Evaluation of recombinant blood group proteins in pre-transfusion and antenatal testing in the RCI laboratory

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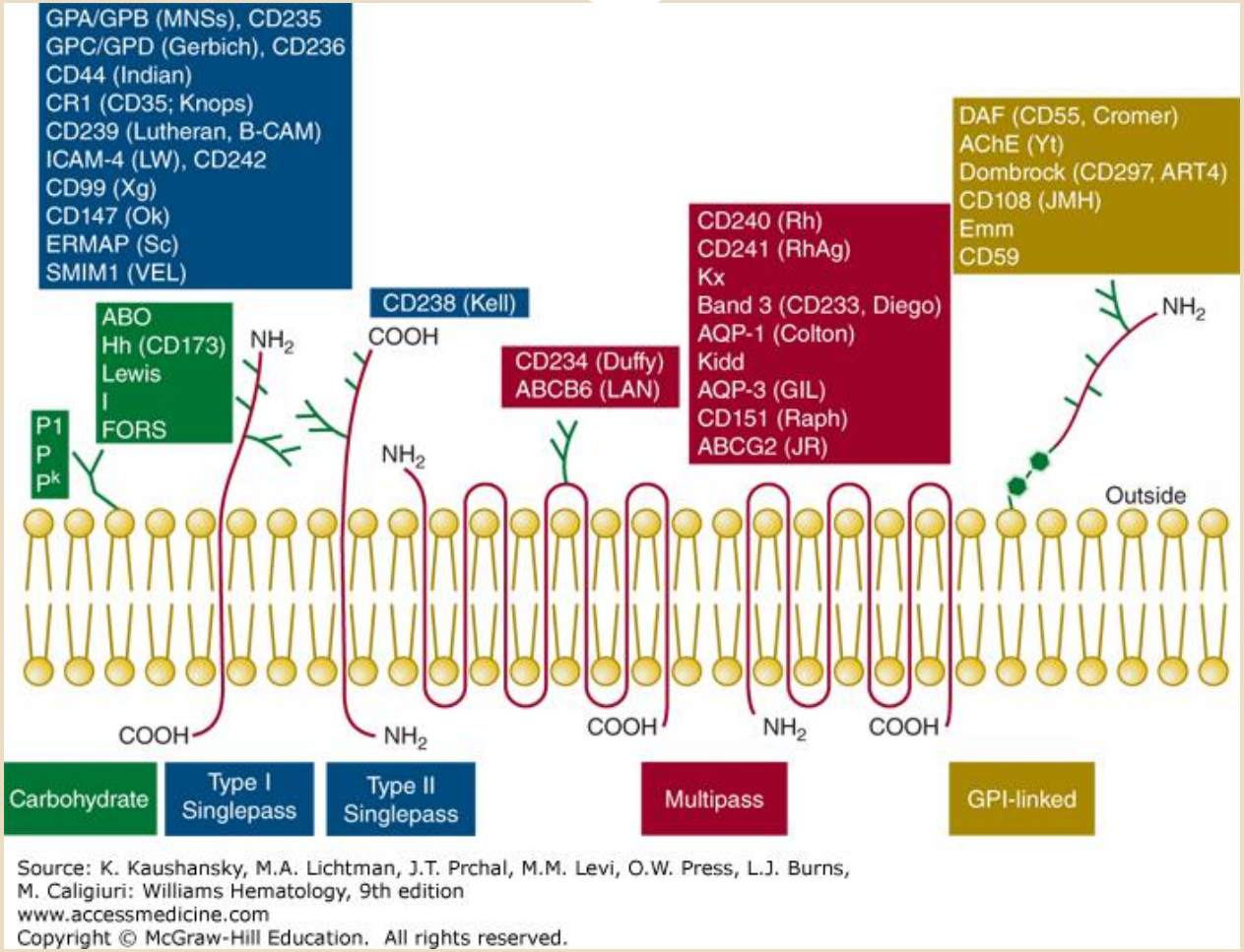
Recombinant Blood Group Proteins (rBGPs)

- Blood Group Antigens – recap
- General Information re rBGPs
- Production of rBGPs
- Background into rBGPs
- Test principle/Assay employed
- Their use in the RCI Laboratory
- Evaluation Study- Results
- Case Study 1
- Case Study 2

Blood Group Antigens - Recap



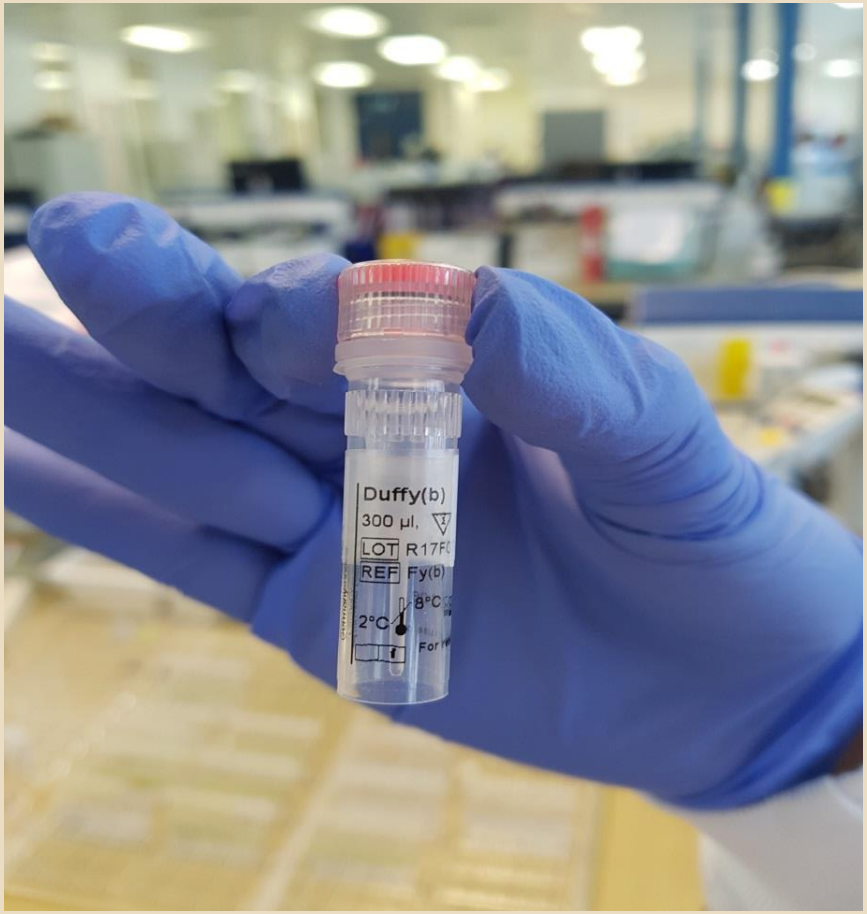
Source: K. Kaushansky, M.A. Lichtman, J.T. Prchal, M.M. Levi, O.W. Press, L.J. Burns, M. Caligiuri: Williams Hematology, 9th edition
www.accessmedicine.com

