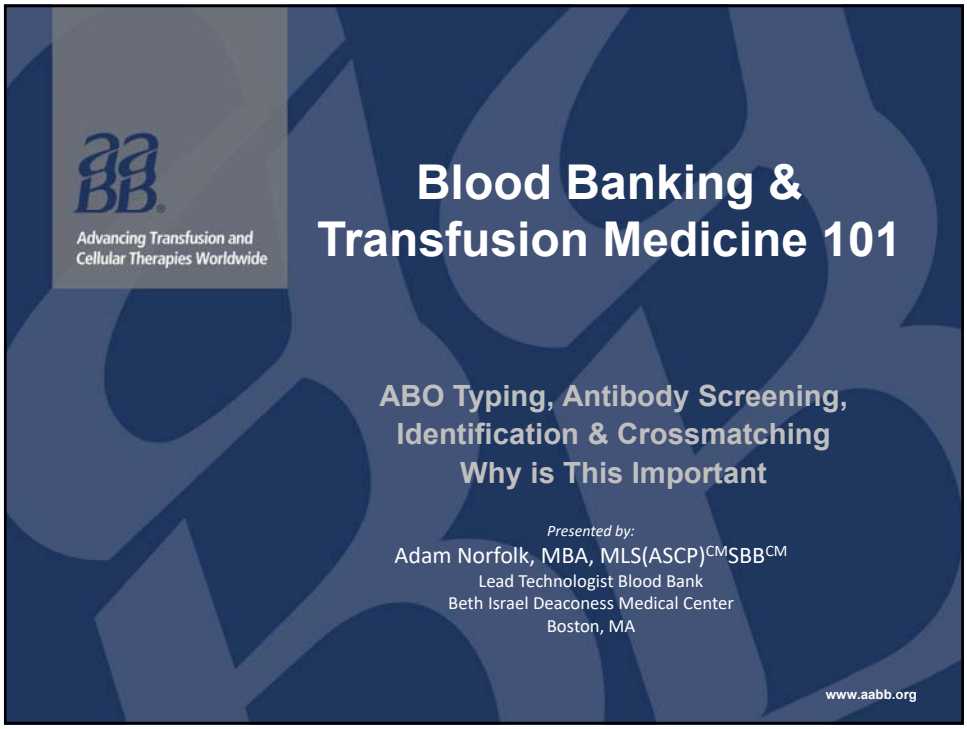

1

Learning Objectives

After participating in this program you should be able to….

- Reveal the major ABO types and their prevalence in the donor and patient population
- Explain what front (forward) and back (reverse) types are and how to perform each
- Describe the purpose of the antibody screen
  - Explain why group O cells are always used in an antibody screen
  - Describe two cell versus three cell screens
- Explain the purpose of antibody identification
- Describe what a crossmatch is
  - Explain the differences between immediate spin, full serological (AHG) and electronic crossmatch

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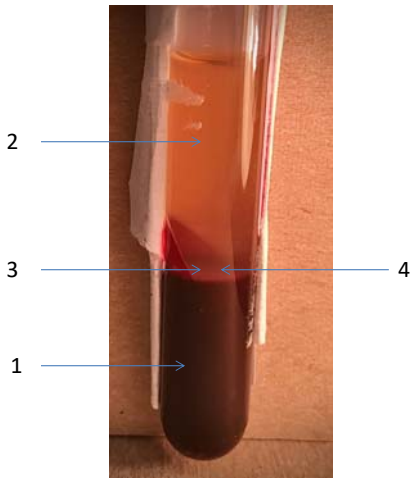
2

What is Blood?

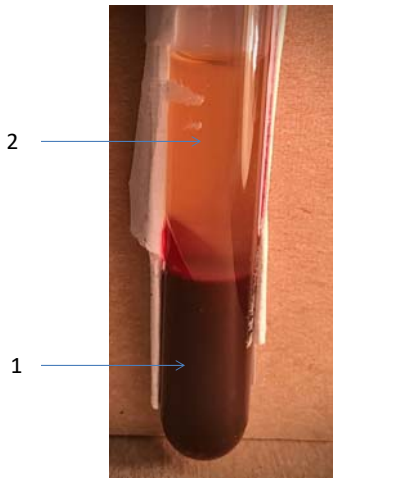
- Blood has 4 major components:
  1. Red Blood Cells (RBCs)
  2. Plasma
  3. White Blood Cells (WBCs)
  4. Platelets



3

What is Blood?

- Blood Bank Tests:
  1. Red Blood Cells (RBCs)
     - Antigens
  2. Plasma
     - Where antibodies are stored



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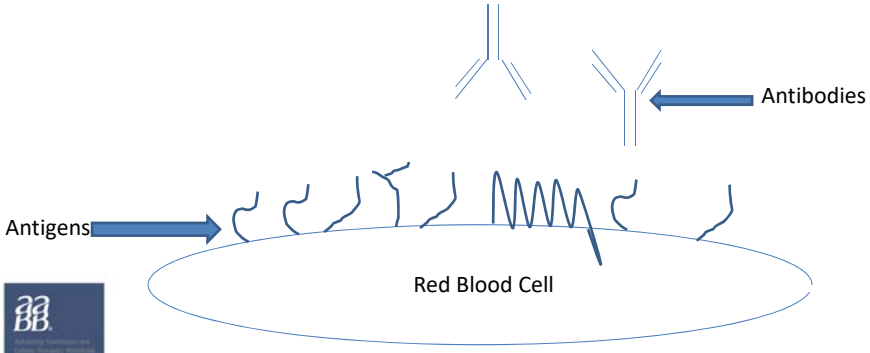
Antigens vs Antibodies

- Antigens if foreign to the body, can stimulate the immune system. Examples:
  - Bacteria
  - Virus
  - RBC
- A stimulated immune system may make antibodies to interact with the antigen.



