**Purpose**

Antibody titration is a semi quantitative method used to determine the concentration of antibody

in a sample. This procedure provides instructions for performing and reporting antibody titration on prenatal samples to identify women with significant levels of antibodies that may lead to hemolytic disease of the fetus and newborn (HDFN).

* ***Note:*** Do not use enhancement techniques, enzyme treated cells or expired cells.
* ***Note:***The titer dilution volume can be modified according to available plasma as long as the ratio remains consistent.

**Procedure:**

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| --- | --- | --- |
| **Step** | **Action** | **Related Documents**  |
| 1 | Confirm sample acceptability.* Anticoagulated samples collected in EDTA are preferred.
* Clotted samples are acceptable.
 | Evaluating Patient Samples and Request Forms |
| 2 | Antibody identification should have been performed on current sample to be titrated.* For samples submitted later in pregnancy after the initial detection of the antibody, the Re-panel Policy should be followed to determine if additional antibodies have developed since the last submitted sample.
* The most recent previously submitted sample from the current pregnancy should be titrated in parallel with the current sample.

1mL of plasma/serum is ideal. More sample may be required if multiple antibodies are present. The remaining sample volume will be frozen and tested in parallel with the next sample submitted. | Guidelines for Antibody IdentificationRe-panel Policy for Antibody Resolution |
| 3 | Frozen samples should be thawed, mixed well and centrifuged to remove any cellular debris before dilutions are prepared. |  |
| 4 | Selection of reagent red cells for titration studies:* For a single antibody, select a cell with the strongest expression of the target antigen.
	+ For Anti-D, use an R2R2 cell (cDE/cDE)
	+ For Anti-K, use heterozygous cell if there is no homozygous
	+ For all other antibodies, use a cell with homozygous expression.
* If the last submitted sample is being titrated in parallel with the current sample, the same cell possessing the target antigen should be used for both samples.
* Ensure there is sufficient quantity of the selected cell for both titrations.
 |  |
| **Step** | **Action** | **Related Documents** |
| 5 | In a Serial 2 fold dilution, each dilution is made using a separate pipette tip. Failure to do so may result in falsely elevated titers due to carry-over.**Note:**The titer dilution volume can be modified according to available plasma as long as the dilution ratio remains consistent. See Table A for volume example.Prepare dilutions as follows:* Label 10 tubes (2 – 11). The patient sample will act as tube 1 (undiluted).
* Deliver 1 volume of saline to each of tubes 2 -11.
* Transfer 1 volume of plasma from patient sample (tube 1) to tube 2 (1:2 dilution)
* Mix the contents in tube 2.
* Load a clean pipette tip.
* Transfer 1 volume from tube 2 to tube 3 (1:4 dilution)
* Continue the same process for all dilutions.
* Set aside tube 11. Use this tube to continue the dilutions if needed.

**Note:** Repeat the process for the last submitted sample if there is one frozen. | Labeling Tubes for Manual Bench TestingRack Set-Up ProcedureTable A: Serial Dilution Tube Set Up and Volume Example |
| 6 | Prepare titer testing tubes as follows:* Label a second set of tubes (1-10).
* Dispense 0.1 mL (100 µL) of undiluted plasma/serum into tube 1.
* Dispense 0.1 mL (100 µL) of each dilution into appropriately labelled tubes (2 – 10).

**Note:** Repeat the process for the last submitted sample if there is one frozen. | Labeling Tubes for Manual Bench TestingRack Set-Up Procedure |
| 7 | The selected reagent cells possessing the target antigen should be mixed well and be at room temperature.* Add **1 drop** of the cells to each tube and mix gently.
 |  |
| 8 | Incubate at 37°C for 60 minutes. |  |
| 9 | Prepare Antibody Titer Worksheet:* + Patient information, Date Tested, Tech ID, and Antibody ID
	+ Enter Specimen date above the appropriate titer grid
 | Antibody Titer Worksheet |
| 10 | Wash the tubes four times with saline. | Washing Red Cell Samples (Manual or Automated) |
| 11 | Add **2 drops** of anti-IgG to each tube. |  |
| 12 | Mix the tubes **immediately** and centrifuge for the posted time in a calibrated serologic centrifuge. |  |
| 13 | Immediately after centrifugation: * Begin by reading tube 10 (1:512 dilution) and work forward to the undiluted tube.
* Read macroscopically, grade and record reactions on the Antibody Titer Worksheet.
* If there is agglutination in tube 10 (1:512 dilution), additional dilutions should be made and tested. Use tube 11 to continue the dilutions.
 | Reading and Grading Tube Hemagglutination ReactionsAntibody Titer Worksheet |
| **Step** | **Action** | **Related Documents** |
| 14 | Validate all negative antiglobulin results:* Add **1 drop** of IgG-coated control cells to all tubes with a negative antiglobulin result.
* Centrifuge for the posted time in a calibrated serologic centrifuge.
* Resuspend the cells, and observe macroscopic agglutination.
* Record results on the Antibody Titer Worksheet.

