**Purpose**

The purpose of this document is to provide the Blood Bank staff with stepwise instructions to perform antibody titrations.

**Principle**

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).

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, the age, and the 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 may be compared by the physician to assess the antibody’s potential clinical impact.

**Scope**

An antibody titration shall be performed on all obstetrical patients with . . .

* Clinically significant antibody(ies).
* Antibodies that are considered to be of varying clinical significance; for example Anti-M and Anti-N.

**Related Procedures**

 The majority of antibody titrations may be performed using the procedures described in this document:

* Table 608-2, *Preparation of a Serial Dilution*
* Table 608-3, *Test Method for Antibody Titration*

However, for the titration of a patient’s multiple antibodies it will be necessary to prepare multiple serial dilutions.

The investigation of HTLA (high titer low avidity) antibodies involves a semi-quantitative titration; for additional information refer to P609, *HTLA / Anti-Bga Investigations.*

**Policies**

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

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.

The technologist shall consult a supervisor or the Medical Director (MD) if there is any question as to whether a titration shall be performed. See also P620, *Interpretation of Antibody Investigations* / *Antibody Titrations as a Tool in the Determination of whether Anti-D Specificity is related to Passive Anti-D due to RhIG Administration, or Alloimmunization****.***

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.

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

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

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.

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.

**Reading and Grading Reactions**

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 P061, *Reading and Grading Test Reactions*.

**Policies Relating to Pipetting Technique**

* 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**.**

**Control Sample Policy for Obstetrical Patients**

* 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).
* 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.

**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 in the serial dilution that is non-reactive. For example:

A serial dilution is made using ten test tubes as described in Table 608-2. 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).

**Patients with Multiple Antibodies**

If multiple unexpected antibodies are present, then a master serial dilution shall be prepared as described in P608, Attachment A, *Preparation of a* *Master Dilution for the Titration of Multiple Antibodies.*  See also *Appropriate Test Cell for Titration,* below.

**Performing a Titration with Fewer than Ten Test Tubes**

Table 608-2 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 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.*

**Titer Screen**

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 1:1dilution is referred to as a “titer screen.”

* If the titer screen is non-reactive, then the titer is reported as less than 1.
* The control sample shall be tested in parallel with a titer screen.
* 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.

**Notification of the Patient’s Physician or of the Blood Bank Medical Director**

* If an Anti-Kell titer is 8 or greater, then the patient’s physician or designee should be notified (each time a titer is performed).
* If the titer of any antibody is 16 or greater (besides Anti-Kell, see above), then the patient’s physician or designee should be notified (each time a titer is performed).
* 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 or designee should be notified (each time a titer is performed).
* Refer to the *Interpretation* section for an example of a twofold titer increase.
* 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 or designee (MD) should be notified at the next daily rounds. The MD will determine whether the patient’s physician should be notified.

**Documentation of Notification**

* The notification of the patient’s physician or designee should be documented as an external message to the antibody titer test in the Blood Bank computer.
* The notification of the Blood Bank Medical Director or designee should be documented as an internal message to the antibody titer test in the Blood Bank computer.

**Appropriate Test Cell for Titration**

Refer to Table 608-1, below, and to the notes following this table to choose an appropriate test cell for the titration.

* **Table 608-1: Appropriate Test RBCs for Antibody Titration**

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibody**  | **Test RBC** | **Antibody**  | **Test RBC** |
| Anti-D | R2R2 | Anti-Fya | Fy(a+b-) |
| Anti-C | R1R1 | Anti-Fyb | Fy(a-b+) |
| Anti-D and Anti-C | R2R2 and r’r | Anti-Jka | Jk(a+b-) |
| Anti-E | R2R2 | Anti-Jkb | Jk(a-b+) |
| Anti-c | rr | Anti-K | K+ k- |
| Anti-c and Anti-E | R2R2 | Anti-k | K- k+ |
| Anti-e | rr | Anti-M | M+ N- |
| Anti-e and Anti-C | R1R1 | Anti-N | M- N+ |
|  |  | Anti-S | S+ s- |
|  |  | Anti-s | S- s+ |
|  |  | Anti-U | S+ s+ |

* The freshest available test cell shall be chosen; expired test cells shall not be used. However, see the following exception for *Anti-Kell Titrations.*
* 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.
* As indicated in this table, test cells are chosen based on homozygous expression of the antigen corresponding to the clinically significant antibody.
* If **multiple antibodies** are present,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 Table 608-1 for the following multiple antibody combinations: Anti-C & D, Anti-c & E, and Anti-e & C.
* **Anti-Kell Titrations**: 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 . . .
* 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.
* Note that it may be possible to obtain an expired homozygous (K+k-) test cell from another facility.
* Note that expired panel cells should be discarded after 3 months, as indicated in P332, *Receipt of Critical Reagents and Review of Manufacturers’ Printed Materials*.
* 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.
* The highest titer result (between the homozygous and the heterozygous test cell) should be reported in the computer.
* A comment shall be documented with the titer results against both test cells, also indicating whether the cells were K+k- or K+k+.
* The control sample should be tested in parallel with both test cells (homozygous and heterozygous), as usual.