Blood Group Antigens –Recap

- 30 blood group systems are known to exist on red blood cells
- Majority are genetically defined as a result of single nucleotide polymorphisms (SNPs) in respective encoding genes
- All but one of the genes encoding the 30 blood group systems have been identified
- This has allowed for the development of recombinant forms of the blood group proteins

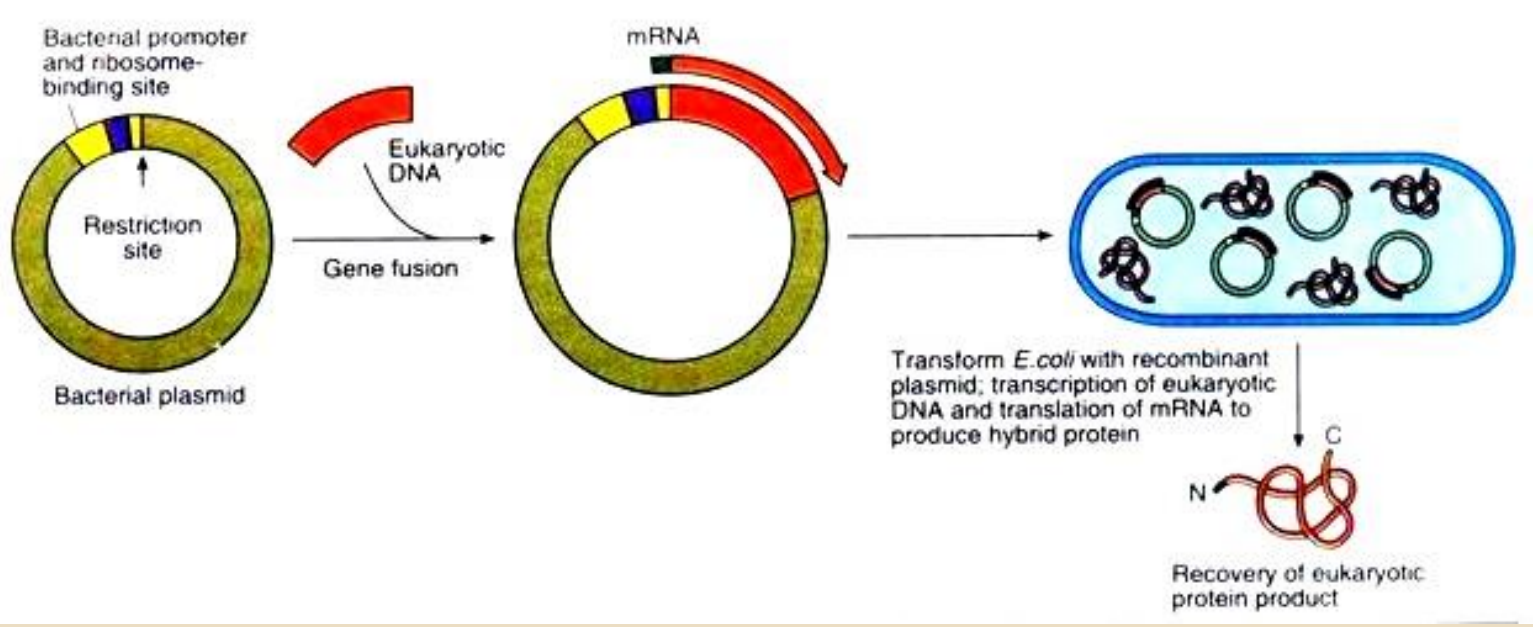
rBGPs- General Information

- Synthetic blood group proteins/antigens
- Supplied by “Imusyn”
- Soluble
- Each vial contains 300ul of rBGP
- Stored (2°-8°c)

