TRAINING UPDATE

Lab Location:	WOMC/SGMC	**Date Implemented:**	1/16/23
Department:	BB	**Due Date:**	1/30/23

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Antibody Identification Procedure and Form

Description of change(s):

1. Antibody Identification Form
    a. The form was revised and has a new look.
    b. The accession number should be the T&S accession number only.
    c. If RhIG was given, we have a space to document whether an antibody screen and ID were completed prior to administration (see below for explanation).
    d. The tech completing the workup must document the date and time of completion.
    e. The tech reviewing the workup must document date and time of review completion.

2. Antibody Identification Procedure
    a. When we call to ask if RhIG was administered, we now have to ask if an antibody screen was performed prior to administration. If yes, what were the results (pos, neg, and Ab ID'd)
    WHY?
    There have been a few cases where BB called an anti-D due to RhIG when the mom had actual anti-D. This caused hemolysis and jaundice in the baby. Investigation revealed that some of the clinics are not performing an antibody screen prior to RhIG administration. The neonatologists want to know if this occurs, so they can watch the baby more closely.
    b. 2nd tech review must be completed within 15 minutes if the patient is in the hospital (inpatient, ED, L&D, OR, etc). The 2nd tech review may be held until the next shift for outpatients as long as there is no transfusion pending. Staff must document the time Ab workup was completed and time the 2nd tech review was performed on the AbID form.

Adventist HealthCare

Shady Grove Medical Center
White Oak Medical Center

Antibody Identification Form

Patient Information			
Patient Name		T&S Accession	
Patient MRN		Collection Date	
Historical Antibodies		Historical Antigen Typing	

Hospital Notification	
Name of person notified	
Date and time of notification	
Tech code	

Obtain Patient History	
What is the patient's pregnancy history?	□ Currently pregnant □ Has been pregnant in the past □ Never pregnant □ Pregnancy history unknown/unavailable
Has the patient received RhIG within the past 90 days?	□ Yes date given: □ No □ RhIG history unknown/unavailable Was an antibody screen done prior to RhIG administration? Y N Antibody (-ies) Identified:
Has the patient been transfused?	□ Yes, within the previous 90 days □ Yes, but not within the past 90 days □ Never transfused □ Transfusion history unknown/unavailable
Has the patient been hospitalized within the past 90 days?	□ Yes Hospital Name: Approximate Date: □ No □ Hospitalization history unknown/unavailable

Antibody Review			
Do all antibody forms contain patient name, medical record number, testing date and testing tech identification?	□ Yes	□ No	□ NA
Is the primary panel within expiration date? Do the antigram lot numbers match the screen/panel lot numbers on the Echo printouts?	□ Yes	□ No	□ NA
Were homozygous cells used to rule out all antibody specificities when available?	□ Yes	□ No	□ NA
Are all positive cells accounted for? If no, was PeG screen performed?	□ Yes	□ No	□ NA
For all new antibodies, are there 3 positive cells and 3 negative cells present for each antibody specificity? For historical antibodies, is there 1 positive cell to rule in?	□ Yes	□ No	□ NA
Was a polyspecific DAT performed and documented in the LIS? Were IgG and C3 DATs reflexed if positive? Was eluate performed if IgG DAT positive?	□ Yes	□ No	□ NA
For all new antibodies, was antigen typing performed? Does not apply if patient has been transfused in previous 90 days unless a pre-transfusion specimen is available.	□ Yes	□ No	□ NA
Was all additional testing performed per SOP? (Example PeG screen/panel, crossmatch, titer, etc).	□ Yes	□ No	□ NA
Is this form filled out completely?	□ Yes	□ No	□ NA

Workup Completed Tech Code/Date/Time:		**2nd Tech Review Completed Tech Code/Date/Time:**	

Billing Review			
Number of Panels Billed		Number of Selected Cells Billed	
Billing Review Performed By:		**Date:**	

Non-Technical SOP

Title	**Antibody Identification**
Prepared by	Stephanie Codina Date: 11/10/2017
Owner	Stephanie Codina Date: 11/10/2017

Laboratory Approval		
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		
Local Issue Date:	Local Effective Date:	

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1. PURPOSE

Unexpected antibodies may be observed by a positive antibody screen, incompatible crossmatch, or ABO discrepancy. When an antibody is detected in serum or plasma, the antibody must be identified to determine its clinical significance. Blood group antibodies are not equally dangerous in transfusion and pregnancy. Antibody identification is accomplished by testing serum/plasma against a panel of red cells having different antigen characteristics, observing the presence or absence of hemolysis and agglutination, and comparing the pattern of reactivity with the antigen profile of the cells. Identification of the specificity of a single antibody is usually possible with a panel of ten to twelve reagent red blood cells. If multiple antibodies are present in the sample, the addition of selected cells may be necessary.

2. SCOPE

This procedure applies to any sample that demonstrates a positive antibody screen.

3. RESPONSIBILITY

All blood bank staff members must understand and adhere to this procedure for antibody identification.

4. DEFINITIONS

None

5. SPECIMEN REQUIREMENTS

5.1 Patient Preparation

Component	Special Notations
Labeling	Patient identification must be confirmed and blood bank armband system utilized. Refer to procedure "Sample Specifications for Blood Bank Testing" for details.

5.2 Specimen Type & Handling

Criteria	
Type -Preferred	Plasma (K_3 EDTA, K_2 EDTA), Whole Blood (K_3 EDTA, K_2 EDTA)
-Other Acceptable	None
Collection Container	Lavender top tube, Pink top tube
Volume - Optimum	10ml of whole blood or 5ml of plasma
- Minimum	4 ml of whole blood or 2ml of plasma
Other Considerations	The specimen will need to be labeled with a blood bank labeling system if used for possible transfusion
Transport Container and Temperature	Same as above, at room temperature
Stability & Storage Requirements	Room Temperature: 24 hours
	Refrigerated (1-10°C): 7 days
	Frozen (≤-20°C): 12 months (unacceptable for whole blood)
Timing Considerations	Test as soon as possible following collection
Unacceptable Specimens & Actions to Take	1) Specimens that are not properly labeled. 2) Specimens with any anticoagulant other than EDTA. 3) Whole blood in serum separator tube (SST). 4) Grossly hemolyzed, lipemic, and/or icteric specimens. 5) Frozen whole blood. Reject specimen. Refer to procedure "Sample Specifications for Blood Bank Testing" for details on recollection and documentation.

Criteria				
Compromising Physical Characteristics	**Condition**	**Slight**	**Moderate**	**Marked**
	Hemolysis	OK	Unacceptable	Unacceptable
	Icterus	OK	OK	Unacceptable
	Lipemia	OK	OK	Unacceptable
Other Considerations	None			

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

6. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used.

6.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
Capture R Ready ID, Capture R Ready ID Extend I, Capture R Ready ID Extend II, Capture R Ready Screen 3	Immucor 66214, 6454, 6455, 6456, 6457, 66813 or equivalent
Capture LISS	Immucor 6420 or equivalent
Capture-R Indicator Cells	Immucor 6428 or equivalent
pHix Buffer Solution	Immucor 5070 or equivalent
Isotonic saline, Certified blood bank saline	Fisher 23535435 or 23062125 or equivalent
Reagent red blood cells, 2-4%	Immucor 2381 (Panoscreen), Immucor, 3023 (Panocell 10), 2332 (Panocell 16), 5020 (Panocell 20), or equivalent

6.2 Reagent Preparation and Storage

Reagent	Capture LISS
Container	11.5ml
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Preparation	Ready to use as supplied.

Reagent	Panoscreen, Panocell 10, Panocell 16, or Panocell 20
Container	3ml each vial
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.

