PROCEDURE

Title: Antigen Typing (*C,c,Cellano,k,E,e,Fya,JKa,JKb,K,Lea,Leb,M,s*)

Procedure #: 2015BLOODBANK73

Institution: Highlands Regional Medical Center

Address: 3600 Highlands Avenue, Sebring Florida 33870

Prepared by: Anita Smith Date: 6/12/2015

Title: Laboratory Administrative Director

Accepted by: _____ Date: 6/12/15

Title: Laboratory Medical Director

Date Patient Testing Implemented: 7/8/2013

Review of procedure every two years

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

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Reviewed by: _____ Date: _____

Discontinued testing date: _____

Highlands Regional MEDICAL CENTER

Policy Name: Antigen Typing | **Department:** Blood Bank

Departmental Review: | **Policy #: B8.8**

INITIATE DATE | **DATE REVIEWED/REVISED** 07/2013; 05/2015 | **PAGE 1 of 3**

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody. Blood grouping antisera detects the presence or absence of antigens on human red cells.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectible, the red cells of all subsequent transfusions to that patient must lack that antigen.

SPECIMEN:
Properly labeled samples in EDTA less than 14 days old may be used for antigen typing.

QUALITY CONTROL:
The reactivity of blood grouping reagents should be confirmed on each day of use by testing with red cells known to be negative and positive for the relevant antigen. Heterozygous cells are preferred for the positive control. If expected results are not obtained after repeated test, antisera may not be used.

REAGENTS:
Commercial typing sera
Anti-IgG
0.9% NaCl for cell suspension and washing
2-4% Reagent screen cells for positive and negative controls

PROCEDURE:
1. Label a 12 x 75mm tube for each unit, patient or control to be tested.
2. Follow manufacturer's product insert for the specific antigen being tested.
3. Record patient, donor and control results on appropriate worksheet.
4. Place the appropriate sticker with the antigen results on the unit.

PROCEDURE NOTES:

1. Patients with anti-E should also be tested for the little-c antigen. In patients with anti-E and negative for little-c antigen, units should be screened for the absence of both antigens. These patients may also produce an anti-c not detected by routine test.

2. When an antibody is considered clinically insignificant (eg, anti-A1, -M, -N, -P1, -Lea, -Leb) and not reactive at 37°C it is not necessary to give antigen negative units to the patient. AHG crossmatch compatibility is required.

3. Antigen typing is questionable when the patient has the antigen of the antibody identified and may require the expertise of a reference lab. These may be caused by:
   a. Donor cells from transfusion within three months.
   b. Patient or donor cells have a positive direct coombs.
   c. Autoantibody present.
   d. Antibody misidentification.

4. To estimate the number of units to screen to find antigen negative donors, divide the number of units needed by the percentage of negative population. For multiple antibodies, multiply the percentages of negative population for each antigen to find the percentage of the population negative for all antigens. The percentage of negative antigen population is determined by the demographics of the donor population.

   Example: Patient has Anti-K, -Jk(b) and needs two units.

   23% of the population is negative for Jk(b); 91% of the population is negative for K.

   $$0.23 \times 0.91 = 0.21$$

   21% of the population is negative for both antigens: 2 units ÷ 0.21 = 9.5

   Approximately 10 units would be screened to find two units negative for both antigens.

REFERENCE:

Antisera reagent inserts
MTS reagent inserts
AABB Technical Manual

Highlands Regional MEDICAL CENTER

Policy Name: C and c Antigen Screen **Department:** Blood Bank

Departmental Review: **Policy #:**

INITIATE DATE **DATE REVIEWED/REVISED** 05/2015

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to the clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient must lack that antigen.

SPECIMEN:
- 2-4% patient red cell suspension

REAGENTS:
- Anti-C /Anti-c antisera (monoclonal)
- Positive and negative screen cells for specified antigen
- 0.9% normal saline

PROCEDURE:
- Label tubes as patient, negative control and positive control.
- Place one drop of Anti-C or Anti-c in a properly labeled tubes.
- Add a drop of a 2 to 4% suspension of patient cells (washed with saline at least twice), negative and positive control.
- Mix contents of tube thoroughly.
- Incubate tubes for 5-15 minutes at 36 to 38°C. Incubating at the maximum time may enhance reactivity.
- Centrifuge then gently agitate tube to suspend the red cell button. Examine for macroscopic agglutination. An optical aid may be used. Record results.