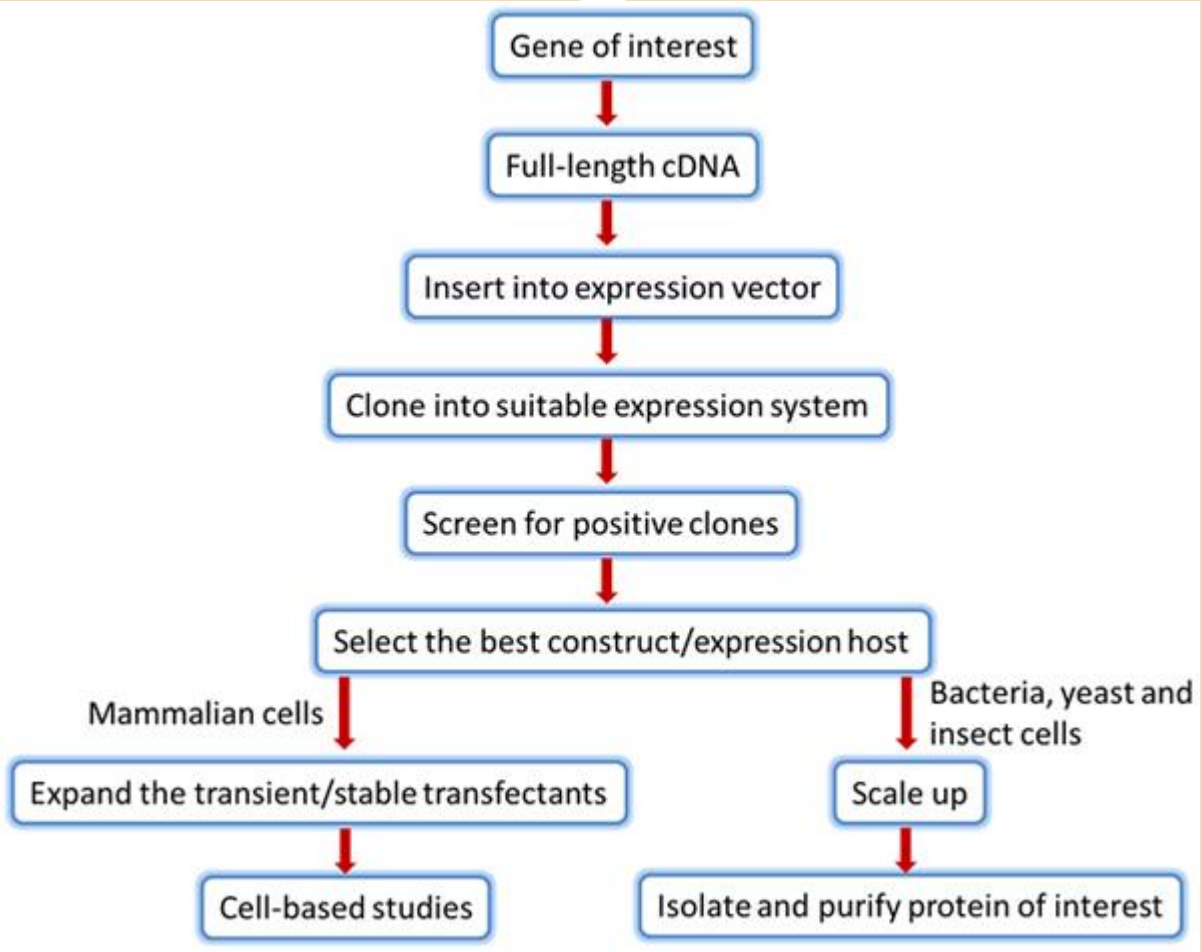


Production of rBGPs

- Recombinant DNA technology used



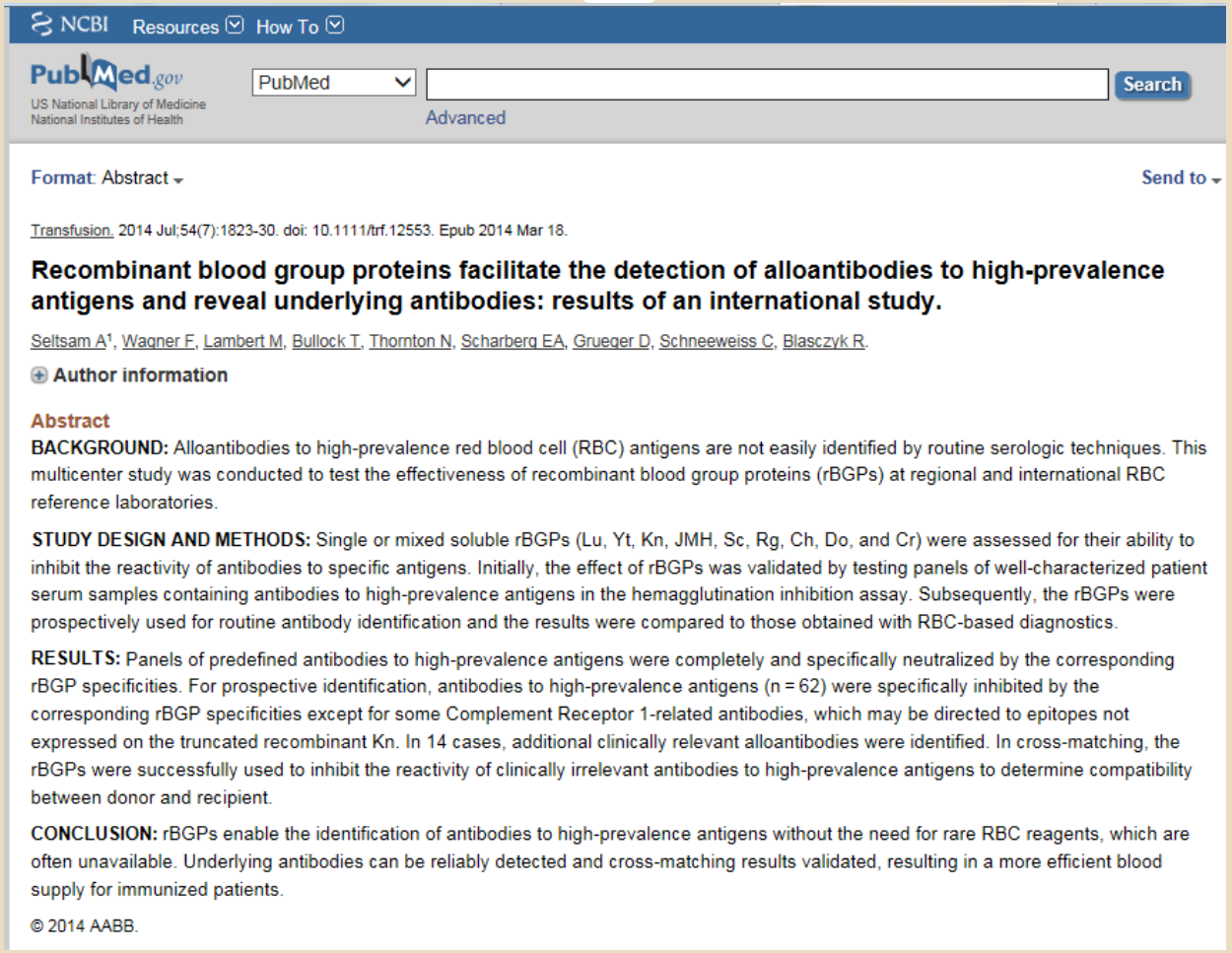
Production of rBGPs



Background of rBGPs

- First introduced in 1996 by Moulds and Rowe
  - Successfully neutralised anti-Knops antibody with soluble CR1 protein (sCR1) produced by recombinant DNA techniques
- Since then the majority of work in the development of rBGPs has been achieved under Axel Seltsam, Hanover based researcher
- Publications: Seltsam *et al* (2003, 2007, 2008, 2009, 2014)

Background of rBGPs



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Transfusion. 2014 Jul;54(7):1823-30. doi: 10.1111/trf.12553. Epub 2014 Mar 18.