5

What is an ABO?

- An *ABO* or *Blood Type* references the ABO blood group system, one of 36 blood group systems![1]
  - Hundreds of antigens on RBCs.
- The ABO blood group system is the most important blood group system.
- The four blood types are: O, A, B, AB

1. Fung M, Eder A, Spitalnik S, Westhoff C. AABB Technical Manual. 19th ed. Bethesda: AABB. 2017; 262 p.

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What is an ABO?

	Type O	Type A	Type B	Type AB
Antigen		A	B	AB
Antibody	Anti-A Anti-B	Anti-B	Anti-A	No antibodies
Transfuse RBC ABO Group	O	A or O	B or O	AB or A or B or O
Transfuse Plasma ABO Group	O or A or B or AB	A or AB	B or AB	AB

7

ABO Prevalence (%) in the US Population

ABO Group	European Ethnicity	African Ethnicity
O	45	49
A	40	27
B	11	20
AB	4	4

AABB Technical Manual, 19 ed. Table 10-1 page 267

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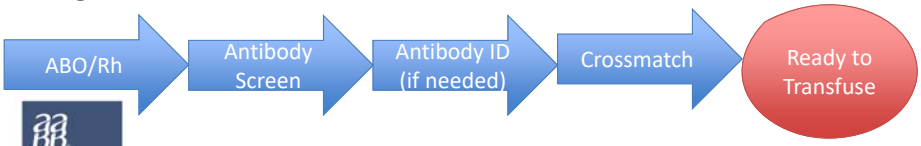
Blood Bank Testing

- Hold
- Type and Hold
  - "Type" or "ABO/Rh" or "Blood Type"
- Type and Screen
  - ABO & Rh Type
  - Antibody Screen
  - Antibody Identification if needed
- Crossmatch



9

ABO & Rh Type

- Front Type
  - Determines which antigens are on the RBCs
  - Reagent ABO antibody bind to patient RBC antigens.

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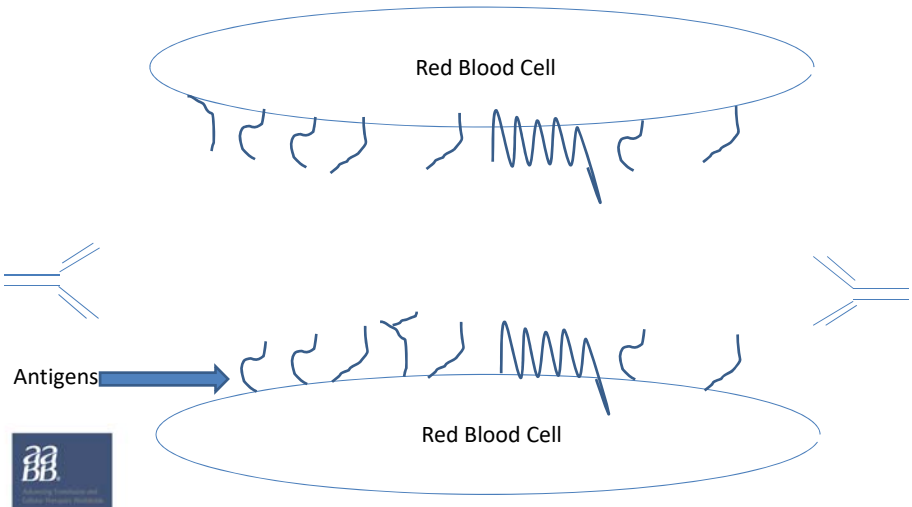
ABO Front Type

1	Place 1 drop of anti-A in a clean, labeled test tube.
2	Place 1 drop of anti-B in a separate, clean, labeled tube.
3	To each tube, add 1 drop of a 2% to 5% suspension (in saline, serum, or plasma) of the red cells to be tested. Alternatively, the equivalent amount of red cells can be transferred to each tube with clean applicator sticks.
4	Gently mix the contents of the tubes; then centrifuge for the calibrated spin time.
5	Gently resuspend the cell buttons, and examine them for agglutination.
6	Read, interpret, and record the test results. Compare the red cell test results with those obtained in the serum or plasma tests.

11

Testing Principles-Agglutination



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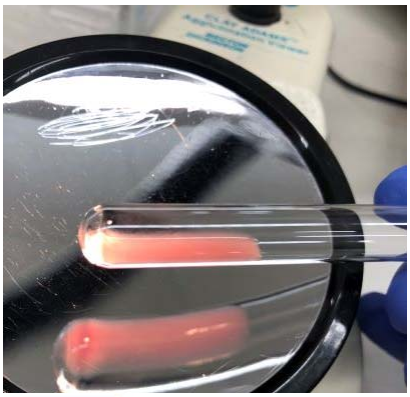
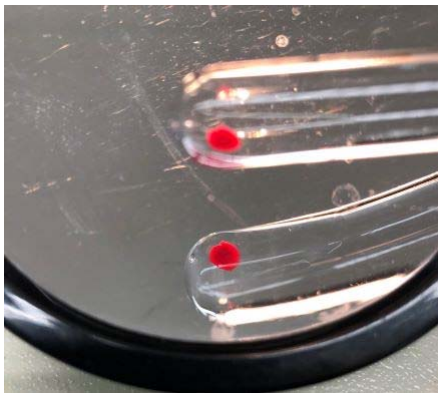
Macroscopic Agglutination

