Status Scheduled PolicyStat ID 11807712

Beaumont

Origination	6/22/2022
Last Approved	6/7/2022
Effective	6/22/2022
Last Revised	6/7/2022
Next Review	6/6/2024
Document Contact	Kelly Sartor
Area	Laboratory-Blood Bank
Applicability	Dearborn

Antibody Titration - Dearborn Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide the Blood Bank staff with policies and procedures to perform antibody titrations.

II. PRINCIPLE:

A. Titration is a semi-quantitative technique used to assess the ability of a known antibody to react with the corresponding antigen. Most frequently, titration is used to evaluate the potential of a clinically significant unexpected antibody in an obstetrical patient to cause hemolytic disease of the newborn (HDN). The investigation of HTLA (high titer low avidity) antibodies also involves a semi-quantitive titration. For additional information refer to Transfusion Medicine policy, *HTLA / Anti-Bg^a Investigations*.

B. Several factors contribute to the difficulty that is associated with the standardization of antibody titration. These factors include technologists' pipetting techniques and the antigenic strength, age, and concentration of the test cell chosen for the titration. To offset these variables, a control sample is frozen and tested in parallel with a subsequent patient sample. The titer result of the current sample and the control sample can be compared by the physician to assess the antibody's potential clinical impact.

III. DEFINITIONS:

A. **Titer Screen**: A titer that is tested with only the 1:1 dilution. A titer screen is used to save time and resources when the antibody titer is expected to be below detectable levels in the test method.

B. **Control Sample**: An obstetrical patient's prior, most recently submitted sample from the

current pregnancy. The control sample is frozen and then thawed when a subsequent sample is received. The control sample is then diluted and tested in parallel with the subsequent sample (the current sample).

C. **Standard Cell Panel**: A commercially prepared panel that usually consists of 11 vials of human RBCs. It is usually performed on patients who do not have a historical antibody record.

D. **Twofold Titer Increase**: When the titer of the current sample is at least four times higher than the titer of the control sample when tested in parallel (The endpoint of the current sample is observed in at least two tubes of the serial dilution higher than the endpoint of the control sample).

E. **Combination Titer**: An antibody titration of multiple unexpected antibodies that will be performed using a test cell that is positive for multiple antigens corresponding to the clinically significant antibodies.

F. **Designee**: A Blood Bank technical director or transfusion medicine fellow.

G. **LIS**: Laboratory Information System.

H. **HIS**: Hospital Information System.

IV. POLICIES:

A. An antibody titration shall be performed on all obstetrical patients with clinically significant antibody(ies).

B. Antibody titrations shall also be performed on obstetrical patients with antibodies that are considered to be of varying clinical significance; i.e., anti-M and anti-N when specifically requested by Medical Director or the patient's obstetrician.

C. The technologist shall consult a supervisor or the Medical Director (MD) if there is any question as to whether a titration shall be performed.

D. Antibody titrations shall generally be performed once per month throughout the patient's pregnancy. If the obstetrician requests titrations to be performed more frequently, then the Blood Bank will do so, but not more frequently than every two weeks.

E. If an antibody titration is ordered and two weeks have not elapsed since the previous titer, then the Medical Director shall be consulted.

F. It is not necessary to perform a titration when the mother is admitted for delivery of the infant.

G. An antibody panel must be performed on the current sample on which the titer is performed in order to exclude the presence of additional unexpected antibodies.

H. 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, to be tested in parallel with a subsequent sample. If possible, avoid freezing the entire volume of patient plasma. This will allow for additional testing to be performed on the current sample, if necessary.

A. 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 reaction shall be graded as described in Transfusion Medicine policy, Reading, Grading, and Recording Test Reactions.