Recombinant blood group proteins facilitate the detection of alloantibodies to high-prevalence antigens and reveal underlying antibodies: results of an international study.

Seltsam A[1], Wagner F, Lambert M, Bullock T, Thornton N, Scharberg EA, Grueger D, Schneeweiss C, Blasczyk R.

Author information

Abstract

BACKGROUND: Alloantibodies to high-prevalence red blood cell (RBC) antigens are not easily identified by routine serologic techniques. This multicenter study was conducted to test the effectiveness of recombinant blood group proteins (rBGPs) at regional and international RBC reference laboratories.

STUDY DESIGN AND METHODS: Single or mixed soluble rBGPs (Lu, Yt, Kn, JMH, Sc, Rg, Ch, Do, and Cr) were assessed for their ability to inhibit the reactivity of antibodies to specific antigens. Initially, the effect of rBGPs was validated by testing panels of well-characterized patient serum samples containing antibodies to high-prevalence antigens in the hemagglutination inhibition assay. Subsequently, the rBGPs were prospectively used for routine antibody identification and the results were compared to those obtained with RBC-based diagnostics.

RESULTS: Panels of predefined antibodies to high-prevalence antigens were completely and specifically neutralized by the corresponding rBGP specificities. For prospective identification, antibodies to high-prevalence antigens (n = 62) were specifically inhibited by the corresponding rBGP specificities except for some Complement Receptor 1-related antibodies, which may be directed to epitopes not expressed on the truncated recombinant Kn. In 14 cases, additional clinically relevant alloantibodies were identified. In cross-matching, the rBGPs were successfully used to inhibit the reactivity of clinically irrelevant antibodies to high-prevalence antigens to determine compatibility between donor and recipient.

CONCLUSION: rBGPs enable the identification of antibodies to high-prevalence antigens without the need for rare RBC reagents, which are often unavailable. Underlying antibodies can be reliably detected and cross-matching results validated, resulting in a more efficient blood supply for immunized patients.

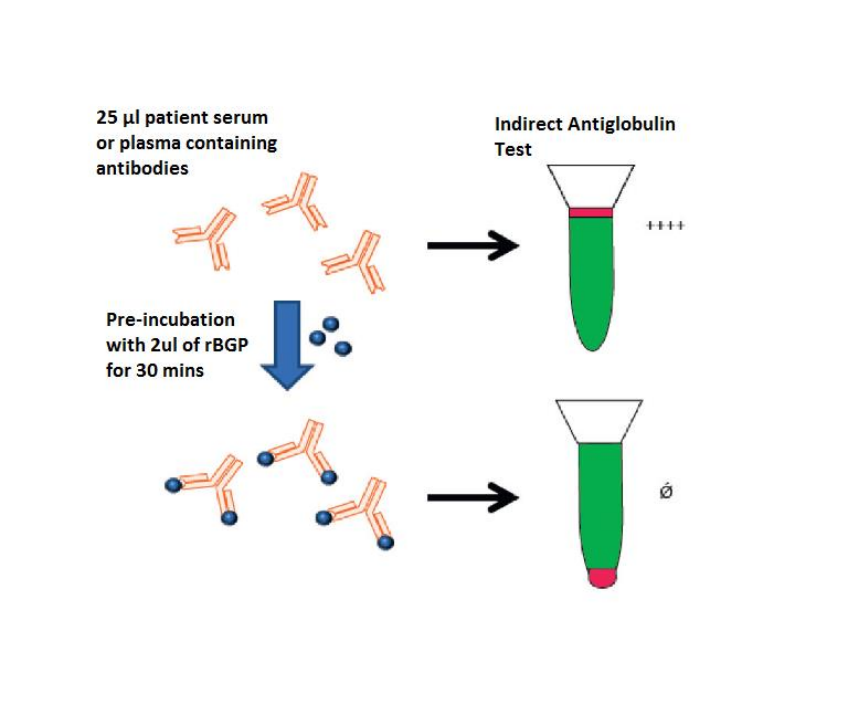
rBGPs Available

REF	Antigen
Ch(a)	C4B*3
CR1	Kn(a), McC(a), Sl(a), Sl3+
CROM	Cr(a+), Tc(a), Dra+, Esa+, IFC+, WES(b), UMC+, GUTI+
Do(a)	Do(a), Hy+, Jo(a+)
Do(b)	Do(b), Hy+, Jo(a+)
Fy(a)	Fy(a)
Fy(b)	Fy(b)
grKba	Js(a), K12+, Ul(a-), K19+, Tou+, K23+, K13+, K22+, K11, Kp(b), Raz+, Vlan+, K, K14, K18+
In(b)	In(b)
JMH	JMH
klkba	Js(a), K12+, Ul(a-), K19+, Tou+, K23+, K13+, K22+, K11, Kp(b), Raz+, Vlan+, k, K14, K18+
Lu(a)	Lu(a), Lu4+, Lu5+, Lu6, Lu8, Lu12+, Lu13+, Lu16+, Lu17+, Lu20+, Lu21+
Lu(b)	Lu(b), Lu4+, Lu5+, Lu6, Lu8, Lu12+, Lu13+, Lu16+, Lu17+, Lu20+, Lu21+
LW(a)	LW(a)
Rg(a)	C4A*3
Sc1	Sc1, Rd-
Xg(a)	Xg(a)
Yt(a)	Yt(a)

Test Principle/ Assay

- Hemagglutination Inhibition Assay (HIA)

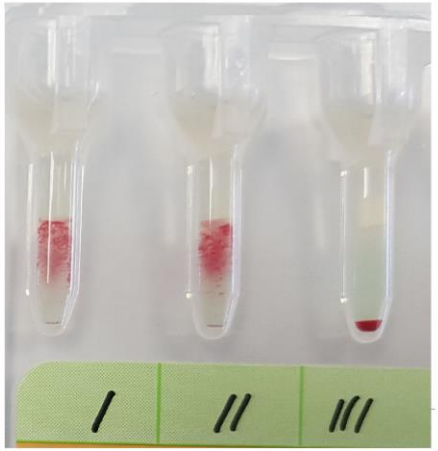
1. 2ul of rBGP to 25ul of plasma
2. Mix and spin (8000g for 5 seconds)
3. Incubate at room temperature (19°C-25°C) for 30mins
4. Include a negative control (2ul of PBS to 25ul of plasma)
5. Perform IAT using reagent panel cells



