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Testing of Eluates by the Gel Method - Dearborn

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document provides instructions to test an eluate by the gel method.

II. CLINICAL SIGNIFICANCE:

A. Red blood cells (RBCs) may be coated with antibody and may have a positive direct antiglobulin test (DAT). An eluate is prepared from these prepared from these coated RBCs using the process described in the procedure, *Preparation of an Eluate*. The RBCs are thoroughly washed to remove all traces of unbound protein using the Working Wash solution, which maintains the association of bound antibody. The washed cells are then suspended in a glycine solution at low pH, which dissociates the bound antibody. After centrifugation, the supernate is separated from the washed RBCs and is neutralized by the addition of a buffering solution. The supernate / eluate is then ready to be tested for antibody activity against a panel of RBCs. Some eluates will demonstrate identifiable antibody activity, some will demonstrate no activity, and others will demonstrate non-specific reactivity. Non-specific reactions are frequently observed in the eluates of patients with warm autoantibodies. Those eluates that demonstrate identifiable antibody activity are most often encountered in hemolytic transfusion reactions and in cases of hemolytic disease of the newborn (HDN).

1. The following is a non-exhaustive list of situations when eluate studies may be performed:
2. To identify antibodies coating cord/newborn RBCs in cases of Hemolytic Disease of the Newborn (HDN).
3. To investigate adverse reactions to transfusions when the post-transfusion Direct Antiglobulin Test (DAT) is positive.
4. To identify the presence of autoimmune hemolytic anemia when the DAT is positive.
5. To detect the presence of Anti-A or Anti-B coating RBCs following the transfusion of ABO incompatible plasma.
6. An eluate may be prepared from a neonatal sample for performing the crossmatch for neonatal exchange transfusion in HDN cases when the mother's and neonate's serum is unavailable. Antibody screening of this eluate may be indicated after consultation with the medical director.
7. In rare situations,after consult with the medical director, an eluate may be be performed to separate

mixtures of antibodies, or to remove surface antibody to allow for cell typing.

III. DEFINITIONS:

A. Standard panel: a commercially prepared panel that usually consists of 11 vials of human RBCs. It is usually performed on patients who do not have a historical antibody record.

B. Selected cell panel: a panel that is pre-selected based on the antigenic profile of the test RBCs.

IV. POLICIES:

Appropriate Test Cells

A. Generally, eluates tested by the gel method should be tested against a standard, commercially prepared panel of 0.8% test cells.

B. The eluate may be tested against a selected cell panel of 0.8% test cells in the following cases when known antibody specificities have been previously identified.

C. The eluate may be tested against a selected cell panel when a smaller than normal of eluate is prepared e.g., neonatal samples. In this case it may be necessary to test the eluate against a smaller number of panel cells, or against a set of screening cells.

D. If a patient develops a positive Direct Antiglobulin Test (DAT) after the transfusion of ABO plasma-incompatible components, then the eluate should be tested against 2 examples each of A1, B, and O test RBCs.

V. SPECIMEN COLLECTION AND HANDLING:

A. The preferred sample is a 6 ml EDTA sample or a cord blood specimen with affixed identifying label. The eluate should be prepared while the sample is still fresh. If a delay in preparing the eluate is necessary, the specimen should be stored at 1-10° C, preferably for not longer than 72 hours. A volume of 1 mL washed RBCs is generally required, but smaller volumes may be sufficient; see procedure: *Preparation of an Eluate*.

B. The prepared eluate may be tested up to seven (7) days after preparation, provided that it is stored at 1-10° C and that turbidity has not developed. Refer to the Gamma Elu-Kit II manufacturer's insert for further information.

VI. REAGENTS:

A. 0.8% test cells; refer to the policy Appropriate Test Cells.

B. MTS Anti-IgG Gel cards, stored at room temperature, 18 °C to 25°C

VII. EQUIPMENT & SUPPLIES:

A. MTS worktable

B. MTS incubator

C. MTS centrifuge

D. Calibrated pipette (electronic or manual)

E. Pipette tips

F. Gauze

VIII. QUALITY CONTROL (QC):

A. An antibody screen of the last wash supernate, obtained during the eluate's preparation, must be tested in parallel with the eluate. The purpose of this antibody screen is to assure that antibody in the eluate has been derived from a bound state on the RBCs and is not merely the result of inadequate washing of the RBCs during the eluate's preparation. This antibody screen should be non-reactive.