Preparation	Resuspend red cells before use by gently inverting each vial several times.

Reagent	Capture R Indicator Cells
Container	11.5ml each
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Preparation	Resuspend red cells before use by gently inverting each vial several times.

Reagent	pHix
Container	200 mL bottle
Storage	18-30°C
Stability	Stable until expiration date on bottle
Preparation	Ready to use. Concentrate is added to saline to create PBS

Reagent	Isotonic Saline
Container	20L or 10L container
Storage	18-30°C
Stability	Stable until expiration date on container until opened. Stable for 30 days once opened and after pHix is added.
Preparation	pHix is added prior to use

Reagent	Capture R Ready ID, Capture R Ready ID Extend I, Capture R Ready ID Extend II, or Capture R Ready Screen 3
Container	Pack Containing a Tray of Strips
Storage	1-30°C
Stability	Stable until expiration date on package as long as humidity indicator is acceptable
Preparation	Ready to use.

7. QUALITY CONTROL

7.1 Controls Used

Controls	Supplier and Catalog Number
Capture-R Positive (Weak) and Negative Control Sera (Manual Capture)	Immucor 66245 or equivalent
corQC (Echo)	Immucor 2400 or equivalent

7.2 Control Preparation and Storage

Refer to the QC control kit insert sheet for preparation, storage and handling instructions.

7.3 Frequency

The positive and negative control sera will be tested on each day of use using the Capture-R RS3 strips.

The positive and negative control sera will be tested against one cell in EVERY BATCH of testing performed using the Capture-R Select strips to help ensure that proper washing and centrifugation have been performed. When a monolayer is prepared in the laboratory, the controls will be tested using the current lot of 2-4% screening cells. The positive control serum will be tested against screen cell 2 and the negative control serum will be tested against screen cell 3.

The Capture-R Ready-ID, Extend I, and Extend II panels contain built-in positive and negative serologic controls to help ensure the indicator cells are not neutralized during testing, the indicator cells act correctly during negative assays and form a button at the bottom of the well, and the wash was adequate. Test specimen is added to the positive control well but not the negative control well. The quality control is documented on the panel antigram. A PI/variance form will be generated if the internal positive and negative controls fail for any reason. The PI/variance form will be reviewed by the Technical Supervisor.

7.4 Tolerance Limits

1. Internal Controls:
    a. The positive control serum must produce a positive result $\geq$ 1+ in strength.
    b. The negative control serum must produce a negative result.
    c. If the internal controls fail for any reason, document the failure on a PI/variance form and provide the completed form to the Technical Supervisor for review.
2. Quality control values must be within acceptance limits before reporting patient results.
3. Reject the run/result(s) if controls exceed acceptable limits.
4. Take action to correct the problems that led to unsatisfactory QC result and document these actions. Problem solving techniques include: reviewing maintenance procedures, checking control material and reagent deterioration, pipetting technique, and verifying equipment performance necessary in order to correct any systemic problem that may exist. If all reagent and instrument checks appear normal, controls and patient specimens must be repeated. Notify a supervisor or designee if controls remain out of range. **Do not report patient results until problems are resolved and controls are acceptable.**
5. If applicable, reanalyze patient results in the failed run or since the last acceptable run to determine whether the patient values are accurate and reliable.
6. All failed runs and/or out of limit controls must be documented.

7.5 Documentation

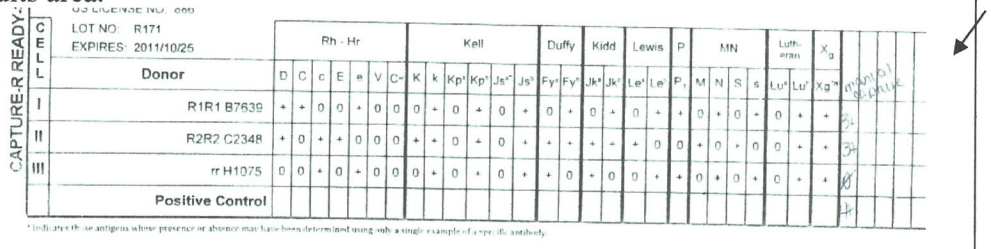
Batch controls performed with Capture-R Select cells are documented on the quality control form and assigned a batch number. The batch number will be assigned using the date of testing and a sequential number to represent the batch identification (MMDDYYYY##). The batch number will be written on the antigram that corresponds to test cell to help ensure that the controls are run with every batch of testing.

8. PROCEDURE

Preparation for Testing

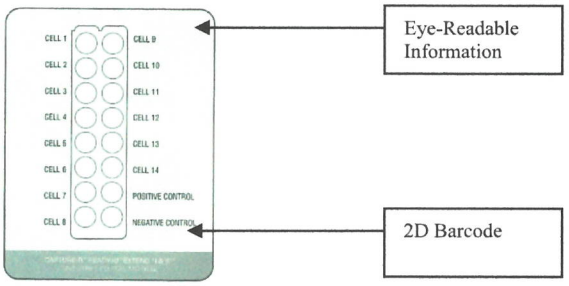
Step	Action
1	Obtain an Antibody Identification Form and complete the top portion by filling in the following information: A. Patient's name B. Patient's medical record number C. T&S specimen accession number D. Date of T&S specimen collection E. Historical antibodies/antigen typing from LIS (see procedure, "Patient History Review.") Note: Indicate if the patient has no historical antigen/antibody data on file by writing "none" or "N/A." F. Document tech code, date, and time to the bottom of the form when the workup is completed.
2	Notify the patient care area that the antibody screen is positive and additional time will be needed to complete the workup if you estimate that it will take >2 hours to provide compatible blood products. A. Offer emergency release blood products if the patient requires red blood cell products immediately. B. Document the notification in the LIS. a. Access the patient in function "Blood Order Processing." b. In the "Add Spec Test" field, press the "Shift" key and press "w" **or** type ";BBCALL" to add a "Called to" field. c. Type 2 semi-colons (;;) and then free-text the name of the person you called and time called. For example, "Notified Nurse Jones of positive antibody screen *date* and *time*." d. Click on the "Save" button.

Step	Action
3	Contact the patient care area and ask nursing staff the following questions. Document the response on the Antibody Identification Form: A. Has the patient been pregnant in the past or is the patient currently pregnant? Note: This question does not have to be asked if the patient is a male or if the patient has a diagnosis of pregnancy in the LIS. a. Check the "Currently Pregnant" box if the patient is currently pregnant. b. Check the "Has Been Pregnant in the Past" box if the patient has previously been pregnant. c. Check the "None" box if the patient has not been pregnant, if pregnancy history is unknown, or if the patient is a male. B. Has the patient received RhIG in the previous 90 days? If yes, was an antibody screen performed prior to RhIG administration? If yes, what were the results of the antibody screen (pos or neg and antibody ID if applicable)? B. Has the patient been transfused at another location? a. If yes, document date and location of last transfusion if available b. If no or unknown, check the "none" box C. Has the patient been hospitalized at another location within the previous 90 days a. If yes, document location b. If no or unknown, check "no" box If the patient has been hospitalized within the previous 90 days **and** the hospital or laboratory is contacted for information, document the phone call and information in the "Hospital Contact/Information" section of the form. **Any historical antibody identified by another laboratory should be entered into the patient's historical file via BAD or BOP and will be honored during transfusion.** A. Enter the antibodies into the blood administrative data file as real antibodies (not a comment). B. Add a comment indicating the hospital at which the antibodies were identified and date of notification.

