to aid in the identification and/or confirmation of antibodies detected in an individual’s serum. Treatment of red cells with papain may significantly enhance the reaction strength of antibodies, including those in the Kell, Cr and Do systems. While many antibodies (except antibodies such as anti-Fy(a), anti-M, anti-n, and anti-I) react strongly, there are occasions when experience is required to detect the difference between very weak specific and non-specific reactions. Non-reactive results (using autologous cells) may be used to confirm that there is no significant antibody activity in the sample.

Antigen Detection

Antigens are destroyed by papain: Fy(a), Fy(b), M, N, s, Fy(x), Fy(r), C2, Ge, Gi, Jka, K, and Rhesus. This reduction in the number of antigen-positive cells in the sample, and the weak or negative reaction, may improve the sensitivity of the test. The reaction strength of certain natural antibodies (such as anti-P1 and anti-Le) may improve if the tubes are re-incubated at 15°C for a further 15 minutes. The reaction strength of some antibodies may be enhanced by the use of LISS-suspended papainised red cells.

Recommended Methods

bioCSL Papain Solution is freely soluble in distilled water and when reconstituted as outlined below, the resultant Papain Stock Solution is equivalent to that of Allen and Stramer. The volume of Celepsol™ used to make the working strength solution may be modified in order to vary the final papain concentration if required.

Reconstitution of Lyophilised Product to Produce Papain Stock Solution

1. Remove the seal and rubber stopper of the bioCSL Papain Solution vial and add 3mL of distilled water.
2. Cap vial with the dropper assembly supplied and allow the vial to stand for 2 minutes to allow reconstitution.
3. Gently mix the contents by swirling and inverting to ensure that all traces of dry material adhering to the neck have been dissolved.

Note: Generally Papain Stock Solution be left for 10 minutes at room temperature to stabilise before use. This stock solution is stable for 28 days at 2° to 8°C.

Loss of activity is caused by oxidation of the product and users performing only a few tests at any one time are advised to aliquot the stock solution into small airtight containers. Any precipitate may be removed by centrifugation, but persistent turbidity is a sign of deterioration and any partly-degraded container showing cloudiness after centrifugation should be discarded.

Antibody Detection

Two-Stage Antibody Screen Tube Technique

1. Prepare a 1/3 working strength Papain Solution by mixing 1 part papain stock solution and 2 parts Celepsol™ (working strength Papain Solution is active for 3 days at 2° to 8°C).
2. Prepare a 5% suspension of test red cells in buffered or unbuffered isosmotic saline, or in bioCSL Celepsol™
3. Add 1 drop of the suspension of test red cells or Reagent Red Blood Cells RRBC to an appropriately labelled clean glass test tube (10/75mm or 12/75mm).
4. Add 1 drop of the working strength Papain Solution to the tube.
5. Gently mix and incubate at 37°C for 5 minutes.
6. Fill tubes with buffered saline and centrifuge at high speed (1000 rcf) for 30 seconds*.
7. Decant the supernatant saline, leaving a button of packed, papain-treated red cells.
8. To this button of red cells, add 2 drops of serum or plasma under test.
9. Gently mix and incubate at 37°C for 5 minutes.
10. Centrifuge at low speed (500 rcf) for 10 to 15 seconds* Read and record results.

Note: * Or centrifuge at a speed and time appropriate for the centrifuge in use.

While many weak antibodies (except antibodies such as anti-Fy(a), anti-M, anti-n, and anti-I) react strongly, there are occasions when experience is required to detect the difference between very weak specific and non-specific reactions. Non-reactive results (using autologous cells) may be used to confirm that there is no significant antibody activity in the sample.

CAT Method

bioCSL Papain Solution is suitable for treating cells that may then be used in the BioRad™ DDAvid™ ID-Micro Typing System (ID-MTS™). Column Agglutination Technology (CAT) Systems. These methods should only be used for antibody investigations. Users should validate controls and technique use.

Two-Stage Antibody Identification Technique for CAT Control

1. Prepare a 1/3 working strength Papain Solution by mixing 1 part papain stock solution and 2 parts Celepsol™ (working strength Papain Solution is active for 3 days at 2° to 8°C).
2. Prepare a 5% suspension of test red cells in isosmotic saline, buffered isosmotic saline or Celepsol™
3. Add 2 drops of serum or plasma under test to an appropriately labelled clean glass test tube (10/75mm or 12/75mm).
4. Add 1 drop of the working strength Papain Solution to the tube.
5. Immediately add 1 drop of the suspension of test red cells or RRBC.
6. Gently mix and incubate at 37°C for 5 minutes.
7. Centrifuge at low speed (500 rcf) for 10 to 15 seconds* Read and record results.

