

# **INTRODUCTION OF THE MMA TEST – A PILOT STUDY**

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South African Blood Transfusion Congress,  
Sun City  
30 August 2017



# MMA???



Accessed online on 10/08/2016 from [www.mutantmma.com](http://www.mutantmma.com)



# MMA???



Accessed online on 10/08/2016 from [www.bleacherreport.com](http://www.bleacherreport.com)



**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<sup>B</sup>, -hr<sup>S</sup>, -Yt<sup>a</sup>, -Lu<sup>b</sup>, -Ge:2, -Ge:3, -Lan, -Gy<sup>a</sup>, -Hy, -Jo<sup>a</sup>, -Jr<sup>a</sup>, -LW, -At<sup>a</sup>, -In<sup>b</sup>, -Mi<sup>a</sup> and -Cs<sup>a</sup>





# 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 unsensitized red blood cells added to the chambers and incubated with the monocyte layer



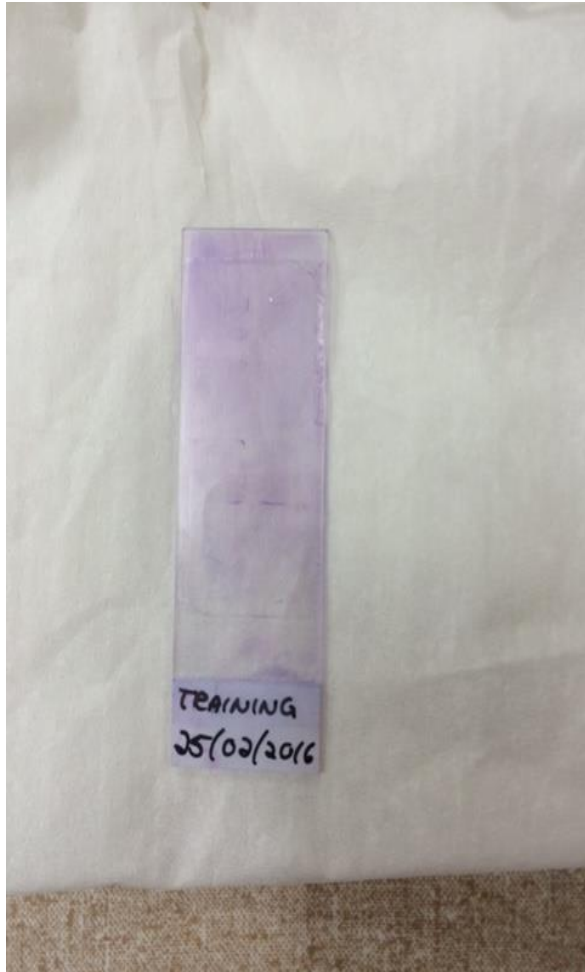
# TEST PROCEDURE



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



Accessed online on 10/08/2016 at [www.ucdmc.ucdavis.edu](http://www.ucdmc.ucdavis.edu)



# INTERPRETATION OF RESULTS

- %R of  $\leq 5\%$  = 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



# SANBS MMA PILOT STUDY

- 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