QUALITY CONTROL:

Reactivity of grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigen. Red blood cells possessing a single dose of the antigen are suitable for the positive control test.

REPORTING RESULTS:

- Agglutination of the red cells constitutes a positive test result and indicates the presence of the relevant antigen.
- No agglutination constitutes a negative test result and indicates the absence of the relevant antigen.

PROCEDURE NOTES:

- Factors that may cause false test results
  - Bacterial contamination.
  - Presence of hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.
  - Improper storage of materials
  - Aged or stored blood specimens may yield weaker reactions
  - Too heavy or light a red cells suspension of the specimen and/or control
  - Improper incubation time and temperature
  - Improper centrifugation
  - Improper examination of agglutination. Vigorous shaking usually cause weak agglutination to disperse causing false negative reaction. Gentle shaking must be carried out to avoid dispersal of weak agglutinates.
  - Enzyme treated red cells must not be used for testing as either red blood cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Always check product package insert for any method modification and product updates.
- False positive reactions may be observed in patients with red cells coated in vivo with IgG molecules (patient with protein abnormalities:ex. multiple myeloma, patient with cold reactive agglutinins).

ANTIGEN FREQUENCIES

The frequencies of each of these antigens in Caucasian population are as follows:

ANTIGEN NOMENCLATURE			FREQUENCY %
Fisher-Race	Weiner	Rosenfield	Caucasian
D	Rh_o	Rh1	85
C	rh'	Rh2	70
E	rh"	Rh3	30
c	hr'	Rh4	80
e	hr"	Rh5	98

REFERENCES:

- Blood grouping reagent Anti-C, Anti-c Series 1 (Monoclonal Reagents); Immucor Gamma; Revised: 01/2006
- AABB Technical Manuel, 17th edition

Highlands Regional MEDICAL CENTER

Policy Name: Cellano (little k) Antigen Screen **Department:** Blood Bank

Departmental Review: **Policy #:**

INITIATE DATE **DATE REVIEWED/REVISED** 05/2015

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to the clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient must lack that antigen.

SPECIMEN:
- 2-4% patient red cell suspension

REAGENTS:
- Anti-k (monoclonal)
- Positive and negative screen cells for specified antigen
- 0.9% normal saline

PROCEDURE:
- Label tubes as patient, negative control and positive control.
- Place one drop of Anti-k in a properly labeled tubes.
- Add a drop of a 2 to 5% suspension of patient cells (washed with saline at least twice), negative and positive control.
- Mix contents of tube thoroughly.
- Incubate tubes for 15 to 30 minutes at 36 to 38°C. Incubating at the maximum time may enhance reactivity.
- Wash cells three times. Decant last saline completely following the last wash.
- Add one or two drops of Anti-Human Globulin (AHG).
- Centrifuge then gently agitate tube to suspend the red cell button. Examine for macroscopic agglutination. An optical aid may be used; however, microscopic reading is not recommended. Record results.

- All negative test must be confirmed by adding check cells. A positive test at this point confirms that active (antiglobulin) was added to the test system and was present when the original test was interpreted as negative.

QUALITY CONTROL:

- Reactivity of grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigen. Red blood cells possessing a single dose of the antigen are suitable for the positive control test.

REPORTING RESULTS:

- Agglutination of the red cells constitutes a positive test result and indicates the presence of the relevant antigen.
- No agglutination constitutes a negative test result and indicates the absence of the relevant antigen.

PROCEDURE NOTES:

- The washing phases of the antiglobulin test must be carried out without interruption, and final test results must be interpreted immediately upon completion of the test.
- Suppressed or diminished expression of certain blood group antigens may conversely give rise to substantially weaker reactions than are observed with the red blood cells normally used for the positive control test. For these reasons, caution should always be exercised when assigning genetic significance on the basis of the test results.
- Factors that may cause false test results
  - Bacterial contamination.
  - Presence of hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.
  - Improper storage of materials
  - Aged or stored blood specimens may yield weaker reactions
  - Too heavy or light a red cells suspension of the specimen and/or control
  - Improper incubation time and temperature
  - Improper centrifugation
  - Improper examination of agglutination. Vigorous shaking usually cause weak agglutination to disperse causing false negative reaction. Gentle shaking must be carried out to avoid dispersal of weak agglutinates.

