Pre-Transfusion Testing

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Pre-Transfusion Testing

- **Purpose**
  - Avoid risks to donor and recipient
  - Meet product specifications

- **Consist of both donor and patient testing.**
  - Donor testing and residual risk of infection
  - Patient testing
Donor Pre-Transfusion Evaluation

- Donor medical history and risk factor assessment
- Infectious disease testing
- ABO and Rh typing
- Test for antibodies to red cell antigens
- Capturing post donation information
Donor Infectious Disease Testing

- Hepatitis B, sAg and anti-core antibody
- Hepatitis C antibody
- HIV 1 and 2 antibodies
- HTLV 1 and 2 antibodies
- Serologic Test for Syphilis
- Nucleic Acid Testing (NAT) for HIV, HCV and WNV
- Detection of Bacteria in platelet products
- CMV antibody for select recipients
## Routine Donor Testing Algorithm

<table>
<thead>
<tr>
<th>Blood Screening Assays</th>
<th>Confirmatory Assays</th>
<th>Supplemental Assays</th>
<th>Referral Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HCV EIA</td>
<td>HIV-1/HCV NAT Discrimination (Chiron)</td>
<td>Anti-HIV-2 EIA</td>
<td>Anti-HIV-2 WB</td>
</tr>
<tr>
<td></td>
<td>RIBA HCV 3.0 SIA</td>
<td>Genetic Systems, Inc.</td>
<td>California Department of Health</td>
</tr>
<tr>
<td></td>
<td>Ab to HCV Strip Immunoblot Assay (Chiron)</td>
<td>• If Non-reactive, no further testing.</td>
<td>Services</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If Reactive, perform HIV-2 EIA WB.</td>
<td>No further testing.</td>
</tr>
<tr>
<td>Anti-HIV-1/2 EIA Plus O</td>
<td>Licensed Anti-HIV-1 IFA Flourognost</td>
<td>Alt Manufacturer</td>
<td>EIA/IFA Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HTLV-I/II EIA</td>
<td>California Department of Health</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If Non-reactive, no further testing.</td>
<td>Services</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If Reactive, sample sent for referral testing.</td>
<td>No further testing.</td>
</tr>
<tr>
<td>Anti-HTLV-I/II EIA</td>
<td>NA</td>
<td>Alt Manufacturer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HTLV-I/II EIA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If Non-reactive, no further testing.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If Reactive, sample sent for referral testing.</td>
<td></td>
</tr>
</tbody>
</table>
## Routine Donor Testing Algorithm

<table>
<thead>
<tr>
<th>Blood Screening Assays</th>
<th>Confirmatory Assays</th>
<th>Supplemental Assays</th>
<th>Referral Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HBC EIA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
| HBsAG ChLIA            | HBsAG Confirmatory Neutralization ChLIA (Abbott Prism)  
                        | HBC ChLIA (Abbott Prism)  
                        | PCR Roche (Referral Testing) |
| NAT HIV-1/HCV          | NAT HIV-1/HCV  
                        | Discrimination (Chiron)  
                        | No further testing | NA |
| NAT WNV                | NA                  | NA                  | WNV Panel Antibody (IgM/IgG)  
                        | ELISA (Sonora Quest)  
                        | No further testing. |
# Routine Donor Testing Algorithm

<table>
<thead>
<tr>
<th>Blood Screening Assays</th>
<th>Confirmatory Assays</th>
<th>Supplemental Assays</th>
<th>Referral Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Syphilis</strong> (Olympus PK-TP)</td>
<td>Syphilis G EIA (Captia)</td>
<td>RPR/Quantitative RPR (Becton Dickinson)</td>
<td>NA</td>
</tr>
<tr>
<td>• If negative, no further testing.</td>
<td>• If positive, supplemental assay performed.</td>
<td>• If negative, no further testing.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If positive, RPR titer performed.</td>
<td></td>
</tr>
<tr>
<td><strong>T. cruzi Ab EIA</strong></td>
<td>NA</td>
<td>NA</td>
<td>T. cruzi Antibody RIPA (Quest Diagnostics Inc. Nichols Institute)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• If positive, no further testing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• If NOT positive, sample sent for Leishmania Antibody IFA (Focus Diagnostics)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No further testing.</td>
</tr>
</tbody>
</table>
The diagram illustrates the risk of infection per unit transfusion over time, with different interventions marked by arrows:

- Donor screening criteria changed (1983)
- Screening for HIV antibody (1984)
- Surrogate screening for non-A, non-B hepatitis (1985)
- Screening for HCV antibody (1986)
- Testing for p24 antigen (1990)
- Nucleic acid testing for HCV/HIV (1999)

The risk of infection per unit transfusion decreases significantly with each intervention, as indicated by the downward trend of the lines for HCV, HBV, and HIV. The reductions are noted as:

- 1/10000000 to 1/1000000
- 1/10000000 to 1/100000
- 1/10000000 to 1/10000
- 1/10000000 to 1/1000
- 1/10000000 to 1/100
- 1/10000000 to 1/100

The final risk levels are:

- 1/58000 to 1/149000
- 1/872000 to 1/1.7 x 10^6
- 1/1.4 x 10^6 to 1/2.4 x 10^6

This graph highlights the effectiveness of various interventions in reducing the risk of bloodborne infections through transfusions.
# Estimates of Known Viral Infectious Disease Risks of Transfusion

<table>
<thead>
<tr>
<th>Virus</th>
<th>Risk per Unit Transfusion</th>
<th>Transmission Rate</th>
<th>Window Period (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV 1&amp;2</td>
<td>1:2,135,000</td>
<td>90%</td>
<td>11 days</td>
</tr>
<tr>
<td>HCV</td>
<td>1:1,935,000</td>
<td>90%</td>
<td>10 days</td>
</tr>
<tr>
<td>HBV</td>
<td>1:205,000</td>
<td>70%</td>
<td>59 days</td>
</tr>
<tr>
<td>HTLV</td>
<td>1:3,000,000</td>
<td>30%</td>
<td>51 days</td>
</tr>
<tr>
<td>WNV</td>
<td>1:350,000</td>
<td>Unknown</td>
<td>11-13 days</td>
</tr>
<tr>
<td>Parvo B19</td>
<td>1:40,000 to 3,000</td>
<td>Low</td>
<td>-</td>
</tr>
<tr>
<td>Hepatitis A/E</td>
<td>1:1,000,000</td>
<td>Low</td>
<td>-</td>
</tr>
</tbody>
</table>

Decreasing Risk

1 in 10,000,000

Risk Decreasing

Death from lightning

1 in 1,000,000

Fatal plane crash

1 in 100,000

Estimated risk per unit transfused of HIV/HCV

1 in 10,000

Fatal auto accident

Risk Increasing

1 in 1,000

Fatal, unexpected drug reaction in hospital

1 in 100

### Estimates of Known NON-Viral Infectious Disease Risks of Transfusion

<table>
<thead>
<tr>
<th>Disease</th>
<th>Agent</th>
<th>Screening/Testing</th>
<th>Risk per Unit Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis</td>
<td><em>T. pallidum</em></td>
<td>Serological assays (Est. 1938; Req. 1958)</td>
<td>No reported cases since 1968</td>
</tr>
<tr>
<td>Malaria</td>
<td><em>Plasmodium</em></td>
<td>Donor screening/deferral</td>
<td>1: 4,000,000</td>
</tr>
<tr>
<td>Chagas Disease</td>
<td><em>T. cruzi</em></td>
<td>Serologic Assay approved by FDA, not yet mandated.</td>
<td>1:2,000 to 1:25,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Parasite reduction in endemic areas.)</td>
<td></td>
</tr>
</tbody>
</table>
Potential or Theoretical Threats