Negative



Positive



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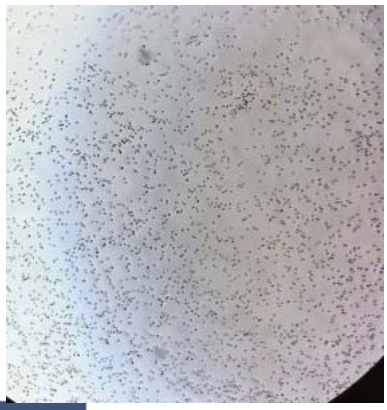
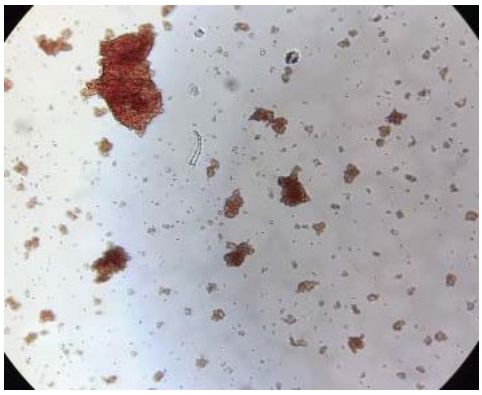
Microscopic Agglutination

Negative



Positive



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ABO Backtype

- Back Type
  - Determines which antibodies are present in the patient
  - Patient's antibody binds to reagent red cell antigens.

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ABO Back Type

1	Add 2 or 3 drops each of serum or plasma to two clean, labeled test tubes.
2	Add 1 drop of A_1 reagent red cells to the tube labeled A_1.
3	Add 1 drop of B reagent red cells to the tube labeled B.
4	Gently mix the contents of the tubes; then centrifuge for the calibrated spin time.
5	Examine the serum overlying the red cell buttons for evidence of hemolysis. Gently resuspend the cell buttons, and examine them for agglutination.
6	Read, interpret, and record test results. Compare serum test results with those obtained in testing red cells.

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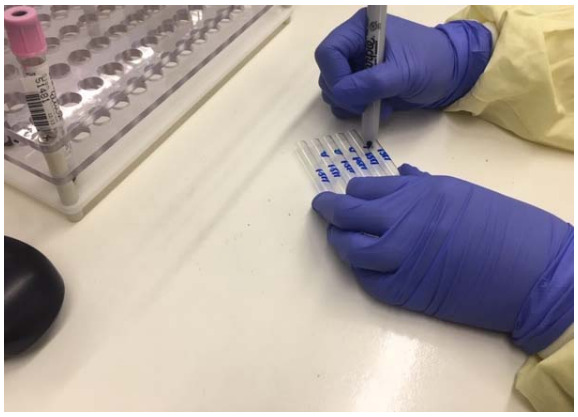
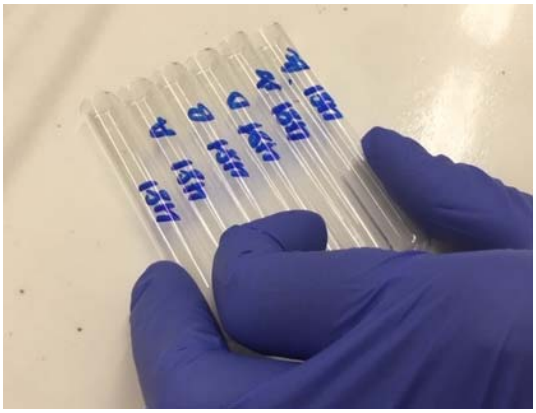
Step 1: Label Tubes



17

Step 1: Label Tubes



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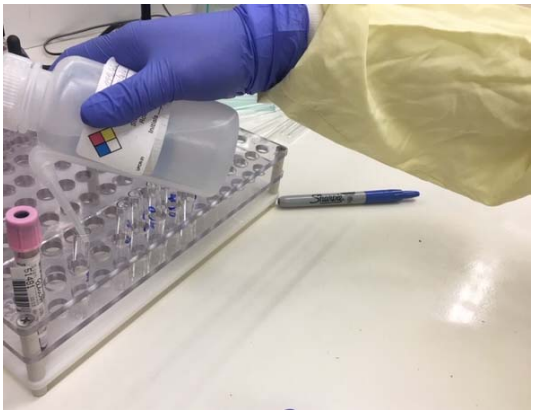
Step 2: Add Saline to Cell Suspension Tube



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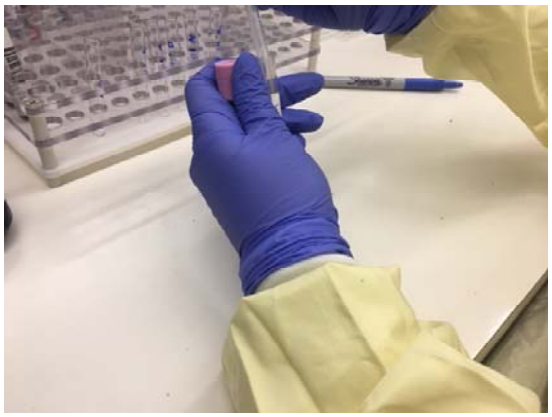
Step 3: Obtain Patient Plasma



