

*Introduction to  
Antibody  
Identification*

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# Objectives

After this presentation the learner will be able to correctly:

1. State the clinical utility of antibody identification.
2. Describe the principle and procedure of the antibody identification tests.
3. Explain heterozygosity and homozygosity as they apply to antibody identification.
4. List allelic pairs in the following blood group systems: Rh, Duffy, Kidd, MNSs.
5. Given patient test results, work through the antibody identification process.

# Antibody Screening and Identification

- Antibody screening is required:
  1. Any intended recipient of transfused blood.
  2. Prenatal testing for obstetric patients:  
evaluates risk of HDFN and candidacy for RhIg
  3. Donors of allogeneic blood and blood products and stem/progenitor cells.
- Further testing (Antibody ID) is required:
  1. If the screen is newly positive
  2. New alloantibody(ies) suspected

# Antibody Identification



# Methods for ABID

- Antibody identification (ABID) can be performed in the following media:
  - **Traditional tube method**
  - **Gel Technique**
  - **Solid Phase Technique**

# Antibody exclusion

- An exclusion procedure can be undertaken through observation of antigens present on reagent cells with which patient sera did not react.
- This means the patient's antibodies are not likely directed against the antigens present on those cells.

# Procedure

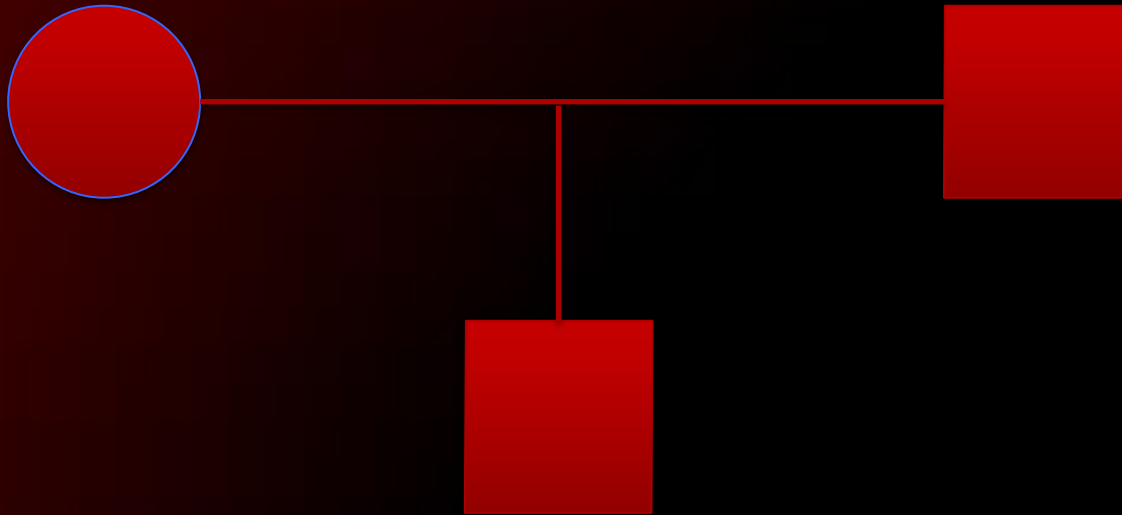
- Go to the first panel cell with a negative reaction, “rule out” or exclude the specificities of antibodies directed against antigens present on the cell.
  - (Rule out when the antigen is positive and the patient did *not* react)
  - Some antibodies demonstrate *dosage*.

# Dosage

- Some antibodies may react so weakly with antigens with heterozygous expression, they might not be detected.
- For antibodies in the following blood groups, it may be prudent to rule out with panel cells that have a homozygous expression of antigen:
  - Rhesus (excluding D)
  - Kidd
  - Duffy
  - MNSs



