Beaumont Laboratory
Royal Oak

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Related Documents: P061, P340, P613, P614, P620

TESTING OF ELUATES BY THE TUBE METHOD (ALTERNATE METHOD)

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Purpose

The purpose of this document is to provide stepwise instructions to test an eluate by the tube method.

Introduction

Once an eluate has been prepared as described in P613, *Eluate Preparation*, it will 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).

Scope

- For a list of situations when eluate studies may be performed, refer to the *Scope* section of P613, *Eluate Preparation*.
- The gel method is the standard method for testing eluates at this facility. Refer to P614, *Testing of Eluates by the Gel Method.*
- Eluates should be tested by the tube method as described in this document only if:
  - The eluate was tested by the gel method, and
  - Non-specific reactions were observed in the gel eluate, and
  - The patient does *not* have a warm autoantibody.

Policies

Appropriate Test Cells

- Generally, eluates tested by the tube method should be tested against a standard, commercially prepared panel of 2% - 4% test cells.
- The elute may be tested against a selected cell panel of 2% - 4% test cells in the following cases:
  - To test for the presence of additional antibodies, when known antibody specificities have been previously identified.
  - When a smaller than normal volume of eluate is prepared (see P613); 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.
  - 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.

Alternate Method

The tube method is the alternate method of testing eluates and shall only be performed as indicated in the *Scope* section of this document.

Definitions

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

Specimen Collection and Handling

The specimen used in this procedure is an eluate, prepared as described in P613, *Eluate Preparation*. The 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.

Reagents

- 2% - 4% test cells; refer to the policy *Appropriate Test Cells.*
- Coombs control cells (IgG coated cells)
- Antihuman globulin (AHG) reagent, monospecific Anti-IgG
- Working Wash Solution: Prepared from concentrated wash Solution by diluting 1 in 10 with laboratory reagent-grade water, as described in P613, *Eluate Preparation*. 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.

Equipment/Supplies

- table top centrifuge
- lighted viewing mirror
- heat block incubator (37°C)
- timer
- automatic cell washer
- 10 x 75 mm test tubes
- disposable pipettes
- gauze

Quality Control

Antibody Screen of the Last Wash Supernate

- An antibody screen of the last wash supernate, obtained during the eluate's preparation (see P613), must be tested in parallel with the eluate. This antibody screen should be nonreactive. 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.
- If the eluate will be tested against group A and B cells, then the last wash must also be tested against group A and B cells (a set of reverse cells).
- Directions to perform this QC are included in Table 615-1, *Testing of Eluates by the Tube Method (Alternate Method).*

Check Cells: IgG coated cells must be added to all AHG phase results that are negative. If a test result with IgG coated cells is negative ~~less than 2+~~, then the test must be repeated.

Forms

F-620, *Special Studies Worksheet*

Procedure

Table 615-1, Testing of Eluates by the Tube Method (Alternate Method)

Step	Action	Notes
1	Proceed from P613, *Eluate Preparation*.	
2	Label 10 x 75 mm test tubes as follows: • Label with the patient's last name. • Label each tube with the identification of the cells against which the eluate will be tested. • Label additional tubes for the last wash quality control (3% Ortho Surgiscreen and/or reverse cells).	Refer to the following: • The *Policies* section / *Appropriate Test Cells* • The *Quality Control* section.
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 3,400 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.**	Eluate was prepared in P613, *Eluate Preparation*.
6	Add 2 drops of the last wash to the dry cell button in each correspondingly labeled tube (3% Ortho Surgiscreen and/or reverse cells).	The last wash supernate should have been saved during preparation of the eluate (See P613).
7	Mix the tubes thoroughly and incubate at 37°C ± 1°C for 15 minutes.	Incubation may be extended up to 30 minutes, and may enhance reactivity.
8	After incubation, wash all tubes as follows: i. Add 5-10 drops Working Wash Solution to the tubes and mix. ii. Centrifuge for 30 seconds at 3,400 rpm. iii. Decant the Working Wash Solution completely and blot the tubes dry.	**CAUTION:** • Do not inadvertently wash with saline in this step. • See the *Reagents* section for directions to prepare the Working Wash Solution.
9	Add 2 drops Anti-IgG AHG reagent to each dry cell button.	
10	Mix well and centrifuge for the AHG time calibrated for the centrifuge.	Refer to P340, *Calibration of Serologic Centrifuges*.
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 F-620, *Special Studies Worksheet*. Do not read microscopically.	Refer to P061, *Reading, Grading and Recording Test Reactions*.

12	Add IgG coated 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. Read, grade, and record the coated cell test results on F-620, *Special Studies Worksheet.*	Coated cells must react positive (any strength) ~~at least 2+~~; otherwise the test must be repeated.
14	Interpret the eluate results.	Refer to the *Interpretation* section.

Limitations

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

Interpretation

Quality Control Interpretation

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.

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 P613, *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 elutes 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.

Eluate Interpretation

Eluates will be interpreted in the same manner as an antibody panel, as described in P620, *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: the eluate is non-reactive, or
2. ENS, 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 a supervisor if necessary.

References

- AABB, *Technical Manual*, current edition.
- Immucor / Gamma ELU-KIT™ II, Manufacturer's Insert, 09/2010.

Authorized Reviewers

Chief, Pathology and Laboratory Medicine
Medical Director and/or Designee, Blood Bank
Manager/Supervisor, Blood Bank

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Document Control

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