Step	Action
4	Select an in-date Capture-R Ready-ID, Extend I, or Extend II panel and the corresponding antigram. Ensure the complete lot number on the panel matches the complete lot number on the panel antigram. Note: The Extend I and Extend II panels often have the same numerical lot number but can be differentiated by the letters "DP" for D-positive (Extend I) or "DN" for D-negative (Extend II). A. The initial antibody identification panel should be performed using the same method by which the antibody was detected. Manual capture and Echo may be used interchangeably. B. An entire panel should be run the first time an antibody is worked up. C. Expedited antibody identification panels may be used for subsequent workups and when: a. We have documented evidence that the patient received RhIG within the previous 3 months. b. Screen cells suggest passive anti-D due to RhIG. c. Note: The procedure outlined in the "Rule-Out" section of this procedure must be followed. D. Rule-out panels may be used when the patient has historical antibodies and has not been transfused within the previous 90 days. a. At least 1 rule-in cell must be tested for each antibody specificity present. i. The cell must be positive for the antigen that corresponds to the antibody being ruled-in and negative for all other antigens that correspond to the patient's other antibodies. ii. This may not be possible for patients with multiple antibodies. Document on the antigram if rule-in cells are not available. b. The procedure outlined in the "Rule-Out" section of this procedure must be followed.
5	Place a patient label on the antigram form or handwrite the following on the form. A. Patient name B. Patient medical record number C. Date of testing D. Tech performing testing The above information must be complete if an aliquot label is used.
6	Document the method used for testing (manual capture or Echo) in the test results area.  CAPTURE-R READY-ID LOT NO: R171 EXPIRES: 2011/10/25 Rh - Hr | Kell | Duffy | Kidd | Lewis | P | MN | Luth-eran | X_g Donor: D C c E e V C- K k Kp^a Kp^b Js^a Js^b Fy^a Fy^b Jk^a Jk^b Le^a Le^b P$_1$ M N S s Lu^a Lu^b Xg^a I R1R1 B7639: + + 0 0 + 0 0 0 + 0 + 0 + 0 + 0 + 0 + 0 + + 0 + 0 + 0 + + 32 II R2R2 C2348: + 0 + + 0 0 0 + + 0 + 0 + + + + + 0 0 + 0 + 0 0 + + 34 III rr H1075: 0 0 + 0 + 0 0 0 + 0 + 0 + + 0 + 0 + 0 + + 0 + 0 + 0 + + 18 Positive Control: 4 manual echo * Indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.

Step	Action
7	Perform a DAT on the test specimen per procedure, "Direct Antiglobulin Test (DAT)."

Testing a Full Panel by Manual Capture

Step	Action
1	Bring the Capture reagents and controls to room temperature (18-30°C).
2	Confirm specimen acceptability and specimen labeling requirements per procedure, "Sample Specifications for Blood Bank Testing."
3	If not already performed, centrifuge the whole blood specimen for 5-10 minutes at 3000-3600 rpm.
4	Remove the required number of Capture-R Ready ID strips from the protective pouch and place in the frame holder. Return all unused strips, dessicant, and humidity indicator to the pouch and reseal. The strip wells are sealed in a foil pouch with a desiccant and humidity indicator. The strip wells should **not** be used if the humidity indicator shows the presence of moisture by turning from blue to pink. The humidity indicator is acceptable if the color of the circle is as blue or bluer than the rectangle. The humidity indicator is UNacceptable if the color of the circle is lighter blue than the rectangle or pink in color. 
5	Check the top of the strip. Do not use the strip if it is not imprinted to show both the test identification (RID) and the lot number. 

Step	Action
6	Place the strip in a frame holder so that the numbers are readable. The 2D barcode will be on the side closest to you.
7	Label the tab of each set of strips with the patient identifiers. At a minimum, this will be the patient's first and last initial or the first 3 letters of the patient's last name. Additional identifiers will be used if needed to differentiate between patient samples.
8	Add 2 drops (100 ± 10 µL) of Capture LISS to each test well. The LISS will be purple when added to an empty test well.
9	Add 1 drop (50 ± 5 µL) of patient plasma to each well. **Do NOT** add patient plasma to the negative control (well 16) of the Capture-R Ready ID, Extend I, or Extend II strip. The LISS will turn blue in the presence of plasma protein. Retention of a purple color may indicate the test plasma or control was omitted from the well.
10	Tap the plate gently to mix and dislodge any bubbles. A. If bubbles remain, try to "pop" them by further tapping. B. If this is not successful, carefully use a wooden stick to pop the bubble. Be sure not to touch or disturb the monolayer.
11	Incubate the strips in the Immucor Incubator P2 at 37 ± 1°C for 20 – 60 minutes.
12	Wash the strips following incubation per one of the following procedures, A. Immucor CSW 100 Capture Strip Well Washer (preferred) B. Manual Wash Techniques (optional)
13	Add 1 drop (50 ± 5 µL) of well-mixed Capture-R Indicator Cells to each of the wells. A. Dispense this reagent by using the dropper at a 45° angle. B. Avoid touching the tip of the dropper. Contamination can neutralize the AHG component.
14	Immediately centrifuge the strips for 2 minutes at 530 rcf.

Step	Action
15	Place the strip on an illuminated surface and examine for the presence or absence of Indicator Red Cell adherence. Grade reactions and record results directly onto the panel antigram form. A. Record the results of the built-in positive and negative process controls on the antigram. a. Document the positive control in the "PC" row. b. Document the negative control in the "NC" row. c. The positive control must be positive (≥ 1+ in strength) and the negative control must be negative or the results of all tests in the batch are invalid. d. If either internal control fails, document the failure on a PI/variance form and provide the completed form to the Technical Supervisor for review. B. Wells can be saved and reread manually for up to 48 hours following testing. a. Cover the wells to prevent evaporation. b. Store the wells in the refrigerator at 1-10°C.
16	Proceed to the "Rule-Out" section of this procedure.

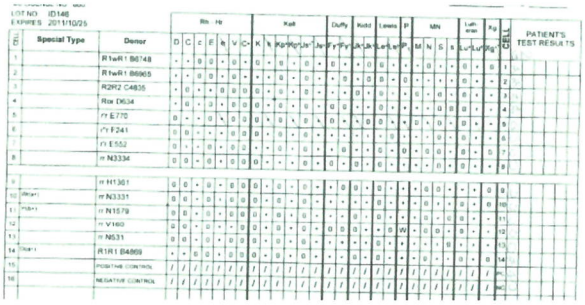
Preparing and Testing Selected Cells in Manual Capture

Step	Action
1	Selected cells can be prepared from reagent red cells using the Capture-R Select strips.
2	Select the cell(s) to be tested using the antigrams. Choose cells that have the antigenic make-up to rule-in or rule-out antibody specificity.
3	Bring all reagents and cells to room temperature (18-30°C). Include 3% screening cells II and III for the positive and negative control material.
4	Remove the required number of Capture-R Select strips from the protective pouch and place in the frame holder. Be sure to include enough wells to perform a positive and negative control. Return all unused strips, dessicant, and humidity indicator to the pouch and reseal. A. The strip wells are sealed in a foil pouch with a desiccant and humidity indicator. The strip wells should **not** be used if the humidity indicator shows the presence of moisture by turning from blue to pink. B. The humidity indicator is acceptable if the color of the circle is as blue or bluer than the rectangle. C. The humidity indicator is UNacceptable if the color of the circle is lighter blue than the rectangle or pink in color.
5	Check the top of the strip. Do not use the strip if it is not imprinted to show both the test identification (SC) and the lot number.

