



△ New York Blood Center Enterprises



**Community
Blood Center**

IRL Experience:
**MLS Rotation in the
Immunochemistry Reference Laboratory**

Student Handbook

innovation • experience • expertise

Required Pre-Rotation Modules

- Print out the handbook for this rotation.
- Access modules at www.nybc.org/educationresources
 - Click on “Laboratory Science Student Resources”
- Complete the following modules and fill out the associated pages in this handbook:
 - **Comparing Blood Bank Methods** (p. 2-5 of this handbook)
 - **ABO Discrepancies** (p. 7 of this handbook)
 - **DATs, Eluates and Rh Nomenclature** (p. 9-14 of this handbook)
 - **Warm Autoantibodies** (p. 15-17 of this handbook)

Learning Objectives

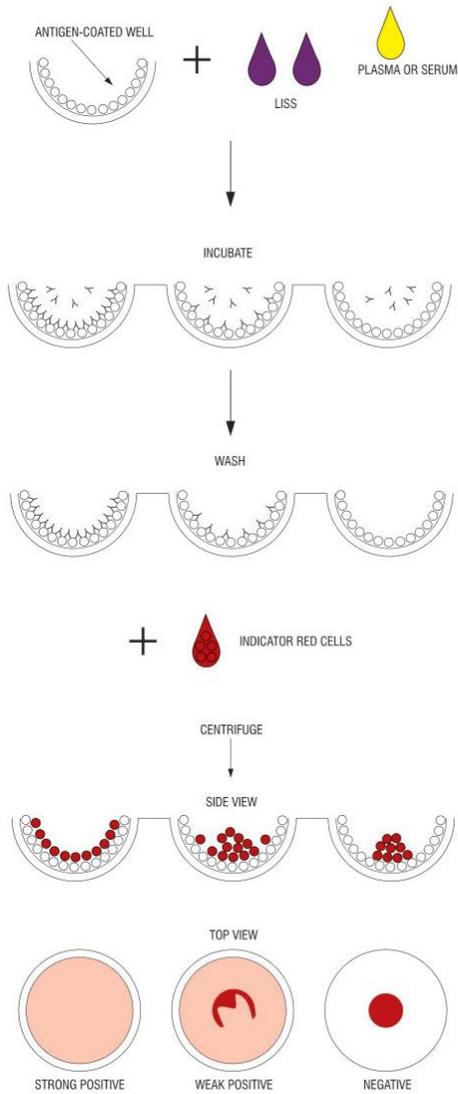
1. Perform antibody testing using the three blood bank methodologies: Tube, gel and solid phase.
 - a. Describe how each methodology works to demonstrate antibodies in patient plasma.
 - b. Compare and contrast gel, solid phase, and tube testing in terms of procedure, interpretation, strengths and limitations.
2. Resolve ABO discrepancies.
 - a. Discuss causes of discrepancies and laboratory methods used to resolve discrepancies of various causes.
3. Perform pre-transfusion testing to rule out alloantibodies in a sample containing warm autoantibody.
 - a. Describe common reactions in a sample containing warm autoantibody.
 - b. Compare and contrast allo- and autoadsorption in terms of criteria for performing, choosing adsorbing cells, treatment of adsorbing cells, and limitations of the procedure
 - c. List transfusion options for patients with warm autoantibody.
 - d. Compare and contrast methods to determine the phenotype of recently transfused patients.

Comparing Blood Bank Methods

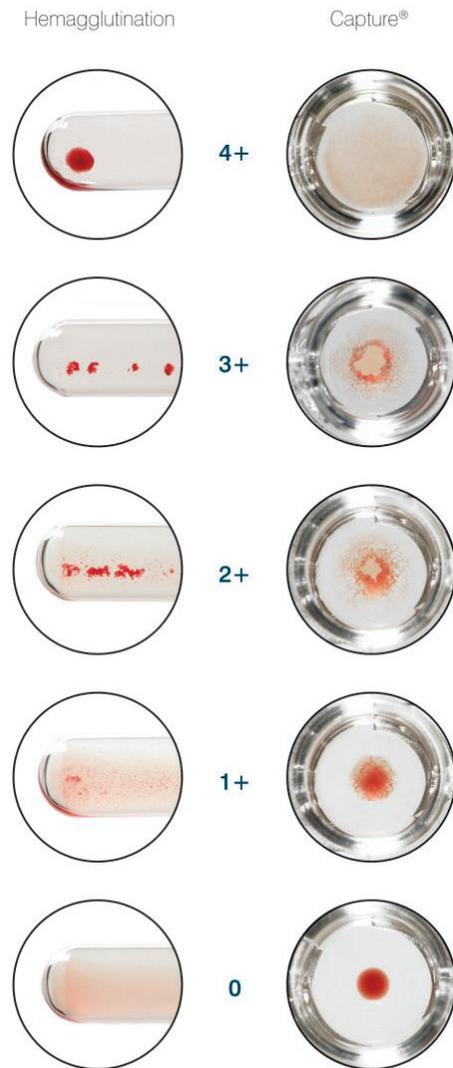
Use the “Comparing Blood Bank Methods” Module to review pages 2-4 and then to complete the chart on p. 5.

