

# Serological Investigations in the Reference Laboratory

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**American  
Red Cross**

# Case History

- 82 year old Caucasian female
- Diagnosis: Severe anemia, CHF, COPD
- Current Hemoglobin: 5.9 g/dL
- Transfusion History: 10 days prior (3 units)
- Previous antibodies: anti-K
- Sample submitted for Antibody Identification and Crossmatch (2 units)
- Pre-transfusion sample included

# Facility Results

- Antibody screen: Positive (PeG)

|     | IS | 37C | AHG |
|-----|----|-----|-----|
| I   |    |     | 1+  |
| II  |    |     | 3+  |
| III |    |     | 3+  |

- DAT: Positive

|         | Poly | IgG | C3 |
|---------|------|-----|----|
| Results |      | neg |    |

# Initial ARC Results

- ABO/Rh

| Anti-A | Anti-B | Anti-D | Rh Cont | A1 Cells | B Cells |
|--------|--------|--------|---------|----------|---------|
| 4+     | 0      | 4+     | 0       | +        | 4+      |

- DAT

| Polyspecific |    |    |    | Anti-IgG |    | Anti-C3 |    |    |    | Saline Ct |    |
|--------------|----|----|----|----------|----|---------|----|----|----|-----------|----|
| IS           | Mi | RT | Mi | IS       | Mi | IS      | Mi | RT | Mi | IS        | Mi |
| 0            | M+ |    |    | 0        | M+ | 0       | 0  | 0  | M+ | 0         | 0  |

# Initial ARC Results

- Antibody Screen

|      |    | PeG | LISS |      |
|------|----|-----|------|------|
|      | IS | IAT | 37C  | IAT  |
| I    | 0  | 3+  | +w   | 2+   |
| II   | 2+ | 3+  | 2+   | 3+   |
| III  | 2+ | 3+  | +    | 3+   |
| Auto | 0  | 0 v | 0    | 0/m+ |

# What's Next?

- ABO/Rh Discrepancy
- DAT positive → Elution
- Antibody Screen
  - IS reactivity
  - 37C reactivity
  - IAT reactivity
- Phenotype

# Phenotype

- Patient was transfused 10 days prior to current sample collection
- Facility sent pre-transfusion sample \*\*\*\*

|          | Anti-C | Anti-E | Anti-c | Anti-e |
|----------|--------|--------|--------|--------|
| Pre-trsf | 4+ mf  | 4+ mf  | 4+ mf  | 3+ mf  |

- Perform cell separation
  - REP (Reticulocyte Enriched Portion)

# Cell Separation (REP)

- Separation of young autologous red cells (i.e. reticulocytes) from transfused RBCs by simple centrifugation in microhematocrit tubes
- Autologous RBCs have a lower specific gravity and therefore concentrate at the top of the microhematocrit tube
- Best if samples collected 3 or more days after transfusion
- Other method: Hypotonic Saline Wash

# Phenotype

| Antisera | -C | -E | -c | -e | -K | Ctl |
|----------|----|----|----|----|----|-----|
| REP      | 4+ | 4+ | 4+ | 3+ | 0  | 0   |

| Antisera | -Fya | -Fyb | -Jka | -Jkb | -S | -s |
|----------|------|------|------|------|----|----|
| REP      | 0    | 2+   | 3+   | 0    | 0  | 3+ |

| Antisera | -M | -N | -P1 | -Lea | -Leb |
|----------|----|----|-----|------|------|
| REP      | 4+ | 3+ | 2+  | 0    | 3+   |

# Possible Antibodies

- Anti-f, -K, Fya, Jkb, S
- Immediate spin antibodies (Cold)
  - None possible
  - What about reactivity seen at IS?