Test Principle/ Assay

Pre-Inhibition
Neat Plasma

Post-Inhibition
Plasma inhibited by rBGP



Post-Inhibition
Plasma + PBS (Control)

3 cell antibody screen

Current Antibody Detection Methods

- Current detection methods for detection of red cell allo-antibodies involve hemagglutination techniques using a panel of pre-typed human RBCs by IAT
- This method of antibody identification however is challenged in the presence of complex antibodies
- In the above cases, referral to an RCI Laboratory is generally required for further investigation

Use of rBGPs

- Investigations involving complex antibodies:
  - Can be both time consuming and labour intensive
  - Rely heavily on patient information such as age and ethnicity along with the use of rare antigen negative red cells
  - Can result in a delay in antibody ID and ultimately patient care
- rBGPs are a novel alternative for elucidating such antibodies

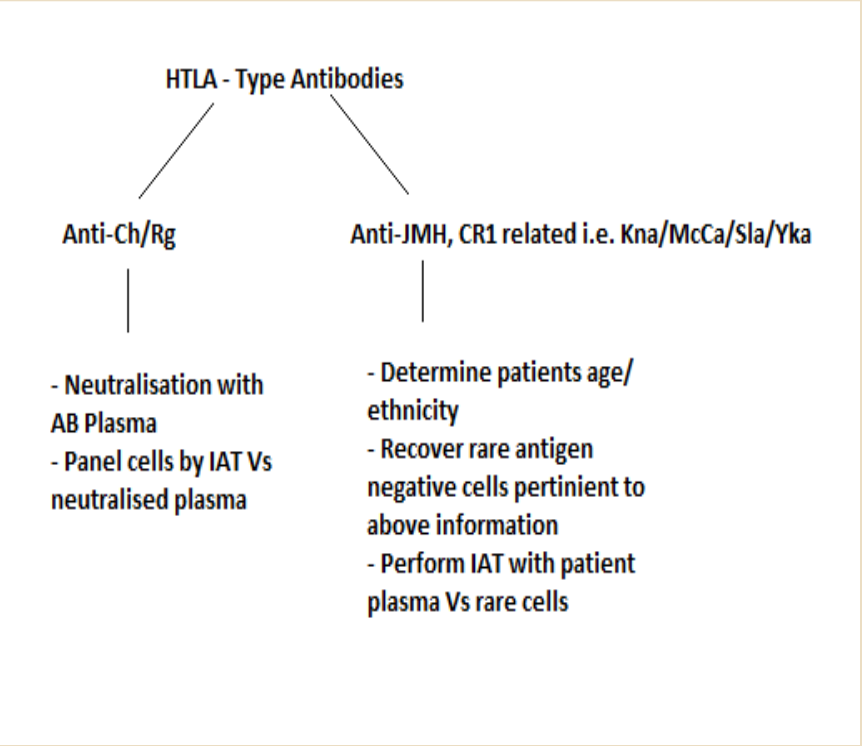
Use of rBGPs

- Complex Antibodies include:
  - High Titre, Low Avidity (HTLA) antibodies
  - Antibodies to high prevalence antigens aka high incidence antibodies
  - Cases of multiple antibodies

High Titre Low Avidity Antibodies

- Demonstrate pan-reactive picture in IAT with various reaction strengths
- They're characterised as being "low avidity" by nature therefore neutralisation techniques can sometimes be ineffective
- Clinically insignificant however, may mask underlying clinically significant antibodies

- Investigation:



Antibodies to high prevalence antigens

- High prevalence antigens are described as antigens which occur at a frequency of over 99% on RBCs
- Serologically pan-reactive usually
- Knowledge of patients ethnicity is key to guide investigation
- Involves the use of rare antigen negative red cells

Ethnic Origin	Sub-Group	'Common' Antibody	Suggested Testing
Caucasian	All	Anti-k	k-
		Anti-Kp^b	Kp^b-
		Anti-Lu^b	Lu(a+b-)
			Lu(a-b-)
		Anti-Co^a	Co^a-
		Anti-Yt^a	Yt^a-
		Anti-Vel	Vel-
		Anti-Lan	Lan-
		Anti-Sc1	Sc:-1,2
		Anti-Sd^a	Sd^a- (Urine inhibition)
	Swedish	Anti-PP_1P^k	PP_1P^k-
	Finns	Anti-Jk3	If patient is Jk(a-b-), then Jk(a-b-) cells
Middle East	Arabs/Jews	Anti-Yt^a	Yt^a-
		Anti-Fy3	Fy(a-b-)
Native N/S America		Anti-Di^b	If patient is Di^a+, then Di^b- cell
Asian	Indian or Bangledeshi	Anti-In^b	If patient is In^b-, then In^b- cells
	Indian	Anti-H	Bombay or Para-Bombay cells
	Polynesians	Anti-Jk3	If patient is Jk(a-b-), then Jk(a-b-) cells
	Papua New Guinea	Anti-Ge3	Ge:-2,-3
	Japan	Anti-PP_1P^k	PP_1P^k-
		Anti-Jr^a	Jr^a-
		Anti-Ok^a	Ok^a-
African	All	Anti-U	If patient is S-s-, then S-s-U- and S-s-U^{wk}
		Anti-Js^b	If patient is Js^a+, then Js^b- cells
		Anti-hr^s	If the patient has 'anti-e' but is e+, type patient with single clone anti-e reagents (see Rare Reference Database), before setting up rare cells
		Anti-hr^B	
		Anti-Hr^B	
		Anti-Fy3	Fy(a-b-) Sl^a+ and Fy(a-b+) Sl^a-
		Anti-Hy	Hy-
		Anti-Jo^a	Jo^a-

Evaluation Study

- Study aims:
  1. Evaluate the efficacy of rBGPs
  2. Evaluate how specific rBGPs are towards their corresponding antibody
  3. Evaluate the effect of antibody strength on its inhibition by rBGPs
  4. Evaluate the possibility of using plasma inhibited by rBGPs in pre-transfusion compatibility testing