Solid Phase Red Cell Adherence Assay (SPRCA)

Test Procedure



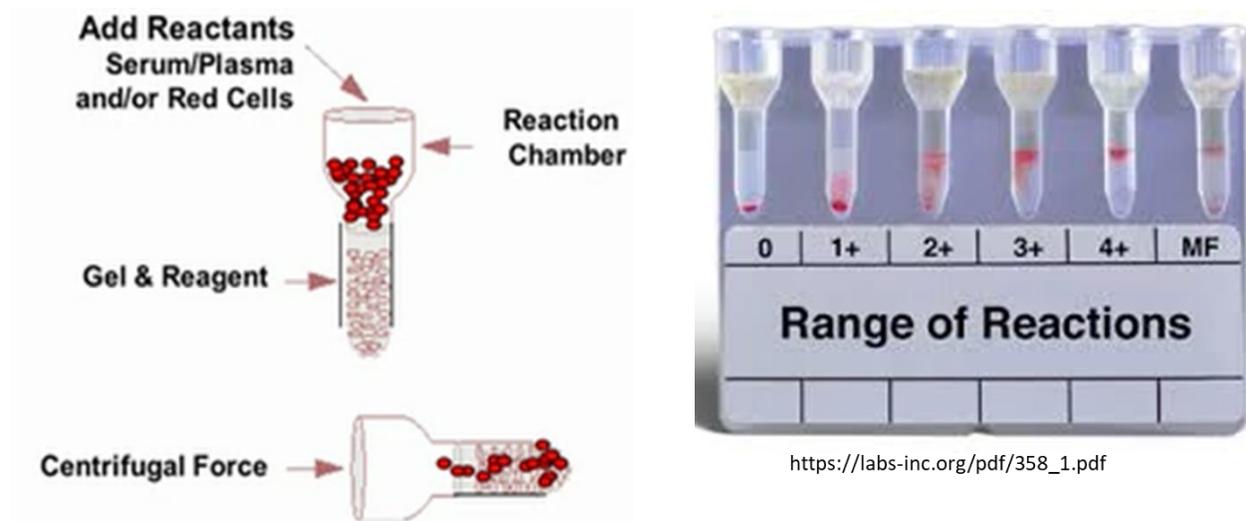
Grading Chart



Solid Phase Red Cell Adherence Assay (SPRCA) continued

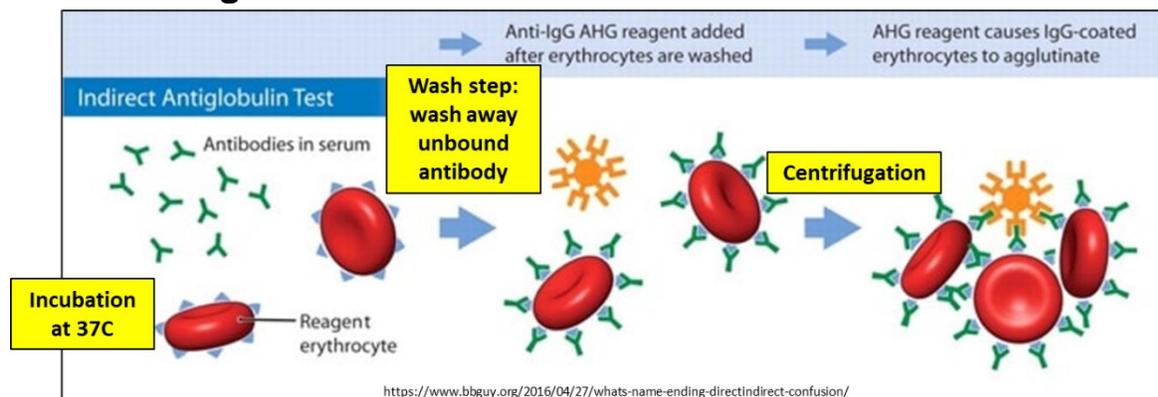
- Antigen coat the well
- Plasma is added to wells, incubated at 37C, antibodies (if present) attach to antigens
- Wash step: unbound antibody is removed
- Indicator cells (RBCs coated with anti-IgG) are added
- Centrifugation
- Interpretation: a tight cell button indicates a negative reaction, effacement of the cell button indicates a positive reaction

