

Supplementary materials

DROP and READ: a paper-based device combined with portable readout for ABO, Rh (D, C, c, E, e) and Mi^a phenotyping

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TableS1. Specific details of four type of paper

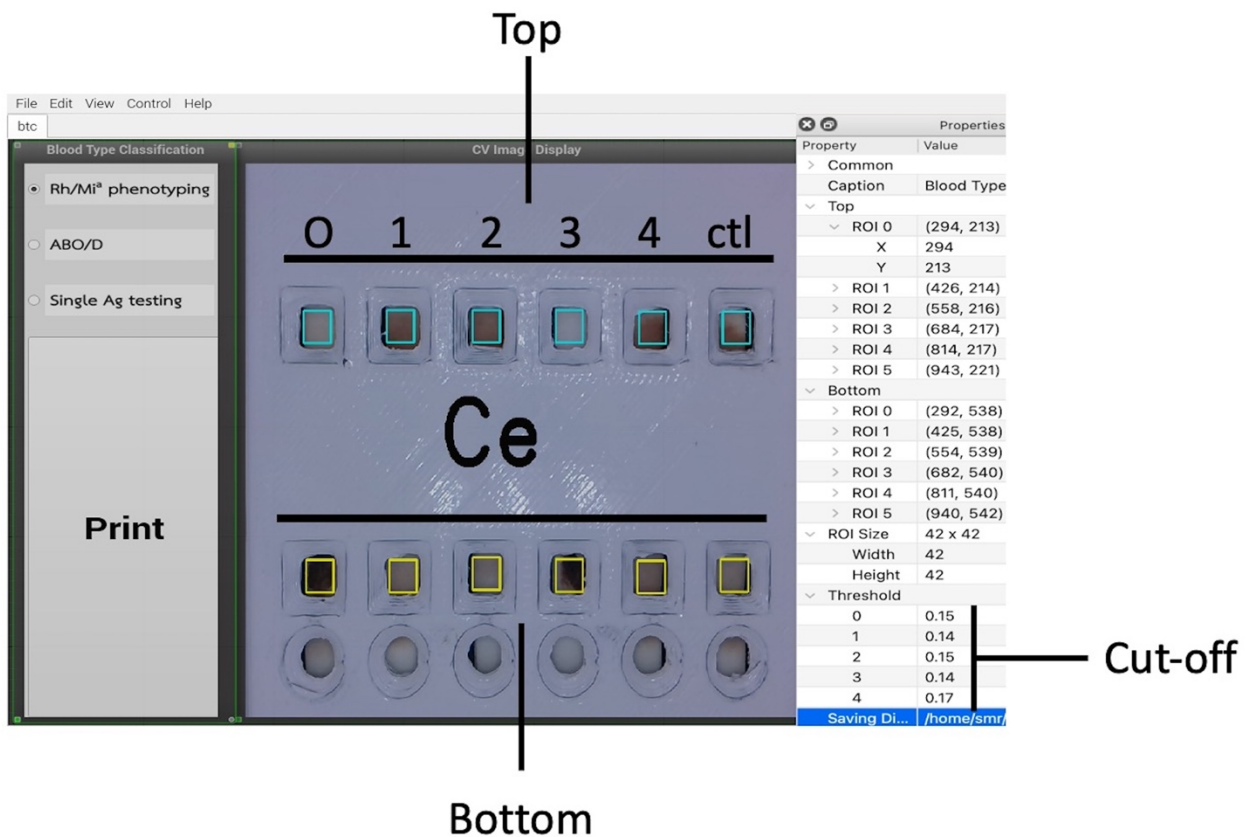
Paper type	Function	Properties	Features and benefits
CF4 (material 100% cotton linter)	Buffer part	Thickness 482 μm @ 53kPA, Wicking Rate 67.3 s/4cm and Water absorption 49.9 mg/cm ²	• Consistent absorbency and wicking rates: Ensure test-to-test reproducibility • Low protein binding: Minimal loss of analyte, so test sensitivity is maintained • Naturally hydrophilic: Rapid rewetting after prolonged storage
Glass fiber fusion 5	Sample and Antibody part, SA part	• Wicking Rate 43.9 s/4cm and Water absorption 42.3 mg/cm²	• Higher level of conjugate release: Less waste means reduced reagent costs • Pad rewets naturally and rapidly every time: Improved consistency