Step	Action
6	Label the strip with the patient and test cell identifiers. A. **Do NOT** test more than one patient's plasma in a single strip (8 wells) to minimize the potential for error. B. Label the tab of the strip with the patient identifiers. At a minimum, this will be the patient's first and last initials or the first 3 letters of the patient's last name. Additional identifiers will be used if needed to differentiate between patients. C. Label the side of each well with the cell identifier. D. Label one well for the positive control "POS" and one for the negative control "NEG."
7	Add 2 drops (100 ± 10 µL) of PBS to each well.
8	Add 1 drop (50 ± 5 µL) of the reagent red blood cell (2-4%) to the corresponding labeled well. A. Add 1 drop of screening cell II to the well labeled "POS" (positive control). B. Add 1 drop of screening cell III to the well labeled "NEG" (negative control). C. DO NOT use red cells that are hemolyzed; red cell fragments will interfere with preparation of the monolayer.
9	Agitate the plates to mix the reagent red blood cells into the PBS to form a suspension.
10	Centrifuge the strips for 5 minutes at 450g.
11	Examine the wells for a red blood cell button following centrifugation. A. Discard the strip if the red cell button is not present. B. Lack of a red cell button may indicate an error with sample addition.
12	Vigorously agitate the plates to remove unattached red blood cells.
13	Wash the strips following agitation per one of the following procedures, A. Immucor CSW 100 Capture Strip Well Washer (preferred) B. Manual Wash Techniques (optional)
14	Examine the monolayer for holes. A. Holes in the monolayer indicate that not enough red cells were available to prepare a proper monolayer, the red blood cells were hemolyzed, or the wash was performed improperly. B. Discard the strips if the monolayer contains holes.
15	Add 2 drops (100 ± 10 µL) of Capture LISS to each test well. The LISS will be purple when added to an empty test well.

Step	Action
16	Add 1 drop (50 ± 5 µL) of patient plasma or Capture control (positive or negative) to each corresponding test well. The LISS will turn blue in the presence of plasma protein. Retention of a purple color may indicate the test plasma or control was omitted from the well.
17	Tap the plate gently to mix and dislodge any bubbles. A. If bubbles remain, try to "pop" them by further tapping. B. If this is not successful, carefully use a wooden stick to pop the bubble. Be sure not to touch or disturb the monolayer.
18	Incubate the strips in the Immucor Incubator P2 at 37 ± 1°C for 20 – 60 minutes.
19	Wash the strips following incubation per one of the following procedures, A. Immucor CSW 100 Capture Strip Well Washer (preferred) B. Manual Wash Techniques (optional)
20	Add 1 drop (50 ± 5 µL) of well-mixed Capture-R Indicator Cells to each of the wells. A. Dispense this reagent by using the dropper at a 45° angle. B. Avoid touching the tip of the dropper. Contamination can neutralize the AHG component.
21	Immediately centrifuge the strips for 2 minutes at 850 rcf. Note: This is different than the centrifugation speed for antibody screen and identification assays.
22	Place the strip on an illuminated surface and examine for the presence or absence of Indicator Red Cell adherence. A. The positive control must be positive (≥ 1+ in strength) and the negative control must be negative or the results of all tests in the batch are invalid. B. Wells can be saved and reread manually for up to 48 hours following testing. a. Cover the wells to prevent evaporation. b. Store the wells in the refrigerator at 1-10°C.
23	Grade reactions and record results immediately. A. Test results are written directly onto the panel antigram that corresponds to the cells antigenic makeup. B. Controls are written on the quality control form. C. The batch identification is written on the panel antigram to link the patient with the batch control.
24	Proceed to the "Rule-Out" section of this procedure.

Rule-Out

Step	Action
1	A rule-out should be performed once the initial antibody identification cells are tested and the corresponding reactions are recorded on the panel sheet. A rule-out consists of crossing off the antigens that did not react with the test sample. A. Only homozygous expressions of each antigen should be ruled out to ensure detection of antibodies showing dosage that may not react with weaker, heterozygous expressions of the antigen. B. The D and P_1 antigens do not demonstrate zygosity. Any cell positive for these antigens may be used for rule-out. C. Kell (K) may be ruled-out using a heterozygous cell. Cellano (k) is a high frequency antigen present on >99% of individuals. Therefore, K+k= cells are rare and cannot be used for routine rule-out. D. C and E may be ruled-out on heterozygous cells in the **presence of anti-D only**. DCe/dCe or dcE/dcE cells are extremely rare and generally not available for testing. E. Low frequency antigens do not normally need to be ruled out. Examples include V, Js^a, Lu^a, Xg^a.
2	Begin at the top of the panel antigram result form. Choose the first non-reactive panel cell. Look at the antigenic makeup of the cell that yielded the non-reactive results and cross off each antigen that demonstrates **homozygous** expression of the antigen by placing a hash mark (/) through the cell reaction **and** the antigen identification at the top of the column. Continue this process until all negative reactions have been checked. 
3	If antibody specificity has not been determined after the panel rule-outs are complete, A. Look for additional rule-out cells using negative reactions obtained in the antibody screen using a screen cell antigram. B. Additional cells may be necessary. Perform additional testing on selected cells by following the testing procedures in the section, "Preparing and Testing Selected Cells in Manual Capture" above. Record results on the panel antigram sheet(s) for the appropriate panel(s). C. Use other techniques as necessary to identify the antibody. Refer to Appendix C.

Step	Action
4	Ensure that all reactions are accounted for once rule-outs are complete and antibody specificities are determined. Additional testing is necessary if extra reactions are noted. A. "Rule-in" each antibody identified for the first time. a. Ensure that you have tested at least 3 cells positive for the antigen corresponding to the suspected antibody. i. All 3 cells must demonstrate positive agglutination with the test serum/plasma to "prove" the antibody is present. ii. Homozygous expressions of the antigen should be tested if the antibody is showing dosage. iii. If more than one antibody is present (current or historical), the rule-in cell must be positive for the antigen that corresponds to the antibody being ruled-in and negative for all other antigens that correspond to the patient's other antibodies. For example, if you suspect a patient has anti-K, anti-Jk^a, and anti-D, you must rule-in the antibodies on 9 cells: 1. 3 cells that are K+, Jk^a=, D= 2. 3 cells that are K=, Jk^a +, D= 3. 3 cells that are K=, Jk^a =, D+ b. Ensure that you have at least 3 cells that are negative for the antigens that correspond to all antibodies identified. All 3 cells must be non-reactive when tested. B. Antigen type the patient for any antigen that corresponds to a newly identified antibody. If an allo-antibody is present, the patient should test negative for the corresponding antigen. Refer to procedure, "Antigen Typing." a. Antigen typing cannot routinely be performed if the patient has been transfused in the previous 90 days. i. Antigen type on the pre-transfusion specimen if available. ii. Document on the antigen typing form and in the LIS that testing was performed on the pre-transfusion specimen. iii. If no pre-transfusion specimen is available, place a comment in the blood bank historical file indicating that antigen typing for a particular antigen should be performed 3 months post transfusion and list transfusion date.
5	Write the name(s) of each antibody identified on the "conclusion" line of the panel antigram sheet.
6	If more than one panel sheet is used, number the sheets in the order of testing (page 1 of x, page 2 of x, etc) to make the workup easier to follow during review. A. The first full panel tested should be labeled as "Page 1 of X." B. The screen antigram sheet should be labeled as "Page 2 of X." C. Echo printouts will follow behind the panel and screen antigrams.
7	Ensure that the identification of each tech assisting with the antibody identification is listed on the antigram sheets.

