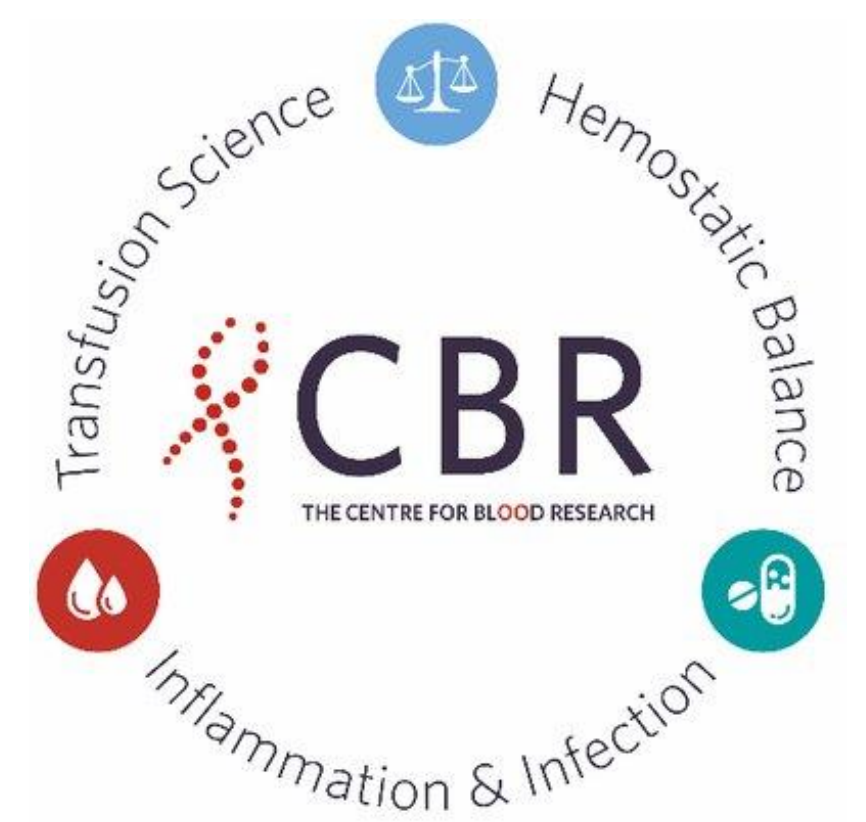




Towards Universal Blood – Enzymatic Conversion of Blood Group A Antigens

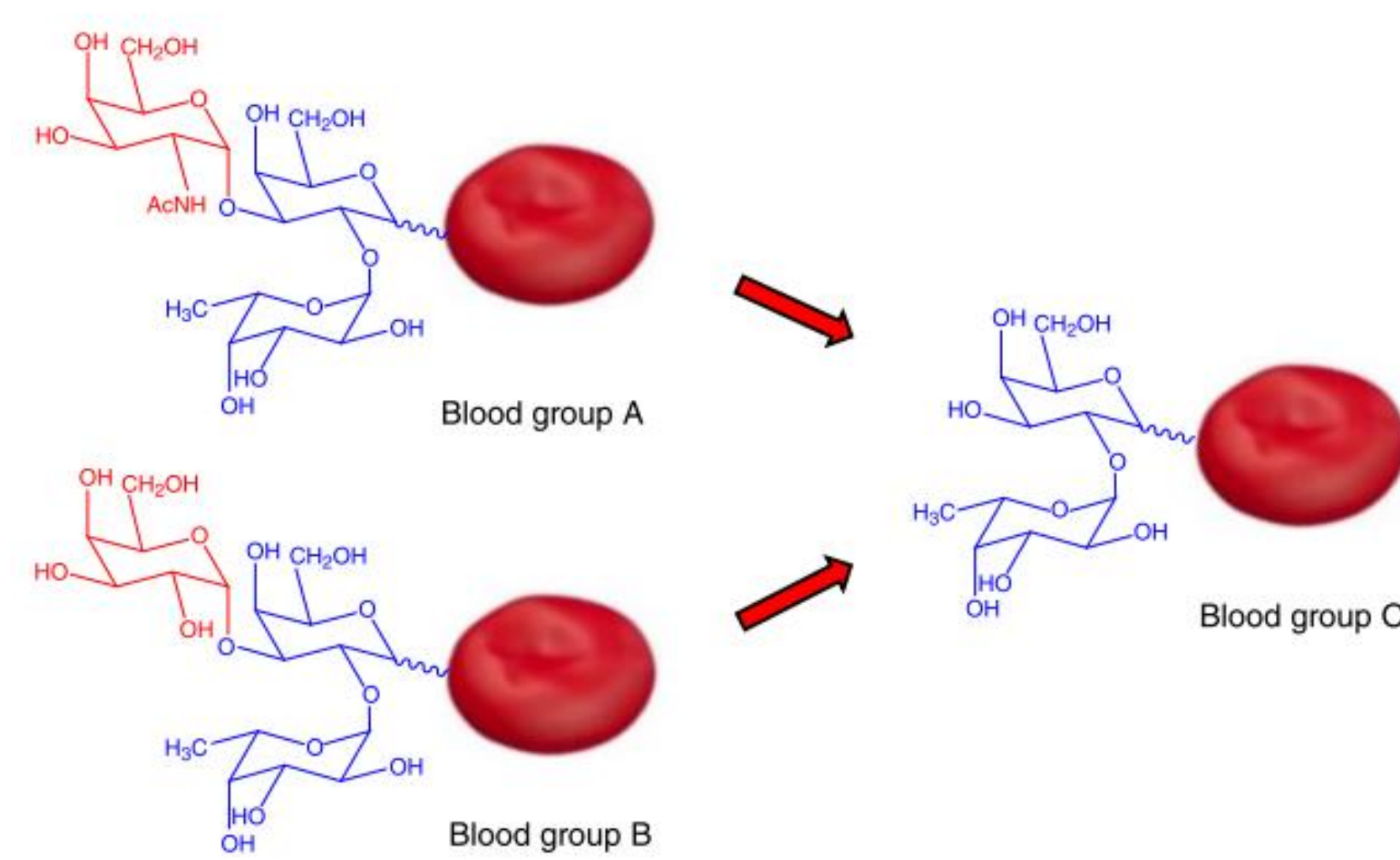
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INTRODUCTION

- Transfusion safety and shortage of specific ABO blood group units are two major challenges present in current transfusion therapy.
- A possible solution to current transfusion challenges is to produce universally transfusable RBCs.
- The ABH blood group specificity is determined by the terminal monosaccharides-*N*-acetylgalactosamine on A group RBCs and galactose on B group RBCs.
- Enzymatic removal of these sugars would uncover universally acceptable H-antigen, which is characteristic of O group RBCs.
- Enzymatic conversion of group A RBCs has lagged behind due to lack of very active glycosidases.