B. 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 of the 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.

C. Control Sample Policy for Obstetrical Patients

1. The control sample is an obstetrical patient's prior, most recently submitted sample from the current pregnancy. The control sample is frozen and then thawed when a subsequent sample is received. The control sample is then diluted and tested, in parallel with the subsequent sample (the current sample).
2. The titer of the current sample, tested in parallel with the control sample, should be within two dilutions of the control sample titer. If the titers of the current and control samples are not within two dilutions, the Medical Director shall be consulted as this may represent a clinically significant increase in the patient's antibody titer.
3. Note that it is not necessary to perform a parallel titer with a control sample when performing an allohemagglutinin titration; see Transfusion Medicine policy, Allohemagglutinin Titration.

D. 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:
    a. A serial dilution is made using ten test tubes. All ten test tubes are reactive; tube # 10 is weak+. The 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).

E. Patients with Multiple Antibodies

1. If multiple unexpected antibodies are present, then each antibody should be titered individually. See the *Appropriate Test Cell for Titration* section of this document.
2. However, in some cases involving multiple unexpected antibodies it may be beneficial to perform a combination titer. These may be performed in situations where the patient's antibody profile makes it difficult to titer out each antibody individually. Combination titers should not be performed unless directed to by the Blood Bank Medical Director.

F. Performing a Titration with Fewer than Ten Test Tubes

1. The *Procedure* below includes directions for preparing serial dilutions using ten test tubes; the dilution of tube # 10 is 1:512. In many cases, it is acceptable to perform a titer with fewer than 10 test tubes in order to save time and resources. For example:
    a. A patient's titer result from the last 3 months / last 3 specimens has been 1:4. It is acceptable to perform a titer with, for example, 6 test tubes, so long as the titer end point requirements are met.

G. Titer Screen

1. If a patient's titer result is anticipated to be or has consistently been less than 1, then it is acceptable to perform a titer using only one test tube (the 1:1 dilution). A titer consisting of only the 1:1 dilution is referred to as a "titer screen".
    a. If the titer screen is non-reactive, then the titer is reported as less than 1.
    b. The control sample shall be tested in parallel with a titer screen.
    c. If the titer screen is reactive, it will then be necessary to repeat the titer with additional dilutions because the end point requirements have not been met.

H. Notification of the Patient's Physician

1. The general indications for notifying the patient's physician are:
    a. If an anti-Kell titer is 8 or greater, then the patient's physician and Blood Bank Medical Director will be notified (each time a titer is performed).
    b. If the titer of any antibody is 16 or greater (besides anti-Kell, see above), then the patient's physician and Blood Bank Medical Director will be notified (each time a titer is performed).
    c. For all antibodies: If the titer of the current sample increases twofold over the titer of the control sample when tested in parallel, then the patient's physician and Blood Bank Medical Director should be notified (each time a titer is performed).
    d. If the titer of the current sample is higher than the titer of the control sample when tested in parallel then the Blood Bank Medical Director will be consulted to determine whether the patient's physician should be notified.

I. Appropriate Test Cell(s) for Antibody Titrations

Antibody	Test RBC		Antibody	Test RBC
Anti-D	R_2R_2		Anti-Fy^a	Fy(a+b-)
Anti-C	R_1R_1		Anti-Fy^b	Fy(a-b+)
Anti-D and Anti-C	R_2R_2 and r'r		Anti-Jk^a	Jk(a+b-)
Anti-E	R_2R_2		Anti-Jk^b	Jk(a-b+)

Anti-D and Anti-E	R_1R_1 and r"r		Anti-K	K+ k-
Anti-c	rr		Anti-k	K- k+
Anti-c and Anti-E	R_2R_2		Anti-M	M+ N-
Anti-e	rr		Anti-N	M- N+
Anti-e and Anti-C	R_1R_1		Anti-S	S+ s-
			Anti-s	S- s+
			Anti-U	S+ s+

