

# Titration

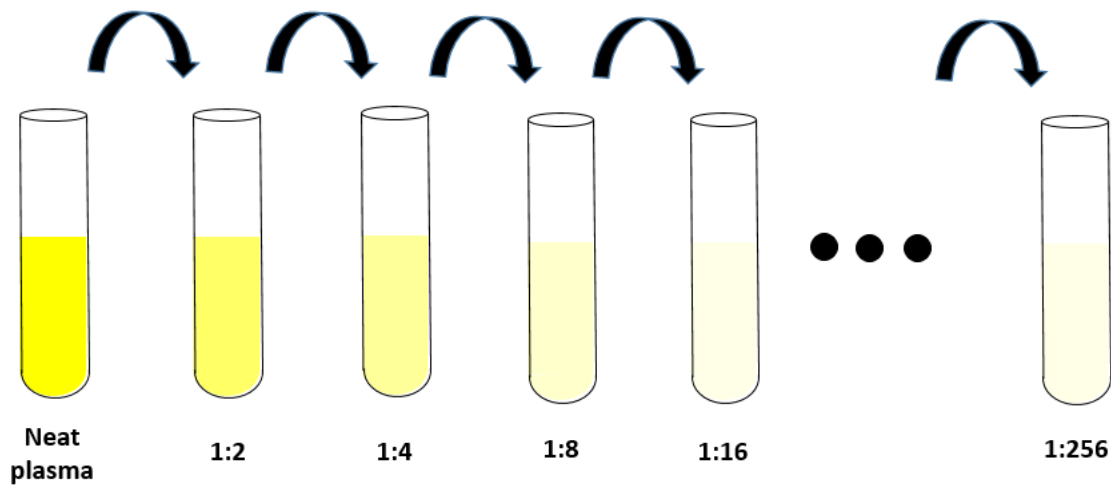
## Objectives:

1. Discuss the most common reasons titration is used in the blood bank.
2. Discuss the reactivity known as HTLA.
3. Interpret titer results.

## Quick Lesson:

An antibody titer is...**a semi-quantitative way to determine the strength of an antibody**

### Step 1: serial dilution of plasma containing antibody



### Step 2: test each dilution with RBCs expressing corresponding antigen

| Titer of plasma containing anti-K | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|-----------------------------------|-------------|-----|-----|-----|------|------|------|-------|-------|
| K+ RBC, IAT                       | 3+          | 3+  | 2+  | 2+  | 1+   | 0    | 0    | 0     | 0     |

### Step 3: endpoint of titer is last dilution with 1+ reactivity (reported as reciprocal of dilution)

| Titer of plasma containing anti-K | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|-----------------------------------|-------------|-----|-----|-----|------|------|------|-------|-------|
| K+ RBC, IAT                       | 3+          | 3+  | 2+  | 2+  | 1+   | 0    | 0    | 0     | 0     |

**Anti-K titer: 16**

## How & why are titers used?

| Utility of Titers in the Blood Bank                 |   |
|---|---|
| <b>Prenatal Titers</b>                              | <ul style="list-style-type: none"> <li>• Noninvasive way to monitor pregnant women with clinically significant antibodies.</li> <li>• If titer increases over time, may indicate that fetal cells are antigen positive, and fetus should be monitored by Doppler ultrasound.</li> </ul>   |
| <b>Serologic Investigation of “HTLA” Reactivity</b> | <ul style="list-style-type: none"> <li>• Characteristic reactivity of some antibodies – see below for more information.</li> </ul>  |
| <b>Isohemagglutinin Titers</b>                      | <ul style="list-style-type: none"> <li>• Titration of anti-A (and/or anti-B) in donor products like group O platelets or group O whole blood that might be transfused to non-group O patient (theory: give “low titer” products to prevent hemolytic transfusion reactions)</li> <li>• Titration of anti-A (and/or anti-B) in recipients of solid organ transplants that are ABO incompatible.</li> </ul> |