# Dosage

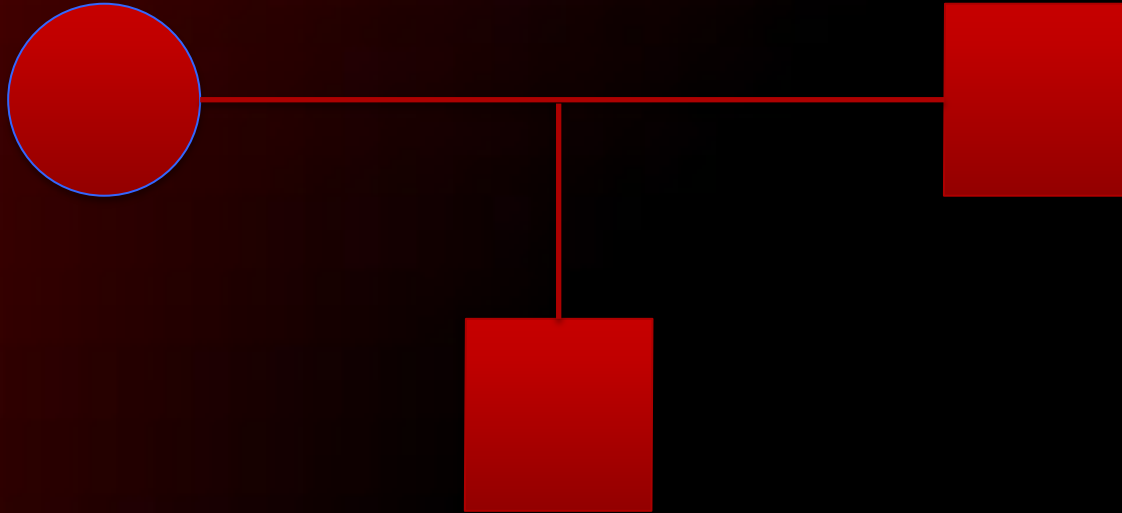


**Heterozygous**

# Dosage

Mother

Father

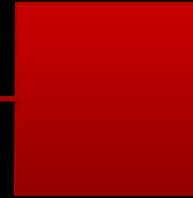
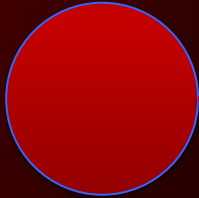