- Enzyme treated red cells must not be used for testing as either red blood cells under investigation or as a source of control red blood cells since it may yield erroneous results.

- Red cells having a positive direct antiglobulin test due to coating of IgG cannot be typed by the indirect antiglobulin technique.
- Enzyme treated red cells must not be used for testing as either red cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Always check product package insert for any method modification and product updates.

PHENOTYPE FREQUENCY

Phenotype	% Frequency	
	Caucasian	African Americans
K+k-	0.2	Rare
K+k+	8.8	2
K-k+	91	98
Kp(a+b-))	Very	0
Kp(a+b+)	2.3	Rare
Kp(a-b+)	97.7	100

REFERENCES:

- Blood grouping reagent Anti-k by IAT; Immucor Gamma; Revised: 10/2010

Highlands Regional MEDICAL CENTER

Policy Name: E and e Antigen Screen **Department:** Blood Bank

Departmental Review: **Policy #:**

INITIATE DATE **DATE REVIEWED/REVISED** 05/2015

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to the clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient must lack that antigen. Patient who is found to be positive for the E antigen and negative for the c antigen must be given blood that are screened negative for the E and c antigen.

SPECIMEN:
- 2-4% patient red cell suspension

REAGENTS:
- Anti-E /Anti-e antisera (monoclonal)
- Positive and negative screen cells for specified antigen
- 0.9% normal saline

PROCEDURE:
- Label tubes as patient, negative control and positive control.
- Place one drop of Anti-E or Anti-e in a properly labeled tubes.
- Add a drop of a 2 to 4% suspension of patient cells (washed with saline at least twice), negative and positive control.
- Mix and centrifuge.
- If test is negative resuspend cells thoroughly and incubate for 15 minutes at room temperature.
- Centrifuge and then resuspend red cells by gently shaking. Examine for macroscopic agglutination. An optical aid may be used. Record results.

QUALITY CONTROL:
Reactivity of grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigen. Red blood cells possessing a single dose of the antigen are suitable for the positive control test.

REPORTING RESULTS:
- Agglutination of the red cells constitutes a positive test result and indicates the presence of the relevant antigen.
- No agglutination constitutes a negative test result and indicates the absence of the relevant antigen.

PROCEDURE NOTES:
- It is best to screen the blood unit for the c antigen if the patient is found to be cE negative.
- Factors that may cause false test results
  - Bacterial contamination.
  - Presence of hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.
  - Improper storage of materials
  - Aged or stored blood specimens may yield weaker reactions
  - Too heavy or light a red cells suspension of the specimen and/or control
  - Improper incubation time and temperature
  - Improper centrifugation
  - Improper examination of agglutination. Vigorous shaking usually cause weak agglutination to disperse causing false negative reaction. Gentle shaking must be carried out to avoid dispersal of weak agglutinates.
  - Enzyme treated red cells must not be used for testing as either red blood cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Always check product package insert for any method modification and updates.

ANTIGEN FREQUENCIES

The frequencies of each of these antigens in Caucasian population are as follows:

ANTIGEN NOMENCLATURE			FREQUENCY %
Fisher-Race	Weiner	Rosenfield	Caucasian
D	Rh_o	Rh1	85
C	rh'	Rh2	70
E	rh"	Rh3	30
c	hr'	Rh4	80
e	hr"	Rh5	98

REFERENCES:

- Blood grouping reagent Anti-E (Monoclonal), Gamma-clone; Immucor Gamma, revised: 10/2007
- Blood grouping reagent Anti-e (Monoclonal Blend), Gamma-clone; Immucor Gamma; revised: 10/2007
- AABB Technical Manual, 17th edition

Highlands Regional MEDICAL CENTER

Policy Name: Fy[a] Antigen Screen | **Department:** Blood Bank

Departmental Review: | **Policy #:**

INITIATE DATE | **DATE REVIEWED/REVISED** 05/2015 | **PAGE 1 of 4**

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to the clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient must lack that antigen.