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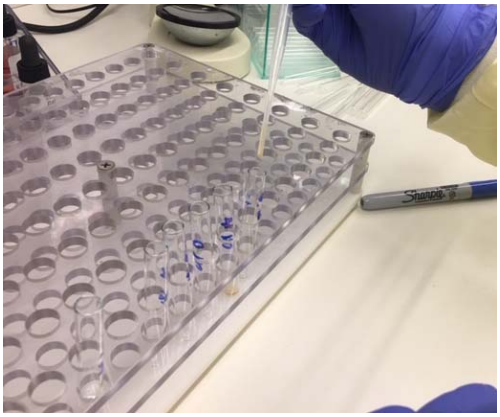
Step 4: Add 2 Drops of Plasma to Backtype Tubes



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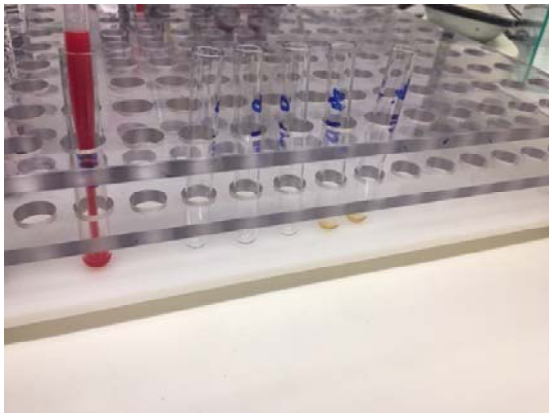
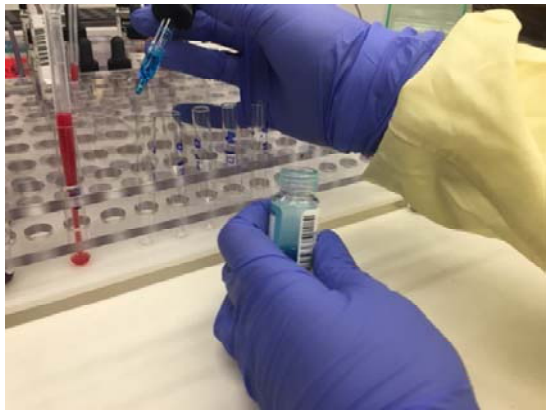
Step 5: Prepare Patient Red Cell Suspension



22

Step 6: Add anti-A



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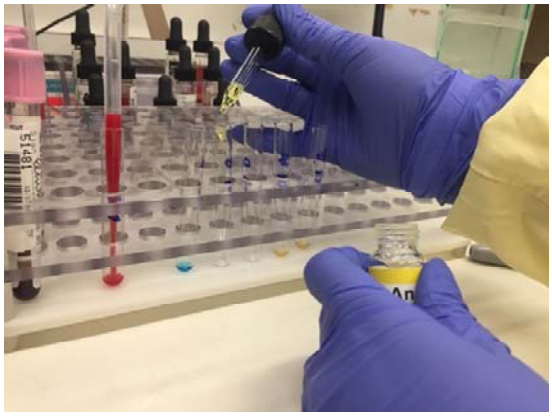
Step 7: Add anti-B



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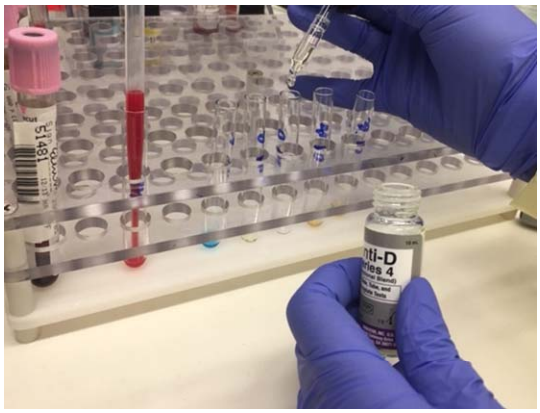
Step 8: Add anti-D



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Step 9: Add Reagent Red Cells to Back Type

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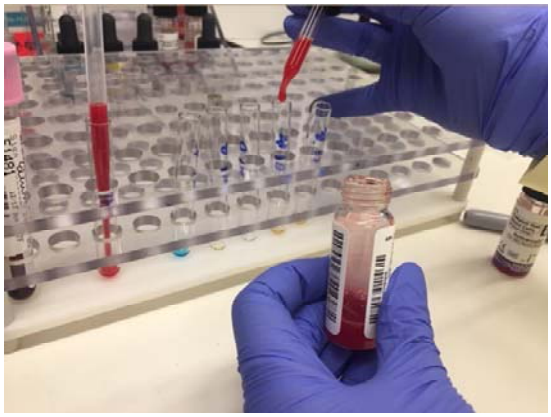
Step 10: Add Reagent A1-cells



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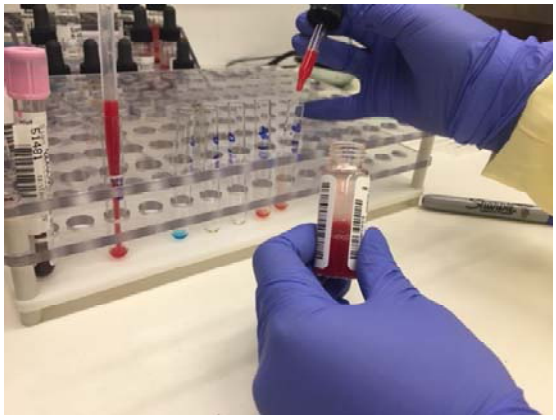
Step 11: Add Reagent B-Cells