Step	Action
8	Have a second technologist review results **prior** to resulting the antibody identification and billing in the computer. A. Ask another tech working the same shift to review the antibody workup. If you are working independently **and** the patient **does not** require transfusion, hold the review until change of shift when the next tech comes in. B. If you are working independently **and** the patient **does** require transfusion, fax the workup to another qualified tech at the sister hospital (SGMC or WOMC) and ask the tech working in blood bank to review the workup for you. He/she must sign and return the Antibody Identification Form to you.
9	Perform additional testing if indicated. A. If the antibody identified is clinically significant and the patient is pregnant, contact the physician to see if an antibody titer should be performed. This does not apply if patient is being tested at the time of delivery. B. Crossmatch blood products on any patient who has a clinically-significant antibody. a. Crossmatch a minimum of 2 units on each patient with a clinically-significant antibody. b. If the physician ordered more than 2 units to be crossmatched, crossmatch the number of units written in the physician order.

Antibody Review (Second Tech Review)

Step	Action
1	Second tech review of antibody identification will take place within 15 minutes of antibody completion for patients assigned to ED, OR, L&D, or any inpatient unit as well as any patient awaiting transfusion. Review of antibody workup will be completed by the next shift for all other patients.
2	Ensure that all antibody forms contain the following information: A. Patient name B. Patient medical record number C. Testing date D. Identification of each tech involved in the testing process
3	Ensure that the primary panel used is in-date (has not exceeded its expiration date).
4	If the screen and/or panel was tested on the Echo, verify that the lot number of strips listed on the Echo printout matches the lot number of strips listed on the antigram.

Step	Action
5	Perform a review of the rule-out process by performing the rule-out and using backwards hashmarks (\) through the cell reaction **and** the antigen identification at the top of the column. Each cell that was used during rule-out will have an "x" in it after the initial rule-out and second tech review. Continue this process until all negative reactions have been checked.
6	Once the rule-out verification has been performed, ensure that: A. All clinically significant antibodies have been ruled-out. B. There are no "extra" reactions that have not been accounted for. If extra positive reactions remain, a PeG screen (and panel if applicable) must be performed to ensure no clinically-significant antibodies remain. C. All antibodies have been ruled out using homozygous antigen expressions with the exception of the following: a. Anti-D and anti-P_1 do not demonstrate zygosity. b. Anti-Kell (K) c. Anti-C or anti-E in the presence of anti-D D. There are 3 positive cells for each antibody specificity (each cell should be negative for antigens that correspond to other antibodies the patient has currently or historically) [New antibodies only] E. There is at least 1 positive cell for each antibody specificity that has been previously identified (each cell should be negative for antigens that correspond to other antibodies the patient has currently or historically) [previously identified antibodies only] F. There are 3 negative cells that lack all antigens to the patient's antibodies [All antibodies]
7	Verify that a polyspecific DAT has been performed and is properly documented in the LIS. A. If the polyspecific DAT is positive, monospecific DATs should be performed. B. An eluate should be performed if indicated per procedure.
8	Verify that antigen typing was performed for all newly identified antibodies. A. Historical antibodies that have been previously antigen typed do not need to be repeated. B. Antigen typing is not normally performed on patients who have been transfused within the previous 90 days. a. Ensure antigen typing was performed on a pre-transfusion specimen, if applicable. b. Ensure that a comment was put in the patient's historical file if antigen typing was not performed.
9	Document the answer to all questions on the Antibody Identification Form. Additional testing is needed if the answer to any question is "No."

Step	Action
10	Verify that all required information is completed on the Antibody Identification Form.
11	Document your tech code, date, and time of review on the form.
12	Enter the antibody identification and billing into the LIS system. A. Charge 1 panel charge for EACH panel performed. B. Charge 1 selected cell for EACH selected cell performed.

Billing Entry Review

Step	Action
1	A supervisor or other trained individual will review all antibody identification panels to ensure proper billing. The supervisor or designee will complete this task within 3 working days of testing.
2	Sign and date the "Billing Review" section of the "Antibody Identification Form." Notify the manager if the billing needs to be updated.

9. REPORTING RESULTS AND REPEAT CRITERIA

The pattern of reactions obtained during the rule-out and rule-in processes will help determine which antibodies are present on the cells.

10. LIMITATIONS OF METHOD

A. Erroneous test results can occur from bacterial or chemical contamination of test materials, inadequate incubation periods, improper centrifugation, inadequate washing of test wells, or omission of test reagents or steps.

B. Contamination of indicator red cells with IgG-containing plasma proteins will neutralize the anti-IgG component of the indicator cells leading to false negative results. Failure of the positive control well is an indication of indicator cell neutralization.

C. Overcentrifugation of tests, following addition of indicator cells, may result in falsely negative or doubtful positive reactions due to the collapse of the adherent indicator layer. Undercentrifugation will lead to falsely positive results.

D. Pure IgG4 subclass antibodies may not be detected by the indicator cells. Pure IgG4 antibodies are very uncommon.

E. The deceleration parameters of the centrifuge in use may affect the type of reactions obtained at the end of the assay.

F. Specimens obtained from tubes containing neutral gel separators may produce falsely positive results in antibody screening and identification tests.

G. The reactivity of the Ready-ID, Extend I, and Extend II reagent red blood cells may diminish over the dating period. The rate at which antigen reactivity is lost is partially dependent on the individual donor characteristics that are neither controlled nor predicted by the manufacturer.

H. The addition of excess indicator cells may result in false negative or doubtful test reactions. The addition of too few indicator cells may cause false positive test results. Indicator cells at temperatures ≤18°C will cause weak, false-positive results.
I. No one test method is capable of detecting all antibodies.
J. Capture-R Ready ID, Extend I, and Extend II panels do not possess all known red blood cell determinants. On occasion it is possible that a serum with known antibody that will not react with any cells.
K. The red blood cells used to prepare the reagents can carry antigens that are not defined by the manufacturer. It is possible to obtain positive reactions that do not correspond to the panel antigram.
L. The genetic background of donors with homozygous phenotypes is not known. These red blood cells are assumed to be homozygous but could have been collected from persons who are genetically heterozygous for the encoding genes.
M. Negative reactions will be obtained if the test specimens contain antibodies present in concentrations too low to be detected by the test method employed.
N. Reactions may be weakened if the saline is too acidic. Saline with a pH between 6.5 - 7.5 should be used.

11. RELATED DOCUMENTS

Form: Capture-R Select Batch Control Form (AG.F138)
Form: Antibody Identification Form (AG.F52)
SOP: Sample Specifications for Blood Bank Testing
SOP: Reagent Quality Control
SOP: Patient History Review
SOP: Direct Antiglobulin Test (DAT)
SOP: Immucor CSW 100 Capture Strip Well Washer (preferred)
SOP: Manual Wash Techniques (optional)
SOP: Antigen Typing
SOP: Crossmatch
SOP: Prewarmed Antiglobulin Technique
SOP: Key to Symbol/Abbreviations for Reaction Grading and Interpretations

12. REFERENCES

1. Standards for Blood Banks and Transfusion Services, current edition. AABB Publishing, Bethesda, Maryland.
2. Berte, L.M. 2007. Transfusion service manual of standard operating procedures, training guides, and competency assessment tools, 2nd ed. AABB Publishing, Bethesda, Maryland.
3. Package Insert for Red Blood Cells Panocell, Immucor, Norcross, GA, Insert Code 316, Current Revision.
4. Package Insert for Capture-R Positive and Negative Control Sera, Immucor, Norcross, GA, Insert Code 352, Current Revision.
5. Package Insert for Capture-R Ready-ID, Capture-R Ready-ID Extend I, and Capture-R Ready-ID Extend II, Immucor, Norcross, GA, Insert Code 369, Current Revision.

