

Kaiser Permanente Medical Center, San Francisco Northern California Region

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Work Instruction			
Title:	TS-Antibody Identification		WI Number SFOWI-0088 Revision: 17
Area:	ment: ohematology eary Blvd SFO Hospital Lab	Document is in the Final Approval Process. 2 - approvals are required	
Type of Document: Work Instruction		Revio	ew Period - 340 Days

#### **PURPOSE**

When clinically significant antibodies are detected, additional testing shall be performed.

- A. Identifying the specificity of significant antibodies makes it possible to test donor blood for the absence of the corresponding antigens(s).
- B. Weakly reactive antibodies may fail to react when donor cells are tested with the prospective recipient's serum, whereas tests with potent reagent antisera may demonstrate the antigen to be present.
- C. In prenatal testing, knowing the specificity and immunoglobulin class of an antibody helps predict the likelihood of Hemolytic Disease of the Newborn (HDN).
- D. The plasma/serum under investigation is tested against a panel of eight or more group O red cells of known antigen composition including an autocontrol.
- E. Commercially prepared panels come with a worksheet that shows the phenotype of each cell.
- F. In patients with previously identified clinically significant antibodies, methods of testing shall be those that identify additional clinically significant antibodies.

#### **REAGENTS**

- A. Antibody Panels
- B. PeG
- C. LISS
- D. Saline
- E. Isotonic saline
- F. Antihuman globulin containing anti-IgG
- G. Screening cells
- H. ID-MTS Gel cards
- I. ID-MTS Diluent 2
- J. timers

#### **SPECIMEN**

- A. EDTA specimen preferable.
- B. Clot specimen acceptable.
- C. Minimum 12 ml (2-3 full 6 mL pink top tubes) for adult or minimum 4 ml for baby if a complex problem must be referred to a Reference laboratory.
- D. If not tested immediately, the sample should be stored in the refrigerator except for patient with cold agglutinins.

## **EQUIPMENT**

- A. Centrifuge capable of separating cells and plasma/serum
- B. 37 °C heat block
- C. Serologic centrifuge
- D. Automatic cell washer
- E. Agglutination viewer
- F. Microscope
- G. Transfer pipettes
- H. 12 x 75 mm test tubes.
- I. ID-MTS Incubator
- J. ID-MTS centrifuge
- K. ID-MTS dispenser
- L. Micropipettes
- M. Water bath

#### **CONTROLS**

- A. Daily Reagent Quality Control.
- B. Carefully match the Lot numbers of all panel cells to the panel cell profile sheet before recording results.

#### **PROCEDURE:**

- A. Perform antibody identification if:
  - 1. **New antibody** is suspected by one or more of the following:
    - a. Positive antibody screen
    - b. Incompatible crossmatch
    - c. Positive direct antiglobulin test
    - d. ABO discrepancy caused by unexpected antibody.
  - 2. Perform antibody studies on subsequent patient specimens if **antibody is previously identified and ANY one of the following applies:** 
    - a. The specimen collection date for the last antibody work-up was 3 days ago. **Repanel shall be done on the 4th day. Day 0 is the day of draw.**
    - b. The specimen collection date for the last antibody work-up was within 3 days but the reaction pattern/phase of the current ABSC does not match the previous antibody work-up.
    - c. The specimen collection date for the last antibody work-up was within 3 days but the reaction strength of the current ABSC is 2 degrees stronger than the previous ABSC using the same methodology.
    - d. Red cell unit that is antigen-negative for patient's known antibodies is now incompatible.
    - e. There is suspicion of clinical hemolysis including unexplained increase in blood needs.
    - f. Requested by Transfusion Service pathologist.

**NOTE:** i) In patients with previously identified antibodies, an abbreviated panel

of selected cells by PeG that rules out the presence of other clinically significant antibodies can be performed. ii) It is recommended that a full antibody work-up is performed if the last work-up was 30 days or longer.