SPECIMEN:
- 2-4% patient red cell suspension

REAGENTS:
- Anti-Fy[a] (monoclonal)
- Positive and negative screen cells for specified antigen
- 0.9% normal saline

PROCEDURE:
- Label tubes as patient, negative control and positive control.
- Place one drop of Anti-Fy[a] in a properly labeled tubes.
- Add a drop of a 2 to 5% suspension of patient cells (washed with saline at least twice), negative and positive control.
- Mix contents of tube thoroughly.
- Incubate tubes for 10 to 15 minutes at 36 to 38°C. Incubating at the maximum time may enhance reactivity.
- Wash cells three times. Decant last saline completely following the last wash.
- Add one or two drops of Anti-Human Globulin (AHG).
- Centrifuge then gently agitate tube to suspend the red cell button. Examine for macroscopic agglutination. An optical aid may be used; however, microscopic reading is not recommended. Record results.

- All negative tests must be confirmed by adding check cells. A positive test at this point confirms that active (antiglobulin) was added to the test system and was present when the original test was interpreted as negative.

QUALITY CONTROL:
Reactivity of grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigen. Red blood cells possessing a single dose of the antigen are suitable for the positive control test.

REPORTING RESULTS:
- Agglutination of the red cells constitutes a positive test result and indicates the presence of the relevant antigen.
- No agglutination constitutes a negative test result and indicates the absence of the relevant antigen.

PROCEDURE NOTES:
- The washing phases of the antiglobulin test must be carried out without interruption, and final test results must be interpreted immediately upon completion of the test.
- Factors that may cause false test results
  - Bacterial contamination.
  - Presence of hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.
  - Improper storage of materials
  - Aged or stored blood specimens may yield weaker reactions
  - Too heavy or light a red cells suspension of the specimen and/or control
  - Improper incubation time and temperature
  - Improper centrifugation
  - Improper examination of agglutination. Vigorous shaking usually cause weak agglutination to disperse causing false negative reaction. Gentle shaking must be carried out to avoid dispersal of weak agglutinates.
  - Enzyme treated red cells must not be used for testing as either red blood cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Red cells having a positive direct antiglobulin test due to coating of IgG cannot be typed by the indirect antiglobulin technique.

- Enzyme treated red cells must not be used for testing as either red cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Always check product package insert for any method modification and product updates.

PHENOTYPE FREQUENCY

Phenotype	% Frequency	
	Caucasian	**Blacks**
Fy(a+b-)	17	9
Fy(a+b+)	49	1
Fy(a-b+)	34	22
Fy(a-b-)	Very Rare	688

REFERENCES:

- Blood grouping reagent Anti-Fy[a] (Monoclonal) Gamma-clone by IAT; Immucor Gamma; Revised: 09/2013
- AABB Technical Manual, 17th edition

Highlands Regional MEDICAL CENTER

Policy Name: Jk[a] Antigen/Jk[b] Antigen Screen **Department:** Blood Bank

Departmental Review: **Policy #:**

INITIATE DATE **DATE REVIEWED/REVISED** 05/2015

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to the clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient must lack that antigen.

SPECIMEN:
- 2-4% patient red cell suspension

REAGENT:
- Anti-Jk[a] /Anti-Jk[b] antisera (monoclonal)
- Positive and negative screen cells for specified antigen
- 0.9% normal saline

PROCEDURE:
- Place one drop of Gamma-clone Anti-Jk[a] or Anti-Jk[b] (monoclonal) into a properly labeled test tube.
- Add one drop of patient red cells suspension (washed with saline), negative and positive screen cells to properly labeled test tubes.
- Mix contents by gently shaking and incubate for 5 to 15 minutes at room temperature 15 to 30°C. Incubating for the upper end of the time range may enhance reactivity.
- Centrifuge test tubes. After centrifugation, immediately resuspend red cells by gently shaking the test tubes and examine for macroscopic agglutination. Negative reactions may be examined with an optical aid; however, **microscopic reading is not recommended.** Record results.
- Test results should be read immediately and interpreted without delay.

QUALITY CONTROL:

Reactivity of grouping reagents should be confirmed on each day of use by testing with red blood cells to be negative and positive for the relevant antigens. Jk^{a+b+} red cells are the most suitable positive control red cells for both anti-Jk^a and –Jk^b (monoclonal). Each reagent is satisfactory for use if it reacts only with antigen-positive red cells.

REPORTING RESULTS:

- Agglutination of the red cells constitutes a positive test result and indicates the presence of the relevant antigen.
- No agglutination constitutes a negative test result and indicates the absence of the relevant antigen.