6. Package Insert for Capture-R Ready Indicator Red Cells, Immucor, Norcross, GA, Insert Code 372, Current Revision.
7. Package Insert for Capture LISS, Immucor, Norcross, GA, Insert Code 363, Current Revision.

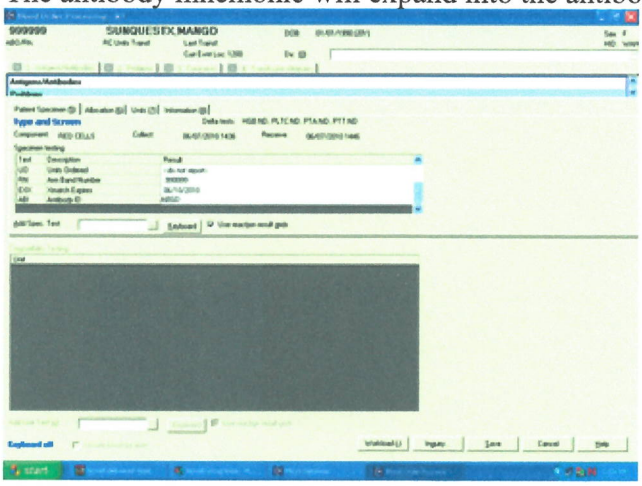
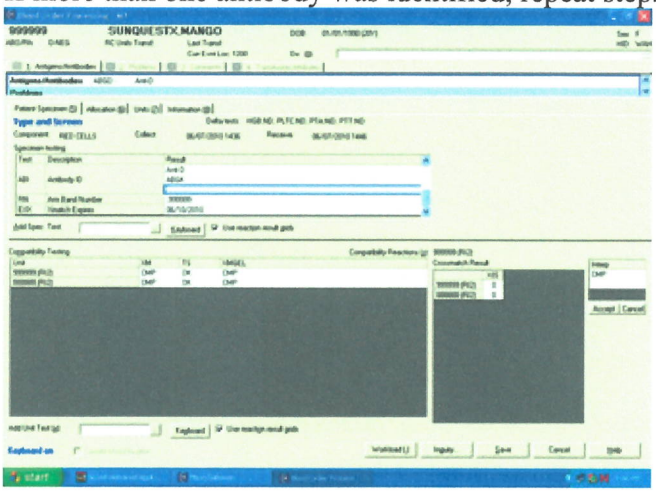
13. REVISION HISTORY

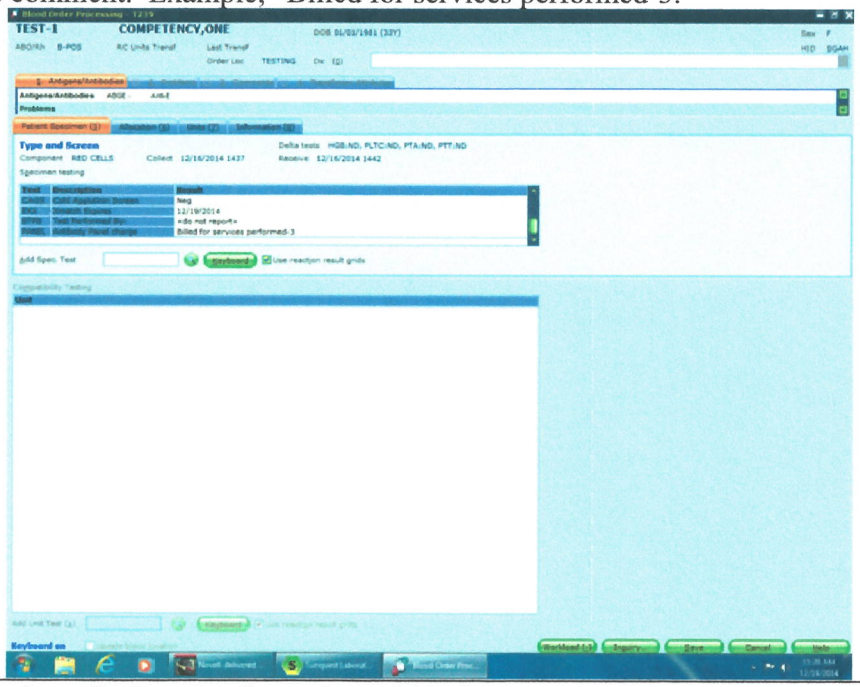
Version	Date	Reason for Revision	Revised By	Approved By
		Supersedes SGAH.BB118.3, WAH.BB110.3		
0	2/11/20	Header: Changed WAH to WOMC Section 12: Updated references App B: Updated LIS antibody code translation table App C: Added specific instructions for documenting RhIG administration	SCodina	Ncacciabeve
1	3/9/22	Removed references to LISS and replaced with PeG for tube testing Footer: Changed prefix to AHC	Scodina	Ncacciabeve
2	1/12/23	Added requirement to ask if RhIG was given and if AbS was performed prior to RhIG administration. Updated procedure to match changes to form. Established TAT for secondary review.	SCodina	NCacciabeve

14. ADDENDA AND APPENDICES

A. Appendix A: LIS Entry and Billing of Antibody Identification
B. Appendix B: LIS Antibody Code Translation Table
C. Appendix C: Guidelines for Antibody Workup

Appendix A
LIS Entry and Billing of Antibody Identification

Step	Action
1	Access the patient in Sunquest function, "Blood Order Processing" and open the T&S test.
2	In the "Add Spec Test" field, add the appropriate tests. A. Type "K" **or** ";ABI" to add the identification panel. B. Type "$" or ";PANEL" to add the panel charge. C. Type ";SCEL" to add the select cell charge.
3	Enter the antibody identification in the "ABI" field. A. Type in a semi-colon (;) B. Type in the LIS antibody code that corresponds to the antibody identified in the patient serum/plasma (refer to appendix B) C. Press the "Tab" key. D. The antibody mnemonic will expand into the antibody name.  E. If more than one antibody was identified, repeat steps A-D. 

Step	Action
4	Bill the number of full panels performed if >1. A. At the "PANEL" prompt, highlight the comment "Billed for services performed." B. Press the "Tab" button once to open a new line. C. Type the semicolon ";" followed by the number of panels to be billed. For example, if 3 panels were run, type ";3." D. Press the "Tab" key until the number to be billed appears on the same line as the comment. Example, "Billed for services performed-3." 
5	Bill the number of selected cells performed if >1. A. At the "SCEL" prompt, highlight the comment "Billed for services performed." B. Press the "Tab" button once to open a new line. C. Type the semicolon ";" followed by the number of panels to be billed. For example, if 3 selected cells were run, type ";3." D. Press the "Tab" key until the number to be billed appears on the same line as the comment. Example, "Billed for services performed-3."
6	Click the "Save" button. **Billing cannot be edited once the save button has been clicked.**