# ABO/Rh Discrepancy

- Reaction with A1 cells (+)
- Possibilities??
  - Rouleaux
    - Probably not; some neg IS reactions with screen
  - Subgroup of A
    - Possibility; test with anti-A1 lectin and A2 cells
  - Cold allo and/or auto
    - Allo – possibility; IS reactivity seen with screen
    - Auto – probably not; no reactivity at IS with auto ctl

# ABO/Rh Discrepancy

- Subgroup of A

|                | Anti-A1 lectin | A2 cells |
|----------------|----------------|----------|
| Pt Cells (REP) | 4+             |          |
| Pt Serum       |                | 0        |

- Pre-transfusion sample

|          | A1 cells | B cells |
|----------|----------|---------|
| Pt Serum | 0        | 4+      |

- Resolution? Put aside for now and come back

# Elution

- Micro + DAT
  - Is it necessary to perform elution?

|          | Eluate<br>(PeG/IAT) | Last Wash<br>(PeG/IAT) |
|----------|---------------------|------------------------|
| I        | 0 v                 | 0 v                    |
| II       | +w                  | 0 v                    |
| III      | 2+                  | 0 v                    |
| A1 cells | +                   | 0 v                    |
| B cells  | +                   | 0 v                    |

# Antibody Screen

|        | Rh-Hr |   |   |   |   |   |    | Kell |   | Duffy           |                 | Kidd            |                 | Lewis           |                 | P              | MNSs |   |   |   | LISS<br>IS / 37 / AHG |
|--------|-------|---|---|---|---|---|----|------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|------|---|---|---|-----------------------|
| Vial # | D     | C | c | E | e | f | Cw | K    | k | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P <sub>1</sub> | M    | N | S | s |                       |
| 1      | +     | + | 0 | 0 | + | 0 | 0  | 0    | + | 0               | +               | +               | 0               | +               | 0               | 0              | +    | + | + | + | 0 / +w / 2+           |
| 2      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | 0               | +               | 0               | 0               | +              | +    | 0 | + | 0 | 2+ / 2+ / 3+          |
| 3      | 0     | 0 | + | 0 | + | + | 0  | +    | + | +               | 0               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 2+ / + / 3+           |

## Antibody Identification

|        | Rh-Hr |   |   |   |   |   |    | Kell |   | Duffy           |                 | Kidd            |                 | Lewis           |                 | P              | MNSs |   |   |   | LISS<br>IS / 37 / AHG |
|--------|-------|---|---|---|---|---|----|------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|------|---|---|---|-----------------------|
| Vial # | D     | C | c | E | e | f | Cw | K    | k | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P <sub>1</sub> | M    | N | S | s |                       |
| 1      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 / 0 / 0 v           |
| 2      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | +               | 0               | 0               | 0               | +              | +    | 0 | 0 | + | 0 / 0 / 0 v           |
| 3      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | +               | 0               | +               | 0               | 0               | +               | +              | +    | + | 0 | + | 0 / 0 / 2+            |
| 4      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | 0               | +               | 0               | 0               | +              | +    | + | 0 | + | 2+ / + / +            |
| 5      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | +    | 0 | + | 0 | 0 / 2+ / 2+           |
| 6      | 0     | + | + | 0 | + | + | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 / 0 / 0 v           |
| 7      | 0     | + | + | 0 | + | + | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 / 0 / 0 v           |
| 8      | 0     | + | + | + | + | 0 | 0  | 0    | + | +               | 0               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 / 0 / 2+            |
| 9      | +     | + | 0 | + | + | 0 | 0  | 0    | + | 0               | +               | 0               | +               | 0               | +               | +              | +    | + | 0 | + | 2+ / + / +            |
| 10     | +     | + | 0 | 0 | + | 0 | 0  | 0    | + | 0               | +               | +               | 0               | +               | 0               | +              | +    | 0 | + | 0 | 0 / 2+ / 3+           |
| 11     | +     | + | + | + | + | 0 | 0  | +    | 0 | 0               | +               | +               | 0               | 0               | +               | +              | +    | + | 0 | + | 2+ / +W / 3+          |