- Transmissible Spongiform Encephalopathies
  - CJD, vCJD
- TTV, SEN-V and HGV
- Human Herpes Virus 6 and 8
- Tick-borne Diseases
- Other Parasitic Diseases
- Other known or unknown viruses
Patient Pre-Transfusion Testing

- Patient identification
- Sample identification
- Patient History Review
- Serologic Testing
  - ABO, Rh type
  - Antibody screen
    - Antibody identification panel
- Compatibility Testing
Specimen Requirements

LABELING
– Patient positively identified – 2 unique identifiers
– Specimen and paperwork appropriately labeled.
– Must have phlebotomist’s initials & date of the draw

VOLUME
– Neonates: 2X 2 ml pink top EDTA BD tubes
– Pediatric/adult patients: 6 ml pink top EDTA

LIFESPAN
– Usually, specimen can only be used within 3 days of collection.
– Exceptions:
  1. Neonates (<4mo) : 4 months
     – Valid throughout the same admission
     – Maternal sample as an alternative
  2. Outpatient, pre-surgical [SDA (Same Day Admission)]: 30 days given no history of transfusion or pregnancy in the last 3 months
Common Blood Bank Orders

**HOLD CLOT**: RBC need is unknown. Specimen is held for 3 days “just in case”. No testing done unless requested.

**TYPE AND SCREEN**: RBC need is possible. Specimen typed for ABO-Rh and screened for RBC antibodies.

**TYPE AND CROSSMATCH**: RBC need likely or definite. T&S performed. Requested # RBCs crossmatched and reserved.

- If pt >4mo until the specimen used for testing is 3 days old.
- If pt <4mo, reserved for the remainder of the admission.

**CHECK TYPE**: 2nd separately drawn specimen to confirm ABO-Rh.

**KEEP AHEAD ORDERS**: Blood Bank ensures that a specified # of RBCs or FP reserved for the patient at all times.
Turn-Around-Times

ROUTINE
- TYPE AND SCREEN
  - ABO-Rh: 15 minutes
  - Antibody Screen: 60 minutes
- CROSSMATCH
  - ~ 15 minutes.
  - Longer if patient has antibodies.

STAT
- Uncrossmatched O-Neg RBCs: 10 minutes
- STAT blood type: 5 minutes
- Uncrossmatched type specific RBCs: 15 minutes
- STAT type and screen: 60 minutes
Check Type

- Method for verification of blood type prior to type-specific RBC transfusion
  - Errors in patient ID/specimen labeling and/or initiating a blood transfusion are 1:15,000 to 1:30,000
  - Comparison of current specimen blood type to historical type or type based on second independently drawn specimen
- UCLA Policy: Second independently drawn blood type sample on previously untyped non-group O patients who require transfusion or are likely to require transfusion
  - Required
    - Non-group O patients
    - All patients admitted to L&D, OR, ICU
  - Exempt
    - Trauma patients with dual banding system
    - Outpatient clinic
Patient Testing:

Check Type

- Two patients on beds in hallway with number above bed
- RN drew patients’ specimens based on location
  - Did not check patient ID
  - Did not label specimens at bedside
# ABO & Rh Compatibility

<table>
<thead>
<tr>
<th>Blood Type</th>
<th>Antigens of RBCs</th>
<th>Antibodies in Plasma</th>
<th>Patient can receive RBCs of group...</th>
<th>Patient can receive plasma of group...</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>No A or B</td>
<td>Anti-A, Anti-B</td>
<td>O only</td>
<td>O, A, B, AB</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>Anti-B</td>
<td>A, O</td>
<td>A, AB</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>Anti-A</td>
<td>B, O</td>
<td>B, AB</td>
</tr>
<tr>
<td>AB</td>
<td>A and B</td>
<td>none</td>
<td>AB, A, B, O</td>
<td>AB only</td>
</tr>
<tr>
<td>Rh pos</td>
<td>D</td>
<td>None</td>
<td>Rh pos or neg</td>
<td></td>
</tr>
<tr>
<td>Rh neg</td>
<td>No D</td>
<td>None</td>
<td>Rh neg*</td>
<td></td>
</tr>
</tbody>
</table>