***Valid control results****: Agglutination of at least grade 2+ must be present or the test results are invalid and the test must be repeated*. | Antibody Titer Worksheet |
| 15 | Analyze the reactions of the IgG-coated RBCs as follows: |
| **If agglutination is…** | **Then…** |  |
| Present | Test is complete. |  |
| Absent | Test is invalid:* + Repeat Steps 6-13
	+ Consider cell washer problem or inactive AHG.
 |  |
| 16 | Results and Interpretation:* The titer is interpreted as the reciprocal of the highest dilution that yields a 1+ macroscopic reaction.
	+ (Example: If the highest 1+ reaction is seen in the 1:32 dilution, the titer is 32.
* Report as “Undiluted” if tube 1 yields a 1+ reaction
* Report as “Too weak to titrate” if tube 1 gives a reaction that is weaker than 1+.
 | Antibody Titer Worksheet |
| **Sunquest Codes** |

|  |  |  |
| --- | --- | --- |
| 1 | Two reportable tests are available:* TTR: Antibody Titer
* TTRRP: Antibody Titer, Previous Sample
 |  |
| 2  | One billable test is available: * TTR2: Additional Antibody Titers
	+ Bills for 2nd titer

If billing for a 3rd titer:* + Tab to next line
	+ Enter “;2”

If billing for a 4th, 5th, etc., enter “ ;3, ;4, etc” |  |
| 3 | One credit test is available:* TTRCR: Antibody Titer, Credit
 |  |
| 4 | Tests are addable to the following batteries. There is no orderable Titer battery:* PREN
* TSCR
* ABSCR
 |  |

|  |  |  |
| --- | --- | --- |
| Step | Action | Related Documents |
| Reporting |
| 1 | TTR * Test is “;;” free text
* Enter antibody name, equals sign “=”, followed by titer result
* Capital letters can stand alone. Enter “LITTLE” for lower case antibody names
* Example: ;ANTI D = 128
* Example: ;ANTI LITTLE E = UNDILUTED
* Example: ;ANTI K = TOO WEAK TO TITER
 |  |
| 2 | TTRRP* Test is “;;” free text
* Enter original sample date followed by antibody name and titer results as described above
* Example: ;12312014 ANTI D = 128
 |  |
| 3 | If multiple titers are reported for each patient:* Place a comma between the results
* Example:
	+ ;ANTI C AND ANTI D = 256, ANTI K = 8
	+ ;ANTI C = 256, ANTI K = 8
 |  |
| Complete Antibody Titer Worksheet and Freeze Specimen(s) |
| 1 | If possible, freeze the current sample in a properly labelled plastic tube and store in the designated area of the freezer.* Indicate whether the specimen was frozen or not on the Antibody Titer Worksheet.
* Update ‘Frozen Titered Antibodies’ excel sheet with sample information, titer result, and storage location.
 | Antibody Titer Worksheet |
| 2 | Check that the Antibody Titer Worksheet is complete:* SQ Reported? Yes or No
* # of titers charged
* Copy to the Medical Director? Yes or No
* Second Tech Review
 | Antibody Titer Worksheet |
| 3 | Place a copy of Antibody Titer Worksheet in the Medical Director’s box for review if HMC patient. UW titers do not require medical director review at HMC.*Note: Do not automatically add an ABPATH to titers. If a consult is indicated, ABPATH can be added and resulted at that time.* |  |
| 4 | UW Titers:Once reviewed, fax a copy of the Antibody Titer Worksheet and the antigram of the cell used in the titer to UW TSL. |  |
| 5 | Worksheet is filed in the patient’s antibody folder and left for the TS Manager review. |  |
| Billing |
| 1 | Test TTR bills for one (1) titerTest TTRRP does not bill. *NOTE: It is illegal to bill for the same test performed twice on one sample.*Test TTR2 bills for additional titers. |  |
| Step | **Action** | **Related Documents** |
| **Billing (continued)** |
| 2 | Examples: |  |
| Number of Titers Performed | Test in BOP | Number of Titers Billed |  |
| 1: Anti-D | TTR | 1 |  |
| 2: Anti-D (frozen sample available) | TTR and TTRRP | 1 |  |
| 3: Anti-D, Anti-K | TTR and TTR2 | 2 |  |
| 4: Anti-D, Anti-K (frozen sample available) | TTR, TTRRP, TTR2 | 2 |  |

**Table A: Serial Dilution Tube Set Up and Volume Example**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tube** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
| **Saline** | 0.2mL | 0.2 mL | 0.2 mL | 0.2 mL | 0.2 mL | 0.2 mL | 0.2 mL | 0.2 mL | 0.2 mL | 0.2 mL |
| **Plasma** | 0.2 mL | 0.2 mL from tube 2 | 0.2 mL from tube 3 | 0.2 mL from tube 4 | 0.2 mL from tube 5 | 0.2 mL from tube 6 | 0.2 mL from tube 7 | 0.2 mL from tube 8 | 0.2 mL from tube 9 | 0.2 mL from tube 10 |
| **Final dilution** | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | 1:512 | 1:1024 Set aside |

Reference:

AABB Technical Manual, Current Edition