PROCEDURE NOTES:

- Factors that may cause false test results
  - Bacterial contamination
  - Improper storage of materials
  - Aged or stored blood specimens may yield weaker reactions
  - Too heavy or light a red cells suspension of the specimen and/or control
  - Improper incubation time and temperature
  - Improper centrifugation
  - Improper examination of agglutination. Vigorous shaking usually cause weak agglutination to disperse causing false negative reaction. Gentle shaking must be carried out to avoid dispersal of weak agglutinates.
  - Enzyme treated red cells must not be used for testing as either red blood cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Always check product package insert. Performance of the antisera is dependent upon adhering to the package insert.

PHENOTYPE FREQUENCY BY ETHNICITY

Phenotype	Caucasians	Blacks	Asians
Jk(a+b-)	26.3	51.1	23.2
Jk(a+b+)	50.3	40.8	49.1
Jk(a-b+)	23.4	8.1	26.8
Jk(a-b-)	Rare	Rare	0.9 (Polynesians)

REFERENCES:

- Blood Grouping Reagent, Anti-Jk[a](Monoclonal),Anti-Jk[b](Monoclonal) Gamma-clone product insert by tube test; Immucor Gamma; revised 09/2013
- AABB Technical Manual, 17th edition

Highlands Regional MEDICAL CENTER

Policy Name: K Antigen Screen | **Department:** Blood Bank

Departmental Review: | **Policy #:**

INITIATE DATE | **DATE REVIEWED/REVISED** 05/2015 |

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to the clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient must lack that antigen.

SPECIMEN:
- 2-4% patient red cell suspension

REAGENTS:
- Anti-K
- Positive and negative screen cells for specified antigen
- 0.9% normal saline

PROCEDURE:
- Label tubes as patient, negative control and positive control.
- Place one drop of Anti-K in properly labeled tubes.
- Add a drop of a 2 to 4% suspension of patient cells (washed with saline at least twice), negative and positive control.
- Mix and incubate for 2 to 10 minutes at room temperature (18 to 30°C). Incubating at the upper end of the time range may enhance reactivity.
- Centrifuge.
- Gently agitate each tube to resuspend red cell button. Read immediately. Examine for macroscopic agglutination. An optical aid may be used. Record results.

QUALITY CONTROL:
Reactivity of grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigen. Red blood cells possessing a single dose of the antigen are suitable for the positive control test.

REPORTING RESULTS:
- Agglutination of the red cells constitutes a positive test result and indicates the presence of the relevant antigen.
- No agglutination constitutes a negative test result and indicates the absence of the relevant antigen.

PROCEDURE NOTES:
- Factors that may cause false test results
  - Bacterial contamination.
  - Presence of hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.
  - Improper storage of materials
  - Aged or stored blood specimens may yield weaker reactions
  - Too heavy or light a red cells suspension of the specimen and/or control
  - Improper incubation time and temperature
  - Improper centrifugation
  - Improper examination of agglutination. Vigorous shaking usually cause weak agglutination to disperse causing false negative reaction. Gentle shaking must be carried out to avoid dispersal of weak agglutinates.
  - Enzyme treated red cells must not be used for testing as either red blood cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- A +1 reaction should not be automatically assumed to carry a diminished expression of K. Red cells producing weak reactions should be further evaluated by direct AHG or in typing test with other anti-K reagents before the K phenotype is assigned.
- Always check product package insert for any method modification and updates.

EXPECTED BLOOD CELL TYPING RESULTS

	BLOOD GROUPING REAGENT		Frequency %	
Phenotype	Anti-K	Anti-k	Whites	Blacks
K+k-	+	0	0.2	Rare
K+k+	+	+	8.8	2.0
K-k+	0	+	91.0	98.0
K-k-	0	0	Extremely Rare	

REFERENCES:

- Blood grouping reagent Anti-K (Human Monoclonal), Immucor Gamma, revised: 08/2007
- AABB Technical Manual, 17th edition

Highlands Regional MEDICAL CENTER

Policy Name: Le^a and Le^b Antigen Screen **Department:** Blood Bank

Departmental Review: **Policy #:**

INITIATE DATE **DATE REVIEWED/REVISED** 05/2015 **PAGE 1 of 4**

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to the clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient must lack that antigen.

