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# Eluates

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Document Type: Procedure

# I. PURPOSE AND OBJECTIVE:

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

# 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 INDICATIONS:

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 cord/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 Supervisor/Manager or Medical Director.
6. In rare situations, an eluate may be performed to separate mixtures of antibodies after consultation with the Supervisor/Manager or Medical Director. The red cells are destroyed by the acid and are no longer suitable for testing.

# IV. DEFINITIONS/ACRONYMS:

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.

C. **RPM**: Revolutions per minute.

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# V. POLICY:

## A. Suspected Transfusion Reactions

1. An eluate should be performed whenever a post-reaction DAT reacts more strongly than the pre-reaction DAT. Refer to Transfusion Medicine policy, Transfusion Reaction Investigation and Workup.

## B. Gel Method vs Tube Method

1. The gel method is the standard method for testing eluates at this facility.
2. Eluates should be tested by the alternative tube method only if the eluate was tested by the gel method and:
    a. Non-specific reactions were observed in the gel eluate, and
    b. The patient does not have a warm autoantibody.

## C. Appropriate Test Cells

1. Generally, eluates should be tested against a standard, commercially prepared panel of 0.8% test cells for the gel method and 3.0% test cells for the tube method.
2. The eluate may be tested against a selected cell panel of test cells in the following cases when known antibody specificities have been previously identified.

a. The eluate may be tested against a selected cell panel when a smaller than normal volume 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.

b. 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 $A_1$, B, and O test RBCs.

# VI. SPECIMEN COLLECTION:

A. The preferred sample is a 6 mL EDTA sample or a cord blood specimen with affixed identifying label. Multiple EDTA microtube (heelstick) samples will be acceptable for neonatal patients if the cord blood sample is not available.

B. 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.

C. 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.

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# VII. 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.

   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.

B. Working Wash Solution: Prepared from concentrated wash Solution by diluting 1 in 10 with laboratory reagent-grade water. Once diluted, the working wash solution 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.

C. Additional Reagents Required for Gel Method:

1. 0.8% test cells; refer to the policy Appropriate Test Cells.
2. MTS Anti-IgG Gel cards, stored at room temperature, 18 °C to 25°C

D. Additional Reagents Required for Tube Method:

1. 2% - 4% test cells; refer to the *Appropriate Test Cells* section of this document.
2. Antihuman globulin (AHG) reagent, monospecific Anti-IgG
3. IgG coated check cells.

# VIII. EQUIPMENT/SUPPLIES:

A. Laboratory reagent-grade water for Working Wash Solution
B. Normal saline
C. 10 x 75 mm, 12 x 75 mm or 13 x 100 mm test tubes and caps
D. Table Top Centrifuge
E. MTS worktable
F. MTS incubator
G. MTS centrifuge
H. Calibrated pipette (electronic or manual)
I. Pipette tips
J. Gauze
K. Lighted Viewing Mirror
L. Heat Block Incubator (37°C)
M. Calibrated Timer
N. Automatic Cell Washer

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# IX. QUALITY CONTROL (QC):

A. Last Wash Neutralization Test (Optional)
The optional Last Wash Neutralization Test may be performed to help assess whether the RBCs have been adequately washed. This test is performed immediately after washing, affording the technologist an opportunity to perform additional washing of the RBCs, if necessary, before the eluate is prepared and tested. The last wash supernate (LW) from the RBCs (prior to elution) will be tested by adding antihuman globulin (AHG) and check cells to a sample of the LW. If the RBCs were inadequately washed, then residual protein in the LW would diminish the reactivity of the AHG. By comparing the degree of agglutination of the last wash supernate to the degree of agglutination in a saline control, the adequacy of washing may be assessed.

B. Antibody Screen of the Last Wash Supernate
An antibody screen of the last wash supernate must be tested in advance or parallel with the eluate testing. 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.This antibody screen must be nonreactive.