Evaluation Study - Results

Table 1 - Evaluation of the efficacy of single recombinant blood group proteins listed below

rBPG used	Total no. samples containing corresponding antibody tested n=	No. of samples successfully inhibited n=	% of samples successfully inhibited
	HTLA Antibodies		
CR1	15	9	60%
JMH	5	0	0%
Ch(a)	3	3	100%
	Antibodies to High Prevalence Antigens		
Lu(b)	2	1	50%
Yt(a)	5	4	80%
In(b)	4	3	75%
klkba (cellano)	3	3	100%
	Other Specificities		
Fy(a)	9	6	66.6%
Fy(b)	2	0	0%
grKba (Kell)	4	3	75%
Total No. Tested	**52**	**32 (61.5%)**	

Evaluation Study - Results

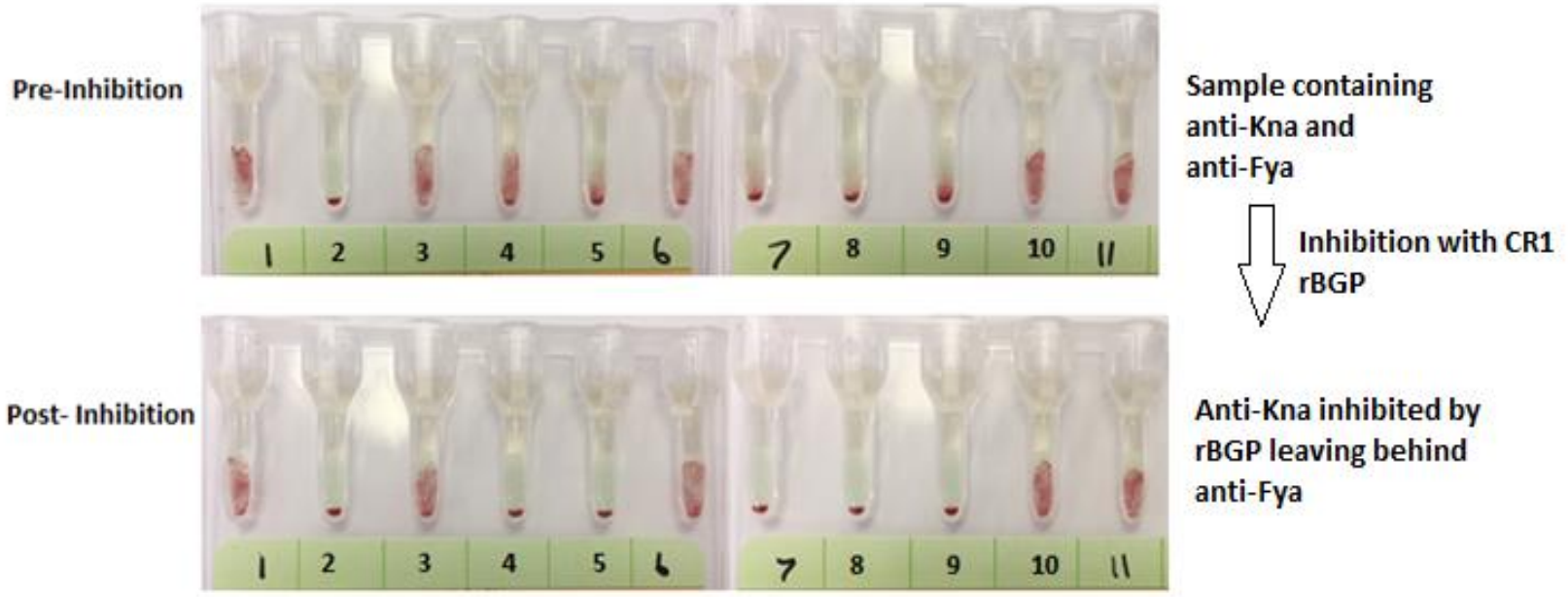
- **Table 2- Evaluation of rBGPs specificity using samples containing multiple antibodies**

rBGP used	Antibodies present in sample	Inhibition Successful (Y/N)	Antibody detected post inhibition
CR1	Kn^a + Fy^a	Y	Anti-Fy^a
Fy(a)	Fy^a+ E	Y	Anti-E
Fy(a)	Fy^a+ D	Y	Anti-D
grKba (Kell)	Fy^a+ K	N	Anti-Fy^a + K
Total No. tested:	**4**	**3 (75%)**	

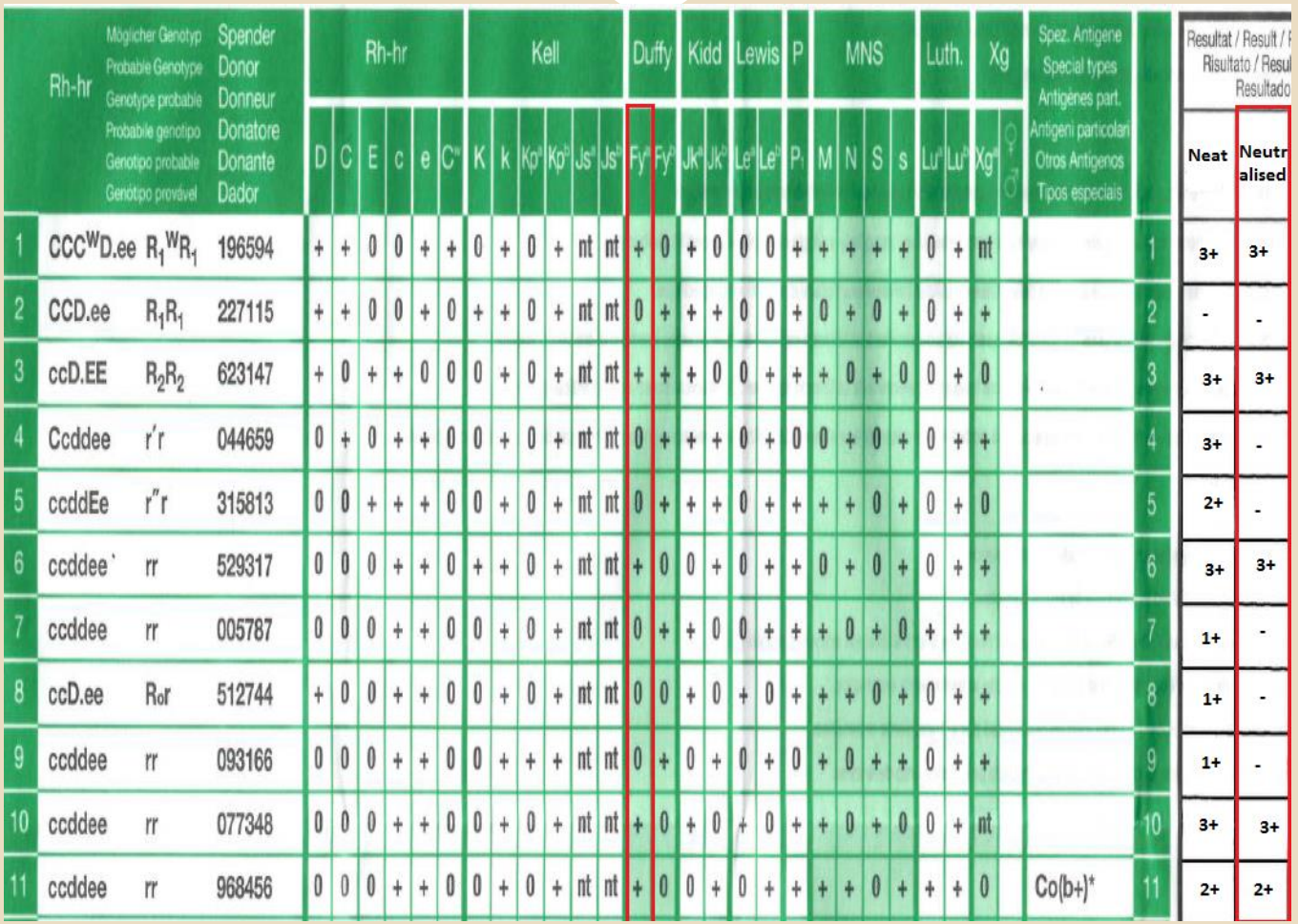
- **Table 3- Further evaluation of rBGP specificity using anti-sera sourced from SCARF versus Chido and Rogers rBGP**

