1. **Principle**

To remove antibody from coated red cells to allow for testing and identification.

Red cells coated with antibody are thoroughly washed to remove unbound protein, using a special wash solution to maintain the association of bound antibody. The washed cells are then suspended in a low pH solution (Acid Eluting Solution) to dissociate the bound antibody. After centrifugation, the supernatant containing any dissociated antibody is separated from the red cells and buffered by the addition of a buffering solution (Base Buffering Solution). The eluate is then ready to be used in antibody detection or identification.

1. **Scope and Related Policies**
	1. In patients with previously identified clinically significant antibodies, the antibody identification shall be performed to exclude any new antibodies.
	2. All reagents shall be used and controlled according to the manufacturers written instructions.9.1
	3. Elution should be performed as described in NRT.005 – Investigation of a Positive Direct Antiglobulin Test.
	4. Elution may be performed at the following times:
		* To investigate a suspected delayed transfusion reaction.
		* To investigate fetal-maternal incompatibility including ABO.
		* To investigate drug-induced phenomena.
		* To resolve multiple antibody specificities present in a single plasma.
		* As a part of a reference laboratory investigation to detect weakly expressed antigens such as in weak subgroups of A.
		* As part of an investigation for warm autoantibodies
		* As requested by a laboratory physician.
2. **Specimen**

EDTA anticoagulated whole blood (preferably less than 72 hours old)

Note: free red cells from a clotted specimen may be used

1. **Material**

**Equipment:** Cell Washer

 Serological centrifuge

 Block for test tubes

**Supplies:** Test tubes – 10 x 75 mm and 12 x 75 mm

 Serological pipettes

 Stopper

 Container for working wash solution

**Reagents:** Acid elution kit containing:

 Wash solution concentrate

 Acid Eluting solution

 Base Buffering Solution

 Manufacturer’s instructions

 Normal saline

 Distilled water

 Anti-IgG

 IgG-coated cells

 3-5% screening cells and/or panel cells

1. **Quality Control**
	1. A DAT that is positive with complement (anti-C3) and negative with IgG will usually result in a negative eluate and therefore usually is not required.
	2. The last wash is tested with the eluate to ensure that the recovered antibody present in the eluate has been released from a bound state on the original cells and not residual unbound antibody remaining as a result of inadequate wash procedure.
	3. The use of cells from a specimen older than 72 hours may be associated with a hemoglobin-stained eluate, and with accompanying difficulty in adjusting the final pH of the eluate for testing.
	4. Prolonged immersion of cells in the acid eluting solution causes hemolysis. The consequent release of hemoglobin into the eluate

 alters the pH and may affect the volume of base buffering solution required to adjust the pH of the eluate.

* 1. A current manufacturer’s insert should be available for referral.
1. **Procedure**

|  |
| --- |
| * 1. If a DAT has not been performed on the current specimen(s), perform a DAT and determine if cells are coated with IgG before preparing the eluate. See RT.007 – Direct Antiglobulin Test.
 |
| * 1. Obtain the working wash solution and check that the solution is clear (no turbidity) and is in-date.

|  |  |
| --- | --- |
| *If* | *Then* |
| the solution is turbid or expired | prepare a fresh working wash solution following the directions in the manufacturers insert. |

 |
| * 1. Centrifuge the specimen for 5 minutes at 3500 rpm or equivalent.
 |
| * 1. Label 7 – 12 x 75 mm tubes with the patient’s family name and identification number. Transcribe the information from the patient specimen label(not from the request form). These tubes will be used throughout the procedure and referred to as “clean, labeled” tubes.
 |
| * 1. After centrifugation, transfer all plasma into a clean, labeled tube in case it is required for further testing.