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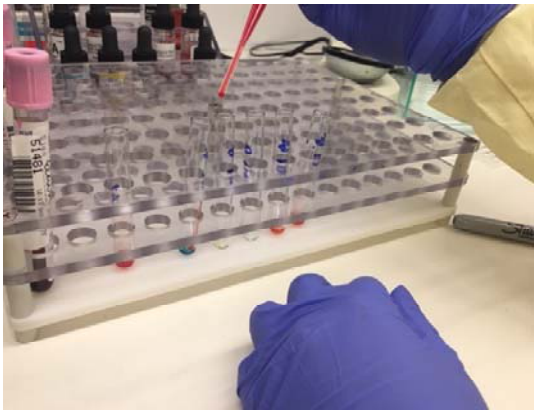
Step 12: Add Patient Cells to Front Type



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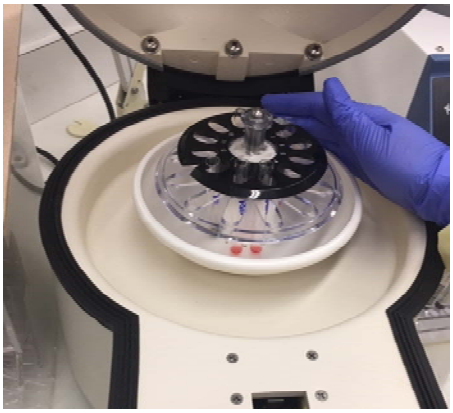
Step 13: Centrifuge



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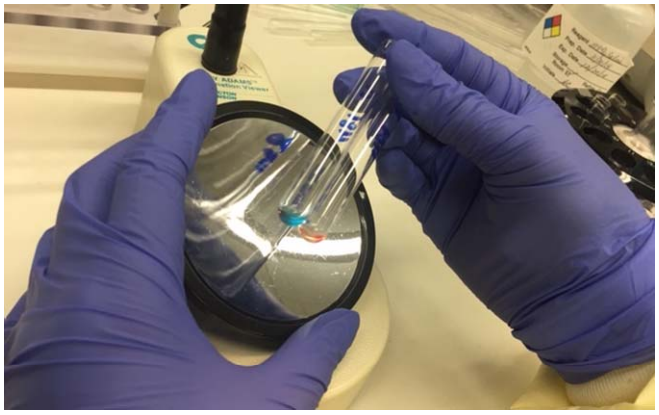
Step 14: Shake, Rattle, and Roll-Front Type



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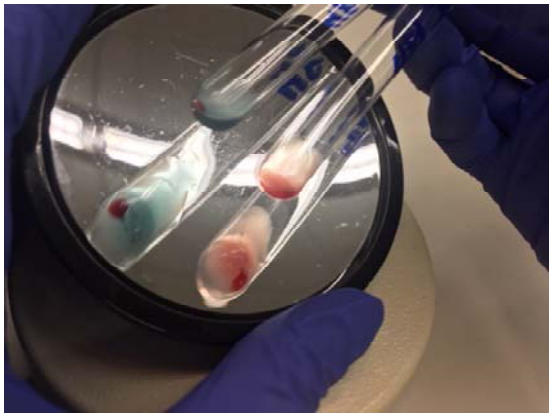
Step 14: Shake, Rattle, and Roll-Front Type



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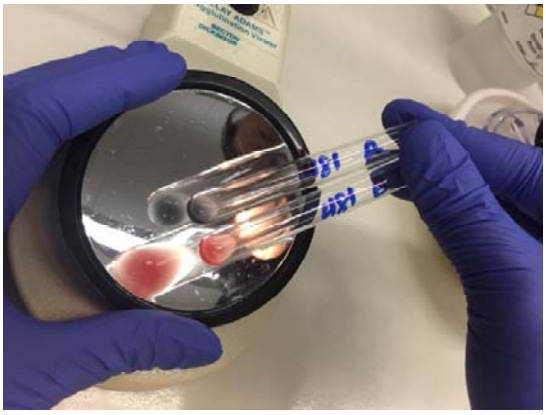
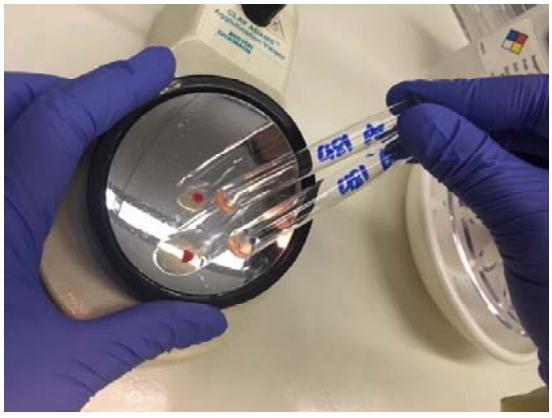
Step 14: Shake, Rattle, and Roll-Front Type