Gel (Column Agglutination Testing)

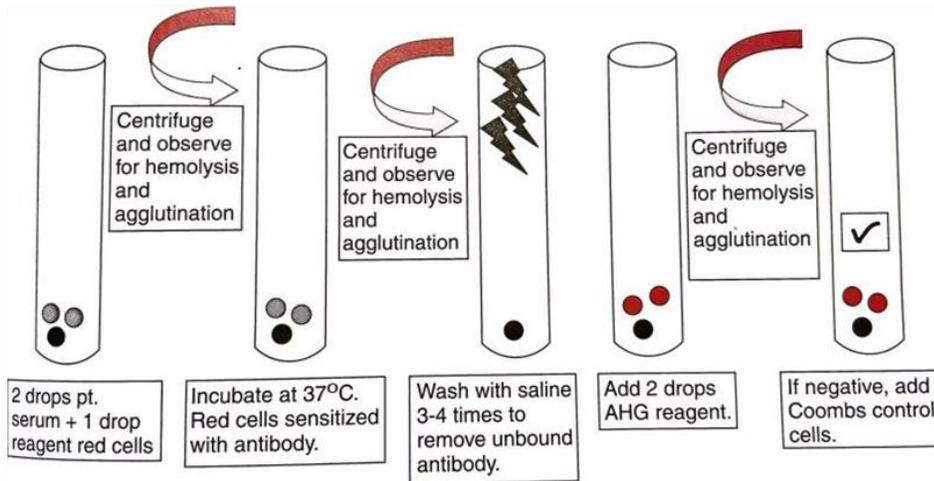


- Plasma and (0.8%) RBC suspension added to reaction chamber
- Incubation at 37C, antibodies (if present) attach to antigens
- Centrifugation pulls RBCs through gel that contains anti-IgG
- RBCs coated with antibody will get stuck in gel; RBCs without coated antibodies fall down to a pellet at the bottom of the column.
- Interpretation: RBCs stuck in the gel indicate a positive reaction; RBCs at the bottom of the column indicate a negative reaction.

Tube Testing



STUDENT HANDBOOK: IRL Experience
Tube Testing continued



Harmening DM. Modern Blood Banking & Transfusion Practices. 5th ed. Philadelphia, PA: F.A. Davis Company; 2005.

- Add plasma and cells to tube; spin and read for immediate spin phase (alternatively, incubate at room temperature before spinning and reading)
- Add enhancement media, if applicable
- Incubate at 37C. Following incubation, spin and read for 37C phase
- Wash
- Add antihuman globulin reagent (anti-IgG)
- Spin and read (may read microscopically)
- To all negative tests, add check cells. Spin and read. Check cells should produce positive result for valid test.

Comparing Blood Bank Methods

Complete this chart prior to arriving at CBC and come ready to discuss.

| Methodology | What does a positive reaction look like? | What does a negative reaction look like? | Tests for IgG, IgM, or both? | Includes incubation at 37C (yes/no)? | Includes centrifugation (yes/no)? | Advantages of this methodology | Disadvantages of this methodology |
|--------------------|---|--|------------------------------|--------------------------------------|-----------------------------------|--------------------------------|-----------------------------------|
| Tube | Antibody coated red cells visibly agglutinate after adding anti-IgG reagent | | | | | | |
| Gel | Antibody coated cells get stuck in gel matrix following centrifugation | | | | | | |
| Solid phase | Indicator cells adhere to the surface of the microwell when antibody is bound to red cell antigens coating the microwell. | | | | | | |

Laboratory Testing: 3 Methods

The table below will be used to record results of testing at CBC.

| | Tube Testing | | | | Gel |
|--------------------|--------------|------|-----|--|-----|
| | IS | LISS | | | IgG |
| | | 37C | IAT | | |
| Screening cell I | | | | | |
| Screening cell II | | | | | |
| Screening cell III | | | | | |

SPRCA testing results will be recorded on a panel provided by the instructor.

