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Preparation of Eluate - Dearborn

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide instructions to prepare an eluate using the Gamma Elu-Kit.

II. PRINCIPLE:

A. Red blood cells (RBCs) may be coated with antibody and may have a positive direct antiglobulin test (DAT). In this procedure RBCs are thoroughly washed to remove all traces of unbound protein using a 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).

III. CLINICAL SIGNIFICANCE:

A. The following is a non-exhaustive list of situations when eluate studies may be performed:

1. To identify antibodies coating cord/newborn RBCs in cases of Hemolytic Disease of the Newborn (HDN).
2. To investigate adverse reactions to transfusions when the post-transfusion Direct Antiglobulin Test (DAT) is positive.
3. To identify the presence of autoimmune hemolytic anemia when the DAT is positive.
4. To detect the presence of Anti-A or Anti-B coating RBCs following the transfusion of ABO incompatible plasma.
5. 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

6. In rare situations, an eluate may be performed to separate mixtures of antibodies. The red cells are destroyed by the acid and are no longer suitable for testing.

IV. POLICY:

A. Test RBCs must be washed thoroughly with the Working Wash solution prior to elution to prevent contamination of the eluate with serum antibody.

B. An eluate should be performed whenever a post-reaction DAT reacts more strongly than the pre-reaction DAT. Refer to *Suspected Transfusion Reaction Investigation Procedure*.

V. SPECIMEN COLLECTION:

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 Table: Eluate Preparation Using the Gamma Elu-Kit II.

VI. REAGENTS:

A. Gamma Elu-Kit II, consisting of the following:

1. Concentrated Wash Solution : Must be diluted 1 in 10 with laboratory reagent-grade water to prepare the Working Wash Solution. Once diluted it may be stored at 1°C to 10°C for as long as it shows no obvious signs of turbidity and is not causing hemolysis of RBCs.
2. Eluting Solution: Stored at room temperature (15°C to 30°C) when not in use. Do not use if markedly turbid.
3. Buffering Solution: Do not use if it is not blue prior to buffering the eluate. Stored at room temperature (15°C to 30°C) when not in use. Do not use if markedly turbid.
4. Antihuman globulin (AHG) reagent, monospecific Anti-IgG
5. IgG coated check cells.

Note: The reagents of the Gamma Elu-Kit II may be interchanged between lots, providing they are in date. Do not use any reagents beyond expiration date.

VII. EQUIPMENT/SUPPLIES:

A. Laboratory reagent-grade water for Working Wash Solution,

B. Normal saline

C. 12 X 75 mm plastic test tube and cap

D. Centrifuge

VIII. QUALITY CONTROL (QC):

A. Antibody Screen of the Last Wash Supernate
An antibody screen of the last wash supernate must be tested in parallel with the eluate testing. This antibody screen should be nonreactive. The purpose of this antibody screen is to assure that antibody

that may be detected in the eluate is not merely the result of inadequate washing / residual antibody from the plasma, but that it has been derived from a bound state on the RBCs.

B. If the eluate will be tested against a and b cells, then the last wash must also be tested against a and b cells (a set of reverse cells).
Refer to Procedure: Testing of Eluates by the Gel Method

IX. PROCEDURE:

A. Obtain the panel sheet for the testing cells and fill in the following information:
   1. Patient's identifying information
   2. Elu-Kit-II Lot # and expiration date
   3. Testing date / technologist's initials

B. Prepare the Elu-Kit Working Wash Solution by adding one volume Concentrated Wash Solution to nine volumes reagent-grade water. Mix thoroughly. Label the Working Wash Solution with the date prepared, expiration date, and lot number.

C. Centrifuge the patient's sample for 10 minutes. Remove as much plasma as possible. The plasma may be transferred to a properly labeled test tube.

D. Transfer an aliquot of packed RBCs to a clean, 12 x 75 test tube labeled with the patient's name. The aliquot should yield at least 20 drops of washed, packed RBCs after washing (steps E and F).
Note: The eluate may be prepared from a lesser or greater volume of RBCs, but the resulting volume of eluate will be affected accordingly.

E. Wash the aliquot of packed RBCs one time with normal saline.

F. Wash the RBCs 4 additional times with the Working Wash Solution to remove all unbound antibody. Save an aliquot of this last wash (LW) solution; do not discard.The LW will be used to perform Quality Control during testing. See Procedure *Testing of Eluates by the Gel Method.*

G. Place 1 ml (20 large drops) of the washed RBCs in a plastic 12 x 75 mm test tube labeled with the patient's name.
Note: The volume of RBCs used in Step D should be equal to the volume of eluting solution.

H. Add 1 ml (20 large drops) of Eluting Solution and mix GENTLY by inverting the tube 4 times. The volume of Eluting Solution should equal the volume of washed RBCs.

I. Immediately centrifuge the test tube for 45-60 seconds at 3,400 rpm. Prolonged immersion in the Eluting Solution causes hemolysis.

J. Transfer the supernatant eluate to a 12 x 75 mm test tube labeled with the patient's name.

K. Discard the RBCs; they are no longer suitable for testing.

L. Add a sufficient volume of Buffering Solution to the separated acid eluate to adjust the pH of the eluate to within the required range (6.4 – 7.6)* for testing (until the eluate turns and remains pale blue). The presence of a blue indicator in the Buffering Solution provides a means to determine that the eluate has been properly adjusted. If the color of the eluate remains yellow after adding 20 drops of the Buffering Solution, continue adding Buffering Solution one drop at a time until a pale blue color persists.
Note: *The volume of Buffering Solution required for this purpose varies with each eluate; refer to the manufacturer's insert for additional information.

M. Mix well, centrifuge, and transfer the supernatant eluate to a 12 x 75 mm test tube labeled with the

patient's name. Place a cap on the eluate sample.
Note: Be sure not to transfer any precipitate or cellular debris. If necessary centrifuge the supernatant twice.

N. Proceed to *Testing of Eluates by the Gel Method* procedure.

X. INTERPRETATION:

A. The eluate is not interpreted until after testing is completed; see Procedure, Testing of Eluates by the Gel Method

XI. SPECIAL NOTES:

A. During eluate preparation, it is essential that processing of the RBCs be efficient and not interrupted. Allowing the RBCs to remain in contact with washing solution may allow antibody to release from the cell surface and move into solution. When the wash solution is discarded, the antibody activity would be lost.

B. Eluates vary both in mechanism and effectiveness in antibody removal. The methods in this procedure are relatively safe and effective for most applications. In rare instances a more potent methodology may have to be used to obtain a satisfactory eluate for testing.

C. Dilute proteins, such as those obtained by elution into saline, are unstable. Eluates should be tested as soon after preparation as possible. Eluates are stable for 7 days if stored at 1-10°C.

D. Failure to render an eluate isotonic or to a neutral pH may cause the RBCs used to test the eluate either to hemolyze or to appear "sticky". Sticky test RBCs, as well as stromal debris, may interfere with the reading of test.

XII. REFERENCES:

A. American Association of Blood Banks Technical Manual, current edition.

B. Immucor / Gamma ELU-KIT II, Manufacturer's Insert, 03/2017

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
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	Kelly Sartor: Supv, Laboratory	8/18/2021

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