33

Step 14: Shake, Rattle, and Roll-Back Type



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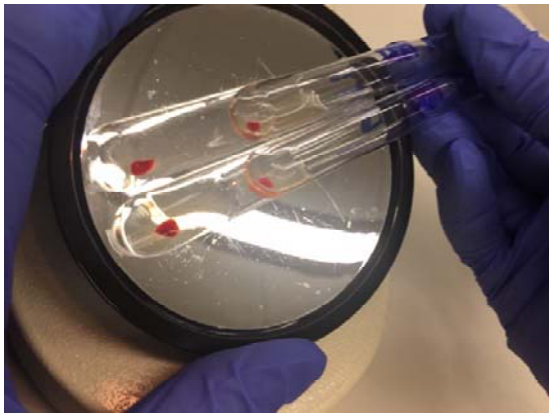
Step 14: Shake, Rattle, and Roll-Back Type



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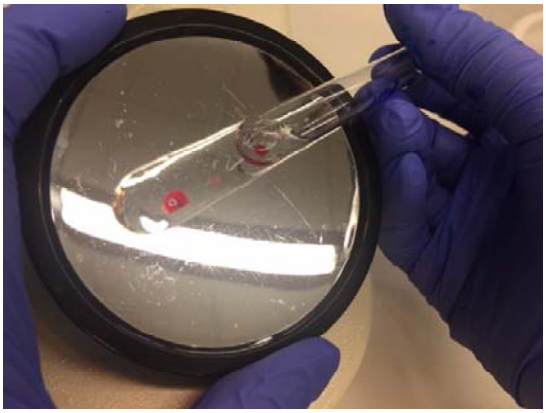
Step 14: Shake, Rattle, and Roll-D Type



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ABO Grouping

Routine ABO Grouping

Reaction of Red Cells with Antisera (Red Cell Grouping)		Reaction of Serum with Reagent Red Cells (Serum Grouping)			Interpre-tation
Anti-A	**Anti-B**	**A_1 Cells**	**B Cells**	**O Cells**	**ABO Group**
0	0	+	+	0	O
+	0	0	+	0	A
0	+	+	0	0	B
+	+	0	0	0	AB

Fung M, Eder A, Spitalnik S, Westhoff C. AABB Technical Manual. 19th ed. Bethesda: AABB. 2017; Table 12-2.

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Detecting RBC Antibodies

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The Antibody Screen

- To detect clinically significant antibodies to non-ABO antigens.
- Reagent red cells must be ABO group O.
- FDA requires the following antigens be present for licensed reagent screens:
  - D,C,E,c,e; K,k; Fy^a,Fy^b; Jk^a,Jk^b; Le^a,Le^b; P1; M,N,S,s

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The Antibody Screen

- Licensed reagent antibody screens have 2 or 3 cells.
  - 2 cell screen
    - Both cells are D-Positive
    - Variable expression of other antigens.
  - 3 cell screen
    - 2 cells are D-Positive and 1 is D-Negative
    - Better differentiation of antibody if detected.
    - Usually offer stronger expression of antigens.

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The Antibody Screen

2-Cell

	RH						MNS				LU		P	Lewis		Kell		Duffy		Kidd		Gel Test
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IAT
1	+	+	0	0	+	0	+	+	+	+	0	+	+	+	0	+	+	+	0	0	+	1+
2	+	0	+	+	0	0	+	0	0	+	0	+	+	0	+	0	+	+	+	+	0	0

3-Cell

	RH						MNS				LU		P	Lewis		Kell		Duffy		Kidd		LISS (Test Tube)		
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	37 C	IAT
1	+	+	0	0	+	0	+	+	+	+	0	+	+	+	0	+	+	+	0	0	+	0	0	2+
2	+	0	+	+	0	0	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	0	0	1+
3	0	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	+	+	+	0	0	0√

Antibody Identification: Art or Science? A Case Study Approach
By Janis R. Hamilton, MS, MT(ASCP)SBB; Susan T. Johnson, MSTM, MT(ASCP)SBB; Sally V. Rudmann, PhD, MT(ASCP)SBB

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Two-Cell Antibody Screen

1. Add 2 drops of patient plasma into 2 clean tubes labeled “I” and “II”.
2. Add 1 drop of screening cell I into tube “I”.
3. Add 1 drop of screening cell II into tube “II”.
4. Add 2 drops of enhancement reagent (PEG). Mix well.
5. Incubate at 37C for 15 minutes.
6. Wash the red cells four times with saline, and completely decant the final wash.
7. Add anti-IgG to the dry red cell button. Mix well.
8. Centrifuge and observe for agglutination. Grade and record the results.
9. Confirm the validity of negative results by adding IgG-coated red cells.

