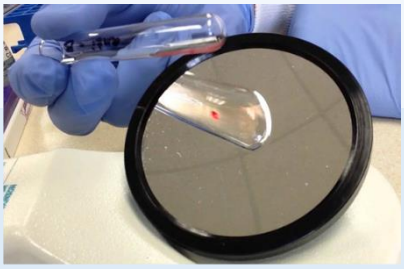
Comparing Blood Bank Methodologies

Objectives:

1. **Describe tube testing, gel testing and solid phase testing in the blood bank.**
2. **Compare and contrast the three methodologies.**
3. **List advantages and disadvantages of each methodology.**

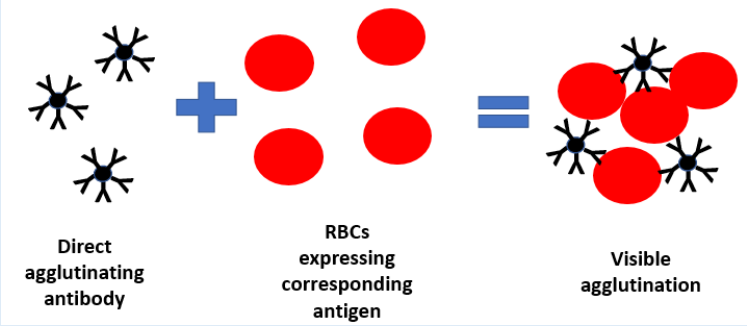
Quick lesson:

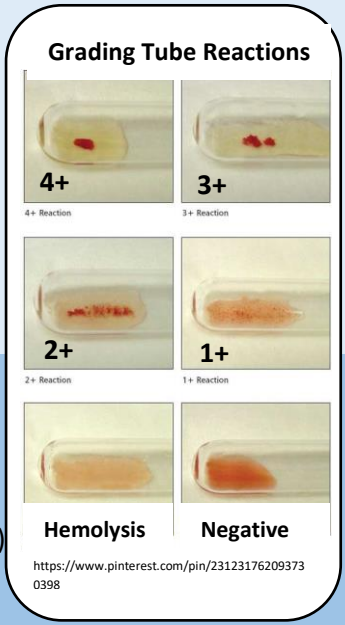
Tube Testing: the "gold standard"



https://www.youtube.com/watch?v=5bEn0HlU_2E

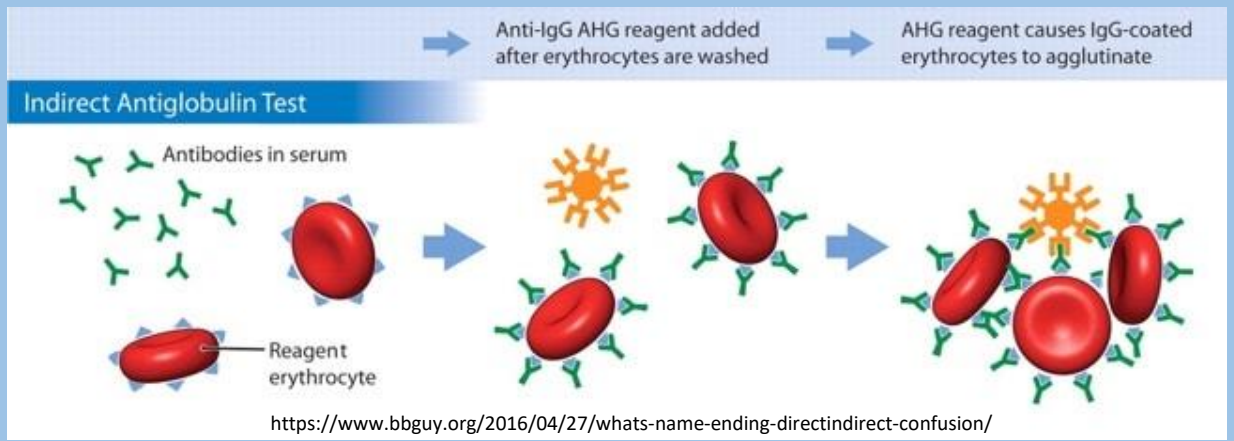
- **Relies on visible agglutination of red cells**
- **Direct agglutination:**
  - Antibody + RBC = visible agglutination
  - "Immediate spin" phase
  - IgM or cold-reacting IgG