Transfer 20 to 25 drops of packed patient red cells from the specimen tube to a clean labeled tube |
| * 1. Wash the packed cells once with saline. The volume of packed red cells should be at least 1.0 mL.
 |
| * 1. Wash the packed cells with the working wash solution an additional four times to remove all unbound antibody.
 |
| * 1. Reserve the “last wash”.
 | * + 1. Label one clean, labeled tube with “last wash” and transfer the last wash into the tube. Centrifuge last wash for 5 minutes at 3400 rpm then transfer to another clean labeled tube. Set aside for parallel testing with eluate.
 |
| * + 1. If plasma antibody is present, or if the DAT was strongly positive (i.e., grade 2 or stronger), the last wash may be tested prior to proceeding with preparation of the eluate (see step 6.10 if time allows). The results should be negative before continuing with preparation of the eluate or the last wash must be set up parallel with the eluate.
 |
| * + 1. Save the tube containing washed cells to prepare the eluate.
 |
| * 1. Prepare the eluate.
 | * + 1. Label one clean, labeled tube with “eluate”.
 |
| * + 1. Pipette 1 mL of patient’s packed washed cells in to this clean, labeled tube (this is usually 20 drops).Note: If there is less specimen available than recommended by the manufacturer, use the maximum amount of packed cells available and adjust the acid eluting solution and base buffering solution accordingly. See procedural Notes 8.7.4, 8.7.5
 |
| * + 1. Add 1 mL (20 drops) or an equivalent amount of Acid Elution Solution as described in the manufacturer’s instructions.
 |
| * + 1. Mix gently by inverting the tube 4-5 times.
 |
| * + 1. Centrifuge immediately for 45 – 60 seconds at 3400 rpm or as recommended by the manufacturer. See Procedural Notes 8.1.
 |
| * + 1. Transfer the supernatant eluate into a clean, labeled tube. Discard the remaining cells.
 |
| * + 1. Add approximately 5 drops of base buffering solution and mix well.
 |
| * + 1. Continue adding the base buffering solution 1 drop at a time, mixing after each drop until a blue color appears and persists upon mixing. See Procedural Notes 8.2 and 8.7.5.
 |
| * + 1. Mix well and centrifuge for 5 minutes at 3400 rpm to remove any precipitate or cellular debris. Transfer the eluate to a clean, labeled tube labeled “eluate”.
 |
| * + 1. Check that the eluate is clear. If cloudiness is noticed, repeat centrifugation. See Procedural Notes 8.3.
 |
| * 1. Test the eluate and last wash (if the last wash was not previously tested) initially with commercial screening cells. If anti-A or anti-B is suspected due to passive infusion of IVIg or plasma products include 3 group A1 or 3 group B cells as applicable according to patient blood group.
 | 6.10.1 Prepare a “dry” red cell button by adding one drop of 3-5% cell suspension to a clean labeled tube. Add 5 - 10 drops of saline. Centrifuge for 60 seconds at 3400 rpm. Completely decant and blot the saline out of the tube to ensure removal of all saline. This is done for all cells that are being tested with the eluate and last wash. |
| 6.10.2 Add two drops of the eluate to the dry cell button. |
| 6.10.3 Add two drops of the last wash to the dry cell button |
| 6.10.4 Mix well and incubate at 37 °C (± 1° C) for 15 minutes |
| 6.10.5 After incubation add 5-10 drops of the working wash solution to each tube. Centrifuge for 60 seconds at 3400 rpm. Completely decant and blot the supernatant wash solution to ensure removal of all residual wash, resulting in a dry red cell button. |
| 6.10.6 Add two drops of anti-IgG, mix gently and centrifuge for 15-20 seconds at 3400 rpm. |
| 6.10.7 Gently resuspend the red cell button and examine for agglutination. Grade and record results. |
| 6.10.8 Negative antiglobulin test results should be appropriately controlled by the addition of IgG sensitized reagent control cells. |
|

|  |  |
| --- | --- |
| *If* | *Then* |
| * 1. The last wash is positive with any of the screening cells
 | The eluate must be repeated, washing the cells 2 additional times or more before preparing the eluate. In this case, it may be desirable to test the last wash before continuing with preparation of the eluate. |
| * 1. The last wash is negative and the eluate is positive with some or all of the screening cells
 | Test the eluate with a panel of cells. See NRT.007 – Antibody Identification for Warm Reactive Antibodies using the eluate instead of plasma. |
| * 1. Both the last wash and the eluate are negative with the screening cells and the last wash is negative with A1 and/or B cells and the eluate is positive with the A1 and/or B cells
 | The cells are coated with ABO antibody(ies) probably due to transfusion of plasma components or derivatives containing anti-A,B, anti-A and/or anti B or with maternal ABO antibody. See 7.0 – Reporting. |
| * 1. The last wash is negative and the eluate is positive with some or all panel cells
 | * + 1. Perform the antibody exclusion. See NRT.008 – Antibody Exclusion. If all cells tested are positive, consider an antibody to a high incidence antigen or a pan-reactive autoantibody or drug induced antibody (refer to Table NRT.010 -1).
		2. If the elution indicates the presence of a clinically significant antibody(ies), select antigen(s) negative donor units for crossmatch.
		3. Report the antibody. See 7.0- Reporting.
 |
| * 1. Both the last wash and eluate are negative with the screening cells and A1 and B cells
 | If applicable, report the results. See Procedural Notes 8.5, 8.7, 8.8 and 7.0 – Reporting. |

 |

1. **Reporting**

The result of the “last wash” must be negative to report a valid elution result. See procedural note 8.6.

