**Objectives**

- Discuss direct agglutination as a means of *in vitro* detection of antigens and antibodies.
- List acceptable specimens for blood type determinations.
- Explain the principle of ABO and Rh testing.
- Explain the principle of the weak D test.
- Describe the reagents used in routine ABO grouping and Rh typing.
- Discuss quality control requirements for ABO and Rh reagents.

**More objectives**

- Discuss tube, microtiter well and gel test platforms.
- Evaluate/ Grade ABO and Rh reactions using conventional reagents.
- Interpret ABO and Rh results when given a set of reactions.

**Visualizing the Reaction**

- **Agglutination**
  - Formation of lattice as antibody binds to antigens on neighboring RBCs.

- **Hemolysis**

**Agglutination**

**Hemolysis**
Detection of Antigens
- Antigens found on RBCs
- Anti-serum = Reagent containing the corresponding antibody

Detection of Antibodies
- Antibodies found in plasma or serum
- Reagent = RBCs known to possess the corresponding antigen

Direct Agglutination

Testing Phases
- IS = Immediate Spin – no incubation period prior to centrifugation; reactants usually at room temperature
- 37°C incubation = reactants brought to 36-38°C for a period of time prior to centrifugation
**Direct Agglutination – Microtiter Plate**

**The Blood Type**
- ABO group
- Rh type
- ABO and Rh tests are almost always performed simultaneously
  - Two separate procedures, as these are two separate blood group systems
  - Procedures are very similar

**ABO Testing**
Test for both antigens on Red Blood Cells (forward grouping) and Antibodies in plasma (reverse grouping)

**Specimen**
- EDTA – most common
- Clot tube
- Centrifuged to separate plasma from RBCs

**Reagents: Anti-serum**
- Contains known antibodies
- Used to detect antigen on RBCs
  - Forward Grouping
  - Front type
- Routinely use anti-A and anti-B
- May be monoclonal or "conventional"

**Polyclonal Vs. Monoclonal**
- Polyclonal reagents = conventional reagents
- Produced by immunizing donors, then collecting the sera containing antibody.
- Contains antibodies against multiple epitopes.
- Monoclonal reagents most frequently used.
- Hybridoma technology used to create a single antibody that is directed against just one epitope.
**Monoclonal Advantages**
- No lot to lot variation in reactivity
- High titers of antibody
- High specificity
- High sensitivity
- Reduced risk of infectious disease

**Monoclonal Disadvantage**
- Antibody is directed against only one epitope, so may get false negative results if patient has an abnormal antigen missing that particular epitope
  - To avoid this, many monoclonal reagents are a blend of several different monoclonal antibodies or a blend of monoclonal and polyclonal antibodies

**Reagent: Red Blood Cells**
- 2-5% suspension of RBCs in a preservative solution
- Known antigen
- Used to detect antibodies in plasma/serum
  - Reverse grouping
  - Back type
- Routine use of A, and B cells
  - Rh Negative to avoid detection of Anti-D in plasma

**Reagent Quality Control**
- A and B RBC reagents are tested against anti-A and anti-B anti-sera daily
  - The A cells should only react with anti-A while the B cells should only react with anti-B
  - Usually expect a strong (4+) reaction with monoclonal reagents
- Includes a visual inspection of the reagents looking for signs of bacterial contamination or improper storage
- Check expiration dates
  - Reagents may be used until the outdate printed on the vial.

**ABO Grouping Tube / Microtiter well**
- Testing performed at "immediate spin" phase
  - Reaction takes place at room temperature
  - Does not require incubation
  - Direct agglutination
- Forward and reverse grouping serve as a check on each other
  - Performed at same time

**Forward Grouping**
- Centrifuge at 3500 rpm. Read, grade, record.
**Introduction to Clinical Immunohematology**

**Blood Type Determinations**

**Forward Grouping – Cellular Level**

- Anti-A
- Anti-B

**Reverse Grouping**

- Centrifuge at 3500 rpm.
- Read, grade, record.

**Reverse Grouping – Cellular Level**

- A₁ Cells
- B Cells

**Interpretation**

- Interpret both the forward and reverse grouping results to determine ABO group
  - Should be in agreement
  - If not, investigate discrepancies

**ABO / Rh - Tube**

**ABO/Rh Typing – Microtiter wells**
ABO/Rh Typing - Gel Method
(Microcolumn Agglutination)

Gel Method

Gel Method – Adding reactants

Gel - Centrifugation

Reading Reactions in Gel

ABO/Rh Typing - Gel Method

Reagent layer
Dextran acrylamide gel

Reaction chamber
**Routine ABO Testing**

<table>
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<th>Reagent Anti-A</th>
<th>Anti-B</th>
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<th>B Cells</th>
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<td>++</td>
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**Note!**

- Testing for the Rh(D) antigen is usually included with ABO forward grouping.
- Together, the ABO and Rh make up what is commonly referred to as the “blood type.”

**Rh Typing**

**D Typing Sera**

- Routine testing is for D antigen only.
- Anti-serum contains antibodies to multiple D epitopes.
- Designed to react at immediate spin phase of testing.
- Low protein reagent most commonly used.

**Anti-D Daily QC**

- Anti-D is tested daily against known Rh Positive RBCs to verify reactivity – Usually expect 3 – 4+ reactions.
- Anti-D may be tested daily against known Rh Negative cells to verify specificity.
- Includes a visual inspection of the reagent and check of expiration date.

**Routine Testing - D Tube Method**

- Centrifuge at 3500 rpm.
- Read, grade, record.

ID

2 - 5% RBCs in saline
Negative Controls for anti-D

- A negative control may be tested in parallel to validate the results with anti-D. This is necessary when:
  - A high protein anti-D reagent is used.
  - The RBCs being tested react with a low protein anti-D reagent along with both anti-A and anti-B.
- The RBCs are tested using an inert reagent in place of the anti-D reagent.
- Reagents that may be used as a negative control include:
  - Rh-hr control
  - 6% Bovine Serum Albumin (BSA)

For example…

When using a low protein monoclonal anti-D

Weak D Test

- Anti-D reagent + 1 drop of 2-5% suspension of individual’s RBCs
- Incubate at 37°C for 15 to 30 minutes
- Wash with saline (x3) to remove unbound antibody
- Add 2 drops of AHG reagent
- Centrifuge, then read for agglutination

Weak D Control

- If the weak D test is positive, a control must be tested. A negative control establishes the validity of a positive weak D test.
- Repeat the weak D test, substituting one of these inert materials for the anti-D reagent:
  - Rh-hr control
  - 6% BSA
- Perform a Direct Antiglobulin Test on the patient’s RBCs
  - Detects RBCs that were coated with antibodies in vivo
**Weak D Controls**

- Control must be negative in order for the positive Weak D test to be considered valid

**Weak D Test – Rh Negative**

**Rh Testing in Gel**

**Weak D Testing in Gel**

**Other Rh Typing Sera**

- Follow manufacturer’s instructions for use
- Controls: Test against an antigen positive cell and an antigen negative cell each day of use.

**Interpreting the Blood Type**

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**Blood Type Determinations**
Grade and Record the Reactions
Then Interpret the Results

Interpretation:
B Rh Positive
(B Rh Pos or B+)

ABO/Rh Typing – Tube

The End