ABO Discrepancies

Use the “ABO Discrepancies” Module to complete this page and come to CBC ready to discuss. Use the choices available in the boxes to explain and resolve the following ABO discrepancies. You may use any answer more than once or not at all. Please note: sometimes, there will be more than one possible explanation and/or more than one way to resolve.

Possible Explanations

- Cold Autoantibody
- Recent Transfusion
- Cold reacting alloantibody
- A subgroup with anti-A1
- BMT (group A+ to O+)
- Rouleaux
- Immunosuppression

Ways to Resolve

- Increase 22C incubation time of back type
- Prewarm back type
- Inquire about patient history
- No further testing required
- Saline replacement of back type
- Warm wash front type
- Increase plasma/cell ratio
- Identify alloantibody
- Test with anti-A1 lectin

| | Front Type: Testing patient cells | | | | | Back Type: Testing patient plasma | | | | | Possible Explanation | Ways to Resolve |
|---|-----------------------------------|------------------|----------|--------|----------|-----------------------------------|----------------------|---------|---------|------------|----------------------|-----------------|
| | Anti-A | Anti-B | Anti-A,B | Anti-D | Rh Cont. | A ₁ cells | A ₂ cells | B cells | O cells | Auto Cont. | | |
| 1 | 4+ | 0 | 4+ | 4+ | 0 | 1+ | 0 | 4+ | 0 | 0 | | |
| 2 | 0 | 4+ | 4+ | 4+ | 0 | 4+ | 3+ | 1+w | 1+w | 1+w | • | • |
| 3 | 0 | 0 | 0 | 4+ | 0 | 0 | 0 | 3+ | 0 | 0 | • | • |
| 4 | 4+ | 2+ ^{mf} | 4+ | 4+ | 0 | 0 | 0 | 0 | 0 | 0 | | • |
| 5 | 1+ | 4+ | 4+ | 4+ | 1+ | 4+ | 4+ | 4+ | 4+ | 4+ | | • |
| 6 | 0 | 4+ | 4+ | 4+ | 0 | 4+ | 4+ | 4+ | 4+ | 0 | | |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | • |

DATs, Eluates & Rh Nomenclature

Use the “DAT, Eluates & Rh Nomenclature” Module to complete pages 9-14.

Name reasons an individual might have a positive DAT:

-
-
-
-
-

What is an eluate?

The eluate procedure begins with WASHING the RBCs. What is the purpose of washing?

The “last wash” is tested as a control. What results are expected when testing the last wash? What if there are unexpected results?

| Reactivity of the Eluate | Possible cause of the positive DAT |
|--|------------------------------------|
| Nonreactive | |
| Panreactive, reactive with autocontrol | |
| Contains alloantibody | |

At CBC you will prepare and test an eluate from the sample provided.

Rh Nomenclature

After completing the online module, complete the table below.

| Common Rh Haplotypes | |
|-----------------------------|----------------|
| Fisher-Race | Wiener |
| DcE | |
| DCe | |
| | R ₀ |
| | r |
| dCe | |
| | r'' |