**Heterozygous**

# Dosage

Mother

Father



*Jka*

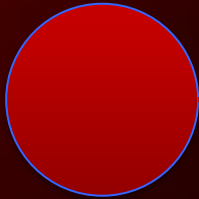


Heterozygous

# Dosage

Mother

Father

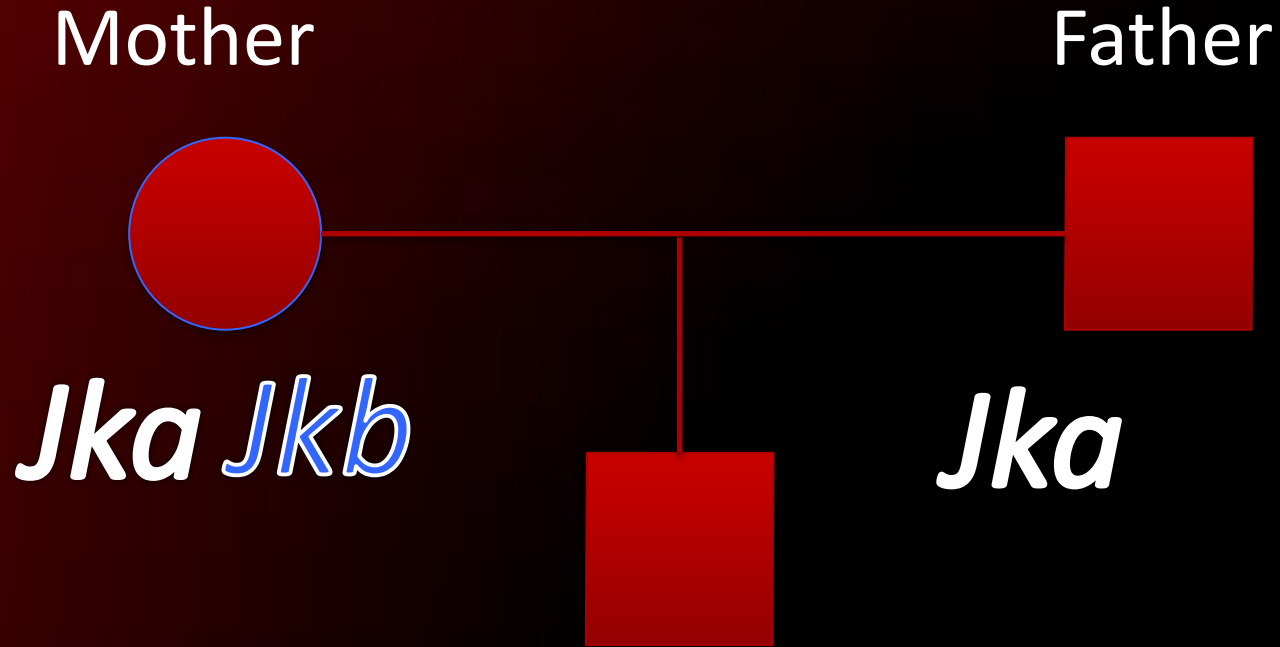


*Jka Jkb*



Heterozygous

# Dosage

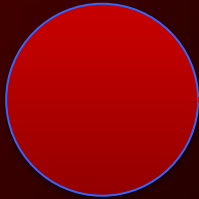


Heterozygous

# Dosage

Mother

Father



*Jka Jkb*

*Jka Jkb*

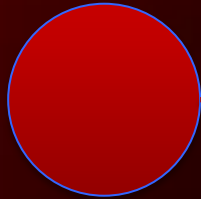


Heterozygous

# Dosage

Mother

Father



*Jkb*



*Jka Jkb*



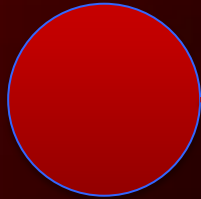
*Jka*