https://www.pinterest.com/pin/23123176209373 0398

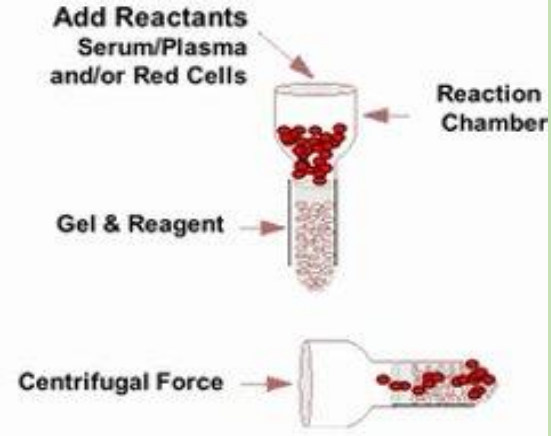
- **Indirect Antiglobulin Test (IAT):**
  - Antibody + RBC
  - Incubation at 37C (antibody coats RBCs)
  - Wash (remove unbound antibody)
  - Add anti-human IgG (antibody to Fc portion of human IgG)
  - Visible agglutination
  - Detection of IgG antibody



https://www.bbguy.org/2016/04/27/whats-name-ending-directindirect-confusion/

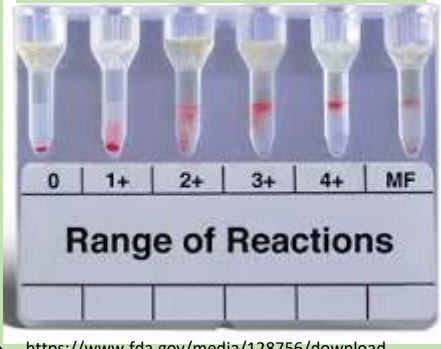
Gel Testing:

- **Relies on antibody-coated red cells getting stuck in gel matrix containing anti-IgG**
- Antibody + RBCs in upper chamber, incubation at 37C
- Centrifugation
- RBCs fall through gel matrix containing anti-IgG
- Antibody-coated cells get stuck in matrix/cells not coated fall to the bottom of column
- Designed to detect IgG



https://quizlet.com/126557723/automation-in-bb-flash-cards/

Grading Gel Reactions



https://www.fda.gov/media/128756/download

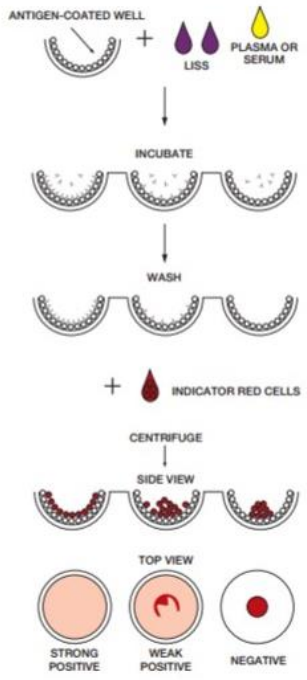
Solid Phase Testing: (SPRCA: solid phase red cell adherence assay)

- Antigens coat microwells
- Plasma and enhancement is added to wells, incubated at 37C, antibodies (if present) attach to antigens
- Wash step: unbound antibody is removed
- Indicator cells (RBCs bound to anti-IgG) are added
- Centrifugation
- Interpretation: a tight cell button indicates a negative reaction, effacement of the cell button indicates a positive reaction

