

“Examination of the feasibility of establishing a peer reviewed external quality assessment scheme for red blood cell antibody eluate investigations”



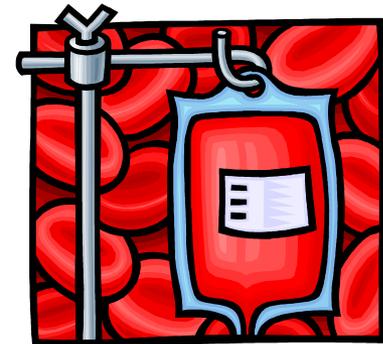
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Antibody Elutions in Blood Transfusion

- Aims to remove antibodies from a sensitised red blood cell, while maintaining the antibody in a usable form for later identification.
- Most commonly used to investigate:
 - Haemolytic Disease of the Newborn
 - Haemolytic Transfusion Reaction
 - Transfusion Reactions due to red cell antibody
 - Auto-Immune Haemolytic Anaemia
- Principles of elution based on breaking the forces holding the Antigen-Antibody complex together:
 - Altering the pH or ionic strength of the suspending solution
 - Using organic solvents or thermal agitation to disrupt the interactions.

Antibody Elutions in Blood Transfusion (2)

- Antibody elution on patient samples are now performed using commercially available kits.
- Two kits are used in the Republic of Ireland, both based on techniques developed by Rekvig and Hannestad (1977) and Bush (1978):
 - Use acid-glycine buffers to elute RBC antibodies.
- Kits allow for standardisation of the reagents and methods, and make the process of eluting antibodies more efficient.

BCSH Guidelines

- New guidelines for pre-transfusion compatibility testing (2012) encourage antibody elutions to be performed when:
 - DAT positive patient who was transfused in the previous month
 - DAT negative but with signs of haemolysis (may occur in ABO HDN)
 - Pallor, fatigue, shortness of breath
 - Bilirubinaemia, increased Lactate Dehydrogenase
 - Red blood cell morphology.
- If guidelines are implemented:
 - Increased antibody elutions performed routinely
 - Increased detection of alloimmunisation
 - Increase in providing fully phenotyped blood for immunised patients.



Irish regulation and EQA

- European Union Directive 2002/98/EC was transposed into Irish law through Statutory Instrument 360 of 2005.
- It stated that all Blood transfusion laboratories must become accredited to continue practice.
- In order to become accredited a laboratory must participate, and do well, in an External Quality Assessment (EQA) scheme for each test included in the accreditation scope, to ensure they are providing a test result to a suitable quality.
- To date, no EQA scheme has been established or introduced in the Republic of Ireland to assess laboratory performance and investigation of antibody elution procedures.
 - Current schemes assess ABO grouping, RhD typing, Antibody detection and identification, crossmatching, RBC phenotyping.
 - Royal College of Pathologists of Australia Quality Assurance Programme (RCPAQAP) provides a general Transfusion scheme, of which around 3 cycles out of 6 annually involve antibody elution as part of the required testing.

Aim of my Research

- If the BCSH standards are implemented, the number of antibody elutions performed routinely could increase dramatically in Rol.
- It would be necessary to establish an EQA assessment of laboratory investigation of antibody eluates to allow for the procedure to be accredited, which a legal requirement for Blood Transfusion laboratories.
- My work aimed to:
 - Develop an experimental model for the production of samples suitable for use in such a scheme
 - Determine the feasibility of establishing such an EQA scheme in the Republic of Ireland, based on a trial.

Questionnaire



- Circulated to approximately 45 Blood Transfusion laboratories in the RoI:
 - 11 stated that they perform antibody elutions in their laboratory.
 - All 11 laboratories agreed to participate in our trial EQA scheme.
 - 6/11 use Kit I
 - 5/11 use Kit II.
- The laboratories stated they use elutions to investigate
 - Transfusion reaction due to red cell antibody (10/11),
 - Haemolytic disease of the newborn (5/11),
 - Autoimmune Haemolytic Anaemia (2/11),
 - Some specifically perform according to the BCSH guidelines (3/11).
- The questionnaire also determined that the vast majority of Blood Transfusion laboratories (50%) believed that 4 EQA test sets per year (each containing two samples) would be sufficient to examine laboratory performance and investigation of antibody eluates.

Optimisation and Validation procedures

1. Equal volumes of two different adsorption cells (AC) with known antigenic profiles were used to produce a pool of RBCs.
2. 2.5mL of packed cells sensitised with chosen antisera (chosen on basis that only one AC population should be positive for antibody's respective antigen). Antiserum to RBC volume ratio calculated.
3. Cells eluted and antibody identified. The antiserum to RBC ratio at which the antibody was clearly identifiable was chosen for possible use in trial. See table below for chosen antisera, and antiserum to RBC volume ratios proven to produce eluates in which antibodies were clearly identifiable.

Antiserum	Anti-S	Anti-Fya	Anti-D	Anti-Jka
Antiserum: RBC	1:6.0	1:3.5	1:12.5	1:6.6

4. Validating sample expiry time.
 - Two weeks post preparation chosen as suitable.
 - Samples produced in optimisation procedures stored and tested over two week period.
 - Eluate strength from stored red blood cells did not vary over the two week period.
5. Comparing results achieved by two commercial elution kits.