Heterozygous

# Dosage

Mother

Father



*Jkb*

*Jka*

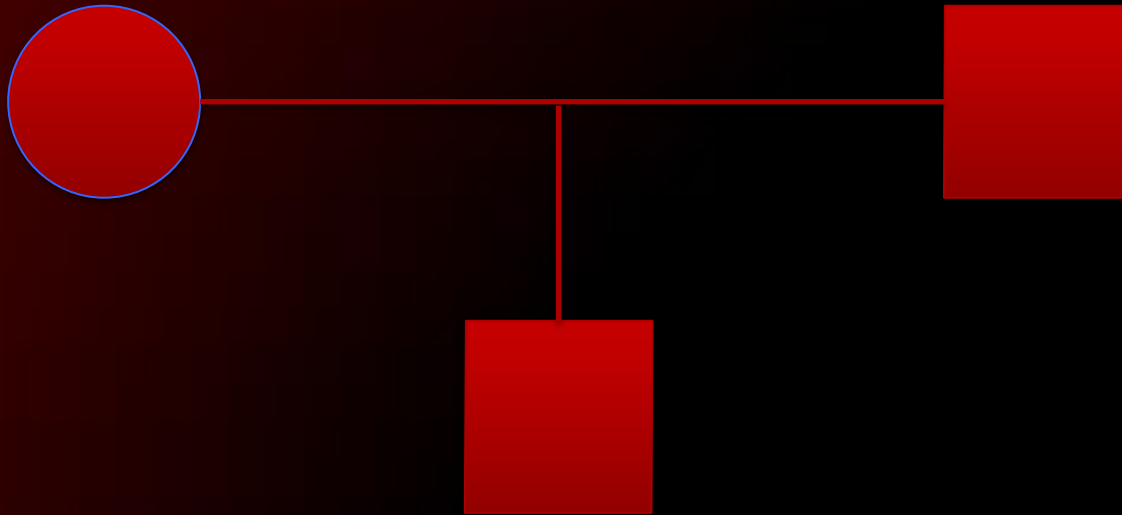


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Heterozygous



# Dosage

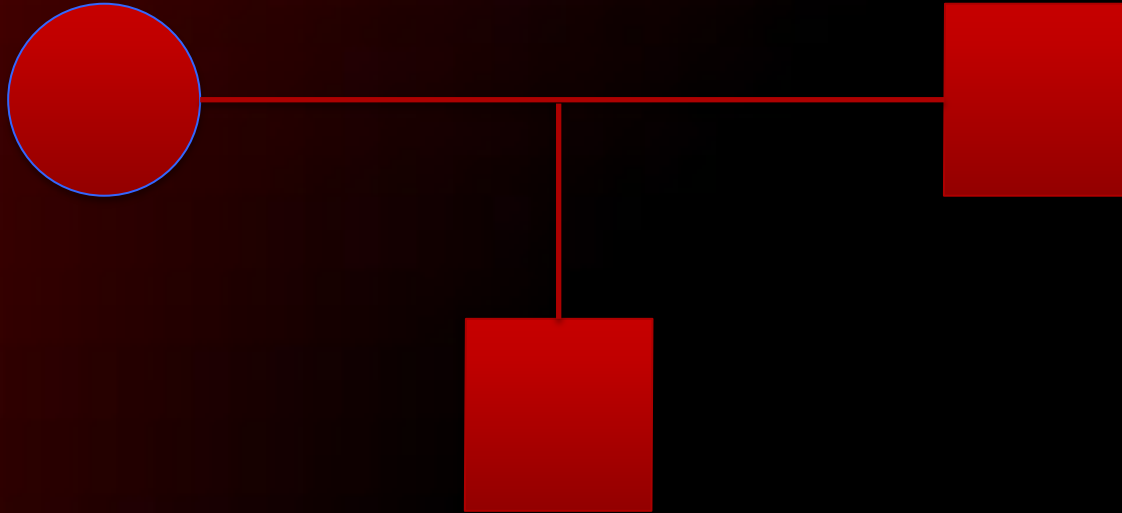


**Homozygous**

# Dosage

Mother

Father

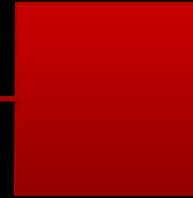
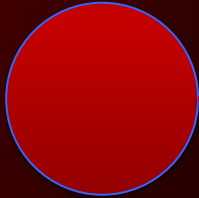


**Homozygous**

# Dosage

Mother

Father



*Jka*

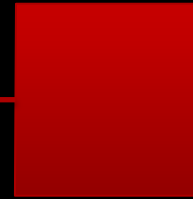
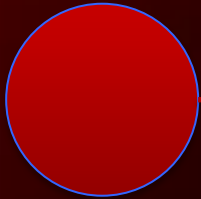


