PROCEDURE
Corewell Health East - Antibody Titration - Blood Bank - All Beaumont Hospitals

This Procedure is Applicable to the following Corewell Health sites:
Corewell Health Beaumont Grosse Pointe Hospital, Corewell Health Beaumont Troy Hospital, Corewell Health Dearborn Hospital, Corewell Health Farmington Hills Hospital, Corewell Health Taylor Hospital, Corewell Health Trenton Hospital, Corewell Health Wayne Hospital, Corewell Health William Beaumont University Hospital (Royal Oak)

Applicability Limited to: N/A

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Functional Area: Clinical Operations, Laboratory

Lab Department Area: Lab - Blood Bank

1. 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 obstetric patient to cause hemolytic disease of the fetus and newborn (HDFN). The investigation of HTLA (high titer low avidity) antibodies also involves a semi-quantitative titration. For additional information, refer to Transfusion Medicine policy, Corewell Health East - HTLA / Anti-Bga Investigation - Blood Bank - All Beaumont Hospitals.

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 if the antibody's strength is changing and its potential clinical impact.

2. Responsibility

Personnel who have completed the competency requirements will perform this testing.

3. Definitions

A. **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).

B. **Standard Cell Panel**: A commercially prepared panel that usually consists of 11 vials of human RBCs.

C. **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).

D. **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.
E. **Designee**: A Blood Bank technical director or transfusion medicine fellow.
F. **BBIS**: Blood Bank Information System
G. **HIS**: Hospital Information System
H. **LIS**: Laboratory Information system
I. **Titer Screen**: A titer that is tested with only the 1:1 dilution.

4. Specimen

The preferred specimen is a 6 mL EDTA sample with an affixed identifying label. The specimen must meet all pertinent criteria for sample acceptability. See Transfusion Medicine policy, Corewell Health East - Triaging And Identifying Acceptable Samples For Testing- Blood Bank - All Beaumont Hospitals.

5. Reagent/Equipment Needed

A. 37°C heat block or water bath
B. Vortex mixer
C. Cell Washer
D. Serofuge
E. Saline
F. 100ul pipette and 50µL pipette
G. Pipette tips
H. 10 x 75 mm or 12 x 75 mm test tubes (site specific, use tubes which were included in serofuge calibration).
I. 13 x 100 ml tubes, optional
J. Test RBC's, Group O, 3-4% suspension
K. Antihuman globulin (AHG)
L. IgG Coated Check Cells

6. Quality Control

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 *Titer End Point Requirements* in the *Results/Interpretation* section of this document.
B. An aliquot of plasma from all patient samples on which antibody titration is performed shall be frozen to be used as the control sample.
C. The control sample will be tested in parallel with the most current sample. The control sample is an obstetric patient's prior, most recently submitted sample from the current pregnancy. The control sample is thawed when a subsequent sample is received. The control sample is then diluted and tested, in parallel with the subsequent sample (the current sample).
D. 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.
E. When the titration is done and the results are confirmed and accepted, the control sample is discarded and the current sample becomes the control for the next titration.
F. Antibody titrations shall be performed on all obstetric patients with a clinically significant antibody(ies).
    1. Antibody titrations shall also be performed on obstetric patients with antibodies that are considered to be of varying clinical significance, i.e., anti-M and anti-N, when specifically requested by the patient's obstetrician. An aliquot of plasma from obstetric patients with an

anti-M or anti-N should be frozen, regardless of whether an antibody titration was performed. In the event the clinician asks for testing at a later date, this specimen can be used as the current or control sample.

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

G. Antibody titrations shall generally be performed once per month throughout the patient's pregnancy with the following exceptions:
   1. 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. If antibody titration is ordered and two weeks have not elapsed since the previous titer, then the Medical Director shall be consulted.
   2. It is not necessary to perform a titration when the mother is admitted for delivery of the infant.
   3. Once a “critical” titer is reached during the current pregnancy (see Section 8.E for titer results that require notification of the patient’s physician), additional titers during the same pregnancy are not indicated. If the obstetrician requests/orders subsequent titrations, the Blood Bank will do so on one more sample (to help confirm the critical result). If titers are ordered beyond this, then the Medical Director shall be consulted.