C. 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) and be non-reactive.

D. Passing QC:

1. Last Wash Neutralization QC: The degree of agglutination in the last wash "LW" tube is greater than or equal to the degree of agglutination in the control "C" tube.
2. 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:

1. Last Wash Neutralization QC: the degree of agglutination in the last wash "LW" tube is less than the degree of agglutination in the control "C" tube.
    a. Perform additional washing to completely remove residual serum antibody/proteins in the last wash supernatant partially neutralized the AHG, leaving less AHG available to react with the IgG coated RBCs.
    b. If degree or reactivity remains weaker than control tube then the antibody coating the RBCs may have 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.
2. 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.
    a. A fresh eluate should be prepared with additional RBCs washing.
        i. 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 is not valid.
        ii. 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.

F. IgG coated control cells must be added to all AHG phase tube results that are negative. If a test result with IgG coated cells is negative, then the test must be repeated.

# X. PROCEDURE:

## A. Eluate Preparation

1. Obtain a *Special Studies worksheet* or the panel sheet for the testing cells or the and fill in the following information:
    a. Patient's identifying information
    b. Elu-Kit-II Lot # and expiration date
    c. Testing date / technologist's initials

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2. 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.
3. Centrifuge the patient's sample and transfer an aliquot of packed RBC to a clean test tube labeled with patients name. The aliquot should yield at least 20 drops of washed, packed RBCs after washing (performed in steps 4 and 5). *Note: The eluate may be prepared from a lesser or greater volume of RBCs, but the resulting volume of eluate will be affected accordingly.*
4. Wash the aliquot of packed RBCs one time with normal saline.
5. 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.**
    a. Last Wash Neutralization Test (Optional)
        i. Label one test tube "LW" for last wash and another test tube "C" for control.
        ii. Add 2 drops of the last wash supernatant to the "LW" tube.
        iii. Add 2 drops of normal saline to the "C" tube.
        iv. Add 1 drop of AHG to each tube.
        v. Add 1 drop of IgG coated check cells to each tube and mix
        vi. Centrifuge both tubes according to calibrated time. Read tubes for agglutination.
        vii. Determine whether the LW neutralization QC has passed or failed.
            A. Passed: The degree of agglutination in the LW tube is greater than or equal to the degree of agglutination in the C tube.
            B. Failed: The degree of agglutination in the LW tube is less than the degree of agglutination in the C tube.
            If the LW neutralization QC has passed, document on the *Special Studies worksheet* and proceed to the eluate preparation procedure.
        viii. If the LW neutralization QC has failed, wash the RBCs at least two (2) additional times with the working wash solution.
        ix. Save an aliquot of the LW supernate for the required QC Antibody Screen of the Last Wash Supernate.
        x. Repeat the procedure above.
        xi. Document the results on the panel antigram or *Special Studies worksheet*.
        xii. If the LW neutralization QC repeatedly fails, despite proper washing technique, refer to the failing QC section of this document.
6. Place 1 mL (20 large drops) of the washed RBCs in a test tube labeled with the patient's name.
7. 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.

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8. Immediately centrifuge the test tube for 45-60 seconds at 3,400 rpm. Prolonged immersion in the Eluting Solution causes hemolysis.
9. Mix well and centrifuge to remove any precipitate or cellular debris, then transfer the supernate eluate to a clean test tube labeled with the patients name. Discard the RBCs; they are no longer suitable for testing.
10. Re-centrifuge the supernate eluate and again transfer the supernatant eluate to a clean test tube labeled with the patient's name. Repeat centrifugation as necessary until the supernate is clear.
11. Add a sufficient volume of Buffering Solution to the separated acid eluate to adjust the pH of the eluate to within the required range for testing (until the eluate turns and remains pale blue).
    a. The volume of Buffering Solution required for this purpose varies with each eluate; refer to the manufacturer's insert for additional information.
    b. The presence of a blue indicator in the Buffering Solution provides a means to determine that the eluate has been properly adjusted.
    c. 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.
    d. The use of pH indicator paper can be used to confirm the required pH range 6.4 – 7.6.
12. Proceed to *Testing of Eluates by the Gel Method* procedure.