### Let’s talk more about...Prenatal titers:

| Titer of plasma containing anti-K  | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|------------------------------------|-------------|-----|-----|-----|------|------|------|-------|-------|
| K+ RBC, IAT<br>Current sample      | 3+          | 3+  | 2+  | 2+  | 1+   | 0    | 0    | 0     | 0     |
| K+ RBC, IAT<br>Last month’s sample | 2+          | 2+  | 1+  | 0   | 0    | 0    | 0    | 0     | 0     |

Prenatal samples are often tested in parallel with a previous sample, as shown above. Here are some reasons why:

- Titer method is highly variable, and there may be tech variation from sample to sample.
- Cells from different individuals can express different amounts of antigen, and the reagent cell used to titer may change from sample to sample. Also, age of the reagent cell used may affect antigen expression.
- If an antibody titer increases >2 tubes, this may be considered a clinically significant titer increase, and may warrant monitoring the pregnancy more closely.

**\*\*While titering can inform whether antibody strength is increasing over time, once it is established that the titer is increasing, it is best to monitor pregnancy by a much more sensitive method than titering.**

**Some things to consider:**

Titer of maternal plasma may depend on:

- Testing method (many times, saline IAT is used)
- Reagent RBC tested (antigen expression is individual-dependent) – also, single dose or double dose of antigen may affect titer
- Tech to tech variability in grading reactions
- Precision of pipetting when preparing serial dilution

**Titer Score:** Another way to measure strength of an antibody.

|                        |    |    |    |    |
|------------------------|----|----|----|----|
| Strength of reactivity | 4+ | 3+ | 2+ | 1+ |
| Score                  | 12 | 10 | 8  | 5  |

**Example: Titer & Score**

| Titer of plasma containing anti-K | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|-----------------------------------|-------------|-----|-----|-----|------|------|------|-------|-------|
| K+ RBC, IAT                       | 4+          | 3+  | 3+  | 2+  | 1+   | 1+   | 1+   | 0     | 0     |
| Titer Score                       | 12          | 10  | 10  | 8   | 5    | 5    | 5    | 0     | 0     |

Anti-K: Titer = 64; Score = 55 (12+10+10+8+5+5+5)

# Prenatal Case Studies

**Case Study #1:** Sample of pregnant patient with anti-c submitted for titer. Titration studies of current sample performed in parallel with sample from 4 weeks ago. Fill in the appropriate scores, and report the titer and score for the current and previous sample.

| Titer                                 | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|---------------------------------------|-------------|-----|-----|-----|------|------|------|-------|-------|
| <b>c+ RBC, IAT</b><br>Current sample  | 3+          | 2+  | 1+  | 1+  | 0    | 0    | 0    | 0     | 0     |
| <b>Titer Score</b>                    |             |     |     |     |      |      |      |       |       |
| <b>c+ RBC, IAT</b><br>Previous sample | 2+          | 2+  | 1+  | 0   | 0    | 0    | 0    | 0     | 0     |
| <b>Titer Score</b>                    |             |     |     |     |      |      |      |       |       |

Current sample: anti-c titer \_\_\_\_\_ Score \_\_\_\_\_

Previous sample: anti-c titer \_\_\_\_\_ Score \_\_\_\_\_

**Case Study #2:** Sample of pregnant patient with anti-K, anti-C, and anti-Fy<sup>a</sup> submitted for titer. Titration studies of current sample performed in parallel with sample from 4 weeks ago. Report the correct titer for each antibody/sample.