## 3. Rh Neg pregnant female suspected of having anti-D:

- a. Perform Gel testing using select cells designated with "@" sign on panel A or C, one R2R2 cell, additional select cells to rule out clinically significant alloantibodies and Autocontrol if ALL of the following apply:
  - i. Suspect that anti-D is passively acquired from RhIg administration.
  - ii. Reaction strength is 2+ or less with D positive screening cells **OR**Negative with R1R1 screening cell **and** reactive 1+ or less with R2R2 screening cell.
  - iii. Antibody screen was negative within the current pregnancy prior to RhIg administration.
  - iv. There is documentation of RhIg administration either in HealthConnect or verification by physician/nurse.
  - v. No recent blood transfusion.
  - vi. No indication on the requisition of recent traumatic event or procedure e.g. amniocentesis, chorionic villi sampling, surgery, etc.

NOTE: If any of the "@" cells is reactive, then a full ABID work-up must be performed.

## b. Perform **full ABID workup** if **ANY** of the following applies:

- i. Patient does not meet criteria for abbreviated workup.
- ii. No history of previous ABSC results.
- iii. Reaction strength is more than 2+ with D positive screening cells.
- iv. No history of RhIg administration.
- v. Recent blood transfusion.
- vi. Recent traumatic event or procedure e.g. amniocentesis, chorionic villi sampling, surgery, etc.
- c. See Documentation section for interpretation.
- 4. For PreOp patients requiring new samples, refer to SFOWI-0112 Processing Blood Units for CVS and OR.

## B. Alerts while ABID is pending

- 1. Alert all blood bank staff that antibody identification is pending and RBCs should not be issued unless in an emergency situation, which has to be approved by attending physician and pathologist on-call.
- 2. Alert floor/MD of the problem immediately if it is a STAT order and the turn around time is 1-2 hours and document notification on paperwork. Complex antibody identification may take one day or more.
- 3. Refer to 'Urgent requirement for Blood and Components' SOP if it is an Emergency Release.

# C. Perform ABID panel and autocontrol using the same method/phase as the antibody screen in general

1. For antibodies previously identified, it is acceptable to run selected

## D. Guidelines for ruling in and ruling out antibody specificity

Antibodies	Minimum Number of Cells Required for Rule
	Out
D	Any 2 cells
C, E, c , e	1 homozygous cell
	2 - 3 heterozygous cells in the presence of
	anti-D
S, s, M, N, Fy <sup>a</sup> , Fy <sup>b</sup> , Jk <sup>a</sup> , Jk <sup>b</sup>	1 homozygous cell
K, k	1 homozygous if not possible then
	2 – 3 heterozygous for anti-K only
f	Any 1 cell
Clinically insignificant P1, Le <sup>a</sup> , Le <sup>b</sup> , Xg <sup>a</sup>	
Clinically significant low frequency C <sup>w</sup> , V, VS, Kp <sup>a</sup>	1 heterozygous cell if possible
Js <sup>a</sup> , Lu <sup>a</sup>	Rule in the low frequency antibody when
, 00 , 10	antigen positive cell is reactive with no other
	pattern.
Clinically significant high frequency k, Kp <sup>b</sup> , Js <sup>b</sup> , Lu <sup>b</sup>	Try to rule out if possible
Questionable significant antibodies, warm reactive	Try to rule out all common clinically
non-specific, HTLA, Warm/Cold autoantibodies	significant underlying alloantibodies.

- 1. An antibody specificity is **'ruled out'** when **cells that are positive** (at least one homozygously) for its corresponding **antigen** (but antigen-negative for other identified antibodies) **are non-reactive.**
- 2. If unable to find a cell with homozygous expression to rule out an antibody specificity, then 2-3 cells with heterozygous expression can be used. However, caution should be used when ruling out certain antibodies known to show dosage e.g. many antibodies in the Rh, MNS, Kidd, Duffy, and Lutheran blood systems, as weak antibody may not react with heterozygous cells.
- 3. **If one antigen remains after crossing out** those antigens that are present on all non-reactive cells, **and the pattern of the antigen matches the pattern of reactivity** obtained, **the specificity of the antibody is tentatively identified**.
- 4. An antibody specificity is 'proven' when 2-3 cells with the corresponding antigen (and do not have the antigens of other identified antibodies or questionable antibodies) react consistently positive and 2-3 cells without the corresponding antigen (and do not have the antigens for other identified antibodies) are non-reactive.
- 5. **If more than one antigen remains** following the elimination, further steps must be taken to **identify possible multiple antibodies**. Positive and negative results that do not fit any of the established patterns for any antigens **may indicate** the presence of **multiple antibodies**, **antibodies to unspecified/low incidence antigens or a weakly reactive antibody**.
- 6. Reasons why **not all antibodies are 'proven' or 'ruled out'** using the guidelines

above, are:

- a. Weak antibodies may not react with cells carrying weak/heterozygous expression or even with some of the homozygous cells. Compare the reactive cells and look for common antigens. Test selected cells that are homozygous positive for the common antigens using enhanced methodology e.g. PeG with increased plasma and longer incubation time.
- b. **Differences in reaction strengths, test methods** and **reactivity phase** might give a clue as to the specificity of the antibody(ies) present.
- c. Other antibodies may be present that displays a reaction pattern indicating a single antibody. Look for exclusion cells to eliminate any other antibodies. Exclusion cells, ideally, are positive for only one of the possible antibodies. If positive, they confirm the presence of that antibody; if negative, they allow you to rule it out. Even if two or more of the possible antibodies are present on a given cell, it should help narrow it down.
- d. **Variable reactions** due to variable antigen expression strength on different cells e.g. P1.
- e. So called HTLA antibodies react weakly with all cells.
- f. Bg (HLA) antibodies react with few cells that seems to have no discerning pattern.
- g. Antibody to low incidence antigen not specified on panel antigrams.
- h. Warm autoantibody or Cold autoantibody with high thermal amplitude reacts with all cells.
- **E. Antigen Typing** (Refer to SFOWI-0096 Antigen Typing for testing instructions). Perform antigen typing, unless the patient has been recently transfused (within the last 3 months).
  - 1. The patient with an alloantibody should type negative for the corresponding antigen.
  - 2. Perform full phenotyping on patients with sickle cell, thalassemia or patients who need chronic transfusion.
  - 3. Phenotype patients who has anti-E or anti-c for both E and c antigens.
  - 4. It is not necessary to retype an antigen if the result is already in the patient 's demographics.
  - 5. Record antigen typing reaction on worksheet and in LIS.
  - 6. Correlate phenotype with ABID results to ensure it confirms the alloantibody(ies).

## F. Perform DAT if the autocontrol is positive

- 1. Inquire transfusion history for the previous 3 months.
- 2. Check medication history for drug that is known to cause **positive** DAT. Refer to SFOWI-0118 Direct Antiglobulin Test.
- 3. If the DAT is positive with anti-IgG, perform an elution on patients who were transfused with red cells within the last 3 months.

## **G.** History and Demographics Inquiry

Perform inquiries of the following:

- 1. Age
- 2. Gender
- 3. Ethnicity
- 4. Diagnosis
- 5. Blood Bank History (antibody, previous transfusions, transfusion reaction)

- a. Check KPHC for Outside Records
- b. If there is no recent history found, ask patient's nurse or provider
- c. If necessary, call the outside hospital for more information.
- 6. Stem Cell or Bone Marrow Transplant History
- 7. Pregnancy History
- 8. Medications and dietary supplements
- 9. IV Solutions e.g. D5W causes rouleaux/hemolysis, Lactated Ringer causes donor RBCs to clot, Lipids may cause difficulty in evaluating test results.

#### H. Documentation

## Reactions must be recorded as they are read.

- Record final ABID and Antigen interpretations on worksheet and verify results in LIS. Also document all pertinent information in LIS and/or Communication binder.
- 2. Comments can be added as Result Comments or Result Notes attached to ABID Interpretation and/or Blood Bank Comments in LIS if needed.
- 3. Use the supplemental worksheet to record additional information i.e. results of different methods of ABSC.
- **4. Attach 'Eluate' as Result Comments** at ABID Interpretation **for** antibody identified by **elution.**
- 5. Select 'Other antibody' as ABID interpretation for inconclusive identification and attach as Result Comments, 'All other clinically significant alloantibodies have been ruled out'.
- 6. Select 'SEE NOTE' as the ABID interpretation for passively acquired antibody.
  - a. Anti-D due to RhIg/IVIg/WinRho. Attach the antibody identity i.e. anti-D, using SF\_RhIg template as Result Comments for: (1) Rh Neg mothers who received RhIg and (2) patients who received IVIg as treatment for ITP.
  - b. Passively acquired maternal antibody in neonates. Attach the antibody identity i.e. anti-Jkb, using SF\_NeoAb template which also include 'Passively acquired maternal antibody' as Result Comments and BB Comments.

