NHS Blood and Transplant

# Case Studies in Red Cell Serology

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Red Cell Reference IBGRL

# Red Cell Reference

- IBGRL red cell reference department investigate referred cases where all cells matched against the patient's plasma / serum are incompatible and blood for transfusion is difficult to find.
- The two main types of serological investigation we receive are:
  - Antibodies to high frequency antigens.
  - Complex mixtures of antibodies.

# Routine Techniques used in Red Cell Reference

- Indirect Antiglobulin Test (IAT)
  - Untreated cells
  - Papain treated cells (for papain sensitive antibodies)
- Direct Agglutination Tests (DAT) at 18ºC and 37ºC

# Case Study 1 - Patient S.S.

- S.S. had pneumothorax with a previous transfusion history.
- Referring lab found S.S. to have an Anti-c reacting by IAT and by saline 20°C.
- They also found additional reactions with a panel of R1R1 cells.

# Initial Approach

- Put up a panel of R1R1 cells (which are c-) to see the reactivity of the additional antibody/ies.
- Include a couple of rr cells to show the presence of the Anti-c.
- Include an auto control to show any autoantibodies present.

# Results

| | LISS IAT | | Papain IAT | | 37°C | | 18°C |
|---|---|---|---|---|---|---|---|
| | LISS | AB | PAPAIN | AB | LISS | PAPAIN | LISS |
| R1R1 | ++ | - | ++++ | - | ++++ | ++++ | +++++ |
| R1R1 | + | - | - | - | ++++ | ++++ | +++++ |
| R1R1 | ++ | - | ++++ | - | ++ | ++++ | +++ |
| R1R1 | ++ | - | +++ | - | ++++ | ++++ | ++++ |
| rr | ++++ | - | +++++ | - | +++++ | +++++ | +++ |
| rr | ++++ | - | +++++ | - | ++++ | +++++ | +++ |
| Self (Auto) | ++ | + | W (mf) | - | W(mf) | ++ | W(mf) |

# Reaction Patterns

| | LISS IAT | | Papain IAT | | 37°C | | 18°C |
|---|---|---|---|---|---|---|---|
| | LISS | AB | PAPAIN | AB | LISS | PAPAIN | LISS |
| R1R1 | ++ | - | ++++ | - | ++++ | ++++ | +++++ |
| R1R1 | + | - | - | - | ++++ | ++++ | +++++ |
| R1R1 | ++ | - | ++++ | - | ++ | ++++ | +++ |
| R1R1 | ++ | - | +++ | - | ++++ | ++++ | ++++ |
| rr | ++++ | - | +++++ | - | +++++ | +++++ | +++ |
| rr | ++++ | - | +++++ | - | ++++ | +++++ | +++ |
| Self (Auto) | ++ | + | W (mf) | - | W(mf) | ++ | W(mf) |

Anti-M
Anti-$P_1$
Anti-c

# Results 2

- Multiple antibodies were suspected from the initial panel.
- A cold reacting antibody and a papain sensitive antibody were found in addition to confirming the known anti-c.
- The additional antibodies detected were identified initially as anti-$P_1$ and anti-M.
- Patient, S.S. was then typed as M-N+, $P_1$-

# Results 3

- Anti-$P_1$ was confirmed, as was the Anti-M, antigen negative cells tested against S.S. plasma to exclude further antibodies.
- Eluate was prepared from self cells and tested, found to be anti-c, eluted from transfused c+ cells.

# Conclusions

- The presence of multiple antibodies were detected in this case study by different serological methods.
- Anti-c was confirmed in both IAT techniques and in DAT tests at 18ºC and 37ºC.
- Anti-M was detected in DAT tests at 18ºC and 37ºC, and it reacted weakly with homozygous cells by LISS IAT.
- Anti-$P_1$ reacted by IAT and was enhanced by papain treatment it also reacted in DAT tests at 18ºC and 37ºC.

# Case Study 2

- Patient R.H. is a 76 yr old male with a fractured NOF.
- Transfusion history shows multiple transfusions.
- Transfusion reactions occurred with last two transfusions.
- The patient's DAT was found to be negative by the referring lab.

# Initial Approach

- Not much sample was sent by referring hospital.
- A three cell screening panel plus an extra rr K+ cell.
- A cell of the same ABO group as patient.
- Self cell.

# Results 1

| | LISS IAT | | Papain IAT | | 37°C | | 18°C |
|---|---|---|---|---|---|---|---|
| | LISS | AB | PAPAIN | AB | LISS | PAPAIN | LISS |
| R1wR1 | ++++ | - | ++++ | - | +++ | ++++ | ++ |
| R2R2 | ++++ | - | ++++ | - | +++ | ++++ | +++ |
| rr | ++++ | - | ++++ | - | +++ | ++++ | +++ |
| rr K+ | ++++ | - | ++++ | - | +++ | +++++ | +++ |
| A cell | ++++ | - | ++++ | - | w | ++ | + |
| Self | - | - | - | - | - | ++ | - |

# Next steps

- There was a strong pan-agglutinin present, reacting by all techniques. The possibility of there being more than one antibody couldn't be ruled out at this stage.
- Test R.H. cells against a panel of antisera to high frequency antigens, including Kna, McCa, Csa, Yka and Vel.

# Results 2

- R.H. typed as Vel neg.
- Put up a couple of Vel- cells against R.H. plasma, these reacted weakly.
- ? Possibility of more than one antibody
- Anti-Vel PLUS something else.
- Included four more Vel- cells.

# Results 3

| | LISS IAT | | | |
|---|---|---|---|---|
| | **LISS** | **AB** | **PAP** | **AB** |
| **Vel-** | (+) | - | (+) | - |
| **Vel-** | + | - | ++ | - |
| **Vel-** | ++ | - | +++ | - |
| **Vel-** | + | - | +++ | - |
| **Vel-** | ++ | ++ | +++ | - |
| **Vel-** | + | - | ++ | - |
| **Pos Control** | ++++ | - | ++++ | - |
| **Self** | - | - | - | - |

# Results 4

- Weak variable reactions were observed with a panel of Vel- cells.
- Looking closer at R.H.'s typing for high frequency antigens patients was Kn(a-/w).
- Typed R.H. with three more examples of anti-$Kn^a$ and found patient to be Kn(a-).
- Found one cell in our Vel- collection of frozen cells which was a known Kn(a-) $M^cC$(a-).
- This cell was negative when tested with R.H. plasma.

# Conclusions

- Two antibodies to high frequency antigens were present.
- R.H. was found to have a combination of anti-Vel and anti-Knops related antibody.
- Anti-Vel is clinically significant so Vel- cells must be selected for transfusion.
- Knops related antibodies are not clinically significant and can be ignored.
- Because we had little plasma to begin with more sample was requested to be referred for MAIEA to confirm that the Knops related antibody was anti-$Kn^a$.

# Learning points

- By looking at reaction patterns it is possible to see what the antibody is likely to be.
- Luckily at IBGRL we have an extensive range of rare cells and sera at our disposal, however it can be difficult to find compatible cells when tricky combinations arise.
- Typing cells with antisera to high frequency antigens is the preferred method when not much serum/plasma is available for testing.
- Choose the appropriate technique.