Note: * Or centrifuge at a speed and time appropriate for the centrifuge in use.

It is essential to add the test red cells to the serum/papain mixture as soon as possible, as prolonged exposure to papain may cause loss of antibody activity and reduction in reaction strength.
One-Stage Antibody Investigation Technique for BioRad™DiaMed™ ID-Micro Typing System (ID-MTS™)

1. Prepare a 1/3 working strength Papain Solution by mixing 1 part Papain Stock Solution and 7 parts Celpresol™ (working strength Papain Solutions are active for 3 days at 2° to 8°C).
2. Prepare a 0.8% suspension of test red cells in BioRad™DiaMed™ ID-Diluent 2™, Celpresol™ LISS or Celpresol™.
3. Appropriately label an ID-MTS™ LISS/Cobas card.
4. Add 25µL of the 1/3 working strength Papain Solution to the appropriate test reaction chambers.
5. Add 50µL of the suspension of test red cells or RBC t to the appropriate reaction chambers.
6. Incubate in the ID-MTS™ System Incubator at 37 ± 1°C for 2 minutes.
7. Add 25µL of serum or plasma under test to the reaction chambers.
8. Incubate for 1 minute (no longer) at 37°C.
9. Centrifuge the card in the ID-MTS™ System Centrifuge at the automatic preset speed setting of the centrifuge.
10. Read both front and back sides of the individual columns for agglutination and/or haemolysis under illumination. Record results.

INTERPRETATION OF RESULTS

It is important to recognize and record that cells have been papain-treated, as antibodies that are destroyed or weakened by papain may not be detected. Agglutination of papain-treated red blood cells by serum indicates that the serum contains antibodies active against blood group antigens present on the test cells.

CONTROLS

When using enzyme techniques for the detection of antibodies or in blood group determinations, the presence of autoantibodies or in vivo sensitized cells may lead to difficulties in the interpretation of false positive results. To assist in the recognition of these conditions an Autocontrol should be performed in parallel with the test samples.

Autocontrol

The Autocontrol should consist of the addition of the appropriate volume of the patient’s serum to 1 volume of the patient’s own cells which have been treated by bioCSL Papain Solution as detailed above.

Positive Control

To ensure that the working strength Papain Solution is sufficiently active, a Positive Control should also be performed in parallel with all tests. The Positive Control should consist of 1 drop of IgG anti-D (diluted to a titre of less than 8) tested against 1 drop of the appropriate suspension of papain-treated RhD Positive red cells.

Activity Control

Lown, Holland and Barr have reported a serum factor that can inhibit the reactions of enzyme reactive antibodies. In order to detect the presence of this factor, it is necessary to control the activity of each negative test in a similar manner to the use of bioCSL AHG Control Cells 3% in the IAT.

To negate each negative test, add 1 drop of serum containing IgG anti-C and anti-e together to a titre of approximately 8 that are active only by the enzyme technique. Leave at 22° to 37°C for 2 minutes, centrifuge and examine for agglutination. A positive result will validate the original negative test result. A negative result is indicative of the presence of the inhibitory factor in the patient’s serum, and invalidates the negative test result. In such cases, the patient’s serum should be inactivated at 56°C for 20 minutes and the enzyme tests repeated.

LIMITATIONS OF PROCEDURE

Antigens such as M, N, S, K, X, Ly, P, E, C, CH, PR, P1, P2, and Tn are destroyed or weakened by the action of papain. Immunoglobulin molecules will be cleaved by papain after extended incubation. For this reason, one-stage methods are not recommended for routine use in antibody screening.

Discrepant results may occur due to:

1. Incorrect technique.
2. Inactivation for incorrect time or temperatures.
3. Presence of gross inactivation.
4. Use of aged blood samples, reagents or supplementary materials.
5. Contaminated blood samples, reagents or supplementary materials.
6. Red cells that have a positive Direct Antiglobulin Test (DAT).
7. Other deviation from the recommended test methods.
8. Incorrect concentrations of red cells.
9. Incorrect reading of results (i.e. failure to detect haemolysis, etc).

PRECAUTIONS

1. The material from which this product was derived is from non-human sources; there is no risk of HIV or HBsAg infection. However, good laboratory practice requires safe handling procedures are used.
2. Turbidity in the reconstituted product may indicate bacterial contamination.
3. The product is derived from human plasma and may contain low levels of heterologous antibodies to human antigens.

REFERENCES


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