# I. Complex antibody problem

If an antibody's specificity is too complex to be determined and/or crossmatch compatible blood cannot be found, send workup to Blood Center of the Pacific (BCP) reference laboratory with supervisor's or manager's approval.

- 1. Call BCP Reference Lab at 415-749-6681 or 415-749-6680 and alert them that specimens are coming.
- 2. Complete a BCP Reference Laboratory Test Request/Billing form.
- 3. Send three properly labeled, signed, dated and timed specimens containing 6 or 7 ml EDTA whole blood; include a pre-transfusion specimen for antigen typing, if available.
- 4. Place the specimens in a double Biohazard plastic specimen bag and put the specimens, the requisition and other pertinent information sheets (print screens of medication history and diagnosis from HealthConnect) in a brown paper bag.
- 5. Close the bag with a tape or staple and write 'BCP Reference Lab' on the paper bag.
- 6. Call taxi or BCP distribution to send a taxi if it is a STAT. If it is a pre-op, leave for BCP driver to pickup.

## J. Delayed Transfusion Reaction

1. A transfusion reaction work-up should be initiated if the patient develops the clinical signs and symptoms of Delayed Hemolytic Transfusion Reaction between 24 hours and 28 days after cessation of transfusion Refer to SFOWI-0121 Delayed Transfusion Reaction SOP.

#### **K.** Antibody Work-up Review

Place worksheets in designated tray for on site supervisor / in-charge to review as soon as possible.

- 1. When serologic inconsistencies are recognized, direct staff to do further testing as needed.
- 2. Verify that antibodies are adequately proven.
- 3. Verify that significant antibodies are adequately ruled out.
- 4. DAT / elution performed as needed.
- 5. Antigen typing performed as needed.
- 6. Verify that comments and transfusion requirements (antigen negative for antibody not ruled out or c-/E- for R1R1 patients with anti-c/E) were entered when appropriate.
- 7. Verify that both crossmatched method and crossmatched units are appropriate.
- 8. Check accuracy and completeness of computer entries.
- 9. Ensure adequate communication and documentation in the communication binder or worksheet.
- 10. Verify that email notification for Pre-Op specimen recollection had been sent.
- 11. File the panel in the completed antibody panel file when TS expires.

## L. Urgent Notification

Notify pathologist and the attending physician immediately if:

- 1. Urgent request for blood before completion of antibody identification.
- 2. Delayed hemolytic transfusion reaction.
- 3. Hemolytic Disease of the Newborn.
- 4. Unable to obtain compatible blood.

#### M. Methods

#### 1. MTS Gel-IgG -primary method

- a. Prepare 0.8% panel/screening cells from 3% screening cells if no 0.8% cells available:
  - i) Label test tubes for panel/screening cells to be tested; including lot number, date and time of preparation.
  - ii) Pipette 100 ul of each cell to its appropriately labeled tube. Add a small volume of MTS Diluent 2 to each test tube for volume.
  - iii) Centrifuge at high speed for 1 minute to pack the red blood cells.
  - iv) Decant the supernatant (a dry cell button is recommended) and then add 200 ul of MTS Diluent 2 to each tube.
  - v) Mix gently. Final cell suspensions should be approximately 0.8% and stable for 24 hours.

## b. Prepare 0.8% autocontrol

- i) Place 1.0 ml of MTS Diluent 2 in a test tube labeled with the test sample identification.
- ii) Add 10 ul of each of the packed red cells from the sample to be tested.

- iii) Mix gently. Final cell suspensions should be approximately 0.8% and are stable for 24 hours.
- c. Refer to 'Antibody Detection Method' SOP for instructions on performing MTS Gel testing.
- 2. **PeG/LISS/Saline IAT panels.** Refer to 'Antibody Detection Method' SOP for instructions.