| Titer                            |                 | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|----------------------------------|-----------------|-------------|-----|-----|-----|------|------|------|-------|-------|
| <b>K+, C-, Fy(a-) RBC</b><br>IAT | Current sample  | 3+          | 3+  | 2+  | 1+  | 1+   | 0    | 0    | 0     | 0     |
|                                  | Previous sample | 2+          | 1+  | 0   | 0   | 0    | 0    | 0    | 0     | 0     |
| <b>K-, C+, Fy(a-) RBC</b><br>IAT | Current sample  | 1+          | 0   | 0   | 0   | 0    | 0    | 0    | 0     | 0     |
|                                  | Previous sample | 1+          | 0   | 0   | 0   | 0    | 0    | 0    | 0     | 0     |
| <b>K-, C-, Fy(+) RBC</b><br>IAT  | Current sample  | 2+          | 1+  | 0   | 0   | 0    | 0    | 0    | 0     | 0     |
|                                  | Previous sample | 2+          | 1+  | 0   | 0   | 0    | 0    | 0    | 0     | 0     |

Current sample: anti-K titer \_\_\_\_\_; anti-C titer \_\_\_\_\_; anti-Fy<sup>a</sup> titer \_\_\_\_\_

Previous sample: anti-K titer \_\_\_\_\_; anti-C titer \_\_\_\_\_; anti-Fy<sup>a</sup> titer \_\_\_\_\_

**Answers: Case Study 1:** Current sample titer= 8/Score= 28  
Previous sample titer= 4/Score= 21

| Titer of patient plasma | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|-------------------------|-------------|-----|-----|-----|------|------|------|-------|-------|
| c+ RBC, IAT             | 3+          | 2+  | 1+  | 1+  | 0    | 0    | 0    | 0     | 0     |
| Current sample          |             |     |     |     |      |      |      |       |       |
| Titer Score             | 10          | 8   | 5   | 5   | 0    | 0    | 0    | 0     | 0     |
| c+ RBC, IAT             | 2+          | 2+  | 1+  | 0   | 0    | 0    | 0    | 0     | 0     |
| Previous sample         |             |     |     |     |      |      |      |       |       |
| Titer Score             | 8           | 8   | 5   | 0   | 0    | 0    | 0    | 0     | 0     |

**Case Study 2:** Current sample anti-K titer= 16, anti-C titer= <2, anti-Fy<sup>a</sup> titer= 2  
Previous sample anti-K titer= 2, anti-C titer=<2, anti-Fy<sup>a</sup> titer= 2

## Let's talk more about...“HTLA”

- **High Titer, Low Avidity** = this characteristic reactivity may help a reference lab identify some antibodies

Here are some blood group systems that have corresponding antibodies that demonstrate HTLA reactivity:

**Knops, Ch/Rg, Cost, JMH antibodies**

HTLA reactivity may provide a clue about the specificity of an antibody

**\*\*\*Reminder:** “HTLA” is not a blood group, but rather describes reactivity. An antibody must be identified by blood group system rather than reporting an “HTLA antibody.”

### Example: HTLA Reactivity

Usually, an antibody decreases in strength with every dilution

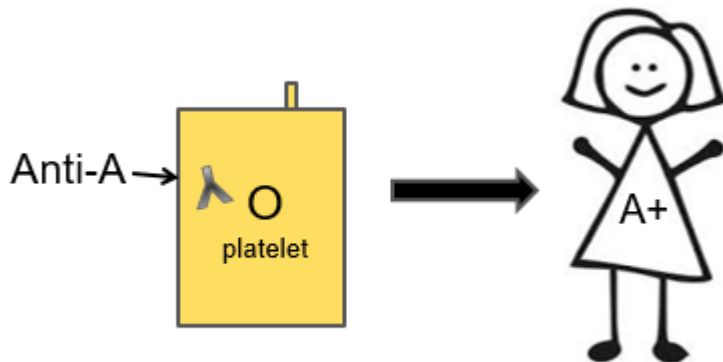
| Titer (tested at IAT)                               | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|---|-------------|-----|-----|-----|------|------|------|-------|-------|
| Normal, strong antibody<br>High titer, high avidity | 4+          | 4+  | 3+  | 3+  | 2+   | 2+   | 1+   | 1+    | 0     |
| Normal, weak antibody<br>Low titer, low avidity     | 1+          | 1+w | 0   | 0   | 0    | 0    | 0    | 0     | 0     |
| High Titer, Low Avidity                             | 1+          | 1+  | 1+  | 1+w | 1+w  | 1+w  | +/-  | +/-   | 0     |

\*\*when performing titers for investigating HTLA reactivity, reactions are read *microscopically* until no reactivity is observed (rather than stopping at 1+).