Warm Autoantibodies

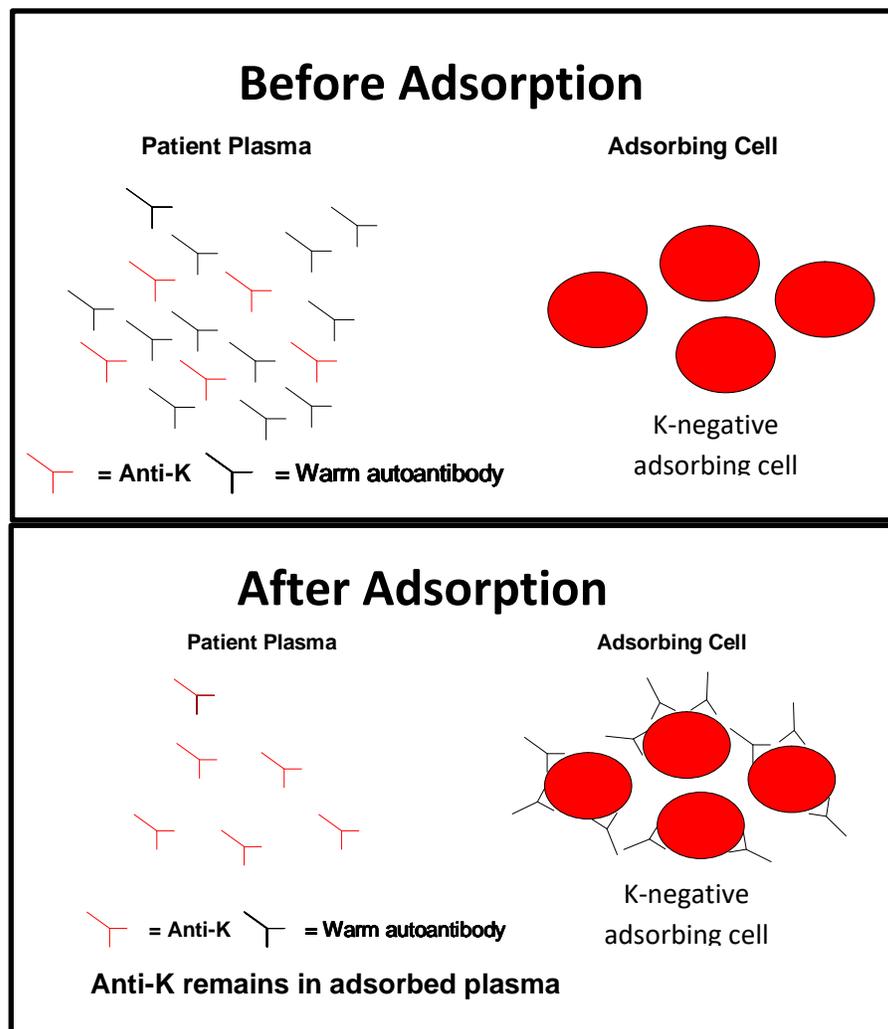
Use the “Warm Autoantibodies” Module to complete pages 15-17.

What are typical reactions for samples containing a strong warm autoantibody?

| Test | | Positive or Negative? |
|-----------------|-----------------|-----------------------|
| Antibody Screen | | |
| Antibody Panel | | |
| Autocontrol | | |
| DAT | Polyspecific | |
| | Anti-IgG | |
| | Anti-complement | |
| | Saline Control | |
| Eluate | | |

****Prepare and test an eluate from the sample provided.**

How are clinically significant alloantibodies to common red cell antigens ruled out in cases of warm autoantibodies that react with all cells tested?



List 2 criteria for performing autoadsorptions

- 1.
- 2.

What is ZZAP? Why do you ZZAP autologous cells prior to adsorption?

When we perform alloadsorptions, we typically use a cell that is phenotypically matched to the patient.

- Describe what we mean by “phenotypically matched.”
- Why do we use phenotypically matched RBCs for adsorbing cells?

What is the biggest risk when doing alloadsorptions?

Why do you ficin-treat adsorbing cells? (Give two reasons)

- 1.
- 2.

Which adsorbing cell would you use for performing alloadsorptions for patients with the following phenotypes? (Adsorbing cells are ficin-treated) **Choose the correct cell(s).**

Adsorbing cells available:

Adsorbing Cell A= R₁R₁, K⁻, Jk(a-)
Adsorbing Cell B= R₁R₁, K⁻, Jk(b-)
Adsorbing Cell C= R₂R₂, K⁻, Jk(a-)
Adsorbing Cell D= R₂R₂, K⁻, Jk(b-)
Adsorbing Cell E= rr, K⁻, Jk(a-)
Adsorbing Cell F= rr, K⁻, Jk(b-)

1. R₁r, K⁺, Fy(a+b-), Jk(a+b-), S+s-

2. rr, K⁻, Fy(a-b-), Jk(a-b+), S-s+

3. R₂R₂, K⁻, Fy(a-b+), Jk(a+b+), S-s+

4. rr, K⁻, Fy(a-b+), Jk(a+b+), S+s+

How do you obtain a phenotype if the patient has been recently transfused?

- **Hypotonic Wash:**
 - **Principle:**
 - **Limitations:**

- **Molecular Phenotype**
 - **Principle:**
 - **Limitations:**

The information below will be covered during your rotation at CBC.

