**HDFN TECHNICAL CASE #4**

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Indian Immunohematology Initiative

**History:** A 37 year old woman in her first pregnancy had a blood group antibody detected on routine prenatal testing at another facility. She was referred to a maternal-fetal medicine specialist at our hospital at approximately 16 weeks gestation. She denied previous transfusion.

## ABO and Rh typing

| <A | <B | A1 cells | B cells | 6% alb | <D | <D/AHG | CCC | Interp |
|---|---|---|---|---|---|---|---|---|
| 4+ | 0 | 3+ | 4+ | | 4+ | | | |

## Antibody Screen

| | Gel |
|---|---|
| SCI | 4+ |
| SCII | 3+ |

## Direct Antiglobulin Test (tube method)

| | Poly | IgG | <C3 |
|---|---|---|---|
| AHG | 0 | 0 | 0 |
| CCC | 2+ | 2+ | 1+ |

## Initial plasma panel

| Lot# 8RA206 | | Rh system | | | | | | Kell | | | | | | Duffy | | Kidd | | Xg | Lewis | | MNSs | | | | P | Lutheran | | Other Typings | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell | Rh | D | C | E | c | e | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ | | Cell | Gel |
| 1 | R1wR1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | + | 0 | + | + | 0 | +s | 0 | + | $C^w$ | 1 | 4+ |
| 2 | R1R1 | + | + | 0 | 0 | + | 0 | + | + | 0 | + | 0 | + | + | + | + | + | 0 | + | 0 | 0 | + | + | + | 0 | 0 | + | | 2 | 4+ |
| 3 | R2R2 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | | 3 | 0 |
| 4 | Ror | + | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | + | 0 | 0 | 0 | + | + | + | + | 0 | + | | 4 | 3+ |
| 5 | r'r | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | +s | 0 | + | | 5 | 0 |
| 6 | r"r | 0 | 0 | + | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | | 6 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | | 7 | 3+ |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | + | 0 | 0 | + | 0 | + | + | 0 | + | | 8 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | 0 | + | + | + | + | 0 | + | | 9 | 3+ |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | + | + | 0 | + | + | + | 0 | + | 0 | + | | 10 | 4+ |
| 11 | R1R1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | | 11 | 4+ |
| Patient | | | | | | | | | | | | | | | | | | | | | | | | | | | | | AC | |

## Additional cells

| | | Rh system | | | | | | Kell | | | | | | Duffy | | Kidd | | Xg | Lewis | | MNSs | | | | P | Lutheran | | Other Typings | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell | Rh | D | C | E | c | e | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ | | Cell | Gel |
| 1 | R1R2 | + | w | + | + | w | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | + | 0 | + | | 1 | 0 |
| 2 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | | 2 | 0 |
| 3 | R1R1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 0 | + | + | + | 0 | + | + | 0 | + | | 3 | 0 |

## HDFN TECHNICAL CASE #4

**Repeat reverse typing,** ficin treated cells, saline/tube method with 2 drops plasma

| | I.S |
|---|---|
| A1 cell | 0 |
| B cell | 4+ |
| Patient (AC) | 0 |

**Selected cell panel, pre-warmed testing** (4 drops plasma, saline suspended RBCs; warmed to 37°C before addition)

| Lot #06776 | | Rh system | | | | | Kell | | | | | | Duffy | | Kidd | | Lewis | | P | MNSs | | | | Lutheran | | Xg | Other Typings | Saline-susp. RBCs, 4 drops plasma | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell | Rh | D | C | c | E | e | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Le^a$ | $Le^b$ | P1 | M | N | S | s | $Lu^a$ | $Lu^b$ | $Xg^a$ | | Cell | IS | RT | 37° | AHG | cc |
| 5 | r'r | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | | 5 | 0 | 0 | 0 | 0 | 2+ |
| 6 | r"r | 0 | 0 | + | + | + | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | 0 | + | + | + | + | 0 | + | + | + | + | | 6 | 1+ | w+ | 0 | 0 | 2+ |
| 7 | rr | 0 | 0 | + | 0 | + | + | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | + | | 7 | 3+ | 3+ | 0 | 0 | 2+ |
| Patient | | | | | | | | | | | | | | | | | | | | | | | | | | | | AC | | | | | |