1. The freshest available test cell shall be chosen; expired test cells shall not be used. However, see the following exception for *Anti-Kell Titrations*.
2. If possible, test cells shall be chosen from antibody screen cell sets, or from panels consisting of 16 or 20 test cells, and not from standard 11- cell panels.
3. As indicated in this table, test cells are chosen based on homozygous expression of the antigen corresponding to the clinically significant antibody.
4. If multiple antibodies are present and each antibody is being titered individually, the test cells used should be positive for only one of the antigens corresponding to the patient's unexpected antibodies and should be negative for all other antigens corresponding to the patient's other unexpected antibodies. Note the exceptions in the table above for the following multiple antibody combinations: Anti-C & D, Anti-D & E, Anti-c & E, and Anti-e & C.
5. If multiple antibodies are present and the Blood Bank Medical Director determined that a combination titer should be performed, select a test cell that has the strongest expression of multiple antigens corresponding to the clinically significant antibodies. If an in-date test cell that matches these guidelines is unavailable, consult the Blood Bank Medical Director to determine the appropriate test cell to use.
6. Anti-Kell Titrations
    a. The appropriate test cell for a Kell titer is an in-date homozygous K+ k- cell. In some cases, an in-date homozygous K+k- test cell may be unavailable. If an in-date homozygous K+k- test cell is unavailable, then:
        i. The titer shall be tested against an expired homozygous (K+k-) test cell, if one is available, AND against an in-date heterozygous (K+k+) test cell.
            A. Note that it may be possible to obtain an expired homozygous (K+k-) test cell from another facility.
            B. Note that expired panel cells should be discarded after 3 months.
        ii. If a homozygous test cell is unavailable (either in-date or expired), then the titer shall be tested against only against an in-date heterozygous (K+k+) test cell.
        iii. The highest titer result (between the homozygous and the heterozygous test cell) should be reported in the computer.
        iv. The antibody card and the **CMTXT** shall be documented with the titer results against both test cells, also indicating whether the cells were K+k-

or K+k+.

v. The control sample should be tested in parallel with both test cells (homozygous and heterozygous), as usual.

V. SPECIMEN COLLECTION AND HANDLING:

The preferred specimen is a 6 ml EDTA sample with affixed identifying label. See Transfusion Medicine policy, Triaging and Identifying Acceptable Samples for Testing, for acceptable alternatives.

VI. EQUIPMENT:

A. 37°C heat block or water bath
B. Vortex mixer
C. Cell Washer
D. Serofuge

VII. SUPPLIES:

A. Saline
B. 100 µL pipette and 50 µL pipette
C. Pipette tips
D. 12 x 75 mm and 13 x 100 mm test tubes
E. Test RBC's, Group O, 3 - 4% suspension
F. Anti-IgG Anti-Human globulin (AHG)
G. IgG Coated Check Cells

VIII. QUALITY CONTROL (QC):

A. IgG coated check cells must be added to all AHG phase results that are negative. If any tube of the titer that requires IgG coated check cells reacts negatively with the IgG coated check cells, then that result is considered invalid. It may be necessary to repeat the titer; see the *End Point Requirements* section of this document.
B. The control sample will be tested in parallel with the most current sample.

IX. BEFORE YOU GET STARTED:

A. Retrieve the control sample (if available) from the freezer and allow it to thaw at room temperature before testing. Document the following on a *Antibody Titers Worksheet:*
    1. Patient's name, medical record number, and birth date. A label that is generated from the Beaker Laboratory Information System may be used for this purpose.
    2. The specificity of the antibody for which the titer will be performed.
    3. The test cell phenotype.

4. The test cell manufacturer, lot number, expiration date, and cell identification number.
5. The collection date of the current and control samples.
6. The technologist's initials and date.

X. PROCEDURE:

Note that the serial dilution of the **current sample** will be made first. The serial dilution of the **control sample** will be made after the serial dilution of the current sample is made.