**Definitions**

* Titer screen . . . a titer that is tested with only the 1:1dilution. 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 described in Table 608-3.
* 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).
* 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.

**Specimen Collection and Handling**

The preferred specimen is a 6 ml EDTA sample with affixed identifying label. See P101, *Triaging and Identifying Acceptable Blood Samples for Testing,* for acceptable alternatives.

**Equipment**

The following equipment is needed for this procedure:

* 37°C heat block or water bath
* Cell Washer
* Serofuge
* Vortex Mixer

**Supplies**

The following supplies are needed for this procedure:

* Saline
* 100 ul pipette and 50 ul pipette
* Pipette tips
* 12 X 75mm Test tubes
* Test RBC’s, Group O, 3-4% suspension
* Anti-IgG Anti-Human globulin (AHG)
* IgG Coated Cells

###### Forms

* F-608, *Antibody Titers Worksheet*

**Quality Control**

IgG coated cells must be added to all AHG phase results that are negative or less than 2+.

If any tube of the titer that requires IgG coated cells reacts less than 2+ with the IgG coated cells, then that result is considered invalid. It may be necessary to repeat the titer; see the policy *End Point Requirements*.

The control sample will be tested in parallel with the most current sample.

See also *Policies Relating to Pipetting Technique* in the *Policies* section.

**Before you get started**

Retrieve the control sample (if available) from the freezer and allow it to thaw at room temperature before testing. Document the following on F-620, *Special Studies Worksheet*:

* 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.
* the specificity of the antibody for which the titer will be performed
* the test cell phenotype; refer to Table 608-1: *Appropriate Test RBCs for Antibody Titration*
* the test cell manufacturer, lot number, expiration date, and cell identification number
* the collection date of the current and control samples
* the technologist’s initials and date

**Procedure**

Follow the steps in Table 608-2, below, to prepare a serial dilution. 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; see steps 17 and 18.

###### Table 608-2: Preparation of Serial Dilutions

|  |  |  |
| --- | --- | --- |
| **Step** | **Action** | **Notes** |
| 1 | Perform the actions indicated in the *Before You Get Started* section.  |  |
| 2 | Label and fill a 12 X 75 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 the current sample date.  | If applicable, see the policy *Performing a Titration with Fewer than Ten Test Tubes.*  |
| 4 | Firmly insert a clean disposable tip onto the 100 uL pipette. | Refer to*Policies Relating to Pipetting Technique* |
| 5 | Depress the pipette plunger and insert the pipette tip into the 12 X 75 mm test tube of saline. |
| 6 | Allow the plunger to **slowly** return to its release position to aspirate 100 uL of saline. Gently wipe the outside of the pipette tip.  |
| 7 | Dispense 100 uL 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.**  |

*Continued Next Page*

###### Table 608-2: Preparation of Serial Dilutions, continued.

|  |  |  |
| --- | --- | --- |
| **Step** | **Action** | **Notes** |
| 8 | Remove the tip from the wall of tube #2 and allow the pipette plunger to return to its release position. | Refer to*Policies Relating to Pipetting Technique* |
| 9 | Repeat steps 5-8 to dispense 100 uL of saline into each of the remaining numbered test tubes (#3 - #11).  |
| 10 | Using a new tip, pipette 100 uL of patient plasma into tube #1, remove and discard tip. |
| 11 | Using a new tip, pipette 100 uL 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 uL 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 uL 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 uL 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.  | See the policy *Titer End Point Requirements* in the *Policies* section.  |
| 17 | Determine whether a control sample is available, and whether testing of the control sample is indicated.  | See the *Control Sample Policy for Obstetrical Patients.*  |
| **If a control sample is . . .**  | **then. . . .** |
| 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 the control sample date.
2. Mix the thawed control sample thoroughly for 5-10 seconds with the Vortex mixer.
3. Proceed to step 18.
 |
| Unavailable, or testing is not indicated | Proceed to step 19.  |
| 18 | Repeat steps 4-16 to prepare a serial dilution of the control sample.  |  |
| 19 | If the titration of multiple antibodies is necessary; repeat steps 1-19 for any additional antibodies. |  |
| 20 | Proceed to Table 608-3, *Test Procedure for Antibody Titration*, to perform the antibody titration using the serial dilutions.  |  |

Follow the steps in Table 608-3, below, to determine the antibody titer result.