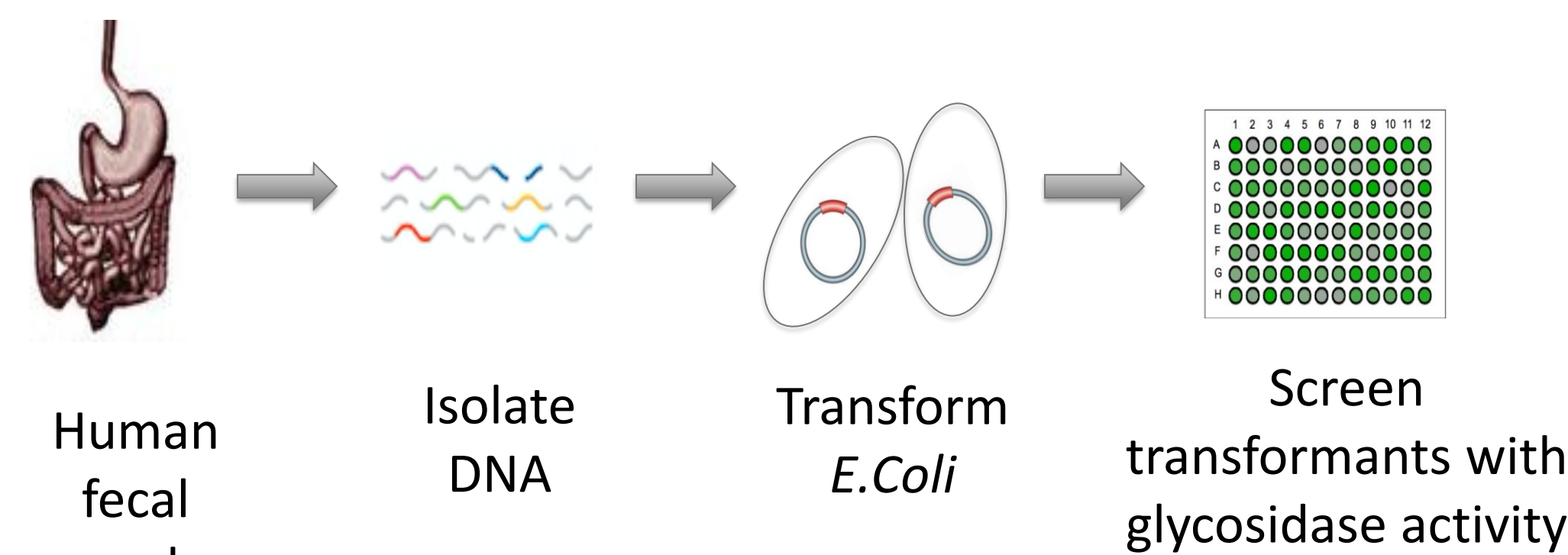
J. Goldstein *et al.* Science (1982), R. Chapanian *et al.* Natural Communications (2014)

OBJECTIVE

- Evaluation of novel enzyme system, with improved kinetic properties and specificities for the A antigen, for the conversion of A group RBCs to universally transfusable O group RBCs.