Paper type	Function	Properties	Features and benefits
blotting paper	flow part	As hemagglutination immunocomplexes are commonly variable and random in size, the release of small immunocomplexes from large pores yields false negative results. In summary, the porosity, wicking flow rate, cellulose paper thickness, and size of the RBC agglutination complexes significantly affected the performance of minor blood PAD typing	In this study, Western blotting paper was chosen as the most suitable substrate in the fabrication of Rh PADs for the antigen phenotyping of RBCs
Absorption pads CF7	Absorption pads at the downstream end of tests control sample flow along the strip	Thickness 1873 μm @ 53kPA, Wicking Rate 35 s/4cm and Water absorption 252.3 mg/cm ² which suitable for high sample volume	• Consistent absorbency: Ensures test-to-test reproducibility • Naturally hydrophilic: Minimal loss of analyte, so

Paper type	Function	Properties	Features and benefits
			test sensitivity is maintained • Wide range of thickness, absorbency and wicking rate: Rapid rewetting after prolonged storage • Minimal Leakage along the strip: No contamination of test result

Based on our previous study (Chomean et al, 2021), The first segment of each strip, buffer pad used CF4 paper. A sample antibody pad (SP) was designed for applying blood samples and coated antibodies using glass fiber conjugate pads. Each SP was immobilized specific antibody and preservative solution (5% sucrose and 5% trehalose). As a control for the detection system, the sixth strip was coated only with a preservative solution. containing 5% sucrose and 5% trehalose. Therefore, RBC agglutination would not occur except in the case of a strong direct agglutination test positive. A Pierce Western blotting paper was used as a flow part for agglutinated or nonagglutinated reactions. The last segment of the paper-based strip, an absorption pad, used CF7 paper (Chomean et al, 2021).

FigureS1. Image processing, grey ROI (region of interest) was measured and then used for formula setting in the OpenCV. The grey ROI was used to measure top channel (blue) and bottom channel (yellow). For interpretation step, F- score was used (Chomean et al. 2023) to separate positive and negative result by optimal cut- off each blood group antigen. For example, to interpret the Rh and Mi^a phenotyping (0=C, 1=c, 2=E, 3=e and 4= Mi^a, respectively), a higher value ($>$ cut off) was positive, whereas a lower value ($\leq$ cut off) was negative. As an example, channel 1 and 3 gave a higher value which indicated a positive result for C and e antigen.



FigureS2. How to use DROP and READ, automation for blood typing reader

Drop&Read procedure



1

1 drop (50 μ l) of whole blood



2

After 10 seconds (ABO,Rh) or 5 min (C,c,E,e and Mia), add 7 drops (350 μ l) of Diluent.



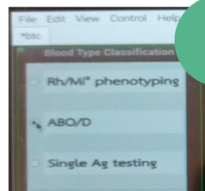
3

Wait 5 minutes, ready to read the results



4

insert the test into Blood group reader



5

select "ABO/D" or Rh/Mia or Single Ag typing as appropriate



6

the result will appear on the screen

7

select "print" to save and print a result

Video link:

https://drive.google.com/drive/folders/1o7UY9aK6NPuZXHfYkNUjr-f7Hmx7-BCG?usp=share_link.

TableS1 Blood group phenotyping results with DROP&READ and references method in 150 blood donors (H059, H072 and H074 were excluded)

