P- 475

RED CELL ANTIBODY INHIBITION USING UNPURIFIED CELL CULTURE SUPERNATANT CONTAINING SOLUBLE RECOMBINANT BLOOD GROUP PROTEINS

Barry Doyle[1], Kay Ridgwell[2], Mark Lambert[1], Frances Green[2], John Crumlish[1], Mary Keenan[1], Sorcha NiLoingsigh[1]
[1]Irish Blood Transfusion Service, Dublin 8, Ireland. [2]DDRS, NHSBT, Filton, Bristol, UK.

Background

The provision of suitable red cell blood components for transfusion and pregnancy can be complicated by the presence of serologically complex red cell antibodies. Recombinant blood group proteins have been shown to have potential in the elucidation of red cell antibodies[1]. Assays using recombinant proteins are available in numerous forms; soluble recombinant proteins that can be used for antibody inhibition, solid phase immobilised recombinant proteins and protein micro-arrays or colour coded microspheres[2].

Aims

The study primarily evaluated the use of cell culture supernatant (CSN), as a source of unpurified recombinant protein in haemagglutination inhibition assays, thus facilitating the identification or exclusion of underlying allo-antibodies. The use of CSN eliminates the protein purification step during recombinant protein production.

Methods

Antibodies to blood group antigens K, k, Kp^b, Js^b, Fy^a, Fy^b, and Lu^b were inhibited using the following recombinant proteins; 3xFLAG-K, 3xFLAG-k, Fy^a-3xFLAG, Fy^b-3xFLAG and Lu^b-3xFLAG protein (K and k proteins also express Kp^b and Js^b). Recombinant proteins were analysed in two forms; 1) CSN containing soluble recombinant protein (CSN-SRP) and 2) purified protein product for comparison. Recombinant proteins were provided by Diagnostics Development, NHSBT Filton, with funding from BIORAD. CSN-SRPs (of unknown concentration) and purified recombinant proteins (of known concentration) were assessed for their ability to inhibit known clinically significant allo-antibodies. All antibodies tested (n=41) were titred to determine their strength and the strongest samples were selected for inhibition. Antibody isotype (IgG/IgM) was also determined by BIORAD monoclonal DAT. Antibody identification was performed by BIORAD IAT pre- and post-inhibition. Both patient and reagent antibodies were tested.

*This work was supported in part by BioRad

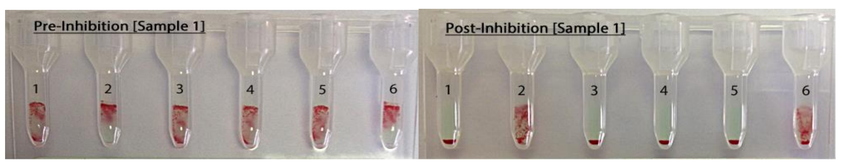
Results

CSN-SRP was extremely effective at inhibiting all patient antibodies tested (titre range from 1 to 512). Samples with an IgM component required a higher volume of CSN-SRP to completely inhibit the antibody. A ratio of 1:10 (v/v) CSN-SRP to plasma, incubated for 10 minutes at 37°C was the optimum method for antibody inhibition. All antibodies were inhibited with CSN-SRP with the exception of one reagent monoclonal anti-K (IgM). The inhibition of selected allo-antibodies facilitated easier identification of other underlying allo-antibodies, especially where multiple antibodies or antibodies to high frequency antigens were present (Table 1, Figures 1, 2, 3 & 4).

Table 1 Antibody Combinations Tested

Sample	Antibodies Present	Antibodies Inhibited	Antibody Detectable Post Inhibition
1	Anti-Kp^b + K	Anti-Kp^b	Anti-K
3	Anti-Fy^a + Jkb + K	Anti-Fy^a	Anti-Jk^b + K
2	Anti-Fy^b + E + K	Anti-Fy^b	Anti-E + K
4	Anti-K + Jk^a	Anti-K	Anti-Jk^a

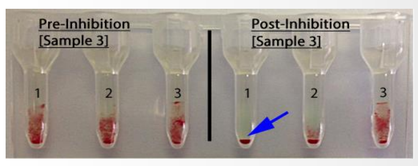
Fig. 1
Sample 1: Pre and Post Inhibition of anti-Kp^b



Anti-Kp^b + underlying anti-K

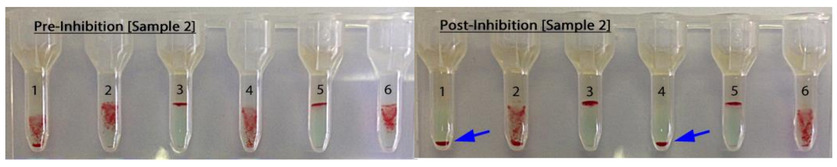
Anti-K detectable post inhibition of anti-Kp^b

Fig. 2
Sample 2: Pre and Post-Inhibition of anti-Fy^a



Post inhibition – Cell 1 negative (blue arrow), only anti-Jk^b is detectable in well 2 and anti-K in well 3.

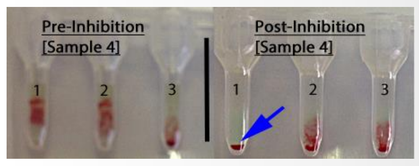
Fig. 3
Sample 3: Pre and Post Inhibition of anti-Fy^b



Pre inhibition - reactivity detectable in columns are due to the following; (1) Anti-Fy^b, (2) Anti-Fy^b+K,(3) Anti-Fy^b+E, (4) Anti-Fy^b, (5) Anti-E, (6) Anti-K

Post inhibition - the anti-Fy^b has been removed and columns 1 and 4 are now negative (blue arrows). The reactivity in columns 2 and 6 are due to anti-K and the reactivity in columns 3 and 5 are due to anti-E.

Fig. 4
Sample 4: Pre and Post inhibition of anti-K



Post inhibition – Column 1 is negative (blue arrow), only anti-Jk^a is detectable in columns 2 and 3.

Conclusions

Successful antibody inhibition using purified protein has been previously reported. This study shows that CSN-SRP is an acceptable alternative eliminating the need for protein purification. The relatively small volume of CSN required for inhibition prevents dilution of other antibodies present in the sample, allowing the effective detection or exclusion of other underlying antibodies. A ratio of 1:10 (v/v) CSN-SRP to plasma, incubated for 10 minutes at 37°C was the optimum method for antibody inhibition.

References

1. Ridgwell, K., Dixey, J. and Scott, M.L. (2007) Production of soluble recombinant proteins with Kell, Duffy and Lutheran blood group antigen activity, and their use in screening human sera for Kell, Duffy and Lutheran antibodies. *Transfusion Medicine*, **17**, 384-394.
2. Seltsam, A. & Blasczyk, R. (2009) Recombinant blood group proteins for use in antibody screening and identification tests. *Current Opinion in Hematology,* **16**, 473-479.

Contact: barry.doyle@ibts.ie