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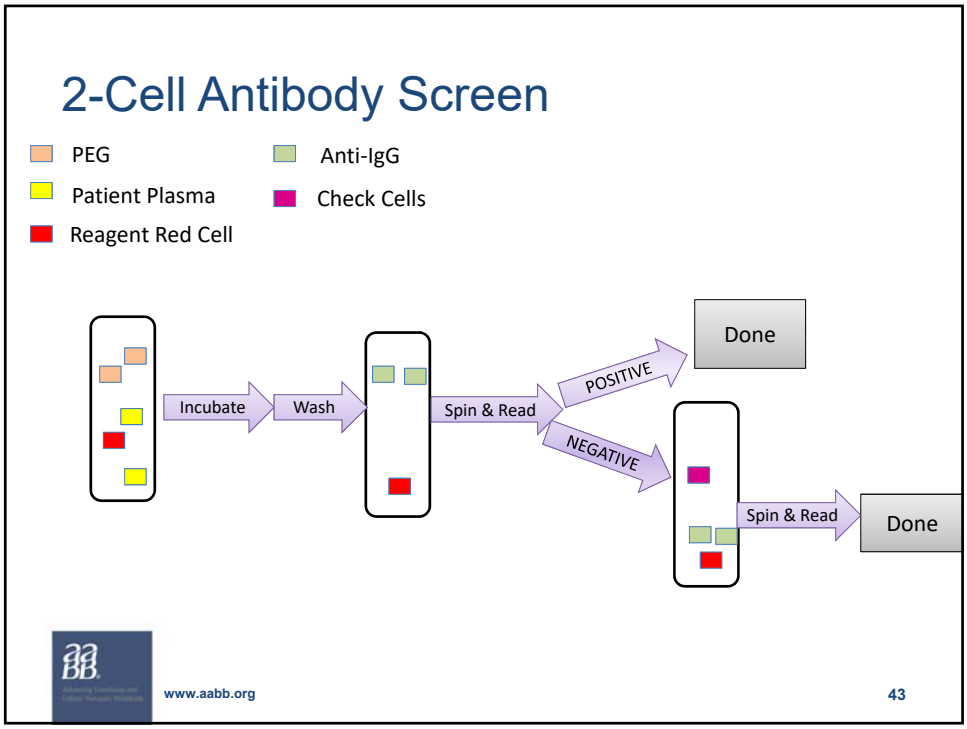

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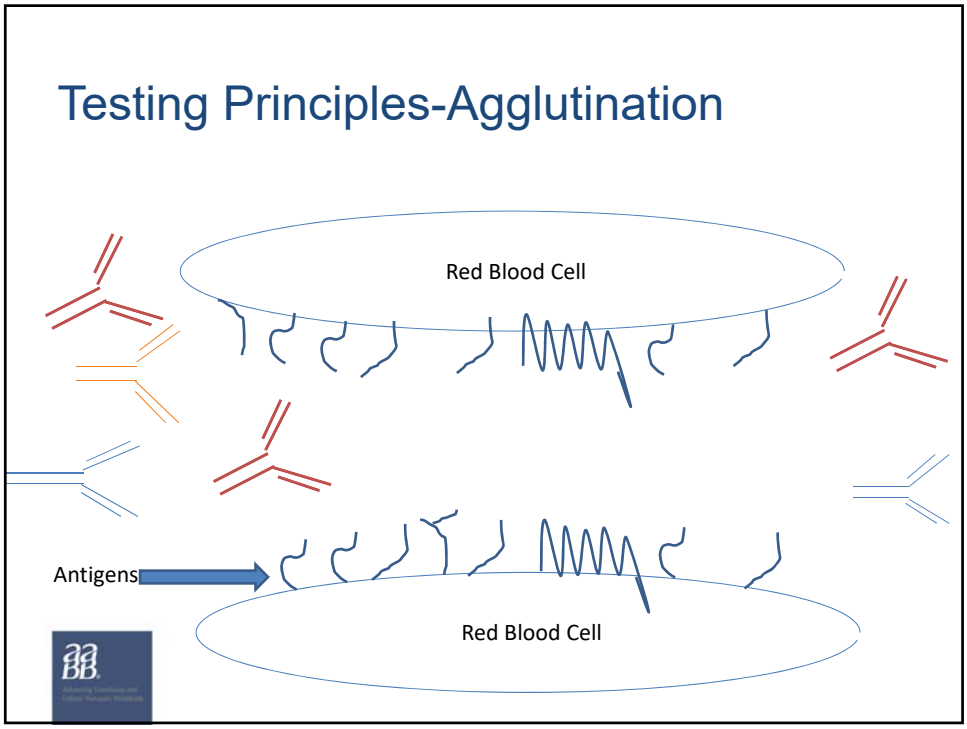

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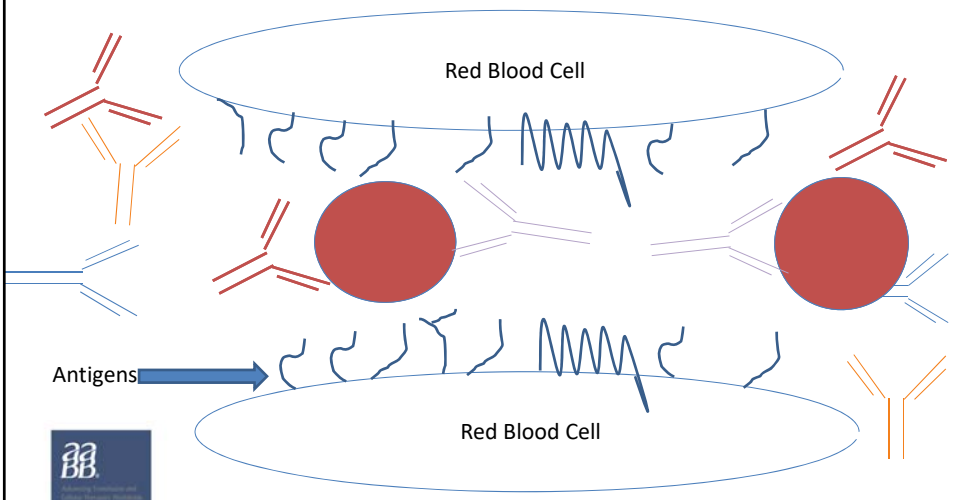
Testing Principles-Agglutination



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Antibody Identification

- Performed if the screen is positive.
- A series of reagent cells, typically 10, 16, or 20 cells, with variable phenotypes for the major blood group systems.
  - Allows for patterns of reactivity to rule in and rule out antibodies.
  - Enhancement media may be added to reduce 37C incubation time from 60 minutes to 15 minutes just like the antibody screen.
- It's a puzzle!