Homozygous

# Dosage

Mother

Father

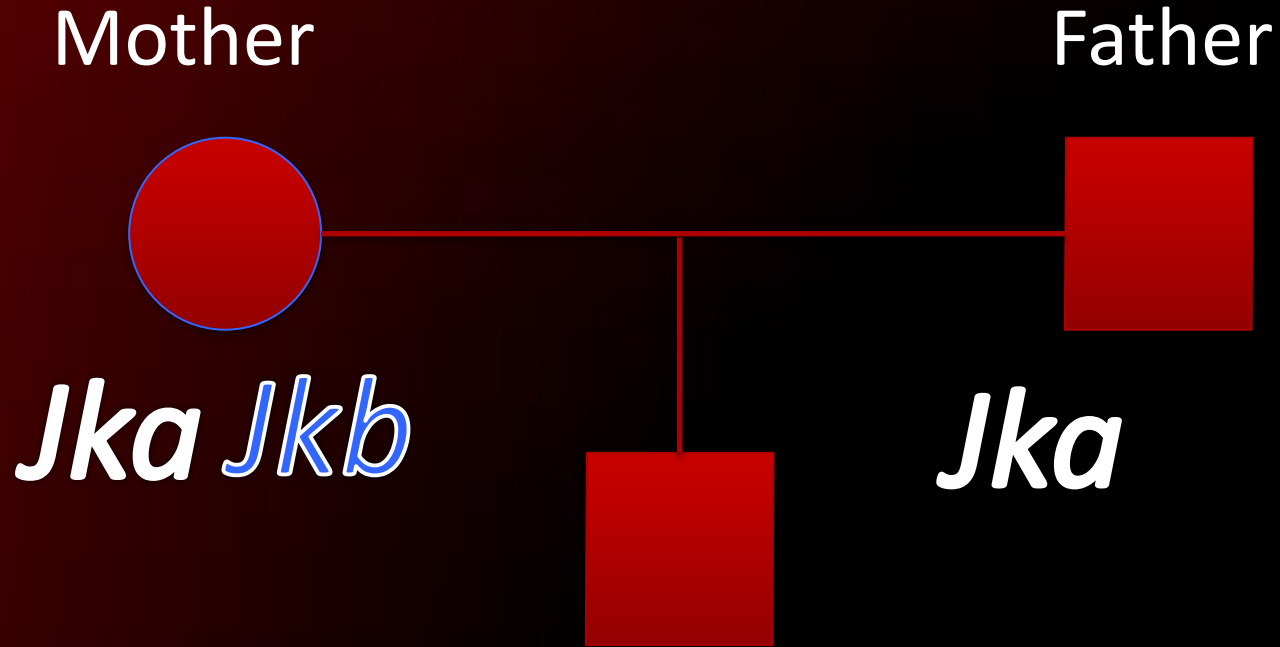


*Jka Jkb*



Homozygous

# Dosage

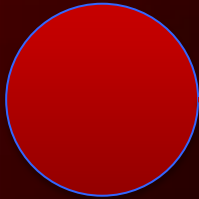


Homozygous

# Dosage

Mother

Father



*Jka Jkb*

*Jka Jkb*

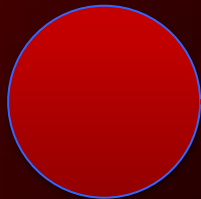


Homozygous

# Dosage

Mother

Father



*Jkb*



*Jka Jkb*



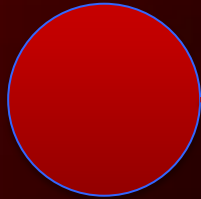
*Jka*

Homozygous

# Dosage

Mother

Father



*Jkb*

*Jkb*



*Jka Jka*

**Homozygous**



# Dosage



- Anti-Jka may not react with a heterozygous “single dose” cell
- It may only react with a cell that has “double the dose” of Jka antigens

# Allelic pairs

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**Rh System**

**C, c**

**E, e**

**Duffy System**

**Fya, Fyb**

**Kidd System**

**Jka, Jkb**

**MNSs System**

**M, N**

**S, s**

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# The next step

- What alloantibody or alloantibodies have not been ruled out?

Anti-E

Anti-Fya

Which of the following is or are most likely?

Look closely at the pattern of reactivity.









# Confirmation steps

- Anti-E is the most likely antibody reacting
- However, we still have not ruled out anti-Fya
- The patient could have anti-Fya underlying the reactions of anti-E

We need to select another cell that is

E antigen negative, and Fy(a+b-)

*HOMOZYGOUS for Duffy A*









# Rule of 3

- Criteria:
  - At least 3 panel cells with E antigen reacted (positive result) with patient's sample
  - At least 3 panel cells lacking E antigen did not react (negative result) with the patient's sample
- Does our example fulfill these criteria?







# Yes!

- Positive for E, patient reacted:

Cells 1, 3, 5, 8

- Negative for E, patient did not react:

Cells 2, 6, 7

Cell 4 can't be used for this because it is E antigen positive

# Rule of 3

- At least 3 true positives and 3 true negatives:

**Following this rule gives us a  $P$  value of 0.05**

**95% chance that the antibody we have identified is correct.**

# Result

- Anti-E identified. All other clinically significant alloantibodies have been ruled out.
- Donor units lacking E antigen should appear crossmatch compatible through the indirect antiglobulin test (IAT).

# In conclusion

- Rules for determining how antibody identification is performed are determined by the facility.
  - ✓ Rule of 2?
  - ✓ 2 in 3 out?
  - ✓ Heterozygous ok if 2
  - ✓ Etc.

# In conclusion

- Some patient antibodies may be so weak, they are only detected with a homozygous cell.
- Antibodies may behave differently in different media:
  - Solid phase?
  - Gel?
  - Tube technique:
    - PeG?
    - LISS?



