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Allohemagglutinin Titration - Dearborn Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide policies and procedures to the Blood Bank staff to perform an allohemagglutinin titration.

II. PRINCIPLE:

Ordinarily, people possess antibodies directed toward the A or B antigens that are absent from their own red blood cells (RBCs). The immunoreactive configurations that confer A and B specificities to molecules of the RBC membrane also exist in other biologic entities widespread in the environment. Immunocompetent persons react to these environmental antigens by producing the ABO antibodies to those antigens absent in their own systems.

III. SCOPE:

A. Allohemagglutinin titrations may be performed on patient samples for several reasons including:
    1. To check for immunodeficiency in young children (9 months to 2 years) suspected to have a disease state associated with absent or abnormally low allohemagglutinins.
    2. To screen the plasma of prospective renal or liver transplant candidates across ABO boundaries.

IV. INTRODUCTION:

A. Immunocompetent persons generally produce ABO antibodies to those ABO antigens absent in their own systems. Therefore, the patient's blood type determines the specificity of the

antibody for which a titer is performed and the test cells used for the titration. For example:

Patient's Blood Type	ABO Antibody for which Titer is Performed	Appropriate Test Cells Used for the Titration(s)
A	Titer not indicated	Not applicable
B	Anti-A	A_2 cells
O	Anti-A and Anti-B	A_2 cells
AB	Titer not indicated	Not applicable

B. The antibody titration is performed using serial dilutions of the patient's plasma as described in Transfusion Medicine policy, Antibody Titration.

V. DEFINITIONS:

A. **Unexpected antibody:** Any antibody (other than naturally occurring anti-A or anti-B that is regularly found in normal serum or plasma) that is currently or was historically present in a patient's sample.

B. **Allohemagglutinins (aka isohemagglutinins):** Naturally occurring antibodies of the ABO system that are directed against the patient's missing ABO antigens.

VI. POLICIES:

A. Patients with Unexpected Antibodies

1. If the patient has any unexpected antibodies, then the A_2 or B test cells used in the allohemagglutinin titration must be negative for all antigens corresponding to the unexpected antibodies.

B. Reading and Grading Reactions

1. It is very important for the technologists to grade the test reactions of an antibody titration consistently. Reactive tubes are graded from weak+ to 4+. Test reactions shall be graded as described in Transfusion Medicine policy, Reading, Grading, and Recording Test Reactions.
2. The end point results of the room temperature titer and AHG titer must be within two tubes. If the results are greater than two tubes apart, then the titer must be repeated by the original testing technologist and a second technologist.

C. Policies Relating to Pipetting Technique

1. A new pipette tip must be used for each tube of the serial dilution.
2. The outside of the pipette tip should be gently wiped after aspiration from one tube, and before dispensing into the next tube. Caution should be used to prevent the removal of any contents from **inside** the pipette tip.
3. When dispensing, the pipette tip should be gently touched to the inside wall of the tube while still depressed so that all contents from the tip are dispensed.

D. Policies Relating to Transplant Patients

1. Allohemagglutinin titers are not routinely tested with a control sample. In some cases, a patient preparing for or having undergone a solid organ transplant will require multiple allohemagglutinin titers. A control sample should be saved and tested as described in Transfusion Medicine policy, Antibody Titration.

E. Titer End Point Requirements

1. The end point is the last tube of the serial dilution displaying macroscopic agglutination. The tube containing the end point must be immediately followed by a tube in the serial dilution that is non-reactive. For example:
    1. An anti-A titration is performed; the serial dilution was prepared as described in Transfusion Medicine policy, Antibody Titration. All ten test tubes are reactive. The allohemagglutinin titer is not reported as 512 because the tube containing the apparent end point is not immediately followed by a tube in the serial dilution that is non-reactive. Because the end point requirements are not met, it will be necessary to prepare and test additional serial dilutions (using tube #11, which was saved).

VII. SPECIMEN COLLECTION AND HANDLING:

The specimen of choice is a 6 mL ETA sample with affixed identifying label, as described in Transfusion Medicine policy, Triaging and Identifying Acceptable Samples for Testing.

VIII. REAGENTS / EQUIPMENT / SUPPLIES:

A. Commercial A_2 reverse typing cells, 3-4% suspension.
B. Anti-IgG Anti-Human Globulin (AHG)
C. IgG Coated Check Cells
D. Normal saline
E. 12 x 75 mm test tubes
F. 13 x 100 mm test tubes
G. 50 µL calibrated pipette
H. 100 µL calibrated pipette
I. 500 µL calibrated pipette
J. Disposable pipette tips
K. Vortex mixer

IX. PROCEDURE:

A. Document the following information on the *Allohemagluttinin Titre Worksheet*:
    1. Patient's name, medical record number, and birth date.

A computer generated label may be used for this purpose.

2. Current sample collection date.
3. Testing date and technologist's initials.

B. Determine the patient's ABO group. Document the following:

1. Patient's blood type.
2. ABO antibody for which titer will be performed.
3. Appropriate test cell manufacturer, ABO type, lot number, and expiration date.

C. Prepare serial dilutions of the patient's sample as directed below.

If the patient is:	Then:
Group A	No further testing is indicated. Proceed to the *Test Resulting* section.
Group B	Prepare a serial dilution of the patient's plasma.
Group O	
Group AB	No further testing is indicated. Proceed to the *Test Resulting* section.

D. To each of the tubes containing the serial dilutions, pipette 50 µL of the appropriate test RBCs. Using proper pipetting technique, remember to use a new tip for each tube and to wipe the pipette tip.