No.	Result from TUH (references method)							Result	No.	DROP&READ							Result	
	ABO	Rhesus					Mi ^a			ABO		Rhesus						Mi ^a
		Rh(D)	C	c	E	e				A	B	D	C	c	E	e		
H001	O	+	+	0	0	+	+	O,Rh+,CCeeMia	H001	0	0	+	+	0	0	+	+	O,Rh+,CCee
H002	A	+	+	+	+	+	+	A,Rh+,CcEe,Mia	H002	+	0	+	+	+	+	+	+	A,Rh+,CcEe,Mia
H003	O	+	+	0	0	+	0	O,Rh+,CCee	H003	0	0	+	+	0	0	+	0	O,Rh+,CCee
H004	B	+	+	0	0	+	0	B,Rh+,CCee	H004	0	+	+	+	0	0	+	0	B,Rh+,CCee
H005	B	+	+	0	0	+	0	B,Rh+,CCee	H005	0	+	+	+	0	0	+	0	B,Rh+,CCee
H006	B	+	+	0	0	+	+	B,Rh+,CCee,Mia	H006	0	+	+	+	0	0	+	+	B,Rh+,CCee,Mia
H007	O	+	+	0	0	+	+	O,Rh+,CCee,Mia	H007	0	0	+	+	0	0	+	+	O,Rh+,CCee,Mia
H008	A	+	+	0	0	+	0	A,Rh+,CCee	H008	+	0	+	+	0	0	+	0	A,Rh+,CCee
H009	O	+	+	0	0	+	+	O,Rh+,CCee,Mia	H009	0	0	+	+	0	0	+	+	O,Rh+,CCee,Mia
H010	O	+	+	+	+	+	0	O,Rh+,CcEe	H010	0	0	+	+	+	+	+	0	O,Rh+,CcEe
H011	B	+	+	0	0	+	0	B,Rh+,CCee	H011	0	+	+	+	0	0	+	0	B,Rh+,CCee
H012	B	+	+	+	0	+	0	B,Rh+,Ccee	H012	0	+	+	+	+	0	+	0	B,Rh+,Ccee
H013	AB	+	+	+	+	+	0	AB,Rh+,CcEe	H013	+	+	+	+	+	+	+	0	AB,Rh+,CcEe
H014	B	+	+	0	0	+	0	B,Rh+,CCee	H014	0	+	+	+	0	0	+	0	B,Rh+,CCee
H015	O	+	+	0	0	+	0	O,Rh+,CCee	H015	0	0	+	+	0	0	+	0	O,Rh+,CCee
H016	O	+	+	0	+	+	0	O,Rh+,CCee	H016	0	0	+	+	0	+	+	0	O,Rh+,CCee
H017	O	+	0	+	+	0	+	O,Rh+,ccEE,Mia	H017	0	0	+	0	+	+	0	+	O,Rh+,ccEE,Mia
H018	O	+	+	0	0	+	0	O,Rh+,CCee	H018	0	0	+	+	0	0	+	0	O,Rh+,CCee
H019	B	+	+	+	+	+	0	B,Rh+,CcEe	H019	0	+	+	+	+	+	+	0	B,Rh+,CcEe
H020	B	+	+	0	0	+	0	B,Rh+,CCee	H020	0	+	+	+	0	0	+	0	B,Rh+,CCee
H021	B	+	+	+	0	+	0	B,Rh+,Ccee	H021	0	+	+	+	+	0	+	0	B,Rh+,Ccee
H022	B	+	+	0	0	+	0	B,Rh+,CCee	H022	0	+	+	+	0	0	+	0	B,Rh+,CCee
H023	O	+	+	0	0	+	0	O,Rh+,CCee	H023	0	0	+	+	0	0	+	0	O,Rh+,CCee
H024	O	+	+	+	0	+	0	O,Rh+,Ccee	H024	0	0	+	+	+	0	+	0	O,Rh+,Ccee
H025	O	+	+	0	0	+	0	O,Rh+,CCee	H025	0	0	+	+	0	0	+	0	O,Rh+,CCee