Name 3 options for transfusion of patients with warm autoantibody and no underlying alloantibodies:

- 1.
- 2.
- 3.

Will crossmatches be compatible?

Procedures

Solid Phase Ready Screen Procedure

1. Add two drops of LISS to each well.
2. Add one drop of positive control to the positive control well. Add one drop of negative control to the negative control well.
3. Add one drop of patient plasma to each of the testing wells (the purple color of the LISS will change to blue).
4. Incubate at 37C for 20-60 minutes.
5. Wash wells 4 times.
6. Add 1 drop of indicator cells to each well.
7. Centrifuge. Refer to job aid for centrifugation specifications.

Gel Testing Procedure

1. Visually inspect gel card and verify the presence of gel and liquid layer above the gel. GEL CARD MUST REMAIN UPRIGHT. Label the card appropriately. Remove the foil from only the wells you will be using.
2. Add 50ul of 0.8% RBC suspension to each appropriate well. The RBC suspension should remain in the upper reaction chamber.
3. Add 25ul of patient plasma.
4. Incubate gel card(s) at 37C for 15 minutes.
5. Centrifuge the gel card for 10 minutes. Read, record, and interpret results.

Tube Testing Procedure

Your instructor can determine if you should include a reading at immediate spin phase and 37C (for LISS testing). This procedure is written to include IAT phase only.

1. Label sufficient tubes for each cell tested.
2. Add 2 drops of plasma (or eluate/or alloadsorbed plasma) to each tube.
3. Add one drop of panel cells or autologous cells to appropriate tubes.
4. Add 2 drops of PEG (or LISS), mix, and incubate at 37C for 10 minutes.
5. Wash 4 times. Add 2 drops of anti-IgG.
6. Centrifuge. Read and record results. Check all negative reactions microscopically.
7. Add 1 drop of check cells to all negative tubes.
8. Centrifuge. Read. Make a check mark beside the IAT result if positive.
9. If negative, repeat IAT procedure.

ABO/Rh Typing

1. Label tubes for front type (anti-A, anti-B, anti-D, Rh control- test anti-A,B and anti-A1 only if necessary to resolve discrepancy) and back type (A₁ cells, A₂ cells, B cells, O cells, autocontrol)
2. Add 1 drop of the corresponding antisera into the front type tubes.
3. Add 2 drops of patient plasma to each back type tube and 1 drop of patient cells to each front type tube.
4. Add 1 drop of corresponding reagent red cells into the back type tubes.
5. Centrifuge for 15 seconds and read.

Eluate Procedure (using acid eluate kit)

1. Wash patient cells one time with saline. Wash 4 additional times with Working Wash Solution.
2. Remove the supernatant from the last wash, saving 1 ml from just above the red cells. Place in a tube labeled "last wash."
3. Add 20 drops of Eluting Solution to 20 drops of washed red blood cells. Gently invert the red cells 4 times to mix.
4. Immediately centrifuge for 45-60 seconds.
5. Transfer the eluate to a clean 12X75 tube.
6. Add Buffering Solution until the eluate turns pale blue.
7. Mix and centrifuge eluate to eliminate debris. Transfer to a clean 12X75 labeled tube.
8. Test the last wash against antibody screening cells and the eluate against a panel by PEG IAT. If reactivity is detected in the last wash, prepare a new eluate after washing the cells more times with Working Wash Solution.

Procedures continued

Alloadsorption Procedure

The number of adsorptions needed to remove the autoantibody is equal to the antibody strength (i.e. an autoantibody reacting 2+ is adsorbed 2 times.)

1. Prepare 2 ml aliquots of appropriate ficin-treated adsorbing cells. Wash until the supernatant is clear.
2. On the last wash, centrifuge for 3 minutes. Remove as much remaining saline as possible (use small glass pipet).
3. To the tube of adsorbing cells, add 1 ml of patient plasma.
4. Mix and incubate at 37C for 10 minutes.
5. Centrifuge the tubes for 3 minutes.
6. Carefully remove the plasma and add to the next tube of adsorbing cells.
7. Repeat steps 2-4 as needed.
8. After final adsorption, remove the serum/plasma and place it into labeled tubes.
9. Test the alloadsorbed plasma against a panel by LISS IAT.



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- New York Blood Center, located in New York City, NY
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