When each sample was tested, it was tested on both kits
Slight variation in strength of eluates depending on antibody but did not affect detection rates of antibody.

Trial EQA scheme

- Anti-Fya and Anti-Jka sensitised cells were chosen for use in the trial.

52mL pool of RBCs was made with two cell populations(Ratio 1:1). The pool was divided into two 26mL aliquots.

7.34mL of Anti-Fy^a antiserum was added to 26mL RBCs make antiserum: RBC ratio of 1:3.55.

3.94mL of Anti-Jk^a antiserum was added to 26mL RBCs make the antiserum:RBC ratio of 1:6.6.

Incubated for one hour at 37°C.

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26mL of Modified Alsevi's solution was added, to equal to volume of RBCs.

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Separated into 4mL aliquots in plastic tubes labelled: Sample 1: 2013-ELU-001.

Separated into 4mL aliquots in plastic tubes labelled: Sample 2: 2013-ELU-002.

- Preparation and Closing date testing included :
 - Polyclonal DAT and monoclonal DAT if required
 - Elution with both commercial elution kits, and
 - Antibody identification on all obtained eluates.
- Trial Packs containing the two samples, exercise instructions and result recording sheets were dispatched to all participants in the IBTS temperature controlled vans within 24 hours of preparation.
- Upon return of all results , each was analysed in relation to :
 - Whether the antibody was identified correctly,
 - Whether there as an association between misidentification and the methods or reagents used
 - Different types of errors incurred
 - Possibility of developing a scoring system based on the results.
- Result reports were issued to all participants 7 days after all results had been returned. Each participant received a report on their own performance and their performance in relation to all other participants.

The Trial results

- With both samples, 9/11 participants identified the correct antibody in the sample. Each sample incurred a procedural error and a technical error.
 - **Sample 1 (Anti-Fya):**
 - One laboratory reported the eluate as negative (found DAT negative also). (Kit II)
 - Other laboratory reported an Anti-Jka.
 - **Sample 2 (Anti-Jka):**
 - One laboratory reported an Anti-C antibody in their eluate. (Kit I)
 - The primary panel and exclusion cells were requested for analysis. Results of the primary panel were inconsistent with the presence of an Anti-Jka, but results of exclusion cells allowed for presence of Anti-Jka.
 - Other laboratory reported an Anti-Fya.
 - The second error in each sample was established to be a transcriptional error.
- Two laboratories returned their results reports after the closing date of the trial.
- All laboratories who incurred errors were given the opportunity to rectify their error, either by reanalysis or by rectifying the transcriptional error.
- A penalty system was chosen as appropriate to mark this scheme, where points are assigned based on errors made. No score was implemented in the scheme. It was used to assign points and to define performance limits.

The Trial results (2)

- The definitions of cumulative penalty scores were set as follows:
 - **0-39** : Satisfactory performance. No action is required.
 - **40-49** : Borderline performance. A review of procedures is required.
 - **≥50** : Unsatisfactory performance. Corrective action should be taken to prevent recurrence.

Proposed Penalty	% participants who achieved expected result	Penalty Score
For each non reactive eluate produced from a positive sample.	≥80%	40
	50-79%	20
	20-49%	10
For each incorrect antibody identified in a reactive eluate.	≥80%	50
	50-79%	25
	20-49%	15
For being unable to identify if interpretation is not agreed with		25
Non-return/ Late return of results.		25

Conclusions

- Both commercial elution kits used in trial achieved similar results for participants.
 - Trial is suitable for all Blood Transfusion laboratories in R.O.I to date.
- Difficulty of assessment suitable to use in EQA scheme.
 - Participants incurred variety of different errors including procedural, technical and late return.
 - Establishment of an online programme for result submission should minimise late returns, therefore any results submitted online after closing date could incur a penalty for the laboratory.
- Results allowed for a penalty scoring system to be adapted to suit this scheme, similar to those used by NEQAS:
 - Allows for performance monitoring of participant laboratories.
 - Not implemented as more trials required to ensure results are consistent, and that limits and penalties are appropriate.
- Methods undertaken to interpret results and issue reports was sufficient and allowed for short turnaround time from submission of results to reporting (7 days).

What's next?

- Variation in detection levels between different pools of RBCs
 - Dosage made it difficult to establish volume ratios that could be carried over from exercise to exercise,
 - Ratios for antibodies such as Anti-Fya will need to be established before every exercise, using the pool of cells to be used for the exercise, as they varied greatly depending on the pool of cell used.
 - Further work required to establish whether ratios set for other antibodies, eg Anti-D can be carried over between exercises as no variation in strength of eluates seen in my research.
- When trials achieve a 100% pass rate, attention will turn to introduce more intricate investigations:
 - Mixed antibodies
 - Differences in detection rates between different elution kits with weak eluates.
- Business plan and accreditation must be addressed by IBTS before scheme could be implemented.
 - ISO 17043:2010: General requirements for the competence of providers of proficiency testing schemes and for the development and operation of proficiency testing schemes.
 - ISO9001:2008: Requirements for a QMS where an organisation is required to demonstrate its ability to provide a product that meets customer and applicable regulatory requirements.

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Questions?