B. If the eluate will be tested against a and b cells, then the last wash must also be tested against a set of a1 and b reverse cells).

C. Directions to perform this QC are included in the procedure section, Testing of Eluates by the Gel Method.

D. Passing QC: The Quality Control- Antibody Screening Test should be non-reactive. If the last wash was tested against a and b cells (a set of reverse cells), then this test should also be non-reactive.

E. Failing QC: If the Quality Control- Antibody Screening Test is reactive (or if the last wash was tested against a and b cells and is reactive), then the eluate generally cannot be interpreted. It may be useful to prepare a fresh eluate, washing the RBCs additional times; see, Eluate Preparation. If the quality control is still reactive, even after preparing a fresh eluate with additional washing, this may indicate that residual serum antibody was present. The eluate may then be considered contaminated, and interpretation may not be valid. However, such reactivity may also occur if the antibody coating the RBCs has low affinity for its corresponding antigen and eluates during the washing process. This may be minimized by washing in 1°C to 10°C Working Wash Solution, although in most cases satisfactory eluates can be made washing at room temperature.

IX. PROCEDURE:

A. Proceed from procedure, Preparation of Eluate.
Note: Verify that supernatant is clear, free of supernatant and cellular debris. If necessary re-centrifuge supernatant additional 5 minutes and transfer the supernatant to a new 12 X 75 mm test tube labeled with the patient's name.

B. Visually inspect gel card(s) before each use. Gel cards should have a clear liquid layer on top of opaque gel. Do not use if gel card show signs of damage.

C. Label the gel card(s) with the following information:

1. The patient's last name.
2. Label each well of the gel card(s) with the identification of the cells against which the eluate will be tested.
3. Label additional wells for the last wash quality control (screen cells I and II and/or reverse cells).

D. Remove the foil seal from the card, exposing only enough wells needed for testing.
Note: Foil should be removed immediately before testing, not more than 1 hour before testing.

E. Pipette 50 µL of well-mixed, 0.8% test cells into the correspondingly labeled wells. If necessary, refer to procedure, *Making a Test Red Cell Suspension.*
Note: Pipette tip should not touch gel card.

F. Add 25 µL of the eluate to the correspondingly labeled wells.
Note:The eluate must be added within 15 minutes after pipetting the test cells.

G. Add 25 µL of the last wash supernate saved during the preparation of the eluate to the wells labeled for screen cells I and II (or for the wells labeled for a and b cells).

H. Incubate the gel cards at 37C for fifteen to thirty minutes (15 – 30 minutes). Note: Incubation time must not exceed 30 minutes.

I. Centrifuge the gel card at the preset conditions specified by the manufacturer for ten (10) minutes @1032 ± 10 revolution per minute (rpm)

J. Read the both front and back of each gel card for agglutination and grade reactions.If necessary, refer to procedure, *Reading and Grading Test Reactions*.

K. Record the graded reactions of the eluate on the panel sheet.

L. Record the graded reactions of the QC (antibody screen of the last wash) on the panel sheet.

M. Interpret the eluate results. Refer to the Interpretation section.

X. INTERPRETATION:

A. Eluates can not be interpreted unless the quality control testing of the last wash is non-reactive. See Quality Control section above.

B. Eluates will be interpreted in the same manner as an antibody panel, as described in procedure *Interpretation of Antibody Investigations*. Most often when a specific alloantibody is identified in the eluate, that same antibody activity is present in the serum. The reactions observed in the gel testing will be interpreted and reported in one of three ways:

1. ENR: if the eluate is non-reactive, or
2. ENS, if the eluate is non-specific, or
3. Reactive, with a red cell antigen specificity. If a specificity is identified, consider the patient's transfusion and medical history, and the DAT results to determine whether the antibody is an autoantibody of apparent specificity, or an alloantibody, or a passively acquired antibody. Consult the Supervisor/Manager or medical director if necessary.

XI. LIMITATIONS:

A. Even though the RBCs used for the elution may have a positive DAT, in some cases no antibody activity will be detected in the eluate. This may be because the IgG coating on the red cells is not directed at RBC antigens, or that the antibody requires certain drugs to be present in the test system for detection.

B. RBCs having a positive DAT attributable to only bound complement will normally yield an eluate showing no antibody reactivity.

XII. REFERENCES:

1. American Association of Blood Banks Technical Manual, current edition.
2. Immucor / Gamma ELU-KITTM II, Manufacturer's Insert, 09/2010.

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
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