H. It is very important for technologists to grade the test reactions of antibody titration consistently. Reactive tubes are graded from weak+ to 4+. Test reaction shall be graded as described in Transfusion Medicine policy, Corewell Health East - Reading, Grading, and Recording Test Reactions - Blood Bank - All Beaumont Hospitals.

I. A new pipette tip must be used for each tube of the serial dilution. 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. 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.

J. If multiple unexpected antibodies are present, then each antibody should be titrated individually. See the *Appropriate Test Cell(s) for Antibody Titrations* section of this document.
   1. 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 in this procedure, or by the Blood Bank Medical Director.

K. Appropriate Test Cell(s) for Antibody Titrations

Table 1:

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 cells shall be chosen; expired test cells shall not be used. See the exception for *Anti-Kell Titrations* below.
2. If possible, test cells should 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 Table 1, 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 titrated individually, the test cells used should be homozygous 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 has 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:
        1) 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) It may be possible to obtain an expired homozygous (K+k-) test cell from another facility.
            b) Expired panel cells should be discarded after 3 months.
            c) If the anti-K titer result is 8 or greater with a heterozygous cell, it is unnecessary to reach out to additional sites for a homozygous cell.
        2) If a homozygous test cell is unavailable (either in-date or expired), then the titer shall be tested only against an in-date heterozygous (K+k+) test cell.
        3) The highest titer result (between the homozygous and the heterozygous test cell) should be reported in the computer.

4) The antibody card or folder (if indicated per site policy) and/or a Patient Profile Note in the BBIS shall be documented with the titer results against both test cells, also indicating whether the cells were K+k- or K+k+.
5) The control sample should be tested in parallel with both test cells (homozygous and heterozygous), as usual.

7. In general, subsequent titers should be performed using cells similar to what was selected per policy previously in order to keep the results consistent (e.g. If R2R2 and r'r cells were tested to titer anti-D and anti-C, do not suddenly switch to R1R1 combination titer. Or if K+k+ heterozygous cells had to be used, keep using heterozygous cells for the duration of the pregnancy).

7. Procedure

Before Starting the Titer:

A. A standard gel antibody screen must be performed.
1. For patients without a history of an antibody, if the screen is negative, then no titration is needed. Add a test result comment to the antibody screen "**antibody titer not indicated, no current or historical antibodies identified**".
2. For patients with a history of an antibody:
a. If the antibody screen is negative, confirm that the screen contained a cell with homozygous expression for any applicable antibody(ies) to be titered. If not, a homozygous cell must be tested as well.
1) If no in date homozygous cell is available, or if the homozygous cell is also negative, the titer will be cancelled as not indicated. A test result comment should be added to the antibody screen "**antibody titer not indicated, Anti-[specificity] not reactive in the current specimen**".
b. If the antibody screen (or homozygous test cell) is positive, an antibody panel must be performed on the current sample on which the titer is to be performed to exclude the presence of additional unexpected antibodies if any of the below statements apply:
1) A panel has not been performed in the last 30 days.
2) The reaction strength of the antibody screen has increased.
3) The antibody screen results do not match the historical antibody(ies).

B. Retrieve the control sample (if available) from the freezer and allow it to thaw at room temperature before testing. Vortex the sample before testing.
1. If the prior testing was completed at another Corewell Health East facility, request the site to Transfer the control specimen.

C. Document the following on the *Antibody Titer Investigation Worksheet*.
1. Patient's name, medical record number, and birth date. A label that is generated from the hospital information system (HIS) may be used for this purpose.
2. The specificity of the antibody for which the titer will be performed.
3. The test cell phenotype. Refer to Table 1: *Appropriate Test Cells for Antibody Titrations*.
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.

D. Titer Screen:
7. 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 interpretation is 0 which corresponds to <1:1.
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.

E. Performing a Titration with fewer than Ten Test Tubes:

8. 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.