DISCOVERY OF NOVEL ENZYME SYSTEM

Metagenomic Screening

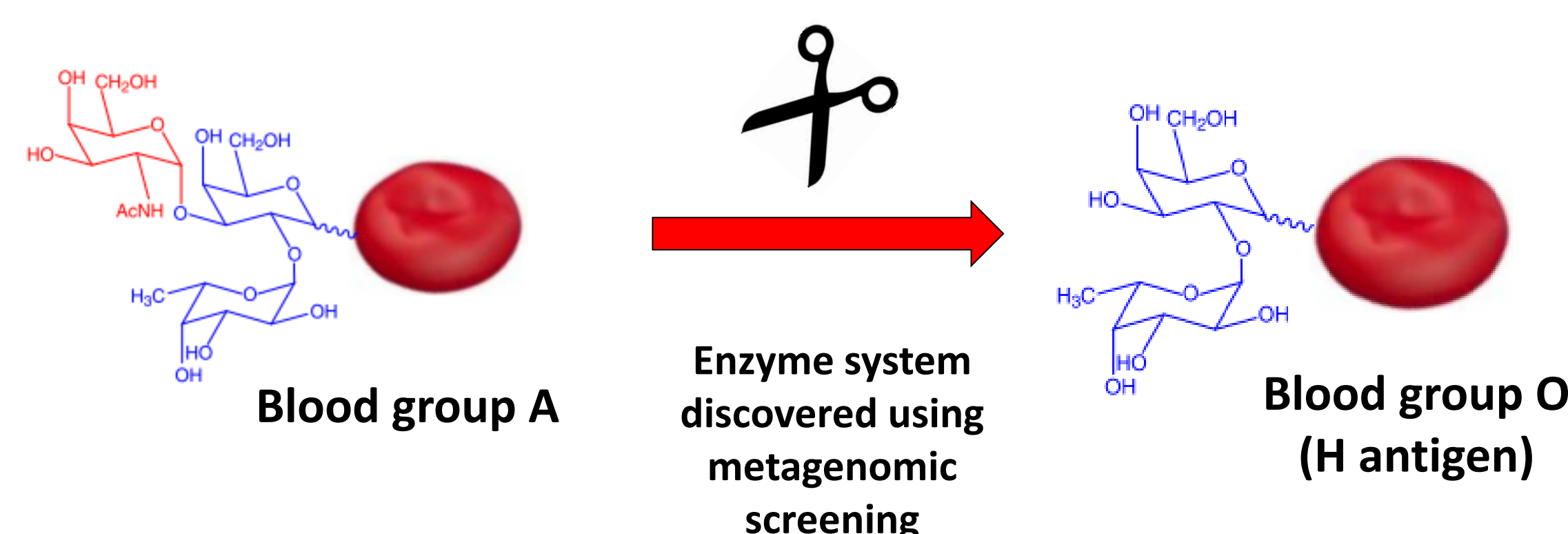


- Performed by Withers group (Chemistry, UBC)

EXPERIMENTAL PLAN

- Type A subgroups A1 and A2 RBCs were collected and treated with novel enzyme system to enzymatically remove A antigens from the RBCs at neutral pH (pH7.4)

A-Enzyme System Converted O (A-ECO) RBCs



ASSAYING REMOVAL OF A ANTIGENS

Micro Typing System (MTS) Results

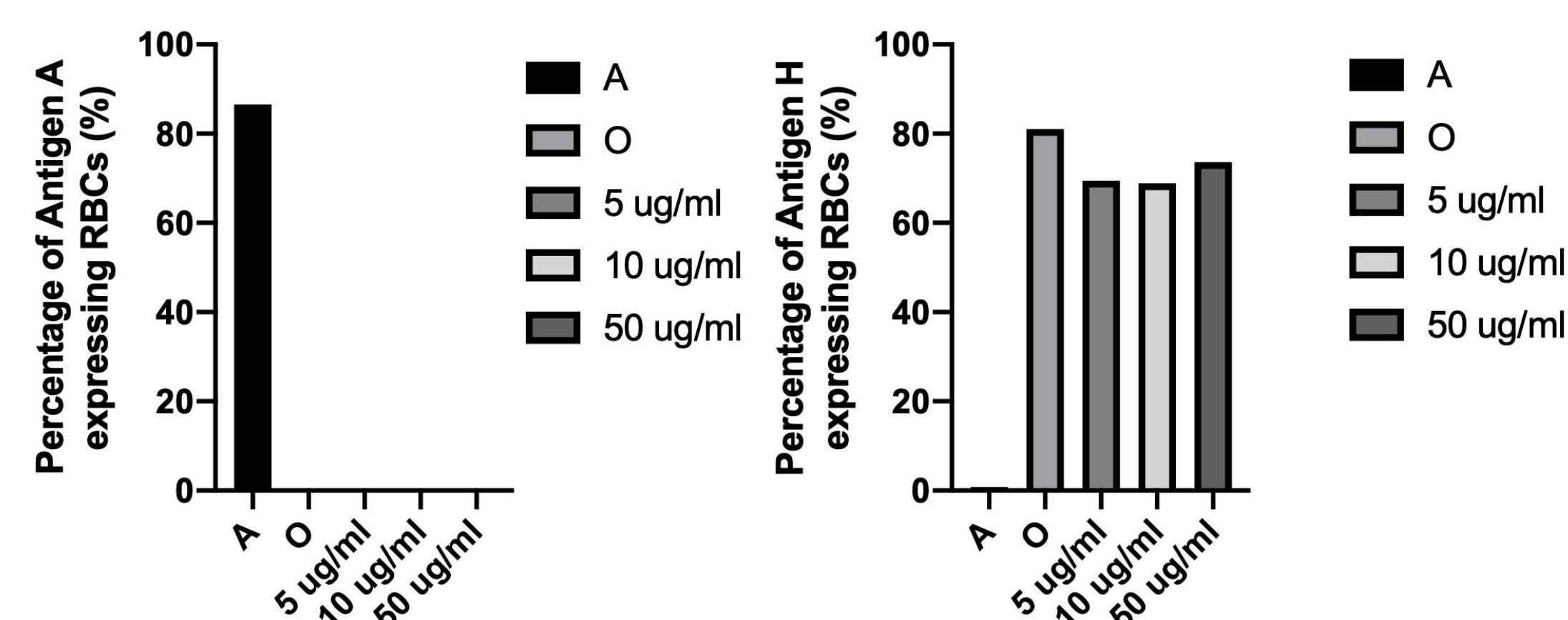
Antibody-conjugated gel columns:

- Large agglutination remain on or near the top of gel interface (4+,3+)
- Smaller agglutination pass partway through the gel, depending on size (2+,1+)
- Non agglutinated RBCs pass freely through the column (0)

Sample	Enz Conc. (ug/mL)	Anti A MTS Score
A RBC cntr	0	4
O RBC cntr	0	0
A-ECO-RBC	10	0

A cntr O cntr A-ECO

Flow Cytometry Results



Compatibility of A-ECO-RBCs

Serologic Crossmatch Data

- Donor sera are provided by the CBS NetCAD Lab

Status	Serum Type	Total # of Sera	RBC subtype A1					RBC subtype A2					
			4	3	2	1	0	4	3	2	1	0	
Control	A+	20	0	0	0	0	20	9	0	0	0	0	9
Treatment	A+	10	0	0	0	0	10	11	0	0	0	0	11
Control	AB+	3	0	0	0	0	3	3	0	0	0	0	3
Treatment	AB+	3	0	0	0	0	3	3	0	0	0	0	3
Control	B+	22	22	0	0	0	0	22	3	2	7	0	10
Treatment	B+	17	0	0	6	7	4	20	0	0	6	4	10
Control	O+	43	40	1	2	0	0	45	39	2	0	1	3
Treatment	O+	42	7	13	11	4	7	44	3	3	15	5	18

- Anti IgG MTS sometimes results in clinically irrelevant positive results. An alternative crossmatch test, a monocyte monolayer assay, considered much more clinically relevant was used to confirm the initial crossmatch data.

Monocyte Monolayer Assay (MMA)

- MMA gives monocyte index (MI) value, which measures phagocytosis of opsonized RBCs by monocytes. MI values ≤ 5-6 % indicate minimal risk of an acute hemolytic reaction of the transfused RBCs.

RBC Donor ID	Status	Anti A MTS	Anti IgG	Monocyte Index (%)
RBCs opsonized with O serum samples				
A1 D13	O control	0	0	0.4
	A control	4	4	61.3
	ECO RBC	0	4	4.3
A1 D36	O control	0	0	3.2
	A control	4	4	77.0
	ECO RBC	0	3	3.2
A2 D22	O control	0	0	2.1
	A control	4	4	70.7
	ECO RBC	0	0	2.1
RBCs opsonized with B serum samples				
A1 D13	O control	0	0	1.2
	A control	4	4	77.3
	ECO RBC	0	2	4.3
A1 D54	O control	0	0	1.2
	A control	4	4	69.1
	ECO RBC	0	2	0.0
A2 D22	O control	0	0	1.2
	A control	4	3	69.1
	ECO RBC	0	0	0.0

CONCLUSIONS AND FUTURE WORK

- Novel enzyme system fully converted A to O RBCs. These A-ECO RBCs showed monocyte index value of < 5%, which is clinically correlated with good *in vivo* circulation of transfused RBCs
- Sera cross matching studies of A-ECO-RBCs are in progress.



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