A. Preparation of Serial Dilutions

1. Perform the actions indicated in the *Before You Get Started* section of this document.
2. Label and fill a 13 x 100 mm test tube with saline.
3. Label a set of 11(12 x 75 mm) test tubes consecutively 1 - 11; also label with the patient's last name and a notation to indicate it is the current sample.
4. Firmly insert a clean disposable tip onto the 100 µL pipette.
5. Depress the pipette plunger and insert the pipette tip into the 13 x 100 mm test tube of saline.
6. Allow the plunger to **slowly** return to its release position to aspirate 100 µL of saline. Gently wipe the outside of the pipette tip.
7. Dispense 100 µL of saline into tube # 2 by depressing the plunger completely. While still depressed, touch the pipette tip to the inside wall of tube #2. **Do not add saline to tube #1.**
8. Remove the tip from the wall of tube #2 and allow the pipette plunger to return to its release position.
9. Repeat steps 5 - 8 to dispense 100 µL of saline into each of the remaining numbered test tubes (#3 - #11).
10. Using a new tip, pipette 100 µL of patient plasma into tube #1, remove and discard tip.
11. Using a new tip, pipette 100 µL of patient plasma into tube #2, remove and discard tip.
12. Using the Vortex mixer, mix the contents of tube #2 approximately 5 - 10 seconds.
13. Using a **clean pipette tip**, transfer 100 µL of the plasma/saline mixture from tube #2 to tube #3. Vortex contents of tube #3 approximately 5 - 10 seconds.
14. Using a **clean pipette tip**, transfer 100 µL of the plasma/saline mixture from tube #3 to tube #4. Vortex contents of tube 4 approximately 5 - 10 seconds.
15. Continue to transfer and Vortex the plasma/saline mixture, from tube to tube as described above, through tube #10.
16. Using a **clean pipette tip**, remove 100 µL of the plasma/saline mixture from tube #10 (or the last tube of the serial dilution) and transfer to tube #11. **Important: Save the last tube (tube #11)** in case the end point requirements are not met.
17. Determine whether a control sample is available, and whether testing of the control sample is indicated.

18. If a control sample is available and testing is indicated:
    a. Label another set of 11 test tubes consecutively 1 - 11 for the control sample; also label with the patient's last name and a notation to indicate it is the control sample.
    b. Mix the thawed control sample thoroughly for 5 - 10 seconds with the Vortex mixer.
    c. Proceed to step 20.
19. If a control sample is unavailable or testing is not indicated, proceed to step 21.
20. Repeat steps 4 - 16 to prepare a serial dilution of the control sample.
21. If the titration of multiple antibodies is necessary; repeat steps 1-20 for any additional antibodies.
22. Proceed to the *Test Procedure for Antibody Titration* procedure below to perform the antibody titration using the serial dilutions.

B. Test Procedure for Antibody Titration

1. Obtain the tubes containing the serial dilutions which were prepared as described in the *Preparation of Serial Dilutions* procedure above.
2. Pipette 50 µL of the appropriate test RBCs to each of the tubes of the serial dilution. Gently agitate the tubes. **Do not add enhancement media.**
3. Incubate all tubes at 37°C for 30 minutes ± 1 minute.
4. Wash all tubes 4 times using the automatic cell washer.
    a. Alternatively, wash manually 4x with large volumes of saline; decant completely after each wash.
5. Add 2 drops of Anti-IgG Anti-Human Globulin (AHG) to each tube. Mix tubes and centrifuge.
6. 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+.
7. Document graded reactions on the *Antibody Titration Worksheet* under the "AG" column.
8. Add IgG coated check cells to all tubes that are non-reactive at the AHG phase. Agitate tubes to mix. Centrifuge according to calibrated time.
9. Gently re-suspend the cell button. Read, grade, and record coated check cell results under the "CC".
    a. Coated cells must react positively at any strength, otherwise the test must be repeated.
10. Interpret the recorded reactions and report the titer results.
11. Discard the control sample (which was used in the parallel testing) ONLY if the Supervisory Review is done and the results are accepted.
12. If sufficient volume is present, freeze an aliquot of the current sample (to be used as a control sample if/when a next sample is received). Label it with the patient's name, ID number and date. Store it at -20°C or colder. Document the aliquot was frozen on *Antibody Titer Worksheet*.
13. Submit the completed antibody titer worksheet and a copy of the panel antigram to the Blood

Bank Supervisor/ Lead Medical Technologist or Blood Bank Medical Director or designee for review and physician notification, if indicated.

