INTRODUCTION OF THE MMA TEST – A PILOT STUDY

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MMA???

Accessed online on 10/08/2016 from www.mutantmma.com
MMA???
MMA = MONOCYTE MONOLAYER ASSAY
THE MONOCYTE MONOLAYER ASSAY

• In vitro assay

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

• Therefore, may be used as a predictor of:
  – The clinical severity of red cell alloantibodies
  – The severity of haemolytic disease of the newborn
INTRODUCTION INTO SANBS

• The MMA test was first put into use in the early 1970’s – but not ever used in SANBS
• Implementation in SANBS Reference Lab will provide an additional tool to patients’ treating physicians regarding the suitability of transfusing their patients with incompatible blood
SANBS APPLICATIONS FOR MMA

• Determining the possible clinical significance of alloantibodies to red blood cell antigens of high frequency where the patient may require transfusion and antigen negative blood is unavailable

• Post transfusion monitoring of cases in which patients with alloantibodies to high frequency antigens have been transfused with incompatible blood due to the lack of compatible or antigen negative blood
SANBS APPLICATIONS FOR MMA

• On request or in studies for determining the possible clinical significance of alloantibodies in haemolytic disease of the foetus and newborn (HDFN) cases

• MMA testing may be applicable when the following antibodies are identified and antigen negative blood is not available: anti-hr^B, -hr^S, -Yt^a, -Lu^b, -Ge:2, -Ge:3, -Lan, -Gy^a, -Hy, -Jo^a, -Jr^a, -LW, -At^a, -In^b, -Mi^a and –Cs^a
TEST PROCEDURE

• Identify the patient’s alloantibody
• Select antigen positive RBC to be used in the test – prior to an incompatible transfusion, this will be a sample of the donor RBC
• Obtain 3 – 4 ACD tubes of whole blood from a healthy, willing monocyte donor
• Centrifuge ACD tubes at 150g for 10 minutes, remove the platelet rich plasma and resuspend in PBS
TEST PROCEDURE

Layer remaining red cell/white cell/PBS mixture onto a solution of Ficoll
TEST PROCEDURE
TEST PROCEDURE

• The monocytes are transferred into a clean test tube and washed
• Sensitized cells are prepared
  – Patient serum (known antibody specificity) and donor RBC (ag+)
• Sensitized and unsensitized cells are washed and tested
TEST PROCEDURE

Monocytes are placed on a glass chamber slide, incubated and the supernatant removed – adherent monocytes remain on the slide.
TEST PROCEDURE

Antibody-sensitized and unsensitised red blood cells added to the chambers and incubated with the monocyte layer
TEST PROCEDURE

The supernatant is removed with a pipette and the chambers separated from the slide.
TEST PROCEDURE

• Unattached RBC are removed by washing and the slide is stained
• Slide is read microscopically
• Percentage of monocytes with adherent and/or phagocytosed RBC is determined.
INTERPRETATION OF RESULTS

Phagocytosis and Moderate Adherence

Accessed online on 10/08/2016 at www.ucdmc.ucdavis.edu
INTERPRETATION OF RESULTS

• %R of $\leq 5\% = \text{Incompatible blood could be given with little risk}$

• %R between 5.1 - 20\% = 33\% of patients may have clinical signs of a reaction

• %R of $> 20\% = 64\%$ of patients may have clinical signs of a reaction
INTERPRETATION OF RESULTS

IMPORTANT:
Although values of $\leq 5\%$ have been associated with only a low risk of an acute haemolytic transfusion reaction, it must be noted that this value does not necessarily guarantee the normal, long-term in-vivo survival of the transfused RBC.
SANBS MMA PILOT STUDY

• Challenge: no prior SANBS experience meant that external training would be required
• Training provided at the American Red Cross, Southern California Region
• ARC protocol brought back to SANBS for implementation
• SANBS Immunohaematology Reference Lab staff trained
SANBS MMA PILOT STUDY

• Series of 3 pilot tests were performed to confirm test performance in our laboratory

• Clinically significant antibodies used
  – 2 X anti-D and 1 x anti-K
  – %R >20% anticipated

• Actual results:
  – 56%, 69% and 44% respectively
The MMA pilot study indicates that the ARC protocol can be successfully implemented in our laboratory.

Full test validation to be completed and documented.

Full rollout of the MMA in the Immunohaematology Laboratory is anticipated later this year.
CONCLUSION

HOWEVER

• The decision to transfuse incompatible blood cannot be based solely on the MMA result – assess clinical need

• Assists the treating physician to make a more informed decision regarding patient treatment
REFERENCES

• Evaluating the Clinical Significance of Blood Group Alloantibodies that are causing Problems in Pretransfusion Testing, Garratty G. Vox Sanguinis 1998; 74 (Suppl. 2): 285 – 290

• 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