The Monocyte Monolayer Assay (MMA)

Background

The Monocyte Monolayer Assay (MMA) is an *in vitro* procedure used to assist in predicting if incompatible blood can be transfused safely to a patient. This testing is useful for antibodies to a high incidence antigen or antibodies for which a specificity cannot be determined, or for those with variable reports of clinical relevance. It can be particularly relevant in cases where antigen negative blood is in limited supply or unavailable, for example the use of Jk(a-b-), Lu(b-) or In(b-) red cells.

Currently there are patients in New Zealand with high incidence antibodies who ideally require antigen negative blood for transfusion. However, due to the extreme rarity of these units, serologically least incompatible red cells can be used with caution. By using the MMA test, we can safely predict the outcome of transfusing least incompatible red cells to these patients.

Alloantibodies to red blood cell antigens can be categorised into three groups as far as clinical significance is concerned: those that are usually clinically significant (e.g. antibodies to Rh, K, FY, JK systems), those that are usually clinically insignificant (e.g. antibodies that do not react at 37ºC), and those that have variable clinical significance (e.g. anti-Yt^{a}, -Ge, -Lu^{b}).

Because red cells lacking high incidence specificities such as anti-Yt^{a}, -Ge, -Lu^{b} and others are rare and should not be wasted, it is important to try to determine which patients require “antigen-negative” blood to avoid an acute haemolytic transfusion reaction.

A particular focus for NZBS implementing the MMA is the effective use of rare red cells. There has recently been a paper published discussing the MMA in relation to transfusing non Jk(a-b-) red cells to a patient with anti-Jk3. With the incidence of anti-Jk3 in New Zealand being reasonably high, and the supply of Jk(a-b-) red cells being requested globally, it would be pertinent to know if the use of Jk(a-b-) red cells is required on all occasions or if this rare blood supply could be utilised more efficiently[1].

Principle of the MMA

Tissue macrophages and peripheral blood monocytes, collectively referred to as mononuclear phagocytes (MP), are mediators of extravascular haemolysis of red cells coated with antibody. The MPs have receptors for the Fc portion of IgG1, IgG3 and the C3b component of complement. Red blood cells coated with these will become attached to MPs, particularly in the liver and spleen, and will be phagocytised. The MMA is intended to be an in vitro model of this in-vivo activity.

The MMA procedure is used to assist in predicting if incompatible blood can be transfused safely to a patient. The test involves using monocytes and patient serum containing the antibody under investigation to ascertain the clinical significance of that antibody. This will support the clinical advice offered when it comes to selecting appropriate red cells for transfusion.

This is a new analytical method for NZBS however the MMA is a well-defined and well-established test in other blood services including the American Red Cross, Philadelphia, Grifols Immunohaematology Centre, Texas and the Swiss Red Cross Kanton Bern, Switzerland.

Description of the Analytical Method

The quantitative analytical method validation protocol used provided a percentage of reactivity within a range established for what is considered to be clinically significant and insignificant when it comes to transfusion, as established by the American Red Cross in Philadelphia.

The monocytes used for testing were pooled from two donors in order to stabilise the reactivity in case of one individual having overactive monocytes. Not all IgG antibodies detected by routine methods (e.g. IAT test) are clinically significant when analysed by means of the MMA, therefore precision testing was required to confirm the reliability of the results.

Reproducibility within a certain range is dependent on the strength of reactivity observed in the MMA. Since the selection of countable fields is subjective, you would expect some variation between counting, even if the tester repeated their own counts at a different time. After 20 years of performing the MMA, the American Red Cross in Philadelphia have ascertained that the coefficient of variation (CV) should be 7-8%. By selecting a 10% reproducibility range during the validation, this allowed for expertise in the test to be established whilst not underestimating the margin of error.

Validation Approach

The MMA is a new qualitative analysis of monocyte reactivity and cannot therefore be compared to any current, validated method of testing. During the validation the parameters evaluated were specificity, a limited approach to accuracy and precision testing including repeatability, intermediate precision, reproducibility and sensitivity.

During the specificity of patient samples, the known and available high incidence antibodies were anti-Rh17, two examples of anti-Lub, anti-Jk3 and anti-k.

Accuracy was a measure of closeness between the measured and real value. Due to the lack of a comparative method for the MMA, accuracy was determined by testing a sample containing a known antibody (anti-MAM) that had already undergone MMA testing by the American Red Cross in Philadelphia. The same sample was referred for testing in 2015 and aliquots of the patients' plasma stored for reference purposes. The results report received from the American Red Cross testing was used as a comparison to determine accuracy during the validation.

The repeatability expresses the precision under the same operating conditions over a short period of time. This was demonstrated by repeating the testing using the same operator on 3 separate occasions.

The intermediate precision is the reproducibility of the assay under a variety of normal, but variable, test conditions. In this case, it was determined by repeating the same test on the same samples, on different days, to ensure the result for each sample remained the same or within 10% of the initial value. The reagents and consumable lots were identical for each test, however the donor monocytes were collected from different donors on each day of testing as freshly collected cells were required.

Reproducibility expresses the precision between laboratories. This was demonstrated by performing the MMA on a stored sample that was previously referred to the American Red Cross in Philadelphia for testing.

The Future

After successful completion of the validation project, NZBS are able to offer the Monocyte Monolayer Assay to clinicians who have patients with high incidence antibodies of known and unknown specificity. Our Transfusion Medicine Specialists (TMS) will be able to offer advice on the level of clinical significance and the likely outcome of transfusion therefore allowing the use of rare phenotyped red cells to be utilised more effectively and issued on a case by case basis.

A concurrent validation will continue for one year after the MMA is implemented in order to review the clinical outcome of all transfusions that proceed as a result of the MMA. This will include requesting repeat samples for MMA testing when transfusion has taken place as well as clinical follow up on any signs and/or symptoms of transfusion reaction. This will allow data to be collected on the success of the transfusion, the accuracy of the MMA and any future implications of not transfusing antigen-negative red cells by means of follow up testing. This may also help identify any detection and quantification limits (LOD and LOQ).

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References

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