Preparation of Serial Dilutions:

A. Perform the actions indicated in the *Before Starting the Titer* section of this document.
B. Label and fill a 12 x 75 mm or 13 x 100 mm test tube with saline.
C. Label a set of 11 (10 x 75 mm or 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.
D. Firmly attach a clean disposable tip onto the 100 µL pipette.
E. Depress the pipette plunger and insert the pipette tip into the 13 x 100 mm test tube of saline.
F. Allow the plunger to slowly return to its release position to aspirate 100 µL of saline. Gently wipe the outside of the pipette tip.
G. 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**.
H. Remove the tip from the wall of tube #2 and allow the pipette plunger to return to its release position.
I. Repeat steps E - H to dispense 100 µL of saline into each of the remaining numbered test tubes (#3 - #11).
J. Using a new tip, pipette 100 µL of patient plasma into tube #1, remove and discard tip.
K. Using a new tip, pipette 100 µL of patient plasma into tube #2, remove and discard tip.
L. Using the Vortex mixer, mix the contents of tube #2 approximately 5 - 10 seconds.
M. 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.
N. 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.
O. Continue to transfer and vortex the plasma/saline mixture, from tube to tube as described above, through tube #10.
P. 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.
Q. Determine whether a control sample is available, and whether testing of the control sample is indicated.
R. If a control sample is available and testing is indicated:
    1. 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.
    2. Mix the thawed control sample thoroughly for 5 - 10 seconds with the Vortex mixer.
    3. Proceed to step T.
S. If a control sample is unavailable or testing is not indicated, proceed to step U.
T. Repeat steps D - P to prepare a serial dilution of the control sample.
U. If the titration of multiple antibodies is necessary, repeat steps A - P for any additional antibodies.
V. Proceed to *Test Procedure for Antibody Titration* below to perform the antibody titration using the serial dilutions.

Test procedure for Antibody Titration:

A. Obtain the tubes containing the serial dilutions which were prepared as described in the Preparation of Serial Dilutions procedure above.
B. 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**.
C. Incubate all tubes at 37°C for 30 minutes ± 1 minute.

D. Wash all tubes 4 times for 12 x 75mm tubes, or 6 times for 10 x 75mm tubes using the automatic cell washer. Alternatively, wash manually 4-6 times with large volumes of saline; decant completely after each wash.
E. Add 2 drops of Anti-IgG Anti-Human Globulin (AHG) to each tube. Mix tubes and centrifuge **according to calibrated time**.
F. 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+.
G. Document graded reactions on the *Antibody Titer Worksheet* or *Special Studies Worksheet* under the "AHG" column.
H. 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.
I. Gently re-suspend the cell button. Read, grade, and record coated check cell results under the "CC" on the *Antibody Titer Worksheet* or *Special Studies Worksheet*. Check cells must react positively at any strength, otherwise the test must be repeated.
J. Interpret the recorded reactions and report the titer results in the BBIS following instructions below in the *Results/Interpretation* section.
K. Discard the control sample (which was used in the parallel testing) ONLY if the Supervisory Review is done and the results are accepted.
L. If sufficient volume is present, freeze an aliquot of the current sample (to be used as a control sample if/when the next sample is received). Label it with the patient's name, ID number, the date of collection, the antibody(ies) tittered, and the result(s). A printed label for the HIS may be used for this. Store it at -20°C or colder. Document the aliquot was frozen on *Antibody Titer Worksheet* or *Special Studies Worksheet*.
M. Submit the completed *Antibody Titer Worksheet* or *Special Studies Worksheet* and a copy of the panel antigram for supervisory review.

8. Results/Interpretation

A. Antibody titers are not read microscopically. Reactive tubes are graded from weak+ to 4+.
B. Titer End Point Requirements: 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 that is non-reactive (negative). For example:
   1. A serial dilution is made, and the tubes are incubated. Tube #5 is 1+ reactive and tube #6 is 0 (negative). The titer is 1:16 because the last tube with agglutination is tube #5.
   2. A serial dilution is made using ten test tubes as described in Table 2. All ten test tubes are reactive; tube # 10 is weak+. The titer is not reported as 1:512 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).
C. 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.

Tube	Dilution / Titer Interpretation in BBIS
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

**If the antibody screen is only positive by the gel method and the saline tube titration results are negative, report the titer as “0” which corresponds to <1:1.

