**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.

**Procedure:**

|  |  |  |
| --- | --- | --- |
| **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.
* Minimum of 1 mL of plasma/serum is required. More sample may be required if multiple antibodies are present.
* Additional 1 mL (or more) to be frozen and tested in parallel with the next sample submitted.
* The most recent previously submitted sample from the current pregnancy should be titrated in parallel with the current sample.
 | * Guidelines for Antibody Identification
* Re-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 homologous
	+ For all other antibodies, use a cell with homozygous expression.
 |  |
| **Step** | **Action** | **Related****Documents** |
| **4****cont** | * Each antibody in a sample with multiple specificities should be titrated separately. Any question regarding the selection of cells should be directed to a lead tech or the Manager.
* 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.
 |  |
| **5** | * Serial 2-fold dilutions of plasma/serum are prepared using 0.5 mL (500 µL) volumes.
* Each dilution is made using a separate pipette tip. Failure to do so may result in falsely elevated titers due to carry-over.
* Prepare dilutions as follows:
* Label 11 tubes (1 – 11).
* Transfer 1 mL of plasma serum from sample tube to tube 1

(undiluted, 1 in 1).* Deliver 0.5 mL saline to each of tubes 2 -11.
* Transfer 0.5 mL of plasma from tube 1 to tube 2 (1 in 2).
* Mix the contents in tube 2. Using a clean pipette tip, transfer 0.5 mL from tube 2 to tube 3 (1 in 4).
* Continue the same process for all dilutions, mixing well after each transfer and using a clean pipette tip for each transfer.
* 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.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tube** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
| **Saline** | 0 | 0.5mL | 0.5 mL | 0.5 mL | 0.5 mL | 0.5 mL | 0.5 mL | 0.5 mL | 0.5 mL | 0.5 mL | 0.5 mL |
| **Plasma** | 1 mL | 0.5 mL | 0.5 mL from tube 2 | 0.5 mL from tube 3 | 0.5 mL from tube 4 | 0.5 mL from tube 5 | 0.5 mL from tube 6 | 0.5 mL from tube 7 | 0.5mL from tube8 | 0.5 mL from tube9 | 0.5mL from tube10 |
| **Final dilution** | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | 1:512 | 1:1024Set aside |

 | * Labeling Tubes for Manual Bench Testing
* Rack Set-Up Procedure
 |
| **6** | * Label another 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.
 |  |
| **Step** | **Action** | **Related****Documents** |
| **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.
* 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
 |  |
| **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.**
* Centrifuge for the posted time in a calibrated serologic centrifuge.
 |  |
| **13** | * Immediately after centrifugation:
* Begin by reading tube 10 (1:512) 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), additional dilutions should be made and tested. Use tube 11 set aside to continue the dilutions.
 | * Reading and Grading Tube Hemagglutination

Reactions* Antibody Titer Worksheet
 |
| **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.

***Valid control results****: Agglutination of at least grade 2+ must be present or the test results are invalid and the test must be repeated*. |  |
| **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.
 |

|  |  |  |
| --- | --- | --- |
| **Step** | **Action** | **Related****Documents** |
| 16 | Results and Interpretation:* The titer is interpreted as the reciprocal of the highest dilution that yields a 1+ macroscopic reaction. (example, if the 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+.
 |  |
| 17 | * Check that the record is complete:
* Date and time of completion,
* Technologist identification, and
* Final clerical check
 |  |
| 18 | * Freeze an aliquot of the current sample (at least 1 mL) in a properly labelled tube to include the date of draw, and store in designated area in the freezer.
 |  |

**Reporting and Billing in Sunquest**

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| --- |
| Sunquest Codes |
| 1. | Two reportable tests are available:* TTR: Antibody Titer
* RPTTR: 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
* TXM
* TSCR
* ABSCR
 |  |
| Reporting  |
| 1. | TTR * Test is “;;” free text (alpha and numeric)
* 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
 |  |
| Reporting (continued) |
| 2. | RPTTR* 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 D = 256, ANTI K = 8
 |  |
| 4. | Place copy of Antibody Titer Worksheet in the Medical Director’s box for review.*Note: Do not automatically add an ABPATH to titers. If a consult is indicated, ABPATH can be added and resulted at that time.* |  |
| Billing |
| 1. | * Test TTR bills for one (1) titer
* Test RPTTR does not bill. *NOTE: It is illegal to bill for the same test performed twice on one sample.*
* Test TTR2 bills for additional titers.
 |  |
| 2 | Examples: |  |
| Number of Titers Performed | Tests in BOP | Number of Titers Billed |  |
| 1: anti-D | TTR | 1 |  |
| 2: anti-D (frozen sample available) | TTR and RPTTR | 1 |  |
| 2: anti-D, anti-K | TTR and TTR2 | 2 |  |
| 4: anti-D, anti-K (frozen sample available) | TTR, RPTTR, TTR2  | 2 |  |
| **Complete Worksheet and Freeze Specimen(s)** |
| 1 | * Freeze available specimen(s).
 |  |
| 2 | * Mark worksheet appropriately:
	+ Specimen frozen? Yes or No
	+ Sunquest reported? Yes or No
	+ Enter number of titers charged
	+ Copy to Medical Director? YES or No
 |  |
| 3 | Worksheet is filed in the patient’s antibody folder and left for the TS Manager review. |  |

Reference:

AABB Technical Manual, Current Edition