SPECIMEN:
- 2-4% patient red cell suspension

REAGENTS:
- Anti-Le^a and/or Anti-Le^b antisera (murine monoclonal)
- Positive and negative screen cells for specified antigen
- 0.9% normal saline

PROCEDURE:
- Label tubes as patient, negative control and positive control.
- Place one drop of Anti-Le^a or Anti-Le^b in a properly labeled tubes.
- Add a drop of a 3 to 4% suspension of patient cells (washed with saline at least twice), negative and positive control.
- Mix and centrifuge.
- If test is negative resuspend cells thoroughly and incubate for 5 to 10 minutes at room temperature (18 to 26°C). Incubating at the upper end of the time range may enhance reactivity.
- Centrifuge and then resuspend red cells by gently shaking. Examine for macroscopic agglutination. An optical aid may be used. **NOTE:** Do not interpret results microscopically. Record results.

QUALITY CONTROL:

- Reactivity of grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigen. Red blood cells possessing a single dose of the antigen are suitable for the positive control test.

REPORTING RESULTS:

- Test and control results must be interpreted immediately upon completion of the test.
- Agglutination of the red cells constitutes a positive test result and indicates the presence of the relevant antigen.
- No agglutination constitutes a negative test result and indicates the absence of the relevant antigen.

PROCEDURE NOTES:

- Factors that may cause false test results
  - Bacterial contamination.
  - Presence of hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.
  - Improper storage of materials
  - Aged or stored blood specimens may yield weaker reactions
  - Too heavy or light a red cells suspension of the specimen and/or control
  - Improper incubation time and temperature
  - Improper centrifugation
  - Improper examination of agglutination. Vigorous shaking usually cause weak agglutination to disperse causing false negative reaction. Gentle shaking must be carried out to avoid dispersal of weak agglutinates.
  - Enzyme treated red cells must not be used for testing as either red blood cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Testing the Lewis antigen from children may not be reliable since said antigen is poorly expressed in children.
- Anti-Le^b may react more weakly with red blood cells of group A_1B and A_1 than with those of group B and O.
- A POSITIVE anti-Le^b reaction on a Le (a+) red cells may represent the Le(a+b+) phenotype. The evaluation of these unusual reactions may require secretor studies.

- The strength of the Lewis antigens may be diminished during pregnancy or in patients with cancer and other diseases.
- Always check product package insert for any method modification and updates.

LEWIS PHENOTYPE FREQUENCY

Phenotype	Whites	African American
Le(a+b-)	22	23
Le(a-b+)	72	55
Le(a-b-)	6	22-30
Le(a+b+)	Rare	Rare

REFERENCES:

- Blood grouping reagent Anti-Le^a(Murine Monoclonal), Gamma-clone; Immucor Gamma, revised: 02/2012
- Blood grouping reagent Anti-Le^b (Murine Monoclonal), Gamma-clone; Immucor Gamma; revised: 02/2012
- AABB Technical Manual, 17th edition

Highlands Regional MEDICAL CENTER

Policy Name: M Antigen Screen **Department:** Blood Bank

Departmental Review: **Policy #:**

INITIATE DATE **DATE REVIEWED/REVISED** 05/2015

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to the clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient must lack that antigen.

SPECIMEN:
- 2-4% patient red cell suspension

REAGENTS:
- Anti-M antisera (Murine Monoclonal)
- Positive and negative screen cells for specified antigen
- 0.9% normal saline

PROCEDURE:
- Label tubes as patient, negative control and positive control.
- Place one drop of Anti-M in a properly labeled tubes.
- Add a drop of a 2 to 4% suspension of patient cells (washed with saline at least twice), negative and positive control.
- Mix and centrifuge.
- If test is negative resuspend cells thoroughly and incubate for 15 minutes at room temperature (20 to 26°C).
- Centrifuge and then resuspend red cells by gently shaking. Examine for macroscopic agglutination. Test result must be interpreted immediately upon completion. An optical aid may be used. Record results.

QUALITY CONTROL:
Reactivity of grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigen. Red blood cells possessing a single dose of the antigen are suitable for the positive control test.

REPORTING RESULTS:
- Agglutination of the red cells constitutes a positive test result and indicates the presence of the relevant antigen.
- No agglutination constitutes a negative test result and indicates the absence of the relevant antigen.