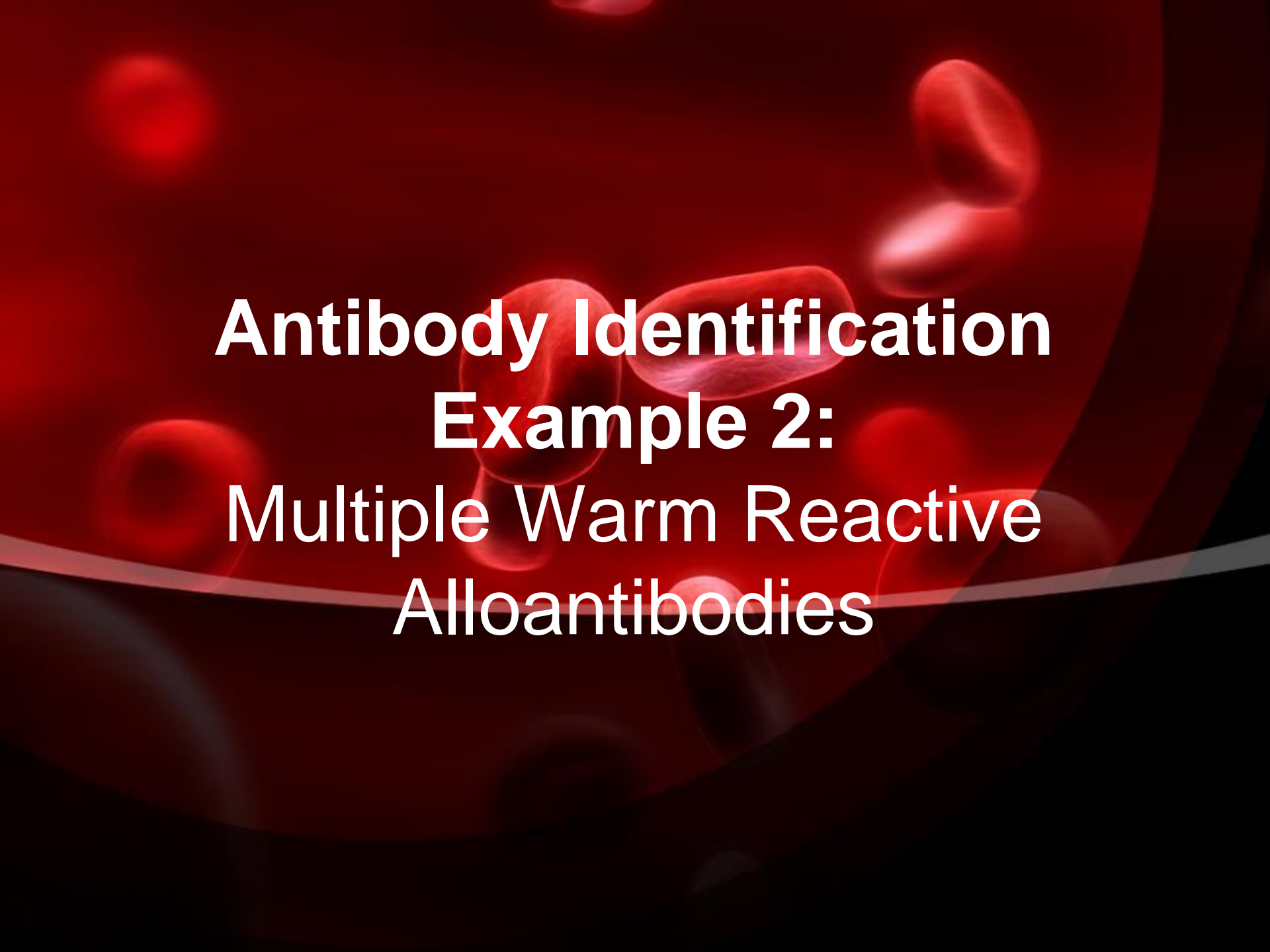
# In conclusion

- With multiple alloantibodies, it is important to prove them *independently* of each other.
- The process of antibody identification can take several hours in order to determine the specificity of the alloantibody(ies).