D. Twofold titer increase:
   1. When the titer of the current sample is at least four times higher than the titer of the contro l 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). The following is an example of a twofold titer increase:
      a. The titer of the current sample is 1:64 and the titer of the control sample is 1:16. 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.

E. Notification of the Patient’s Physician:
   1. If an anti-K titer is 1:8 or greater, then the patient’s physician and the Blood Bank Medical Director will be notified (once per pregnancy).
   2. If the titer of any antibody is 1:16 or greater (besides anti-K, see above), then the patient’s physician and the Blood Bank Medical Director will be notified (once per pregnancy).
   3. 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 the Blood Bank Medical Director should be notified (once per pregnancy).
   4. Document the notification in a Patient Profile Note in the BBIS.

F. Test Reporting:
   1. Ensure the proper test is ordered in the BBIS.
      a. Obstetric titers are ordered in the LIS as Antibody Titer (LAB275), ordered in the BBIS as test battery: Ab 1 Titer. If two or more alloantibodies are being titrated and/or a previous sample is also being titrated, order an additional antibody titer in the BBIS for each antibody as described in the chart below. Note: Do not cancel any duplicate titer orders until all applicable results have been entered and results have interfaced to the HIS.

Antibody Titer	Test Name in BBIS	Test Battery Name
Antibody 1	Ab 1 Titer	TITER
Antibody 1- Previous Sample	Ab 1 Pre Titer	ABPRE1TIT
Antibody 2	Ab 2 Titer	TITER2
Antibody 2 – Previous Sample	Ab 2 Pre Titer	AB2PRETIT
Antibody 3	Ab 3 Titer	TITER3
Antibody 3- Previous Sample	Ab 3 Pre Titer	AB3PRETIT
Antibody 4	Ab 4 Titer	TITER4
Antibody 4- Previous Sample	Ab 4 Pre Titer	AB4PRETIT

2. For patients with multiple antibodies: Check notes and previous test results in BBIS (include ALL sites by unchecking box for "Only display tests at this location") and confirm which antibody has been resulted as Antibody 1, Antibody 2, etc. Make sure to result with the same designation as previously resulted. Add a Patient Profile Note in BBIS (if not previously added) to indicate the Antibody Titer naming convention for the patient's antibodies, i.e. Ab 1 Titer = Anti-E, Ab 2 Titer = Anti-Fya.
3. Result the antibody titration(s) in the BBIS system.
   b. Both the specificity of the antibody titrated, and the endpoint reaction of the titration are documented separately in the result entry fields.
   c. The endpoint reaction is the value that is documented for the titer value.
   c. If the antibody screen is only positive by gel method and the saline tube titration results are negative, report the titer as 0 which correlates to <1:1.
   d. For previous sample results, add the collection date of the specimen as a free text result comment.
   e. Only one titer is performed for the combinations Anti-c, E and Anti-e, C. When resulting, add a free text result comment indicating that only one test cell was used and indicate the phenotype of the cell.
4. These results will interface to the HIS.

9. 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.
   4. Samples from Trenton, Taylor, and Wayne Blood Banks that require antibody titration will be sent to Dearborn or Royal Oak for testing.
   5. If a titer is ordered and one has been completed in the last 2 weeks, the test will be cancelled in EPIC with the following comment:"**Test cancelled per protocol. Testing performed in the last 2 weeks**".
   6. In the event that a titer is required for a rare antibody where there are no cells available, the titer will be sent to the reference lab. The results will be given to the Manager, Supervisor, or Lead Medical Technologist for resulting.

10. Revisions

Corewell Health reserves the right to alter, amend, modify, or eliminate this document at any time without prior written notice.

11. Procedures Superseded and Replaced: This procedure supersedes and replaces the following procedures as of the effective date of this procedure: [33840 Corewell Health East – Antibody Titration – Farmington Hills]

12. References

A. AABB, Technical Manual, current edition.
B. 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.
C. Harmening, Denise M., Modern Blood Banking and Transfusion Practices, Third Edition,1994.

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9. Keywords
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