1. Do not add enhancement media.

E. Gently agitate the test tubes.

F. Incubate at room temperature for 30 minutes ± 1 minute.

G. Centrifuge tubes according to calibrated time.

H. In the order of highest dilution to lowest, gently re-suspend the cell buttons to read and grade reactions at the room temperature phase. Reactive tubes are graded from weak+ to 4+. Document the graded reactions on the *Allohemagluttinin Titre Worksheet* under the "RT" column.

1. Do not read tubes microscopically.

I. Incubate all tubes for 30 minutes at 37°C ± 1°C.

J. Wash the tubes in an automatic cell washer for 4 cycles.

1. Alternatively, wash 3 - 4 times with large volumes of saline. Decant completely after the last wash.

K. Add 2 drops of anti-IgG Anti-Human Globulin (AHG) to each tube. Mix tubes and centrifuge.

L. Read and grade the tubes in order of highest dilution to lowest dilution. Do not read microscopically. Reactive tubes are graded from weak+ to 4+.

M. Document the graded reactions on the *Allohemagluttin Titer Worksheet* under the "AG" column.

N. Add IgG coated check cells to all tubes that are negative at the AHG phase. Agitate tubes to mix. Centrifuge according to calibrated time.

O. Gently re-suspend the cell button. Read, grade, and record coated cell test results under the

"CC" column on the *Allohemagluttin Titre Worksheet.*

P. Interpret the recorded reactions.

Q. Save an aliquot of the patient plasma in the freezer.

X. INTERPRETATION AND REPORTING:

A. Grading Reactions

1. Antibody titers are not read microscopically. Reactive tubes are graded from weak+ to 4+. For additional information refer to Transfusion Medicine policy, Reading, Grading, and Recording Test Reactions.

B. End Point

1. The end point corresponds to the last tube of the serial dilution displaying macroscopic agglutination. The tube containing the end point must be immediately followed by a tube in the serial dilution that is non-reactive.

C. Titer Result

1. The titer result is the reciprocal of the end point. For example:
    a. The last tube of the serial dilution displaying macroscopic agglutination is tube #8, which corresponds to the 1:128 dilution. Tube #9 is non-reactive. The end point is 1:128. The allohemagglutinin titer is 128.

2. The following table indicates the dilutions that correspond to the labeled tubes of the serial dilution, and the titer results that should be reported if the end point is observed in that tube.

Serial Dilutions		
Tube	**Dilution**	**Titer**
1	1:1	1
2	1:2	2
3	1:4	4
4	1:8	8
5	1:16	16
6	1:32	32
7	1:64	64
8	1:128	128
9	1:256	256
10	1:512	512

D. Titer > 512

1. If all ten tubes of the serial dilution are reactive, the titer is not reported as 512 because the end point requirements are not met; the tube containing the apparent end point is not immediately followed by a tube in the serial dilution that is non-reactive. Because the end point requirements are not met, it will be necessary to prepare and test additional serial dilutions (using tube #11, which was saved).

E. Result Reporting

1. A technologist will enter the allohemagglutinin titer results in the Blood Bank computer and will ensure that they interface to the HIS. The report shall indicate the following:
    a. The anti-A titer results (if applicable) at both phases.
    b. The patient's blood type.
    c. The sample collection date.
    d. The date of testing.
2. If the patient's blood group is A or AB, then the report should indicate that titration of anti-A and anti-B is not indicated.
3. The allohemagglutinin titer results will be documented:
    a. On the *Allohemagluttinin Titer Worksheet.*
    b. As a comment in the Blood Bank computer.

XI. NOTES:

A. An aliquot of plasma from all patient samples on which an antibody titration is performed shall be frozen. The frozen sample aliquot will be thawed and used as the control sample, if necessary to be tested in parallel with a subsequent sample.

B. It is not necessary to test the allohemagglutinin titer in parallel with a control sample. For additional information about parallel testing with a control sample, refer to Transfusion Medicine policy, Antibody Titration and the *Policies Relating to Transplant Patients* section of this document.

XII. REFERENCES:

1. AABB, *Technical Manual*, current edition.
2. AuBuchon, J.P., de Wildt-Eggen, J., and Dumont, L.J., *Reducing the Variation in Performance of Antibody Titrations, Vox Sanguinis* (2008) 95, 57-65.
3. Harmening, Denise M., *Modern Blood Banking and Transfusion Practices*, Third Edition, 1994.

Attachments

Allohemaglutinin Titer Worksheet

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	6/7/2022
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Supv, Laboratory	6/6/2022
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	6/6/2022
	Kimberly Geck: Dir, Lab Operations B	6/4/2022
	Kelly Sartor: Supv, Laboratory	6/3/2022
	Kelly Sartor: Supv, Laboratory	6/3/2022

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ALLOHEMAGLUTININ TITER WORKSHEET

Name:		A1 Test cell: mfg, lot# and exp.	
MRN:		A2 test cell: mfg, lot# and exp.	
DOB:	ABO/Rh:	B test cell: mfg, lot# and exp.	
ABO antibody(ies) for which titer is performed:		Coombs Control Cells: mfg, lot# and exp.	
Tech:	Date:	Sample Date:	

		A1 Titer			A2 Titer			B Titer		
		RT	AHG	CC	RT	AHG	CC	RT	AHG	CC
1	1:1									
2	1:2									
3	1:4									
4	1:8									
5	1:16									
6	1:32									
7	1:64									
8	1:128									
9	1:256									
10	1:512									
11	1:1024									
12	1:2048									

Titer Result:		RT	AHG
	A_1		
	A_2		
	B		

Reviewed By: _____ Date: _____