* 1. If an antibody is identified in the eluate, report: “Eluted Antibody Anti - …. (name of the antibody).”
	2. If the eluate reacted with all test cells, report “Reactivity with all cells tested no specificity”.
	3. If the eluate is negative against the screening, A and B cells and/or panel cells, report: “Negative: No antibodies demonstrable in the eluate.” See Procedural Notes 8.5, 8.7.
1. **Procedural Notes**
	1. After centrifugation, the cells should be packed at the bottom of the tube. There should not be a line of red cells formed along the side of the tube. If a cell line is seen along the side of the tube, the centrifugation time should be extended until all the cells are packed at the bottom of the tube.
	2. The volume of base buffering solution required for this purpose varies with different eluates, depending on a number of factors, the most prominent being the extent to which hemolysis has occurred during the eluting step. The blue color indicates a pH range of 6.5 to 7.5.
	3. Particles may cause false positive results when the eluate is tested.
	4. The eluate may be tested immediately or refrigerated up to seven days and tested if no turbidity is observed during storage.
	5. When the DAT result is weakly positive, sensitivity of the antibody detection in the eluate may be enhanced by increasing the number of drops of eluate per test (e.g., 3-4 drops). In this case, record the number of drops of eluate used and use the same number of drops when testing the last wash.
	6. Most of the time a positive last wash is due to insufficient washing. The possibility should be considered that a positive “last wash” could be due to the dissociation of bound antibody during the washing. Washing using the working wash solution at 4ºC may minimize this.9.2
	7. The yield of antibody obtained upon elution from coated cells is dependent on the following variable factors:
		1. The amount of antibody bound to the cells.
		2. The degree of dissociation of antibody that occurs during the washing procedure.
		3. Red cells that have a positive DAT due to bound complement alone will usually yield an eluate showing no antibody activity.
		4. Excess dilution of the eluate can occur from using less than 1.0mL of sensitized cells or from the addition of excessive amounts of reagent in adjusting the pH. This may limit the activity of the eluate.
		5. Incorrect adjustment of the pH in the eluate may cause hemolysis of the red cells in subsequent testing.
	8. If the antibody was passively acquired from a blood component or plasma derivative, report: “Positive DAT probably due to a recent transfusion of blood components. This patient may be experiencing a delayed hemolytic transfusion reaction due to passive transfer of anti-\_\_\_\_\_ [*name(s) of the antibody(ies)*].”
2. **References**
	1. Standards for Hospital Transfusion Services Version 3 – February 2011, Canadian Society for Transfusion Medicine, 5.3.1.1.
	2. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 470-480.
	3. Manufacturer Insert. ELU-KIT™.PLUS. Red Cell Elution System. Dominion Biologicals Limited, DBL-26 revised 03/2011.
	4. Judd WJ. Methods in immunohematology. 3rd ed. Bethesda, MD: 2008: 128-131
3. **Revision History**

|  |  |
| --- | --- |
| **Revision Date** | **Summary of Revision** |
| March 1, 2014 | * Revised manual name
* Renumbered and/or revised wording in sections 2.0, 5.0 and 6.0
* Revised wording to include “3-5% screening cells and/or panel cells” in section 4.0
* Section 5.1- revised wording to include “A DAT that is positive with complement (anti-C3) and negative with IgG will usually result in a negative eluate and therefore is not required.”
* Section 6.1- changed RT. 004 to RT. 007
* Changed “label 6” to “label 7” in section 6.4
* In section 6.9.2, revised wording to include “and adjust the acid eluting solution and base buffering solution accordingly.”
* Revised list of references to include latest versions/editions.
 |
| April 7, 2016 | * Added 12 x 75 mm test tubes to section 4.0 *Supplies*
* Changed 10 x 75 mm test tubes to 12 x 75 mm in section 6.4
* Revised wording to include “Transfer 20 to 25 drops of packed patient red cells from the specimen tube to a clean labeled tube” in section 6.5
* Revised wording to include “Centrifuge last wash for 5 minutes at 3400 rpm then transfer to another clean labeled tube. Set aside for parallel testing with eluate” in section 6.8.1
* Revised wording to include “for 5 minutes at 3500 rpm” in section 6.9.9
* Revised wording in section 6.10
 |

Table NRT.010 -1

Various drugs are known to be associated with a positive DAT

|  |  |  |  |
| --- | --- | --- | --- |
| MECHANISM | DRUG | IMMUNOGLOBULIN CLASS | ACTIVITY |
| Drug Adsorption | Penicillins,Cephalosporins | IgG (sometimes C3 also) | React with drug-coated RBCs but not untreated RBCs |
| Immune Complex | Phenacetin, quinidine, third generation cephalosporins antihistamines | C3 (sometimes IgG also) | Serum reacts with RBCs only in the presence of the drug; eluate nonreactive |
| NonimmunologicProteinAdsorption | Cephalothin | IgG + C3 + albumin, etc. | Serum may contain low titre anti-drug antibody; eluate nonreactive |
| Autoimmunity | α-methyldopa(Aldomet), procainamide | IgG (rarely C3 also) | React with normal RBCs in absence of the drug |

References specific for drug induced positive DAT.

1. Reid M, Lomas-Francis C, The Blood Group Antigen Facts Book 2nd Edition, Academic Press, 2004.
2. CSTM Bulletin Vol.4, Sept 1992.
3. Roback JD, ed. AABB, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 519-522.