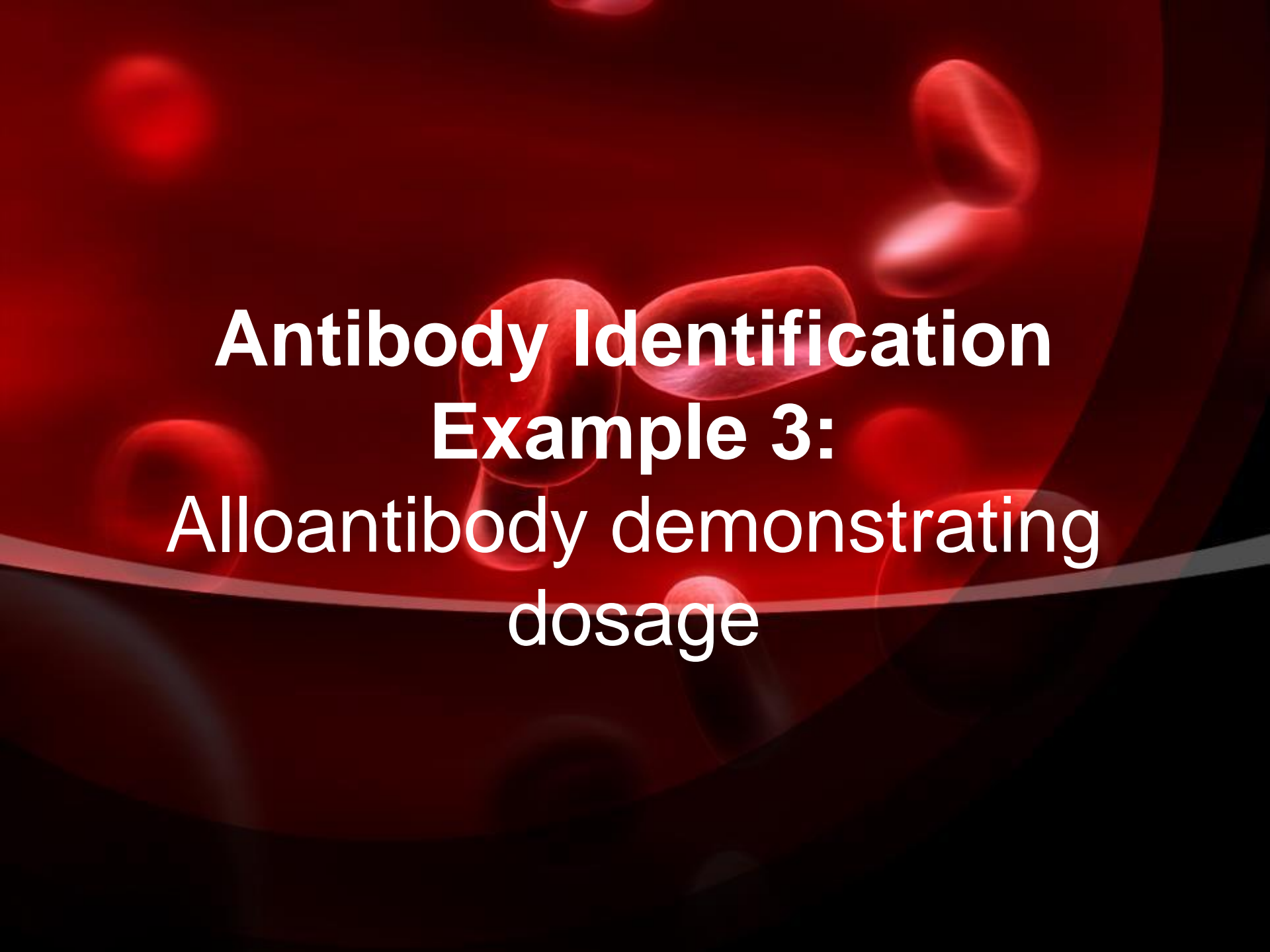


A microscopic view of several red blood cells, which are biconcave discs, floating in a dark red fluid. The cells are illuminated from the side, creating a bright rim and a darker center. The background is a deep red color with some darker, out-of-focus areas.

**Antibody Identification**  
**Example 1:**  
**Cold Reactive Alloantibody**

A microscopic view of several red blood cells, which are biconcave discs, set against a dark red background. The cells are slightly out of focus, creating a sense of depth. The lighting highlights the texture and color of the cells.

**Antibody Identification**  
**Example 2:**  
**Multiple Warm Reactive**  
**Alloantibodies**

A microscopic view of several red blood cells, which are biconcave discs, floating in a dark red fluid. The cells are illuminated from the side, creating a bright rim and a darker center. The background is a deep red color with a subtle gradient.

**Antibody Identification**  
**Example 3:**  
**Alloantibody demonstrating**  
**dosage**

*Thank you!*