rBGP	Anti-Sera	Inhibition Successful (Y/N)
Ch(a)	Anti-Chido	Y
Ch(a)	Anti-Rogers	N
Rg(a)	Anti-Chido	Y
Rg(a)	Anti-Rogers	N

Sample containing anti-Kna with underlying anti-Fya



Sample containing anti-Kna with underlying anti-Fya



	Rh-hr	Möglicher Genotyp / Probable Genotype / Genotype probable / Probable genotipo / Genotipo probable / Genotipo provável	Spender / Donor / Donneur / Donatore / Donante / Dador	Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth.		Xg		Spez. Antigene / Special types / Antigènes part. / Antigeni particolari / Otros Antigenos / Tipos especiais		Resultat / Result / Risultato / Resultado	
				D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P$_1$	M	N	S	s	Lu^a	Lu^b	Xg^a	♂ ♀			Neat	Neutralised
1	CCC^{W}D.ee	$R_1{}^WR_1$	196594	+	+	0	0	+	+	0	+	0	+	nt	nt	+	0	+	0	0	0	+	+	+	+	+	0	+	nt			1	3+	3+
2	CCD.ee	R_1R_1	227115	+	+	0	0	+	0	+	+	0	+	nt	nt	0	+	+	+	0	0	+	0	+	0	+	0	+	+			2	-	-
3	ccD.EE	R_2R_2	623147	+	0	+	+	0	0	0	+	0	+	nt	nt	+	+	+	0	0	+	+	+	0	+	0	0	+	0			3	3+	3+
4	Ccddee	r′r	044659	0	+	0	+	+	0	0	+	0	+	nt	nt	0	+	+	+	0	+	0	0	+	0	+	0	+	+			4	3+	-
5	ccddEe	r″r	315813	0	0	+	+	+	0	0	+	0	+	nt	nt	0	+	+	+	0	+	+	+	+	0	+	0	+	0			5	2+	-
6	ccddee	rr	529317	0	0	0	+	+	0	+	+	0	+	nt	nt	+	0	0	+	0	+	+	0	+	0	+	0	+	+			6	3+	3+
7	ccddee	rr	005787	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	+	0	0	+	+	+	0	+	0	+	+	+			7	1+	-
8	ccD.ee	R$_{or}$	512744	+	0	0	+	+	0	0	+	0	+	nt	nt	0	0	+	0	+	0	+	+	+	0	+	0	+	+			8	1+	-
9	ccddee	rr	093166	0	0	0	+	+	0	0	+	+	+	nt	nt	0	+	0	+	0	+	0	+	0	+	+	0	+	+			9	1+	-
10	ccddee	rr	077348	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	+	0	+	0	+	+	0	+	0	0	+	nt			10	3+	3+
11	ccddee	rr	968456	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	0	+	0	+	+	+	+	0	+	+	+	0		Co(b+)*	11	2+	2+

Evaluation Study - Results

- **Table 4 - Evaluation of the effect of antibody strength on inhibition by rBGPs**

Sample	Antibody Titre (Anti- Fya)	Successful Inhibition (Y/N)
Sample 1	2	Y
Sample 2	4	Y
Sample 3	8	N
Sample 4	128	Y
Sample 5	256	Y
Sample 6	512	Y

Evaluation Study - Results

- **Table 5- Pre-transfusion compatibility testing over an eight day period using six plasma samples inhibited by rBGPs**

rBGP used		Day 1	Day 2	Day 3	Day 6	Day 7	Day 8
Fya	Sample1	C	C	C	C	C	C
	Sample 2	C	C	C	C	C	C
	Sample 3	C	C	C	C	C	C
	Sample 4	C	C	C	C	C	C
K	Sample 5	C	C	C	C	C	C

- **“C” denotes compatible**

Evaluation Study - Conclusion

- Efficacy of rBGPs
  - Overall out of 52 samples tested , 61.5% were successfully inhibited
  - HTLA Antibodies- 52.2% successfully inhibited
  - Antibodies to high prevalence antigens -78.5% successfully inhibited
- rBGP specificity
  - rBPGs have been shown to be specific to their corresponding antibody and allow for differentiation between closely related antibodies
- Antibody strength does not have an effect on its inhibition
- Inhibited plasma can be used in pre-transfusion compatibility testing