**Patient antigen phenotype**

| | Rh system | | | | Kell | | | | Duffy | | Kidd | | Lewis | | MNSs | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | E | c | e | K | k | $Kp^a$ | $Js^a$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | I | H | $A_1$ | |
| Patient | 4+ | 4+ | 0 | 4+ | | | | | | | | | | | | | 0 | 4+ | | | | | |
| Pos control | 4+ | 4+ | 4+ | 4+ | | | | | | | | | | | | | 4+ | 4+ | | | | | |
| Neg Control | 0 | 0 | 0 | 0 | | | | | | | | | | | | | 0 | 0 | | | | | |

**Titration** (IAT test with 2 drops plasma, 1 drop saline-suspended target RBCs, 30' incubation at 37°C, then addition ofAHG)

| ANTIBODY: Anti-M | | | EDC: ((day 280) | | | Tech: | | | Date tested: (Day 89) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample dilution, Reaction strength at AHG phase: | | | | | | | | | | | | | | |
| Sample date: | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | 2048 | 4096 | 8192 | Titer |
| New: (day 89) | 0 | 0 | w+ | 1+ | 2+ | 1+ | w+ | | | | | | | | |
| New: (repeat) | 0 | 0 | vw+ | 2+ | 2+ | 1+ | w+ | vw+ | 0 | 0 | 0 | | | | |
| Cell type: R1R1, M+N+ | | | Manufacturer: | | | | | Lot number: | | | | Comment: | | | |

**HDFN TECHNICAL CASE #4**

**Questions:**

1. What is the probable identity of this antibody? Is any further workup needed to prove it? Why were the additional 3 gel test panel cells run after the initial 11 cell panel?

2. What was the cause of the ABO discrepancy? How was this resolved?

3. What is the indirect antiglobulin test (IAT) titer of this antibody? Why was the titer repeated?

4. The tube IAT was initially negative (see 37$^{o}$ and IAT reactions, tube test panel #06776), but in the titration the antibody reacted in the IAT? Can you explain this discrepancy?

5. Are you concerned that this pregnancy might be affected by hemolytic disease of the fetus and newborn? If so, what would you do to investigate this possibility?

**HDFN TECHNICAL CASE #4**

Additional specimen was obtained, and the plasma was incubated with an equal volume of 0.01 M dithiothreitol (DTT). Titration was then repeated with DTT-treated plasma, as well as with a typical, cold-reactive anti-M serum for a control.

**Titration with DTT treated serum:** (AHG test with 2 drops plasma, 1 drop saline-suspended target RBCs, 30' incubation at 37°C)

| **ANTIBODY: Anti-M** | | **EDC: ((day 280)** | | | **Tech:** | | | | **Date tested: (Day 90)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Sample dilution, Reaction strength at AHG phase:** | | | | | | | | | | | | |
| | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** | **1024** | **2048** | **Titer** |
| **Patient serum tx'd with DTT** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | | | | | **0** |
| **Dilution control: (serum + PBS)** | **vw+** | **vw+** | **w+** | **w+** | **w+** | **vw+** | **0** | 0 | | | | | **32** |
| **Control study with anti-M from a blood donor** | | | | | | | | | | | | | |
| **Donor serum tx'd with DTT** | **0** | **0** | **0** | | | | | | | | | | **0** |
| **Dilution control: (serum + PBS)** | **2+** | **w+** | **0** | | | | | | | | | | **4** |
| **Cell type: R1R1, M+N+** | **Manufacturer:** | | | | | **Lot number:** | | | | **Comment:** | | | |

**Questions:**

6. What is your conclusion regarding the likelihood of this antibody to produce HDFN? Why is the titer of the dilution control interpreted as "32" rather than "16"?

7. Would you recommend any other testing related to this problem during this pregnancy?

**HDFN TECHNICAL CASE #4**

The first child did not have jaundice or other problems at birth. The mother became pregnant again 2 years later. Her titer, determined at another laboratory, increased from 32 on her first visit to 128 over several months. Samples were submitted to our laboratory for study at 27 weeks, 4 days gestation. The serologic findings are shown below.