PROCEDURE NOTES:
- Factors that may cause false test results
  - Bacterial contamination.
  - Presence of hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.
  - Improper storage of materials
  - Aged or stored blood specimens may yield weaker reactions
  - Too heavy or light a red cells suspension of the specimen and/or control
  - Improper incubation time and temperature
  - Improper centrifugation
  - Improper examination of agglutination. Vigorous shaking usually cause weak agglutination to disperse causing false negative reaction. Gentle shaking must be carried out to avoid dispersal of weak agglutinates.
  - Enzyme treated red cells must not be used for testing as either red blood cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Always check product package insert for any method modification and updates.

PHENOTYPE FREQUENCY

Phenotype	% Frequency	
	Whites	African Americans
MM	28	26
MN	50	44
NN	22	30

REFERENCES:

- Blood grouping reagent Anti-M (Murine Monoclonal), Gamma-clone; Immucor Gamma, revised: 10/2007
- Blood grouping reagent Anti-N (Murine Monoclonal), Gamma-clone; Immucor Gamma; revised: 10/2007
- AABB Technical Manual, 17th edition

Highlands Regional MEDICAL CENTER

Policy Name: Little s Antigen Screen **Department:** Blood Bank

Departmental Review: **Policy #:**

INITIATE DATE **DATE REVIEWED/REVISED** 05/2015

PURPOSE:
To prevent a secondary immune response, patients with clinically significant antibodies who require transfusion must receive red cells that have tested negative for the antigen corresponding to the antibody.

POLICY:
A patient with an identified antibody is tested for the absence of the corresponding antigen at the initial antibody workup. Red cell units to be transfused must be tested for the absence of antigens corresponding to the clinically significant antibodies detected in a positive antibody panel. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient must lack that antigen.

SPECIMEN:
- 2-4% patient red cell suspension

REAGENTS:
- Anti-S or Anti-s antisera
- Positive and negative screen cells for specified antigen
- 0.9% normal saline

PROCEDURE:
- Label tubes as patient, negative control and positive control.
- Place one drop of Anti-S and/or Anti-s antisera in a properly labeled tubes.
- Add a drop of a 3 to 4% suspension of patient cells (washed with saline at least twice), negative and positive control.
- Mix contents of tube thoroughly.
- Incubate tubes for 10 to 15 minutes at 36 to 38°C. Incubating at the maximum time may enhance reactivity.
- Wash cells three times. Decant last saline completely following the last wash.
- Add one or two drops of Anti-Human Globulin (AHG).
- Centrifuge then gently agitate tube to suspend the red cell button. Examine for macroscopic agglutination. An optical aid may be used; however, microscopic reading is not recommended. Record results.

- All negative test must be confirmed by adding check cells. A positive test at this point confirms that active (antiglobulin) was added to the test system and was present when the original test was interpreted as negative.

QUALITY CONTROL:

- Reactivity of grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigen. Red blood cells possessing a single dose of the antigen are suitable for the positive control test.

REPORTING RESULTS:

- Agglutination of the red cells constitutes a positive test result and indicates the presence of the relevant antigen.
- No agglutination constitutes a negative test result and indicates the absence of the relevant antigen.

PROCEDURE NOTES:

- The washing phases of the antiglobulin test must be carried out without interruption, and final test results must be interpreted immediately upon completion of the test.
- Factors that may cause false test results
  - Bacterial contamination.
  - Presence of hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.
  - Improper storage of materials
  - Aged or stored blood specimens may yield weaker reactions
  - Too heavy or light a red cells suspension of the specimen and/or control
  - Improper incubation time and temperature
  - Improper centrifugation
  - Improper examination of agglutination. Vigorous shaking usually cause weak agglutination to disperse causing false negative reaction. Gentle shaking must be carried out to avoid dispersal of weak agglutinates.
  - Enzyme treated red cells must not be used for testing as either red blood cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Red cells having a positive direct antiglobulin test due to coating of IgG cannot be typed by the indirect antiglobulin technique.

- Enzyme treated red cells must not be used for testing as either red cells under investigation or as a source of control red blood cells since it may yield erroneous results.
- Always check product package insert for any method modification and product updates.

PHENOTYPE FREQUENCY

Phenotype	% Frequency	
	Whites	African American
S+s-	11	3
S+s+	44	28
S-s+	45	69
S-s-U-	0	<1
S-s-U+(wk)	0	<1

REFERENCES:

- Blood grouping reagent Anti-S/Anti-s by IAT; Immucor Gamma; Revised: 07/2007
- AABB Technical Manual, 17th edition