**ANTIBODY TITRATION**

**PRINCIPLE**

Titration is a semi-quantitative means of measuring the amount of antibody in a patient’s serum. Serial dilutions of the antibody are reacted with a constant volume of the specific red blood cells and the results are expressed as the reciprocal of the highest dilution in which agglutination is observed. Titration studies are most frequently performed in the following situations:

1. Prenatal studies — If the mother has a significant antibody, a titer should be performed to determine

 possible hemolytic disease of the newborn.

2. HTLA Antibodies — Especially in the case of anti-leukocyte antibodies. The titer in this case will be a

 micro to a weak positive reaction. (Refer to anti-Leukocyte titration procedure.

**SPECIMEN COLLECTION**

No special preparation of the patient is required prior to specimen collection. Blood should be drawn by an aseptic technique. Serum or EDTA plasma may be used for testing within three (3) days of collection. Store samples at 2 – 8 C until testing can be done. **After testing is done, freeze an aliquot of the sample for subsequent testing.**

**REAGENTS/SUPPLIES**

1. Appropriate RBC suspension from either the screening cell I or II, or the panel cells.2. Patient’s serum.

3. Frozen aliquot of patient sample from previous testing.

4. Pipette measuring 100 ul and 50 ul

5. Saline (isotonic saline).

**PROCEDURE**

1. Select rbcs from a cell panel or screening cell I/II containing the antigen corresponding to the identified

 antibody. When possible select a homozygous rbc. (Ex: If Anti-E was identified, select a cell containing

 the E antigen)

2. Use a serial dilution for testing. Either label test tubes according to the serum dilution factor.

 [ex: 1 (1 : 1 dil); 2 (1 : 2 dil); 4 (1 : 4 dil); 8 (1 : 8 dil); 16 (1 : 16 dil); etc.] or according to the chart below.

 The number of tubes used for the titer is determined by antibody screen strength. **Always save the last**

 **tube in case a higher dilution must be prepared.**

Tube 1 1 : 1 Dilution Tube 6 1 : 32 Dilution

Tube 2 1 : 2 Dilution Tube 7 1 : 64 Dilution

Tube 3 1 : 4 Dilution Tube 8 1 : 128 Dilution

Tube 4 1 : 8 Dilution Tube 9 1 : 256 Dilution

Tube 5 1 : 16 Dilution Tube 10 1 : 512 Dilution

3. Add 100 ul of saline to all of the tubes except the first one. (Tube 1 is serum only.)

4. Add 100 ul of the serum to be tested to tube #1 and #2.

5. Mix the serum/saline solution in tube #2 well.

6. Using a clean pipette tip, transfer 100 ul of the mixture from tube #2 to tube #3. Transfer 100 ul from

 tube #3 to tube #4 and so on. Repeat this procedure using a clean pipette tip for each transfer. Place

 the discard from the last tube into another tube and save in case a higher dilution needs to be tested.

7. To each tube, add 50 ul of the rbc suspension. Mix the tubes well.

8. Incubate the tubes for 30 minutes in the 37 C incubator.

9. After incubation, resuspend the mixture from each tube and wash 3 times in saline.

10. Add 2 drops of Anti-IgG AHG to each tube. Mix well and centrifuge all tubes.

11. Starting with the last tube, examine each tube macroscopically for agglutination. Grade the positive

 tubes and record reactions.

**Interpretation of Results**: The titer is reported as the highest dilution exhibiting a 1+ agglutination reaction. The titer is reported as the reciprocal of the dilution. (i.e. A 1:4 dilution is reported as a titer of 4). If less than a 1+ is all that is seen, report the titer as <1.

**Note:** Freeze an aliquot of the patient's sample for future Testing.

**NOTE**: A change in titer of 1 tube is not significant; however, a fourfold rise (at least 2 tubes) is considered a significant change.

Roback, J. (2011). Technical manual (17th ed.). Bethesda, Md.: AABB.

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