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Antibody ID Antigram

	RH						MNS				LU		P	Lewis		Kell		Duffy		Kidd		SP
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IAT
1	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	+	+	2+
2	+	+	0	0	+	0	+	+	+	0	0	+	0	0	0	0	+	+	+	+	+	2+
3	+	0	+	+	0	0	0	+	+	+	0	+	0	0	0	0	+	+	+	0	+	2+
4	+	0	0	+	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	0	+	2+
5	0	+	0	+	+	+	+	+	0	+	0	+	+	+	0	0	+	+	0	+	0	0
6	0	0	+	+	+	+	+	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0
7	0	0	0	+	+	+	+	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0
8	0	0	0	+	+	+	+	+	+	+	0	+	0	0	+	0	+	+	0	+	0	0
9	0	0	0	+	+	+	+	0	+	0	0	+	+	+	0	0	+	0	+	+	+	0
10	0	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0
11	+	+	0	0	+	0	+	+	+	+	0	+	0	+	0	+	+	0	+	+	+	2+

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Crossmatching Blood

How do we detect incompatibilities?

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Crossmatch

- Immediate-Spin
  - Serological method to detect ABO incompatibility
- Computer/Electronic
  - Alternative way to verify ABO compatibility
- Antiglobulin
  - Serological method to detect RBC compatibility to non-ABO clinically significant antibodies.

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Immediate-Spin Crossmatch

- Donor red cells and patient plasma are mixed, spun, and read.
- Can be used as the sole crossmatch method only if the recipient has no present or previously detected, non-ABO clinically significant antibodies.
- Failure to follow procedure can result in failure to detect ABO-incompatible RBCs.

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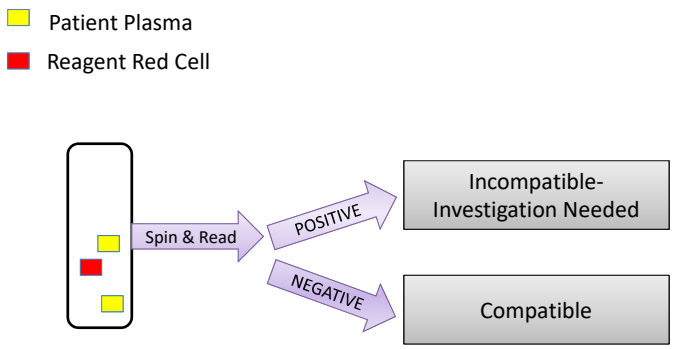
Immediate Spin Crossmatch



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Computer/Electronic Crossmatch

- Can be used if:
  - System is validated to ensure only ABO-compatible RBCs are given.
  - Two determinations of the patient's ABO group are made
  - The system contains the DIN, component name, ABO & Rh group, the confirmed donor unit's ABO group, two unique patient identifiers, the patient's ABO, Rh, ABS result and compatibility interpretation.
  - A method to verify correct entry of data before release of blood.
  - System contains logic to alert users of discrepancies or incompatibility.

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Antiglobulin Crossmatch

- Used for patient with historical or currently detected non-ABO clinically significant antibodies.
- RBC must lack relevant antigens the antibodies are directed to.
- Crossmatch mixing donor RBC and patient plasma, a 37C incubation, followed by the AHG test.
  - Enhancement media may be added to reduce 37C incubation time from 60 minutes to 15minutes just like the antibody screen and antibody ID!
- Not a substitute for the IS crossmatch.

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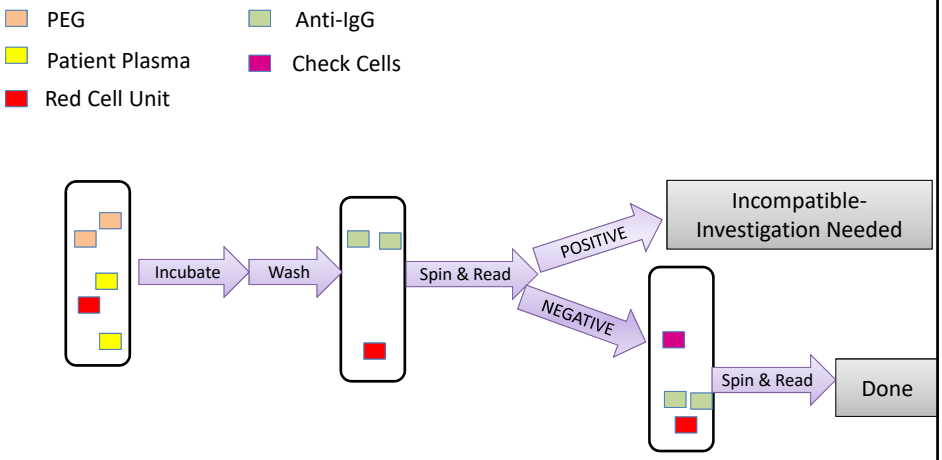
Antiglobulin Crossmatch



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Why Is This Important?

- Transfusing blood is the most common procedure performed in the USA.
- Support fetal development, surgeries, cancer treatments, CMO patient's.
- National Security
- Failure to provide safe transfusions can be fatal.

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Questions?

Contact
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