THE MONOCYTE MONOLAYER ASSAY:

A CASE STUDY

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Background

What is the Monocyte Monolayer Assay (MMA)?

• In vitro assay

• Considered to be representative of in vivo survival of sensitized red blood cells

• Therefore, may be used as a predictor of:
  o The clinical severity of red cell alloantibodies
  o The severity of haemolytic disease of the newborn
Background

• Determine possible clinical significance of alloantibodies:
  o To high frequency RBC antigens where antigen negative blood is unavailable
  o In HDFN cases – on request or when participating in studies

• Post transfusion case monitoring:
  o Patients transfused with incompatible blood due to the lack of antigen negative blood
Background

• First developed in the 1970's
• Results used as additional information for the treating doctor when antigen negative units are not available:
  o Not a stand-alone test, entire case must be reviewed
• Not for use for patients with non-specific antibodies – allo-antibody specificity must be identified
MMA Test Procedure

- Isolate monocytes from a fresh blood sample
- Layer the monocytes onto a chamber slide and allow to adhere
MMA Test Procedure

• Sensitize the incompatible RBC for transfusion with the patient’s antibody +ve plasma
• Add the sensitized cells to the adhering monocytes in the chamber slide
• Incubate at 37°C to facilitate binding of sensitized cells and monocytes
• Remove unbound cells by washing and stain slide
MMA Test Procedure

- \( \leq 5\% \): incompatible blood may be given with little risk
- \( 5.1 - 20\% \): 33\% of patients may have clinical signs of a reaction
- \( >20\% \): 64\% of patients may have clinical signs of a reaction

Accessed online on 10/08/2016 at www.ucdmc.ucdavis.edu
Case Study – Patient X

- 75 year male, with chronic renal failure
- History of multiple transfusions
- First presented as a problem crossmatch case ± 2011
- Multiple red cell antibodies: anti-D, -C, -E, -K, -Fya, -Yta
- Group O, Rh Negative
Case Study

• Blood not readily available for this patient
• No compatible donors in South Africa
• Screen for compatible blood - D, C, E, K, Fy^a and Yt^a
  antigen negative blood is required
  o Yt^a is a high frequency antigen
  o Anti-Yt^a reagent not readily available
Case Study

• 2 x units sourced from International Rare Donor Registry
  o Not sustainable for ongoing transfusion requirements
• Least incompatible blood issued, the use of IV-IG recommended
  o Units confirmed negative for the D, C, E, K, and Fy\textsuperscript{a} antigens
  o Most likely Yt\textsuperscript{a} antigen positive
Case Study

• Incompatible transfusions well tolerated by Patient X
• IV-IG is extremely expensive, costs often not covered by medical aid
• Is the IV-IG really necessary?
  o Clinical significance of the anti-Yt\textsuperscript{a} antibody?
  o Will the antibody cause increased RBC destruction if incompatible units are transfused?
Nature of the Case

• MMA
  o Considered to be representative of in vivo survival of sensitized red blood cells
  o Therefore, may be used as a predictor of the clinical severity of red cell alloantibodies

• Patient X’s previous tolerance of incompatible blood transfusions
  o Expected MMA result of low clinical significance
## MMA Results

<table>
<thead>
<tr>
<th></th>
<th>Anti-D &amp; D Pos cells</th>
<th>Anti-D &amp; D Neg cells</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Pos control</td>
<td></td>
<td></td>
<td>50.5%</td>
</tr>
<tr>
<td>Neg control</td>
<td></td>
<td></td>
<td>7%</td>
</tr>
<tr>
<td>Cell 1+ patient</td>
<td></td>
<td></td>
<td>13.5%</td>
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<tr>
<td>serum</td>
<td>D-, C-, E-, K-,</td>
<td></td>
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<tr>
<td></td>
<td>Fy(\alpha)-, Yt(\alpha)+</td>
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<tr>
<td>Cell 2 + patient</td>
<td></td>
<td></td>
<td>8%</td>
</tr>
<tr>
<td>serum</td>
<td>D-, C-, E-, K-,</td>
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<tr>
<td></td>
<td>Fy(\alpha)-, Yt(\alpha)-</td>
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</tbody>
</table>
Interpretation of MMA Results

• Positive and negative controls required with each MMA test
• Control results may be unpredictable due to unknown reactivity between RBC and monocytes
• The %R of the negative control and Cell 2 suggests non-specific background reactivity of donor monocytes to the sensitized RBC.
Conclusion

• The actual %R of Cell 1 not conclusively determined, however:
  o %R of these incompatible cells demonstrates that the anti-Yt\(a\) is of low severity

• Consistent with his tolerance to previous Yt\(a\) incompatible transfusions.
  o Yt\(a\) positive RBC may again be considered
Conclusion

• Performed by SANBS Reference Laboratory
• Not available in emergency cases
• Only when compatible blood is not available
• Provide extra information to the treating doctor:
  
  o Patient’s clinical condition and urgency of transfusion requirement is always the driving factor behind the decision to transfuse incompatible blood
Acknowledgements

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• Staff of the Immunohaematology Reference Laboratory

References

• A retrospective analysis of the value of monocyte monolayer assay results for predicting the clinical significance of blood group alloantibodies. Arndt PA, Garratty G. Transfusion 2004; 44: 1273 – 81

• Monocyte Monolayer Assay; Special Immunohaematology Laboratory, American Red Cross Services, Southern California Region, Pomona, CA
Thank you