Grading Solid Phase Reactions

https://www.trasci.com/article/S1473-0502(12)00070-5/pdf

Solid Phase Methodology



Compare and Contrast Methodologies

Methodology	What does a positive reaction look like?	What does a negative reaction look like?	Volume of plasma required (per test)	% RBC suspension utilized	Tests for IgG, IgM, or both?	Includes incubation at 37C (yes/no)?	Includes a wash step (yes/no)?	Utilizes centrifugation (yes/no)?	Advantages of this methodology	Disadvantages of this methodology
Tube	Antibody coated red cells visibly agglutinate after adding anti-IgG reagent	No agglutination	100ul	3%	IgG & IgM (IS & IAT phases)	Yes	Yes	Yes	Gold standard, testing conditions easily manipulated, differentiates IgG from IgM	Requires competence of techs, tech time, subjective scoring
Gel	Antibody coated cells get stuck in gel matrix following centrifugation	Cells at pellet at bottom of column	25ul	0.8%	Designed for IgG. IgM may be detected due to size of IgM molecule	Yes	No	Yes	Small sample volume, not as subjective of grading, very sensitive, can be automated	Very sensitive, rouleaux interferes as there isn't a wash step, requires 0.8% RBC suspension
Solid phase	Indicator cells adhere to the surface of the microwell when antibody is bound to red cell antigens coating the microwell.	Cells in solid pellet in well	50ul	3%	Designed for IgG	Yes	Yes	Yes	Can be automated, sensitive	Subjective grading

Further Discussion:

1. Which **description and picture** best describe the possible result of forgetting to incubate the gel card prior to centrifugation?

   a. False Positive 

   b. False Negative 

   c. False Positive 

   d. False Negative 

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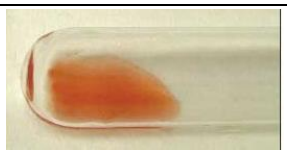
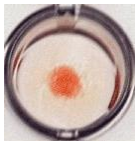
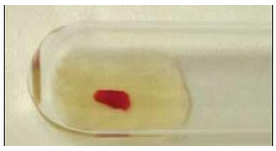
2. What is the purpose of the "wash" step in tube and solid phase testing?
   a. To enhance antibody-antigen reactivity
   b. To remove unbound antibody
   c. To decrease incubation time
   d. To wash off antibody that is bound to red cells

3. Which row of pictures is matched with the correct interpretation (tube, solid phase, gel and interpretation are correct)?

	Tube testing	Solid phase testing	Gel Testing	Interpretation
a.				Negative
b.				2+
c.				4+
d.				Negative

Further Discussion: Answers

1. d. During incubation, antibody (if present) binds to RBCs. If this binding doesn't occur, antibody won't be detected (false negative). The RBCs won't get stuck in gel matrix, and will fall to the bottom of column.
2. b. Both tube testing and solid phase testing include a addition of a reagent that could be neutralized by unbound antibody (in tube testing, anti-IgG: in solid phase testing, indicator cells). The wash phase removes plasma and any unbound antibody from the test system, so that these reagents react with the antibody that is bound to antigen (on RBCs or on the microwell). Gel testing has no wash step.
3. a. All reactions in row a are negative reactions. In rows b, c and d, the gel reaction is incorrect. Row b gel reaction is 4+. Row c gel reaction is negative. Row d gel reaction is 2+.

Assessing Understanding

1. **In tube testing, which of the following best describes the difference between the immediate spin (IS) phase and the IAT phase?**
    a. IS phase usually detects warm reacting antibody; IAT phase usually detects cold reacting antibody
    b. IS phase usually detects IgM; IAT phase usually detects IgG
    c. IS phase may indicate clinically significant reactivity; IAT phase often indicates insignificant reactivity
    d. IS phase detects alloantibody; IAT phase detects autoantibody
2. **Which of the following best represents sample volume requirements for each methodology?**
    a. Gel>Solid phase>Tube
    b. Tube>Solid phase>Gel
    c. Solid phase>Gel>Tube
    d. Solid phase>Tube>Gel
3. **Which of the following is an important disadvantage of gel testing?**
    a. Cannot be automated
    b. Requires large sample volume
    c. Very subjective reaction grading
    d. Rouleaux can interfere with detection of IgG antibody

Answers: 1. b; 2. b; 3. d