Appendix B
LIS Antibody Code Translation Table

Code	Translation
AA1	Anti-A1
ABG	Anti-Bg
ABGC	Anti-C
ABGD	Anti-D detected
ABGE	Anti-E
ABGG	Anti-G
ABGI	Anti-I
ABGM	Anti-M
ABGN	Anti-N
ABGS	Anti-S
ABGV	Anti-V
ACEL	Anti-cellano
ACHDA	Anti-Chido (a)
ACOB	Anti-Colton (b)
ACW	Anti-Cw
ADOA	Anti-Dombrock (a)
ADRH	Anti-D detected, possibly due to RhIG administration
AFYA	Anti-Fy (a)
AFYB	Anti-Fy (b)
AGOA	Anti-Go (a)
AH	Anti-H
AHE	Anti-Henshaw
AHIA	Antibody to high incidence Ag
AHRB	Anti-hrB
AIH	Anti-IH
AJKA	Anti-Jk (a)
AJKB	Anti-Jk (b)
AJSA	Anti- Js (a)
AJSB	Anti- Js (b)
AKEL	Anti-Kell
AKPA	Anti- Kp (a)
AKPB	Anti- Kp (b)
ALEA	Anti- Le (a)
ALEB	Anti- Le (b)
ALIA	Antibody to low incidence Ag
ALTF	Anti-f
ALTI	Anti-i
ALUA	Anti-Lu (a)
ALUB	Anti-Lu (b)
AP1	Anti- P1
ASAR	No significant antibodies found
ASDA	Anti-Sd (a)
ASMC	Anti-little c
ASME	Anti- little e

Code	Translation
ASMS	Anti-little s
ATJA	Anti- Tj (a)
AU	Anti-U
AWIN	Anti-D due to Win Rho D
AWRA	Anti- Wr (a)
AXGA	Anti-Xg (a)
AYTA	Anti-Yt (a)
AYTB	Anti-Yt (b)
ASME	Anti- little e
CAA	Cold auto antibody
EAA1	Anti-A1 found to be coating cells
EABGC	Anti- C found to be coating cells
EABGD	Anti-D found to be coating cells
EABGE	Anti-E found to be coating cells
EABGG	Anti-G found to be coating cells
EABGS	Anti-S found to be coating cells
EACEL	Anti-Cellano found to be coating cells
EADRH	Anti-D, possibly due to RhIG, found to be coating cells
EAFYA	Anti-Fy (a) found to be coating cells
EAFYB	Anti-Fy (b) found to be coating cells
EAJKA	Anti-Jk (a) found to be coating cells
EAJKB	Anti-Jk (b) found to be coating cells
EAJSA	Anti- Js (a) found to be coating cells
EAJSB	Anti- Js (b) found to be coating cells
EAKEL	Anti-Kell found to be coating cells
EAKPA	Anti- Kp (a) found to be coating cells
EAKPB	Anti- Kp (b) found to be coating cells
EAM	Anti-M found to be coating cells
EAN	Anti-N found to be coating cells
EAP1	Anti-P1 found to be coating cells
EASMC	Anti-c found to be coating cells
EASME	Anti-e found to be coating cells
EASMS	Anti-s found to be coating cells
EAU	Anti-U found to be coating cells
EAWIN	Anti-D due to Win Rho D found to be coating cells
EINCL	No antibody found to be coating cells
ENHAN	Antibody to enhancement media
NAAB	No new antibodies detected
NEL	No antibody detected in eluate
NSC	Non-specific cold antibody
PEL	Panagglutinin found to be coating cells
PLA1	Platelet antibody
WAA	Warm auto antibody

Appendix C
Guidelines for Antibody Workup

SEROLOGIC EVALUATION OF PASSIVELY-ACQUIRED ANTIBODIES

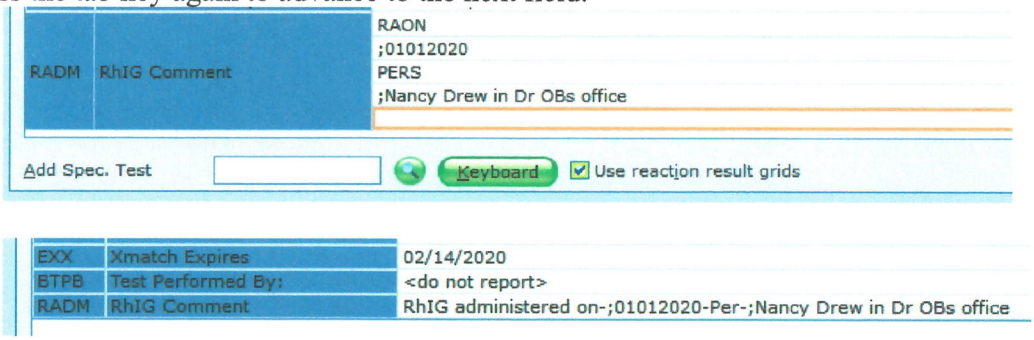
Antibodies can be passively acquired via injection, infusion, or transfusion. Blood bank staff members must be able to determine if an antibody is passive or active based on patient history.

Medications that are known to cause passive transfer of antibodies:

- Rh Immune Globulin (RhIG)- A sterile solution containing IgG anti-D for use in preventing Rh immunization.
    - RhIG demonstrates like anti-D.
    - Rh-negative and weak D positive women generally receive RhIG during and following pregnancy.
    - Rh-negative women and men may receive RhIG following an Rh-positive platelet transfusion or other exposure to Rh-positive red cells.
    - RhIG generally reacts at strengths $\leq$2+ in strength. However, titer should never be used to differentiate active from passive anti-D.
    - RhIG can be differentiated from real D by determining if it has an IgM component; RhIG only contains IgG anti-D. Refer to the reference lab if it is necessary to determine if a patient is making real anti-D.
    - RhIG has a half-life of 30 days and will fall below detectable levels within a few months.
- WinRho- A form of RhIG used to treat immune thrombocytopenia (ITP). WinRho binds to D antigen sites in Rh-positive individuals and mimics an autoantibody with D specificity. WinRho should be suspected when an Rh-positive individual demonstrates the appearance of an autoantibody with D specificity **especially** when the individual has a low platelet count or diagnosis of ITP.
    - WinRho is seen in Rh-positive patients with a diagnosis of ITP or thrombocytopenia.
    - Serologically, WinRho generally appears with the following results:
        - Rh positive
        - DAT positive
        - Antibody in plasma anti-D (due to WinRho)
        - Antibody in eluate anti-D (due to WinRho)
        - Other antibodies such as anti-A, -B, -C, and –E may also be seen in patients who have received RhIG.
- Immune Globulin- Concentration of plasma immunoglobulins used to treat congenital immunodeficiencies or viral exposures or to provide prophylaxis for certain viral exposures. Immune globulin comes in different forms including IVIG, anti-lymphocyte globulin, and anti-thymocyte globulin.
    - IVIG is routinely given to patients with the following diagnoses:
        - Primary or secondary immune deficiencies
        - Immune cytopenias
        - Presumed immune disorder
        - Other immunologic conditions
    - IVIG can (rarely) convey sufficient antibodies to cause a positive DAT.

When a passively-acquired antibody is suspected:

1. Obtain the patient's medication history. This can be done by calling the patient care area or accessing the information in the patient's electronic medical record. Document on the antibody identification form.
2. Enter the correct antibody code into the LIS.
    a. ADRH is anti-D detected, possibly due to RhIG administration
    b. EADRH is anti-D, possible due to RhIG, found to be coating cells
    c. AWIN is anti-D due to WinRho D
    d. EAWIN is anti-D due to Win Rho D found to be coating cells
    e. AIVIG is anti-D due to IVIG
    f. Other passive antibodies must be typed freetext into the LIS
3. When a passive antibody is detected, a comment must be added.
    a. Freetext the comment for IVIG administration.
    b. Follow this procedure to enter the comment for RhIG or WinRho D administration. The comment must be entered in this fashion or it will not cross to Cerner.
    c. In the "Add Spec Test" box, type a lowercase "r" for the "RADM" field to open.
    d. In the RADM box, type a lowercase "s" to add the "RAON" comment.
    e. Press the tab key to move to the next line. Press the semi-colon twice to open the field for a freetext comment. Then, enter the date on which the patient received RhIG or WinRho D.
    f. Press the tab key to move to the next line. Then, type a lowercase "p" for the "PERS" field to open.
    g. Press the tab key to move to the next line. Press the semi-colon twice to open the field for a freetext comment. Then, enter the person who provide the date of RhIG/WinRho D administration. It is acceptable to type "Cerner" in this box if the information was obtained from the electronic medical record.
    h. Press the tab key again to advance to the next field.