XI. INTERPRETATION:

A. Twofold titer increase:

1. When the titer of the current sample is at least four times higher than the titer of the control sample when tested in parallel. (The endpoint of the current sample is observed in at least two tubes of the serial dilution higher than the endpoint of the control sample). Following is an example of a twofold titer increase:

    a. The titer of the current sample is 64 and the titer of the control sample is 16. The endpoint of the current sample is observed in test tube # 7, and the endpoint of the control sample is observed in test tube # 5, which is at least two tubes higher in the serial dilution. This is a twofold titer increase.

B. The following table indicates the dilutions that correspond to the labeled tubes of the serial dilution, and the titer result that should be reported if the end point is observed in that tube; see *End Point*, below.

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

C. Grading Reactions

1. Antibody titers are not read microscopically. Reactive tubes are graded from weak+ to 4+.

D. 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. See the *Titer End Point Requirements* section of this document for additional information.

E. 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 # 4, which corresponds to the 1:8 dilution. Tube # 5 is non-reactive. The end point is 1:8. The titer result is 8.

XII. LIMITATIONS:

A. The following may influence the validity of test results:
    1. Technical variability can greatly influence the titration results.
    2. Careful pipetting technique is essential. The failure to change pipette tips may lead to erroneous results.
    3. The age, phenotype, and concentration of the test RBCs may affect titer results.

XIII. TEST REPORTING:

A. The technologist will enter the antibody titration results in the Blood Bank computer as described in the Blood Bank CDM, Resulting Antibody Titers. This report will indicate the following:
    1. The titer result(s) of the current sample,
    2. The titer result(s) of the control sample,
    3. The date of the control sample,
    4. The date of the current sample, and
    5. The specificity of the antibody(ies).
B. This result will interface to the HIS.

XIV. NOTES:

A. The Antibody Titer test code (TITR) is a profile test code that automatically includes tests for the current sample titer and the control sample titer. If a control sample is unavailable, then the Control Titer will be canceled in SoftBank using the Blood Bank CDM - Cancelling Orders in SoftBank.
B. If multiple antibodies are present, then additional Titer test codes (TITR2, TITR3) orders must be added to the accession order and resulted separately so that all test results are available in the HIS.
C. Only one titer is performed for the combinations Anti-c, E and Anti-e, C. The canned messages TITR1 and TITR2 will be used to report that a single titer was performed; these canned messages also indicate the phenotype of the test cell.

XV. 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

Antibody 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	5/31/2022
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	5/31/2022
	Kimberly Geck: Dir, Lab Operations B	5/30/2022
	Kelly Sartor: Supv, Laboratory	5/27/2022
	Kelly Sartor: Supv, Laboratory	5/27/2022

Beaumont HEALTH

Beaumont Laboratory

ANTIBODY TITER WORKSHEET

Name:	Homozygous Test Cell#
MRN:	Mfg:
DOB: ABO:	Lot#:
Antibody(ies):	Exp:
Tech: Date:	*Attach Antigram sheet from panel cell used for titer.*

Titer		Results							
		Current Sample		**Control Sample**		**Current Sample**		**Control Sample**	
		Anti-		Anti-		Anti-		Anti-	
		AHG	CC	AHG	CC	AHG	CC	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:	Titer reviewed by: Tech: _____ Date Reviewed:_____
Sample aliquot frozen: Y_____ N_____ If no aliquot frozen, why? _____	Does the Physician or Medical Director need to be notified? Y____ N____ Who was notified? _____ Date and time of notification: _____