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Improving the Provision of Blood for Patients with Auto-Immune Haemolytic Anaemia

Mark Lambert

Irish Blood Transfusion Service, National Blood Centre, James Street, Dublin 8, Ireland

Irish Blood Transfusion Service
Seirbhís Fuilaistriúcháin na hÉireann

Background

Patients with auto-immune haemolytic anaemia, and other auto-immune disorders, usually have free auto-antibody in their plasma. This free auto-antibody will react with all reagent cells used for antibody investigation and usually both IAT-panels and enzyme-treated panels.

For antibody investigation and the transfusion of the correct blood, this can be problematic. Underlying allo-antibodies are present in 28% of AIHA patients seen in Ireland [unpublished data]. However they cannot be readily detected without the use of specialised serological techniques.

These techniques involve the removal of auto-antibody by either auto-adsorption or allo-adsorption, to allow detection of any potential underlying allo-antibody.

Different methods of adsorption have been described for the removal of auto-antibody including the use of papanised cells, ZZAP-treated cells and addition of PEG. Standard incubation time published is usually 30 minutes at 37°C.

Standard methods using papanised or ZZAP-treated cells are time consuming, both from the point-of-view of cell preparation and adsorption incubation time. The use of PEG in adsorption has resulted in the loss of allo-antibodies during the adsorption procedure.

The Red Cell Investigation Laboratory at the Irish Blood Transfusion Service (IBTS) is the main Red Cell Referral Laboratory in Ireland. A major role is the investigation of patients with auto-immune haemolytic anaemia, involving adsorption of auto-antibody by either auto- or allo-adsorption.

Allo-adsorptions at the IBTS are currently performed using O R_1R_1 and O rr red cells matched for Kidd, Duffy and MSs antigens, and prepared for adsorption by papanisation each time they are required. Incubation is for 30 minutes at 37°C [AABB Technical Manual 2008].

This procedure can be time-consuming, leading to delays in transfusing AIHA patients. From sample receipt to unit despatch, a patient requiring 3 allo-adsorptions will take ~215 minutes.

Aims

The aim was to investigate the possibility of changing the overall protocol for allo-adsorption, to include pre-papanisation of cells, change of adsorption technique and incubation time. The new adsorption technique described by Chiaroni *et al.* in 2003 is referred to as LISS-Addition Method (LAM).

Methods

Samples from patients with AIHA were collected for approximately one year (only those with sufficient volume were collected). Samples included a range of required-adsorptions (x1 to x9), a range of samples containing allo-antibodies and auto-antibodies with 'specificity'. 56 samples from patients with AIHA were collected (2 requiring adsorption with untreated cells). 3 samples from antibodies to high incidence antigens were included (anti-Yt^a (x2), anti-In^b).

Methods

Materials used:

- Day 3 Red Cell unit (04333 - IBTS)
- Papain (Palerm, Diagast)
- Modified Alsever's Solution (Inverclyde Biologicals)
- LISS (Inverclyde Biologicals)
- PBS pH 7.0 (Inverclyde Biologicals)
- Bio-Rad LISS-Coombs Cards (Bio-Rad)

A SAG-M red cell unit was taken on day 3 (O D-C-E-K-Jk(a-b+) Fy(a-b+) M+N-S-s+). 100ml was divided into 20ml aliquots for untreated adsorption. The remaining red cells were washed in PBS and papanised. The papanised cells were stored in Modified Alsever's solution until unit expiry. When required red cells were washed with PBS before use.

All adsorptions were performed using the LAM described by Chiaroni *et al.* Patient plasma and LISS were adding to packed cells at a ratio of 1:1:1. This was mixed and incubated at 37°C for 10 minutes for the first adsorption. After the 10 minute incubation the plasma-LISS-cell mixture was centrifuged using a DiaCent-12 (1000g for 2 minutes). No additional LISS was added for subsequent adsorptions. Testing was performed by Bio-Rad IAT column agglutination technology (CAT).

Adsorptions were continued on samples until free auto-antibody was removed from plasma (established by testing after each adsorption with a 3-Cell Screen).

Results

The mean adsorptions per sample using the IBTS method (requiring >1 adsorption) was 3.5. Using the LAM the mean adsorption for these same samples was 1.85. The average 'adsorption time' per sample (>1 adsorption) was **reduced from 112 minutes for IBTS method to 22 minutes for LAM**.

Mean IBTS Adsorptions (>1) = 3.5
Mean LAM Adsorptions (>1) = 1.85

Mean Overall Adsorptions IBTS = 2.85
Mean Overall Adsorption LAM = 1.63

The identification of underlying allo-antibodies was also enhanced. Table 1 shows the allo-antibodies detected following adsorption, including five ***not*** detected using the IBTS adsorption (***bold italics***).

Adsorption of antibodies to high incidence antigens was successful using untreated cells. Two Anti-Yt^a and a single anti-In^b were each cleared by one allo-adsorption.

Table 1. Allo-antibodies detected following allo-adsorption of auto-antibody.

	IBTS Adsorption		LAM Adsorption	
Sample	Adsorption	Specificity	Adsorption	Specificity
1	6	C	6	C
2	9	c	3	c
3	8	S	4	S
4	1	E	1	E+***C^w***
5	1	E	1	E
6	2	D	1	D
7	5	C+K+Kp^a	2	C+K+Kp^a
8	1	Lu^a	1	Lu^a
9	2	None Detected	1	***C^w***
10	5	None Detected	3	***Jk^a***
11	3	None Detected	1	***C***
12	2	None Detected	1	***C***

Results

No samples required additional adsorptions using LAM compared to IBTS adsorptions. Only 6 of 40 samples (>1 adsorptions) required the same number; all other samples required less adsorptions using LAM.

10 minute incubation times proved as effective as 30 minute incubation. Other investigators have also described effective removal of auto-antibody following adsorption with 10 minute incubation [Stamps *et al.* 1999; Leger *et al.* 2011].

Conclusion

Although other investigators using tube-IAT report no significant reduction in the number of adsorptions needed, the major benefit is seen when using CAT [Magtoto-Jocom *et al.* 2011; Chiaroni *et al.*, 2003].

Adopting LAM, a 10-minute incubation and pre-papanised cells will significantly improves turn-around-time for AIHA patients. Allo-antibody detection is also enhanced. The example given above will now take ~115 minutes, almost half the time. This protocol has been introduced at the Irish Blood Transfusion Service.

Turn-Around Time Estimations

Step	IBTS Adsorption	LAM Adsorption
Registration	5	5
Separation	5	5
ABO/Rh	20	20
Routine Identification*	40	30
Adsorption	35 x 3	16
Adsorption Identification and Compatibility Testing	30	30
Labelling	10	10
Total	**215 minutes**	**115 minutes**
	(3 hr 35 min)	(1 hr 55 min)

References

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Contact Details: E-mail: mark.lambert@ibts.ie, Phone: +353-1-4322972; Web: www.giveblood.ie