4. Crossmatch per crossmatch procedure.

WARM AUTOANTIBODIES WITH BROAD UNDETERMINED SPECIFICITY

Warm autoantibodies with broad undetermined specificity present special problems for antibody identification and blood transfusion. These antibodies often agglutinate all red blood cells with which they are tested, interfering with both pre-transfusion testing and crossmatching. This type of antibody should be suspected when all cells are positive on the antibody screen, antibody panel, and eluate (if tested).

Initial Workup:

1. Initial panel positive with all or most cells tested
2. DAT positive (Do NOT perform eluate if sending the sample to ARC and patient has been transfused; ARC will perform in this situation)
3. Send to ARC for workup and phenotyping (if blood needed)
4. Notify the patient care area or caregiver that there will be an extended time delay
5. Notify the pathologist if blood is needed immediately
6. Crossmatch incompatible or least-incompatible units pre-screened for patient by ARC. A physician must sign for incompatible/least-incompatible units using the emergency release form.

Subsequent Workups:

1. Initial panel positive
2. Perform a screen/panel with PeG
    a. Complete workup if no reactivity or identifiable reactivity exists
    b. Send to ARC if undetermined reactivity persists
3. DAT workup
4. Eluate if necessary per criteria
5. Crossmatch least incompatible blood products.

COLD ANTIBODIES/AUTOANTIBODIES

Cold antibodies are those that optimally react at temperatures between 4 and 25°C and can be auto-or allo- in nature. These antibodies rarely cause destruction of transfused red cells because body temperature is closer to 37°C. However, the antibodies cause problems with testing, because they interfere with ABO typing and can mask the reactions of clinically significant antibodies. A cold antibody should be suspected when weak reactivity is seen on a large number of cells but all clinically significant antibodies can be ruled out. Examples of cold antibodies include:

Anti - A1	Anti - M
Anti - P1	Anti - N
Anti - Le(a)	Anti - I
Anti - Le(b)	Anti - H; IH

Workup:

1. Initial panel and additional cells if necessary rule out all clinically significant antibodies
2. DAT and workup if necessary per criteria
3. Perform an immediate spin antibody screen to see if a pattern results. Perform an antibody panel at immediate spin if applicable.
4. Perform PeG testing to rule-out all clinically significant antibodies.
5. Give AHG XM compatible blood products.
    a. If an IgG crossmatch is compatible on the Echo or in Manual Capture but the immediate spin crossmatch is positive due to a strong cold antibody, carry the immediate spin crossmatch through the AHG phase using PeG enhancement.

   b. The PeG AHG procedure will rule out ABO incompatibility while manual capture and Echo will not.
6. Refer to procedure, "ABO Discrepancies" if applicable.
7. Refer to procedure, "Prewarmed Antiglobulin Technique" if applicable.

SUSPECTED SEROLOGIC TRANSFUSION REACTION

Serologic transfusion reactions occur when a patient becomes alloimmunized to an antigen present on the transfused cells. Serologic transfusion reactions should be suspected when a patient who has been transfused in the previous 3 months presents with an identifiable antibody in the eluate. The antibody may or may not be present in the serum/plasma. The blood bank is often the first service to identify this type of transfusion reaction.

Workup:

1. Initial panel and additional cells if necessary for identification of antibody
2. DAT workup
3. Eluate workup and panel
4. Pull segments from transfused donor cells (if available) and antigen type
5. Antigen type patient's pre-transfusion sample if available (If pre-transfusion sample is not available, place a note in the patient history indicating antigen typing must be performed 3 months post-transfusion)
6. Perform a transfusion reaction investigation using the current sample and pre-transfusion sample (if available). Leave the area for vital signs blank and indicate the transfusion reaction was identified by blood bank personnel.
7. Consult a pathologist. The pathologist will notify the treating physician and help manage patient symptoms if necessary.

ROULEAUX

Rouleaux is caused by unusual properties in a patient's serum that can aggregate red cells and mimic agglutination. This aggregation is not due to antibodies but to a change in the surface charge on the red cell. Rouleaux can occur as a result of abnormal concentrations of serum proteins in disease states such as multiple myeloma, Waldenstrom's macroblogulinemia, cirrhosis, and hyperviscosity syndrome or as a result of intravenous injections such as high molecular weight dextran, polyvinylpyrrolidone (PVP), hydroxethylstarch (HES), or fibrinogen. Rouleaux is characterized by refractile, shiny clumps that often resemble a "stack of coins" microscopically in tube testing. Rouleaux can interfere with any test combining red cells with patient serum/plasma in any test that does not contain a wash phase.

Workup:

1. Rouleux is generally not seen in capture assays or after the wash phase in manual tube methodology.
2. Rouleaux in tube can be confirmed microscopically and by using the saline addition/replacement technique.

POSITIVE ANTIBODY SCREEN WITH NEGATIVE ANTIBODY PANEL

Occasionally, a patient will have a positive antibody screen and a negative antibody panel. This is most often the result of an antibody directed towards a low-frequency antigen but can be the result of testing error.

Workup:

Look up the extended cell typing for the positive cell to determine if the cell contains a previously identified low-frequency antigen.

1. If the cell does have an identifiable antigen site, test 2 additional cells containing the antigen to rule-in the antibody
2. If the cell does not have an identifiable antigen site, have a different tech repeat the screen (automation is considered one tech)
    a. If the results repeat positive, treat this antibody as an antibody to a low-incidence antigen and give AHG XM compatible red blood cells
    b. If the results are negative, the antibody screen can be reported as negative, but the antibody screen should be repeated for all samples tested in the same batch as the initial positive screen when manual testing is performed. This will ensure that the specimens were not switched and another sample was positive.

POSITIVE ANTIBODY WITH NO DISCERNIBLE SPECIFICITY

Occasionally an antibody will be encountered with no discernible specificity. This can occur for many different reasons. Patients who have received human-derived solutions such as RhIG, anti-lymphocyte globulin, anti-thymocyte globulin, and such can have antibody carryover from the donor. In addition, patient's who are recently sensitized can have blood group antibodies that have not yet declared specificity. In generally, antibodies will no discernable specificity will be positive on a few cells but all clinically significant antibodies will be ruled out.

Workup:

1. Rule out all clinically significant antibodies; the remaining reactivity must have no discernable pattern
2. Perform PeG screen and panel as indicated to ensure no pattern emerges
3. Report antibody as "ASAR."
4. An eluate workup may be necessary if the patient has been recently transfused; consult a supervisor or pathologist
5. Perform AHG crossmatch on all units considered for transfusion and only issue units that are completely compatible.

CAPTURE ANTIBODIES

It is common to detect antibodies using Capture methodology (including testing on the Galileo Echo) that will not react by other methods. This is due to crypt antigens on the donor red cells that are exposed during the drying process.

Workup:

1. Perform the initial antibody panel using the same methodology as antibody screen testing.

2. If all cells tested are positive at similar strengths, run the antibody screen using a different methodology. **Workup the antibody using routine methods if the positive results are reacting at different strengths or if you have negative reactions mixed in with positive reactions.**
    a. If the alternate methodology yields a negative screen, the antibody is generally considered insignificant and due to the Capture reagents.
    b. If the alternate methodology demonstrates positive results, work-up the antibody using the alternate method and refer to a reference lab per routine procedure.