**Table 608-3: Test Procedure for Antibody Titration**

|  |  |  |
| --- | --- | --- |
| **Step** | **Action** | **Notes** |
| 1 | Obtain the tubes containing the serial dilutions which were prepared as described in . . . * Table 608-2, *Preparation of a Serial Dilution*
 | Serial dilutions of the current sample and the control sample may have been prepared; see the policy *Control Sample Policy for Obstetrical Patients.* |
| 2 | Pipette 50 ul of the appropriate test RBCs to each of the tubes of the serial dilution. Gently agitate the tubes. **Do not add enhancement media** | Refer to the policy *Appropriate Test Cell(s) for Antibody Titrations.*  |
| 3 | Incubate all tubes at 37°C for 30 minutes + 1 minute.  |  |
| 4 | Wash all tubes 4 times using the automatic cell washer.  | 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. | If necessary, refer to P317*, Centrifuge RPM Check.* |
| 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+. | If necessary, refer to P061, *Reading and Grading Test Reactions*.  |
| 7 | Document graded reactions on F-608, *Special Studies Antibody Titers Worksheet* under the “AG” column.  |  |
| 8 | Add IgG coated cells to all tubes that are non-reactive or less than 2+ at the AHG phase. Agitate tubes to mix. Centrifuge according to calibrated time. | If necessary, refer to P317*, Centrifuge RPM Check.* |
| 9 | Gently re-suspend the cell button. Read, grade, and record coated cell results under the “CC” column on F-620.  | Coated cells must react at least 2+; otherwise the test must be repeated*.*  |
| 10 | Interpret the recorded reactions and report the titer results.  | See *the Interpretation and Test Reporting* section, below. |
| 11 | Freeze an aliquot of the current sample (to be used as a control sample if / when a subsequent sample is received).  | See the *Notes* section, below, for additional information.  |

**Notes**

**After Testing is Complete / Freeze an Aliquot of the Current Sample**:

If sufficient volume is present, freeze an aliquot of patient plasma from the current sample to be used for parallel testing with a subsequent sample. If possible, avoid freezing the entire volume of patient plasma in case additional testing is required on the current sample. Label with patient name, hospital number, and sample date; an LIS label may be used for this purpose. Store the labeled aliquot at -20°C or colder (in the frozen aliquot rack in freezer #9). Document that the aliquot of the current sample was frozen on F-620, *Special Studies Worksheet.*

**Limitations**

The following may influence the validity of test results:

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

**Interpretation**

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). Following is an example of a twofold titer increase:

*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.*

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 |

 **Grading Reactions**

Antibody titers are not read microscopically. Reactive tubes are graded from weak+ to 4+. For additional information refer to P061, *Reading and Grading Test Results.*

**End Point**

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 policy *Titer End Point Requirements* for additional information.

**Titer Result**

The titer result is the reciprocal of the end point. For example:

*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.*

**Test Reporting**

The technologist will document the titer results of the current sample and the control sample in the Blood Bank computer record as a patient comment, and on the *Patient Antibody Card*.

The technologist will also enter the antibody titration results in the Blood Bank computer as described in the *BBCDM / Antibody Problems / Antibody Titration.*

This report will indicate the following:

* the titer result(s) of the current sample,
* the titer result(s) of the control sample,
* the date of the control sample,
* the date of the current sample, and
* the specificity of the antibody(ies).

This result will interface to the HIS (hospital information system).

**References**

* American Association of Blood Banks, *Technical Manual*, sixteenth edition.
* 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.
* Harmening, Denise M., *Modern Blood Banking and Transfusion Practices*, Third Edition,1994.

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Transfusion Services System Medical Director

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Transfusion Services Dept Manager

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| Signature | Date | **Revision #** |  | **Related Documents****Reviewed/****Updated** |
| Revised by: Kelly Sartor | 02/05/2020 | 01 | * The uniform AHG method has been adopted.
* The requirement to make a primary dilution if the titer result is > 512 has been eliminated.
* Several policies have been added.
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| Approved By: Dr. J.T. Powers | 02/23/2020 |  |  |
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