## 3. Multiple antibodies

# The following are testing options that can aid in identification of multiple antibodies or suspected antibody to a high incidence antigen:

- a. Perform full antigen phenotyping if no transfusion within the last 3 months.
- b. Use ficin treated cell panel. See manufacturer's insert.

  Antigens that are usually denatured or altered by proteolytic enzymes are M, N, S, s, Fy<sup>a</sup>, Fy<sup>b</sup>, Yt<sup>a</sup>, Ch, Rg, Pr, Tn, Mg, Mi<sup>a</sup>/Vw, Cl<sup>a</sup>, Je<sup>a</sup>, Ny<sup>a</sup>, JMH, some Ge, In<sup>b</sup>. Antigens that are usually enhanced by enzyme are Rh, P, I, Kidd and Lewis.
- c. Use DTT treated cells. DTT treated cells will not react with antibodies in the Kell blood group system, most antibodies in the Knops system or most examples of anti-LW<sup>a</sup>, -Yt<sup>a</sup>, -Yt<sup>b</sup>, -Do<sup>a</sup>, -Do<sup>b</sup>, -Gy<sup>a</sup>, -Hy, and -Jo<sup>a</sup> (send out to BCP).
- 4. **Warm reactive non-specific antibody or HTLA** (significant antibodies are difficult to rule out). Testing options include:
  - a. Perform Saline IAT without enhancement. Refer to 'Antibody Detection Method' SOP for instructions.
  - b. Perform full antigen phenotyping if no transfusion within the last 3 months.
  - c. Use enzyme treated cells. See manufacturer's insert.
  - d. Use DTT treated cells (send out to BCP).
  - e. HTLA titer with and without plasma neutralization (send out to BCP).

#### 5. **Rouleaux**

- a. Saline replacement is used when the appearance of the resuspended cells suggests rouleaux formation (stacks of coins) after immediate spin or RT incubation. Caution: Saline replacement may disperse agglutination of weak cold reacting antibody.
  - i) Recentrifuge the plasma/cell mixture.
  - ii) Remove the plasma, leaving the cell button undisturbed.
  - iii) Replace the plasma with an equal volume of saline (2 drops).
  - iv) Resuspend the cell button gently and observe for agglutination. Rouleaux will disperse when suspended in saline, whereas true agglutination will remain.

#### 6. Cold-reactive antibodies

- a. Test screening cells and auto control at IS, RT, 4°C. If necessary, test panel cells and group O cord cells also.
  - i) Add 1 drop of each 2-5% cells into its appropriately labeled tube.
  - ii) Add 2-3 drops of plasma to each tube.
  - iii) Mix and centrifuge at calibrated speed and time.

- iv) Examine for macroscopic agglutination or hemolysis if using serum.
- v) Record results on antigrams and/or supplemental worksheets.
- vi) Determine if antibody specificity can be assigned based on the reaction pattern. If not, proceed to the next step.
- vii) Mix and incubate for 15 minutes at room temperature (RT).
- viii) Centrifuge and examine for macroscopic agglutination or hemolysis if using serum.
- ix) Record results on antigrams and/or supplemental worksheets.
- x) Determine if antibody specificity can be assigned based on the reaction pattern. If not, proceed to 4°C incubation for 15 minutes.
- xi) Centrifuge and examine for macroscopic agglutination or hemolysis if using serum.
- xii) Record results on antigrams and/or supplemental worksheets.
- b. Prewarmed technique with anti-IgG. Refer to SFOWI-0087 'Antibody Detection Method' SOP for instructions. *Note:* Prewarmed technique should be used with caution as it has been shown to result in decrease reactivity of clinically significant antibodies and caused weak antibodies to be missed.
- c. Cold autoadsorption with or without enzyme treated cells (send out to BCP).
- d. REST adsorption can be performed (send out to BCP).
- e. Cold Agglutinin Titer is performed in Hematology Lab.
- f. Thermal amplitude studies at 37°C, 30°C, RT, 4°C (send out to BCP).