* Women of child bearing age and patients with allo-anti-D
## Type & Antibody Screen

<table>
<thead>
<tr>
<th>ABO-Rh cells</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>A1 cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Donor 1

<table>
<thead>
<tr>
<th>Rh</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>C</td>
<td>c</td>
<td>K</td>
<td>k</td>
<td>Fya</td>
<td>Fyb</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Donor 2

<table>
<thead>
<tr>
<th>Rh</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- Antibody Screen/Indirect antiglobulin test (IAT): Detects in-vitro binding of antibody and RBCs by using AHG.
Indirect Antibody Screen (IAT)

Anticorps d’un sérum humain reconnaissant un antigène érythrocytaire.

Incubation des globules dans le sérum humain.

Complexes antigène-anticorps à la surface des globules (GRS).

Ajout de l’AGH après lavage des GRS.

Agglutination car les globules rouges sont sensibilisés.
RBC Agglutination & Grading

4+ Reaction
3+ Reaction
2+ Reaction
1+ Reaction
Hemolysis
Negative reaction
Application of the IAT

- RBC antibody screen and identification
- Weak D testing
- Antibody titration
- IgG Crossmatching
- Typing of erythrocytes antigens
Causes of False Negative IAT

- Failure to wash RBCs adequately
- Delay in adding AHG reagent or expired AHG reagent
- Too little serum added/too much reagent RBCs added
- Under- centrifugation
- Improper incubation temperature or time
Causes of False Positive IAT

- Sensitized patient RBCs (positive DAT with allo or auto antibodies)
- Contamination of saline with materials that can cause spontaneous aggregation of RBCs (eg, colloidal silica from glass bottles)
- Over centrifugation
- Improper AHG reagent
# Type & Antibody Screen

<table>
<thead>
<tr>
<th>ABO-Rh cells</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>A1 cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>O pos</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DONOR</th>
<th>Rh</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MNS</th>
<th>Gel IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

- **Rh**: Rh factor
- **Kell**: Kell factor
- **Duffy**: Duffy factor
- **Kidd**: Kidd factor
- **Lewis**: Lewis factor
- **P**: P factor
- **MNS**: MNS factor
- **Gel IgG**: Gel IgG test results

The table above summarizes the Type & Antibody Screen results for different donors, including the Rh, Kell, Duffy, Kidd, Lewis, P, and MNS factors. The Gel IgG test results are also indicated.
# Antibody Identification Panel

<table>
<thead>
<tr>
<th>DONOR</th>
<th>Rh</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MNS</th>
<th>Gel IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2</td>
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<td>+</td>
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<tr>
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<td>+</td>
<td>0</td>
<td>+</td>
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<td>+</td>
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</tr>
<tr>
<td>6</td>
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<td>+</td>
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</tr>
</tbody>
</table>

AC
# Antibody Identification Panel

<table>
<thead>
<tr>
<th>DONOR</th>
<th>Rh</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MNS</th>
<th>Gel IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>C</td>
<td>E</td>
<td>c</td>
<td>e</td>
<td>K</td>
<td>k</td>
<td>Fy&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
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<td>0</td>
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</tr>
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<tr>
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<tr>
<td>6</td>
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<td>7</td>
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</tr>
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<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

**AC**

**DAT:** PS = 0/0  Ct = 0/0
### Antigen Frequencies within Caucasian Donor Populations

#### Rh System
- **D**
- **C**
- **c**
- **E**
- **e**
- **f**
- **V**
- **Cw**
- **K**
- **k**
- **Kp**
- **Kp**
- **Js**
- **Js**
- **Fy**
- **Fy**
- **Jk**
- **Jk**
- **S**
- **s**