## Antibody Identification

|        | Rh-Hr |   |   |   |   |   |    | Kell |   | Duffy           |                 | Kidd            |                 | Lewis           |                 | P              | MNSs |   |   |   | LISS<br>IS / 37 / AHG |
|--------|-------|---|---|---|---|---|----|------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|------|---|---|---|-----------------------|
| Vial # | D     | C | c | E | e | f | Cw | K    | k | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P <sub>1</sub> | M    | N | S | s |                       |
| 1      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 / 0 / 0 v           |
| 2      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | +               | 0               | 0               | 0               | +              | +    | 0 | 0 | + | 0 / 0 / 0 v           |
| 3      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | +               | 0               | +               | 0               | 0               | +               | +              | +    | + | 0 | + | 0 / 0 / 2+            |
| 4      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | 0               | +               | 0               | 0               | +              | +    | + | 0 | + | 2+ / + / +            |
| 5      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | +    | 0 | + | 0 | 0 / 2+ / 2+           |
| 6      | 0     | + | + | 0 | + | + | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 / 0 / 0 v           |
| 7      | 0     | + | + | 0 | + | + | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 / 0 / 0 v           |
| 8      | 0     | + | + | + | + | 0 | 0  | 0    | + | +               | 0               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 / 0 / 2+            |
| 9      | +     | + | 0 | + | + | 0 | 0  | 0    | + | 0               | +               | 0               | +               | 0               | +               | +              | +    | + | 0 | + | 2+ / + / +            |
| 10     | +     | + | 0 | 0 | + | 0 | 0  | 0    | + | 0               | +               | +               | 0               | +               | 0               | +              | +    | 0 | + | 0 | 0 / 2+ / 3+           |
| 11     | +     | + | + | + | + | 0 | 0  | +    | 0 | 0               | +               | +               | 0               | 0               | +               | +              | +    | + | 0 | + | 2+ / +W / 3+          |

## Eluate - Antibody Identification

| Vial # | Rh-Hr |   |   |   |   |   |    | Kell |   | Duffy           |                 | Kidd            |                 | Lewis           |                 | P              | MNSs |   |   |   | ELUATE<br>PeG<br>AHG |
|--------|-------|---|---|---|---|---|----|------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|------|---|---|---|----------------------|
|        | D     | C | c | E | e | f | Cw | K    | k | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P <sub>1</sub> | M    | N | S | s |                      |
| 1      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 v                  |
| 2      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | +               | 0               | 0               | 0               | +              | +    | 0 | 0 | + | 0 v                  |
| 3      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | +               | 0               | +               | 0               | 0               | +               | +              | +    | + | 0 | + | 2+                   |
| 4      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | 0               | +               | 0               | 0               | +              | +    | + | 0 | + | 0 v                  |
| 5      | +     | 0 | + | + | 0 | 0 | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | +    | 0 | + | 0 | 0 v                  |
| 6      | 0     | + | + | 0 | + | + | 0  | 0    | + | 0               | +               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 0 v                  |
| 8      | 0     | + | + | + | + | 0 | 0  | 0    | + | +               | 0               | +               | 0               | 0               | +               | +              | 0    | + | 0 | + | 2+                   |
| 9      | +     | + | 0 | + | + | 0 | 0  | 0    | + | 0               | +               | 0               | +               | 0               | +               | +              | +    | + | 0 | + | 0 v                  |
| 10     | +     | + | 0 | 0 | + | 0 | 0  | 0    | + | 0               | +               | +               | 0               | +               | 0               | +              | +    | 0 | + | 0 | 0 v                  |
| 11     | +     | + | + | + | + | 0 | 0  | +    | 0 | 0               | +               | +               | 0               | 0               | +               | +              | +    | + | 0 | + | 0 v                  |
| 12     | 0     | 0 | + | 0 | + | + | 0  | +    | + | 0               | 0               | +               | 0               | 0               | +               | +              | +    | 0 | 0 | + | 0 v                  |

# Summary of Antibodies

- Serum/Plasma
  - Anti-K and Anti-Jkb (IS, 37C and AHG)
  - Anti-Fya (AHG)
  - Anti-S (37C and AHG)
- Eluate
  - Anti-Fya
  - Unidentified reactivity (screen cell II with +w reaction)

# Resolving the Blood Type

- Still have to figure out why we have the (+) reactivity in the reverse
- Identified anti-K and –Jkb at IS; could one of those be interfering?
- To resolve: find A and B cells that are neg for K and Jkb; retest with patient serum

|          | LC48651   | KK71059   |
|----------|-----------|-----------|
|          | A+ K-Jkb- | B+ K-Jkb- |
| Pt serum | 0         | 4+        |

# Finding Compatible Units

- Frequencies (neg)
  - K – 91%
  - Fya – 35%
  - Jkb – 27%
  - S – 45%
- Formula
  - $(.91)(.35)(.27)(.45) = 0.03869 \times 100 = 3.8\%$
  - Would need to screen  $\sim 50$  random units to find 2

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