#### 7. Warm autoantibodies

- a. Perform IAT test using LISS and/or Saline without enhancement. Refer to SFOWI-0087 Antibody Detection Method SOP for instructions.
- b. Warm autoadsorption (W.A.R.M) can be performed if no transfusion within the last 3 months (send out to BCP).
- c. Full antigen phenotype will be helpful in ruling out alloantibody and providing phenotypically matched donor blood if needed (if patient has been recently transfused send out to BCP BioArray HEA<sup>TM</sup> DNA-based assay can be performed).
- d. Warm alloadsorption if patient has been multiply transfused within the last 3 months (send out to BCP).
- e. Check medication history for drug that is known to cause **positive** DAT. Refer to SFOWI-0118 Direct Antiglobulin Test.

Algorithm to manage warm auto antibody

	Patients with suspect	May interfere with the ability to	Send out to Reference Lab if
	warm autoantibodies.	detect underlying alloantibodies. Perform a complete automated	need to rule out underlying clinically significant
		panel, LISS ABSC, and if LISS ABSC is reactive, perform Saline ABSC. Include Auto Control.	alloantibodies in transfused patients or patients with history of antibody. Order 'BB SO Ag Type' and 'BB Ref Lab WkUp'.
	Patients with current	Provide units in accordance with	Enter Antigen Negative
	identified warm	Reference Lab recommendations if	recommendation as Special
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autoantibodies.	workup was sent out.  Provide least incompatible crossmatched units using the appropriate XM EXT method if workup was performed in-house.  NOTE: Other Special Requirements may apply for chronically transfused patients.	Requirement and any appropriate BB Comments if needed. Result 'BB SO Ag Type' and 'BB Ref Lab WkUp'.
Patients who no longer demonstrate warm autoantibodies, and no historical/current alloantibodies.	Notify supervisor to review and remove recommendation or requirements.	OK to perform EXM if the patient qualifies.

# N. Crossmatch requirements

Immediate Spin Crossmatch will be perform concurrently with AHG crossmatch except when using LISS Ortho Antibody Enhancement Solution. Refer to SFOWI-0089 'Compatibility Testing' SOP.

NOTE: Antigen negative RBCs should be given for clinically significant (i) probable antibody(ies) or (ii) antibody(ies) that cannot be ruled out on the current work-up due to lack of exclusion cells because of the antigen frequency or presence of multiple antibodies.

<b>Antibodies Specificity &amp; Significance</b>	Crossmatch Requirements
Clinically significant:	i. IgG-XM compatible.
D, C, E, c, e, f, G, K, S, s, Fy <sup>a</sup> , Fy <sup>b</sup> , Jk <sup>a</sup> ,	ii. RBCs must be antigen-negative.
Jk <sup>b</sup> ,	iii. Patient who has anti-E or anti-c and types
	negative for both antigens should be given
	E-c- RBCs.
	iv. Give c negative RBCs for anti-f.
	v. Give C negative RBCs for anti-G.
Clinically significant high frequency	i. IgG-XM compatible.
antigen:	ii. Order antigen negative RBCs from BCP.
k, Kp <sup>b</sup> , Js <sup>b</sup> , Lu <sup>b</sup> , U	
Clinically significant low frequency	i. IgG-XM compatible.
antigen;	ii. Give antigen negative RBCs if anti-sera is
C <sup>w</sup> , V, VS, Kp <sup>a</sup> , Js <sup>a</sup> , Lu <sup>a</sup>	available.
Questionable significance:	i. IgG-XM compatible or least incompatible,
HTLA (Kn, McC, Sl <sup>a</sup> , Yk <sup>a</sup> , Ch, Rg,	signed by attending physician for approval
JMH, Cs <sup>a</sup> ), Warm reactive non-specific,	of transfusion.
Warm/Cold autoantibodies	
Clinically insignificant:	i. IgG-XM compatible.
M, N, P <sub>1</sub> , Le <sup>a</sup> , Le <sup>b</sup>	ii. M negative RBCs should be provided for
	<b>neonates</b> , anti-M has been implicated in
	rare cases of HDN.
ABO antibodies:	i. IS-XM compatible group O RBCs.

A1	<ul><li>ii. IgG-XM compatible group A RBCs.</li><li>ii. Group O or A RBCs need to be</li></ul>
*Neonates	phenotyped A <sub>1</sub> negative to avoid override in LIS.
	*Refer to SFOWI-0105 Neonatal Transfusion for neonates.