Usually, weak antibodies have very low titer

In HTLA reactivity, weak reactions persist over multiple dilutions

**Let's talk more about... isohemagglutinin titers**



**Isohemagglutinin titers = titers of anti-A & anti-B**

**Titerting anti-A in group O platelets.**

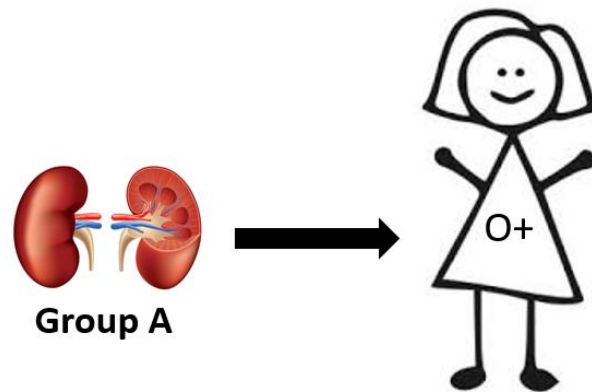
- Donor units with very high titer of anti-A, transfused to group A patients, have been implicated in hemolytic transfusion reactions.
- If a donor platelet has a high titer of anti-A, it may not be appropriate to transfuse to a group A patient.

**IN CASES LIKE THIS, TITERS ARE PERFORMED ON THE BLOOD PRODUCT**

**Non-ABO identical Solid Organ Transplant**

- Another application of isohemagglutinin titers.
- In this example, the patient's anti-A may contribute to graft rejection of the group A organ.
- Isohemagglutinin titers of transplant patients may be monitored to determine eligibility to receive a non-ABO identical organ.

**IN CASES LIKE THIS, TITERS ARE PERFORMED ON THE PATIENT**



# Assessing Understanding:

1. Which of the following antibodies demonstrates “HTLA” reactivity?

| Titer    | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|----------|-------------|-----|-----|-----|------|------|------|-------|-------|
| Plasma 1 | 4+          | 3+  | 3+  | 2+  | 2+   | 1+   | 1+   | 1+    | 0     |
| Plasma 2 | 1+          | 1+  | 0   | 0   | 0    | 0    | 0    | 0     | 0     |
| Plasma 3 | 1+          | 1+  | 1+  | 1+w | +/-  | +/-  | +/-  | +/-   | +/-   |
| Plasma 4 | 2+          | 2+  | 1+  | 1+  | 0    | 0    | 0    | 0     | 0     |

- a. Plasma 1
- b. Plasma 2
- c. Plasma 3
- d. Plasma 4

2. In which of the following cases would it be helpful to perform isohemagglutinin titers on a blood product?

- a. Group O whole blood being transfused to a group O recipient
- b. Group A platelet being transfused to a group O recipient
- c. Group O platelet being transfused to a group A recipient
- d. Group B platelet being transfused to a group O recipient

3. What is the appropriate way to report the following prenatal titers? Anti-E and anti-c were detected in the patient’s plasma, and titers were requested.

| Titer         | Neat plasma | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|---------------|-------------|-----|-----|-----|------|------|------|-------|-------|
| E+,c- RBC IAT | 2+          | 1+  | 0   | 0   | 0    | 0    | 0    | 0     | 0     |
| E-,c+ RBC IAT | 3+          | 3+  | 2+  | 2+  | 1+   | 0    | 0    | 0     | 0     |

- a. Anti-E: 1:2; anti-c: 1:16
- b. Anti-E: 2; anti-c: 16
- c. Anti-E: 16; anti-c: 2
- d. Anti-E: 32; anti-c: 64

Answers: 1. c 2. c 3. b