**ABO and Rh typing**

| <A | <B | A1 cells | B cells | 6% alb | <D | <D/AHG | CCC | Interp |
|---|---|---|---|---|---|---|---|---|
| 4+ | 0 | 1+ | 4+ | | 4+ | | | |

| | A1 cells | B cells | Pt. cells |
|---|---|---|---|
| Ficin treated RBCs | 0 | 4+ | 0 |

**Antibody Screen**

| | Gel |
|---|---|
| SCI | |
| SCII | |

**Direct Antiglobulin Test (tube method)**

| | Poly | IgG | <C3 |
|---|---|---|---|
| AHG | 0 | 0 | 0 |
| CCC | 2+ | 2+ | 1+ |

**Selected cell panel**

| | | Rh system | | | | | | Kell | | | | | | Duffy | | Kidd | | Xg | Lewis | | MNSs | | | | P | Lutheran | | Other Typings | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell | Rh | D | C | E | c | e | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ | | Cell | Gel |
| 1 | R1wR1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | 0 | + | 0 | + | + | 0 | + | $C^w$, $Lan^+$, JMH+ | 1 | 0 |
| 2 | R1R1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | 0 | 0 | + | Bg+ | 2 | 4+ |
| 3 | R1wR1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | 0 | + | $C^w$ | 3 | 0 |
| 4 | R2R2 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | + | 0 | + | + | +s | 0 | + | | 4 | 4+ |
| 5 | R2R2 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | | 5 | 0 |
| 6 | r'r | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | +s | 0 | + | | 6 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | | 7 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | 0 | + | + | + | 0 | + | + | + | + | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | | 8 | 0 |
| Patient | | | | | | | | | | | | | | | | | | | | | | | | | | | | | AC | |

**Selected cell panel, pre-warmed testing** (4 drops plasma, saline suspended RBCs; warmed to 37°C before addition)

| Lot #51877 | | Rh system | | | | | | Kell | | | | | | Duffy | | Kidd | | Lewis | | P | MNSs | | | | Lutheran | | Xg | Other Typings | 4 drops/saline | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell | Rh | D | C | c | E | e | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Le^a$ | $Le^b$ | P1 | M | N | S | s | $Lu^a$ | $Lu^b$ | $Xg^a$ | | Cell | 37° | AHG |
| 1 | R1wR1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | + | + | + | 0 | + | + | + | 0 | + | 0 | + | + | $C^w$ | 1 | 1+ | 1+ |
| 2 | R1R1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | + | + | 0 | 0 | + | + | 0 | + | + | + | 0 | 0 | + | 0 | + | 0 | | 2 | 4+ | 4+ |
| 3 | R1R1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 3 | 0 | 0 |
| 4 | RzR1 | + | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | 0 | + | + | + | 0 | 0 | + | 0 | + | + | | 5 | 4+ | 4+ |
| 5 | R2R2 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | | 6 | 0 | 0 |

**HDFN TECHNICAL CASE #4**

**Titration with DTT treated serum:** (AHG test with 2 drops plasma, 1 drop saline-suspended target RBCs, 30' incubation at 37°C)

| **ANTIBODY: Anti-M** | **EDC: ((day 280)** | | | | **Tech:** | | | | **Date tested: (Day 90)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Sample dilution, Reaction strength at AHG phase:** | | | | | | | | | | | | |
| | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** | **1024** | **2048** | **Titer** |
| **Patient serum tx'd with DTT** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | | | | **0** |
| **Dilution control: (serum + PBS)** | **3+** | **3+** | **3+** | **2+** | **2+** | **1+** | **w+** | vw+ | 0 | | | | **128** |
| **Cell type: R1R1, M+N+** | **Manufacturer:** | | | | | **Lot number:** | | | | **Comment:** | | | |

| **DTT control studies** | **Immediate spin testing** | |
|---|---|---|
| | **Group A, M-neg donor RBCs** | **Group B, M-neg donor RBCs** |
| **DTT-treated patient serum** | **0** | **0** |
| **Patient serum + saline** | **0** | **4+** |

**Questions:**

8. What do the DTT control studies demonstrate?

9. Would you make any other recommendations regarding testing or management during this pregnancy?

**FOLLOW-UP AND COMMENT:**

The second child also did well, without jaundice or anemia in the newborn period.

In pregnant women with anti-M our laboratory performs a pre-warmed test as shown above. DTT treatment is only performed if the patient's anti-M reacts at 37°C or in the IAT.