#### PROCEDURE NOTE(S)

- A. If plasma is not sufficient for an IgG autocontrol, perform DAT.
- B. **False Negatives** may be due to the following:
  - 1. Bacterial or chemical contamination of the reagent cells.
  - 2. Inappropriate plasma/serum to cell ratios.
  - 3. Inadequate incubation time or temperature.
  - 4. Undercentrifugation.
  - 5. Antibody titer below detection.
  - 6. Inadequate washing of red cells.
  - 7. Weakly reactive antibody lacks a definitive pattern.
  - 8. Improper storage of test materials.
  - 9. Omission of antiglobulin or plasma/serum.
  - 10. Antibody to a low incidence antigen.
  - 11. Interrupted testing.
  - 12. The reactivity of reagent red cells may diminish over the dating period. The rate at which antigen reactivity is lost is partially dependent upon the individual donor characteristics that are neither controlled nor predicted by the manufacturer.
- C. **False Positives** may be due to the following:
  - 1. Antibody to a reagent constituent. Washing the cells should eliminate that problem.
  - 2. Overcentrifugation.
  - 3. Contaminated reagents or samples.
  - 4. Use of wrong reagent.

## **REFERENCE:**

- A. AABB Technical Manual, current edition, Bethesda, MD.
- B. AABB, Standards for Blood Banks and Transfusion Services, current edition, Bethesda, MD.
- C. Reagents manufacturer's inserts.

#### **Associated Documents:**

**External Documents** 

**Associated Documents:** 

SFOWI-0087 -- TS-Antibody Detection Method

SFOWI-0089 -- TS-Compatibility Testing

SFOWI-0096 -- TS-Antigen Typing

SFOWI-0082 -- TS - Performing ABO Grouping & Investigating ABO Grouping Discrepancies

SFOWI-0112 -- TS-Processing Blood Units for CVS and OR

SFOWI-0121 -- TS-Delayed Transfusion Reaction

SFOWI-0118 -- TS-Direct Antiglobulin Test

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#### **Documents Generated:**

# **Document Revision History:**

Revision: 17 Date Created: 09/21/2005 Last Approval Date: 01/23/2019 Date of Last Revision: 04/24/2019

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#### **Reason for Change:**

Revision:	Sec/Para Changed	Change Made:	Date
1	Approver	New Lab Director	01/01/07
1	Method	Include cold and warm autoantibodies.	1/1/07
2	Approver	New Lab Director	7/1/07
3	Procedure	Selective cell panel used for previously identified antibod(ies) or anti-D due to RhIG	12/26/08
4	Procedure, A.2 f,g	Clarified work-up related to anti-D due to possible RhIG.	3/4/10
5	Approver	New Lab Director	6/1/2011
6	Procedure Procedure B.3. Procedure D.6.b. Procedure D.8 Procedure D.9.f. Procedure E. Procedure E.6.a. Procedure E.6 and 7.  Procedure E.7.c.  Procedure Notes C. and D. Procedure Notes B. Associated Documents	Reformatted sentences, paragraphs and sections. Corrected spelling and grammatical errors.  Added reference for emergency release.  Added situations when to do full phenotyping.  New section for documentation instructions.  Added 'If it is a pre-op'.  Deleted instructions for MTS Gel testing.  Added instructions for testing at IS, RT and 4°C.  Added caution statement.  Removed instructions to perform cold autoabsorption, warm autoabsorption, RESTabsorption, EGA and Chloroquine treatment.  Added 'in ruling out alloantibody and providing phenotypically matched donor blood if needed (send out to BCP - BioArray HEA™ DNA-based assay can be performed if patient has been recently transfused).  Reformatted paragraphs and added more possible reasons for false negatives. Removed some improbable reasons for false positives .  Added 'except when using LISS Ortho Enhancement Antibody Solution' and reference to Compatibility Testing SOP.  Added documents.	11/3/2011
7	Procedure D.2.  Procedure D.12.  Procedure A.3. and D.9.e.f.g.  Procedure A.3.a.v. and 3.b.iv.  Procedure 3.b.v.  Procedure A.3.NOTE  Procedure A.2.c.	Added instruction for using 2-3 heterozygous cells to rule out an antibody specificity.  Changed instructions to place ABID worksheets in designated tray.  Added new instructions for interpretations.  Added transfusion history requirement for Rhlg panel qualification.  Added trauma as a criteria for Rhlg panel disqualification.  Added NOTE to perform full work-up if any "@" cell is reactive.  Added another criteria for ABID repanel sooner than 1 month.	6/28/12
8	Approver Procedure A.2.a, b, c Procedure A.2.Note Procedure D.9.g.i.	New Lab Director. Changed from one month to 7 days for ABID repanel. New. Removed BB Comments.	2/19/13
9	Approver Procedure A.2.  Procedure E.5.	New BB Medical Director. Change frequency of ABID repanel from 7 days to 3 days per recommendation of Regional BB Subgroup. Deleted 'routine incubation' and replace with 'immediate spin or RT	8/5/13