Case Study One

- Female 70yrs old
- Phenotype: R1r K-, s-, Fya-, Jkb-
- Previously identified HTLA-type antibody which was successfully inhibited by AB plasma
- Referral on this occasion: serology changed and neutralisation with AB plasma no longer successful
- 2 units requested

Case Study One

- Serology on referral:

Test/Panel Number	1	2	3	4	5	6	7	8	9	10	11
Enzyme Panel	-	0.5+	-	0.5+	-	-	0.5+	-	-	0.5+	-
IAT Panel	2+	2+	2+	3+	3+	2+	2+	3+	3+	2+	2+
Neutralised + IAT	2+	2+	2+	3+	3+	2+	1+	3+	2+	2+	2+
PBS Ctl + IAT	2+	2+	2+	3+	3+	2+	1+	3+	2+	2+	2+

- Increased ratio of AB plasma to patient plasma from 2:1 to 3:1 and tested against 3 cell screen by IAT

Test/Panel Number	1	2	3
Neutralised (3:1) + IAT	-	-	-
PBS Ctl (3:1) + IAT	3+	3+	2+

Case Study One

- Supplementary to this – rCh protein was also used at a ratio of 2µl of rBGP: 25µl of patient plasma however did not result in successful antibody inhibition
- The ratio of rBGP: Plasma was increased to 6µl:25µl (0.24:1)
- This plasma was used in an 11 cell IAT - resulted in successful antibody inhibition with additional reactivity remaining

Test/ Panel	1	2	3	4	5	6	7	8	9	10
Inhibited Plasma (6:25) + IAT	-	1+	0.5+	0.5+	0.5+	-	0.5+	-	-	-
PBS Ctl (6:25) + IAT	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+

- Cells 2, 3, 4, 5 & 7 = Fy^a +

Case Study One

- Inhibited plasma was also used in pre-transfusion compatibility testing

Unit Number	RT (Neat)	IAT (Neat)	rBGP	PBS Ctl
Unit 1	0.5+	3+	0.5+	2+
Unit 2	-	2+	-	2+
Unit 3	-	3+	-	2+
Unit 4	-	2+	-	2+

- Conclusion:
  - Could not exclude E or Fya
  - Concluded: HTLA type antibody of chido specificity
  - Chido antibodies are not known to cause Tx rxn or HDN
  - Patient received E-, K-, Fya- serologically least incompatible red cells

Case Study One

- By using rBGP in this case:
  - Allowed us to exclude clinically significant antibodies for this patient without having to rely on plasma diluted at a ratio of 3:1
  - Allowed us to crossmatch units required and gave confidence in issuing these units
  - Ultimately their use resulted in safer provision of units for the patient

Case Study Two

- Female, 89yrs old
- Hb: 8.2gr/dl
- Phenotype: R1R2 K-
- Investigation and 2 units requested urgently

Case Study Two

- Investigation:

Test/Panel	1	2	3	4	5	6	7	8	9	10	11
Enzyme Panel	4+	4+	4+	4+	-	4+	-	4+	-	4+	4+
IAT Panel 1	3+	4+	3+	3+	-	3+	-	3+	-	3+	3+
IAT Panel 2	3+	4+	3+	3+	3+	NT	4+	3+	3+	3+	NT
IAT Panel 3	3+	2+	3+	4+	4+	3+	3+	3+	-	2+	2+
IAT Panel 4	3+	3+	4+	3+	-	2+	3+	3+	2+	3+	
IAT Panel 5				2+							

- Cell 9 IAT Panel 3 = Dob-

Case Study Two

- Anti-Do^b suspected
- HIA performed with rDo^b protein and patients plasma
- Allowed for quick identification of anti-Do^b present

Test/panel	1	2	3	4	5	6	7	8	9	10	11
Enzyme Panel	4+	4+	4+	4+	-	4+	-	4+	-	4+	4+
IAT Panel	3+	4+	3+	3+	-	3+	-	3+	-	3+	3+
rDob inhibited plasma + IAT	-	4+	-	2+	-	4+	-	0.5 +	-	-	-
PBS Ctl + IAT	3+	4+	3+	3+	-	3+	-	3+	-	3+	3+

Positivity in cells 2 & 6 = Anti-K

Positivity in cells 4 & 8 = Possible white cell antibody

Case Study Two

- Recombinant proteins allowed for quick identification of anti-Do^{b} in patients sample as opposed to relying on recovering rare red cells which is time consuming
- Occurrence of Do^{b} = 82% Caucasians
- No antigen typing reagents exist to type for Do^{a} or Do^{b} – need to molecular type- therefore units are not typed for Dombrock antigens
- Transfusion protocol for Do^{b} is “Crossmatch compatible @ 37°C”
- Because we had identified Dob quickly – allowed time to screen 30+ units, of 30+ units screened – 3 compatible units were acquired

Unit Number	RT	IAT	rDo^{b}	PBS Ctl
Unit 1	-	-	-	-
Unit 2	-	-	-	-
Unit 3	-	-	-	-

Case Study Two

- By using rBGP in this case:
  - Allowed fast identification/confirmation of anti-Dob present in patient sample
  - Allowed faster exclusion of clinically significant antibodies
  - Avoided the need to recover rare Dob- cells to exclude
  - Allowed for screening of units for the patient
  - Greatly reduced overall investigation time and provision of units to the patient

rBGPs Potential/ Looking forward

- Disadvantages
  - They are expensive
  - CE marked in Germany only at present

rBGPs Potential/ Looking forward

- Advantages:
  - They have the potential to greatly reduce time taken for antibody identification
  - Quick and easy to use
  - Have relatively long expiration dates (1 year approx)
  - Reduce reliance on rare red cells when dealing with complex antibodies

rBGPs Potential/ Looking forward

- The use of rBGP cocktails would be of great benefit
  - For Example using a “High Incidence” cocktail containing rBGPs pertinent to the Irish population (e.g. k, Kpb, Lua, Yta, Sc1)
  - “HTLA” cocktail (JMH, Kna, McCa, Yka)

  - Further Evaluation required

Conclusion

- rBGPs are not effective 100% of the time and so cannot replace current methods
- Are useful to guide antibody investigation strategy
- They may be implemented as supplementary to current validated methods
- Findings from the use of rBGPs will be supportive of results obtained with validated methods

Thank you!

QUESTIONS?