#### Table:
| Ab | D | C | c | E | e | f | V | Cw | K | k | Kp | Kp | Js | Js | Fy | Fy | Jk | Jk | S | s |
|----|---|---|---|---|---|---|---|----|---|---|----|----|----|----|----|----|----|---|---|
| %  | 15| 30| 20| 70| 2 | 35| 99| 98| 91| 0.2| 99| 0.1| 99| 0.1| 35| 17| 23| 26| 50| 10|

- When anti-E and anti-K are detected, E and K – RBCs units are required, 63% of donors are E and K antigen negative.
IAT for IgG Crossmatch

- Patient’s plasma is incubated with donor RBCs at 37 C for a period of time, then centrifuged and examined for hemolysis or agglutination
- Wash 3-4 times to remove all unbound free serum globulins
- Add polyclonal antihuman globulin
- Observe agglutination formation
Direct Antiglobulin Test (DAT)

- Detects antibodies bound to erythrocytes in vivo.
Application of the DAT

• Hemolytic transfusion reactions workup (acute or delayed) – post transfusion blood sample
• Hemolytic disease of the fetus/newborn – cord blood or newborn blood sample
• Investigation of autoantibodies (for possible autoimmune hemolytic anemia)
• Medication induced antibody or complement binding
## Eluate

<table>
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<tr>
<th>DONOR</th>
<th>Rh</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MNS</th>
<th>LW</th>
<th>Gel IgG</th>
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Reasons for False Positive Reaction

• Specimen collected in serum separator tube or red-top tube (agglutination of RBCs by gel or clot)
• Specimen collected in 5-10% dextrose IV line (dextrose cause in vitro complement fixation)
• Patient is septic or specimen is contaminated by bacteria (T activation causing pan agglutination)
• Saline is contaminated with colloidal silica from glass bottles or dirty glassware
• Potent agglutinins such as strong cold agglutinins
• Improper procedure: over-centrifugation
• Over-incubation with enzyme-treated cells
• Improper use of enhancement reagents
Reasons for False Negative Reaction

- Misinterpretation in testing: weak positive can be misinterpreted as negative, use microscope for better observation
- Improper reagent:
  - Expired reagent?
  - Storage temperature out of range?
  - Contaminated reagent?
- Improper procedure:
  - Failure to add antiglobulin reagents
  - Improper washing: neutralization of AHG reagent by proteins in the sample not washed
  - Improper centrifugation
- Saline: pH too low? temperature?
- Serum/cell ratio too low
- IgA or IgM coating RBCs (DAT only detects IgG or C3d)
Enhancers/Potentiators

- Albumin: Reduces negative charges of RBCs, allow RBCs to come closer
- LISS: Reduces zeta potential to allow increased antibody uptake by RBCs (RBCs negatively charged, Ab positively charged)
- PEG: Excludes water from around RBCs, increases antigen-antibody binding.
  - Enhances IgG antibody detection (including warm autoantibody), but weakens ABO and Lewis antibody reactions
- Enzymes: Removes sialic acid residues → decreasing negative charges from RBCs- allow RBCs to come closer (similar to albumin) → only enhance ABO, Lewis, Rh, Jka, Jkb antibodies and destroys Duffy, MNS antibodies
Effect of Enzymes on Antigens

- Common enzymes: ficin, papain, bromelin.
- Destroys
  - Duffy, MNS,
  - Chido, Rodger, JMH, York,
  - Pr, Tn, In, Xg, Gerbich, Cromer
- Enhances
  - Rh antigens
  - Jka, Jkb
  - ABO, I, P1, Lewis
- No effect:
  - Kell, Lutheran, s, U
DTT (Dithiothreitol)

- Destroys Kell antigens
- Destroys IgM antibodies by reducing disulfide bond, will leave IgG antibodies intact for identification or titering
Rules to live by……

• Don’t panic
• Follow routine procedures
• Be consistent and systematic
• Document all tests and results
• Never hesitate to ask for help
• Identify and use your resources
• Consider what is “safest” for the patient!