	Procedure A.2.Note Procedure A.4.	incubation. Caution: Saline replacement may disperse agglutination of weak cold reacting antibody.'  Added recommendation to perform full Ab work-up if previous work-up was >= 30 days.  New. Added reference to SFOWI-0112 for surgery patients.	
10	Procedure E. Procedure E.6.a. Procedure E.6.b. Procedure D. Procedure I. Procedure O.  Procedure F.  Procedure H. Purpose A. Specimen C. Procedure O. Table Associated Documents	Added Rule Out Table. Added even with some homozygous cells. Added test methods and reactivity phase. New. Added anti-P1, HTLA, HLA Ab and Ab to low incidence Ag, warm & cold autoAb. New. Added use of expired panel cells. Changed from primary panel sheet to worksheet. Moved Crossmatch requirements from Procedure Notes. Added to give E-c- RBCs to patient with anti-E or anti-c who types negative for both antigen and to give c negative for anti-f. Added NOTE to given antigen negative for probable or not ruled out Ab. Added to phenotype patient for E and c antigens when anti-E or anti-c is identified. New section for History and Demographics Inquiry. Deleted 'more accurately'. Revised. Added 'minimum' and '-3 full 6ml' and 'tubes'. Added ABO Ab, significance and Xm requirement for anti-M in HDN. Added SFOWI-0089.	10/16/13
11	Procedure A.3.b. Procedure L.6, 7 & 10	New. Added instructions for performing mini Rhlg panel when only R2R2 screening cell is reactive <2+.  New. Added to review tx requirements/comments, crossmatch and PreOp email.	10/3/14
12	Procedure D.	Deleted use of expired panel cells for ABID workup. Move E. to D.	12/14/15
13	Procedure J.	Added instructions to compare reactive cells for common antigen(s) and test select cells with enhanced methods.  Deleted instructions to initiate DTR work-up based only on serologic findings.	2/22/16
14	Approver	New CLIA Director.	9/28/16
15	Specimen D.  Procedure A.2.NOTE  Procedure A.3.a.  Procedure M.7.  Procedure F.2. and M.7.e.	Revised instructions not to store samples in the fridge if patients have cold agglutinins.  Added PeG as the methodology of choice for abbreviated ABID rule-out panel on patients with previously identified antibody.  Added one R2R2 cell, additional select cells to rule out clinically significant allo antibodies and Auto Control by Gel to RhIg workup for both scenarios of suspected anti-D (RhIg): 1) <=2+ for Sc1 & Sc2 and 2) Neg with Sc1 and <=1+ for Sc2.  Added Algorithm Table for managing warm auto Ab workup.  Added to check medication Hx for drug that causes positive DAT.	3/21/17
16	Crossmatch Requirements Table	Clarified that M negative RBCs should be given to neonates.  Due to recent change in Anti-A1 designation to clinically significant in Millennium, group O and group A RBCs should be A1 negative to avoid override in LIS.  Added antigen negative requirement for anti-G.	12/12/18
17	Approver Procedure G.5	New Lab Director and Transfusion Service Director. Added instructions to check Outside Records for BB history in KPHC.	4/19/19

## **Notification List:**

# **Approvals:**

#### First Approver's Signature

Name: Sarah C Cherny/CA/KAIPERM Title: Transfusion Service Medical Director

## Second Approver's Signature

Name: Elizabeth M Hosfield/CA/KAIPERM Title: Chief of Pathology; CLIA Director

#### **Document History Section**