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# B. Testing of Eluates by the Gel Method

1. Verify that supernatant is clear, and free of cellular debris. If necessary re-centrifuge supernatant additional 5 minutes and transfer the supernatant to a clean test tube labeled with the patient's name.
2. 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.
3. Label the gel card(s) with the following information:
    a. The patient's last name.
    b. Label each well of the gel card(s) with the identification of the cells against which the eluate will be tested.
    c. Label additional wells for the last wash quality control (screen cells I and II and/or reverse cells).
4. 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.
5. Pipette 50 µL of well-mixed, 0.8% test cells into the correspondingly labeled wells. If necessary, refer to Transfusion Medicine policy, *Making a Test Red Cell Suspension*. Note: Pipette tip should not touch gel card.
6. 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.

7. 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).
8. Incubate the gel cards at 37°C for fifteen to thirty minutes (15 – 30 minutes). Note: Incubation time must not exceed 30 minutes.
9. Centrifuge the gel card at the calibrated time and speed.
10. Read the both front and back of each gel card for agglutination and grade reactions. If necessary, refer to Transfusion Medicine policy, Reading and Grading Test Reactions.
11. Record the graded reactions of the eluate on the panel sheet.
12. Record the graded reactions of the QC (antibody screen of the last wash) on the panel antigram or *Special Studies worksheet*.
13. Interpret the eluate results. Refer to the *Interpretation* section.

# C. Testing of Eluates by the Tube Method

1. 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 test tube labeled with the patient's name.
2. Label a clean test tubes with the following information:
    a. The patient's last name.
    b. Label each tube with the identification of the cells against which the eluate will be tested.
    c. Label additional tubes for the last wash quality control (3% Ortho Surgiscreen and/or reverse cells).
3. Place 1 drop of panel cells, 3% Ortho Surgiscreen, or reverse cells (if indicated) in the correspondingly labeled tubes.
4. Wash the test cells with 5-10 drops of normal saline. Centrifuge for 30 seconds at 3400 RPM, decant the saline, and blot the tubes dry.
5. Add 2 drops of the eluate to the dry cell button in each correspondingly labeled tube. **Do not add LISS**.
6. Add 2 drops of the last wash supernate (saved during the preparation of the eluate) to the dry cell button in each correspondingly labeled tube (3% Ortho Surgiscreen and/or reverse cells).
7. Mix the tubes thoroughly and incubate at 37°C ± 1°C for 15 minutes.
   Note: Incubation may be extended up to 30 minutes, and may enhance reactivity.
8. After incubation, wash all tubes with the Working Wash Solution as follows:
    a. Add 5-10 drops Working Wash Solution to the tubes and mix.
    b. Centrifuge for 30 seconds at 3400 RPM.
    c. Decant the Working Wash Solution completely and blot the tubes dry.
9. Add 2 drops Anti-IgG AHG reagent to each dry cell button.

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10. Mix well and centrifuge for the AHG time calibrated for the centrifuge.
11. Resuspend the cells by shaking gently. Read, grade, and record the graded reactions of the eluate and of the last wash at the AHG phase on the cell antigrams. Do not read microscopically.
12. Add 1 drop of IgG Coated control cells to all AHG phase results that are negative. Agitate tubes to mix and centrifuge according to calibrated time.
13. Gently resuspend the cell button and read, grade, and record the control cell test results on the panel cell antigrams or special studies worksheets.
    a. Control cells must react positive (any strength); otherwise the test must be repeated.
14. Interpret the eluate results.

# XI. INTERPRETATION:

A. Eluates can not be interpreted unless the quality control testing is considered passing. See the *Quality Control* section above.

B. Eluates will be interpreted in the same manner as an antibody panel, as described in Transfusion Medicine policy, Antibody Identification.

C. Most often when a specific alloantibody is identified in the eluate, that same antibody activity is present in the serum. 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.

D. The reactions observed in the gel testing will be interpreted and resulted in one of two ways:
   1. NEG: If the eluate is non-reactive, absence of agglutination when the eluate is tested against appropriate red cells
   2. POS: If the eluate is reactive, exhibits agglutination when tested against appropriate red cells.
      a. If a specificity is identified add a result comment indicating the antibody which was recovered and identified.
      b. If no specificity is identified add a result comment "Eluate is non specific with test RBCs"

# XII. 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.

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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.

E. 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.

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

# XIII. REFERENCES:


A. AABB, *Technical Manual*, current edition.

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


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## Approval Signatures

| Step Description | Approver | Date |
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