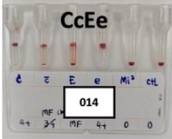
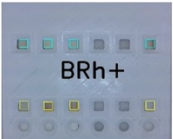
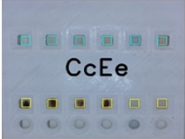
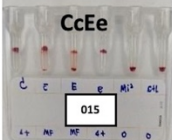
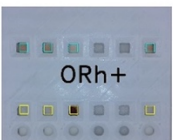
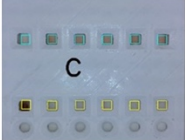
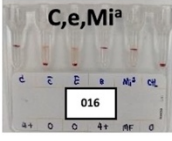
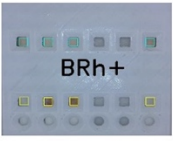
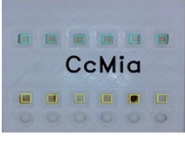
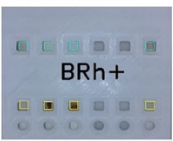
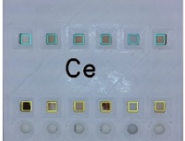
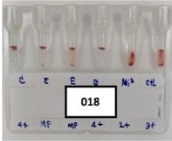
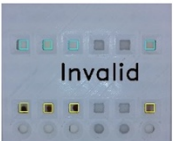
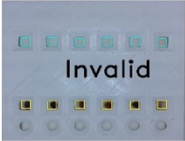
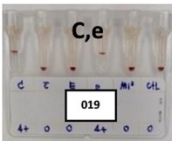
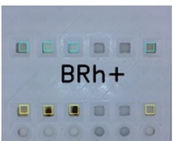
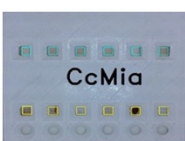
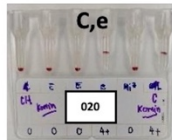
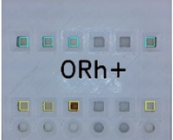
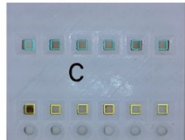
No.	Result from TUH (references method)							Result	No.	DROP&READ								Result
	ABO	Rhesus					Mi ^a			ABO		Rhesus					Mi ^a	
		Rh(D)	C	c	E	e				A	B	D	C	c	E	e		
H053	A	+	+	0	0	+	0	A,Rh+,CCee	H053	+	0	+	+	0	0	+	0	A,Rh+,CCee
H054	A	+	+	0	0	+	0	A,Rh+,CCee	H054	+	0	+	+	0	0	+	0	A,Rh+,CCee
H055	O	+	+	0	0	+	0	O,Rh+,CCee	H055	0	0	+	+	0	0	+	0	O,Rh+,CCee
H056	B	+	+	+	+	+	0	B,Rh+,CeEe	H056	0	+	+	+	+	+	+	0	B,Rh+,CeEe
H057	B	+	+	0	0	+	0	B,Rh+,CCee	H057	0	+	+	+	0	0	+	0	B,Rh+,CCee
H058	B	+	+	+	+	+	0	B,Rh+,CcEe	H058	0	+	+	+	+	+	+	0	B,Rh+,CcEe
H060	B	+	+	0	0	+	0	B,Rh+,CCee	H060	0	+	+	+	0	0	+	0	B,Rh+,CCee
H061	O	+	+	0	0	+	0	O,Rh+,CCee	H061	0	0	+	+	0	0	+	0	O,Rh+,CCee
H062	B	+	+	0	0	+	0	B,Rh+,CCee	H062	0	+	+	+	0	0	+	0	B,Rh+,CCee
H063	O	+	+	0	0	+	0	O,Rh+,CCee	H063	0	0	+	+	0	0	+	0	O,Rh+,CCee
H064	B	+	+	+	+	+	0	B,Rh+,CcEe	H064	0	+	+	+	+	+	+	0	B,Rh+,CcEe
H065	O	+	+	+	+	+	0	O,Rh+,CcEe	H065	0	0	+	+	+	+	+	0	O,Rh+,CcEe
H066	B	+	+	0	+	+	0	B,Rh+,CCEe	H066	0	+	+	+	0	+	+	0	B,Rh+,CCEe
H067	A	+	+	0	0	+	0	A,Rh+,CCee	H067	+	0	+	+	0	0	+	0	A,Rh+,CCee
H068	O	+	+	+	0	+	0	O,Rh+,Ccee	H068	0	0	+	+	+	0	+	0	O,Rh+,Ccee
H069	B	+	+	0	0	+	0	B,Rh+,CCee	H069	0	+	+	+	0	0	+	0	B,Rh+,CCee
H070	O	+	+	+	+	+	+	O,Rh+,CcEe,Mia	H070	0	0	+	+	+	+	+	+	O,Rh+,CcEe,Mia
H071	B	+	+	+	+	+	0	O,Rh+,CcEe	H071	0	+	+	+	+	+	+	0	O,Rh+,CcEe
H073	O	+	+	0	0	+	0	O,Rh+,CCee	H073	0	0	+	+	0	0	+	0	O,Rh+,CCee
H075	O	+	+	0	0	+	0	O,Rh+,CCee	H075	0	0	+	+	0	0	+	0	O,Rh+,CCee
H076	A	+	+	0	0	+	0	A,Rh+,CCee	H076	+	0	+	+	0	0	+	0	A,Rh+,CCee
H077	O	+	+	0	0	+	0	O,Rh+,CCee	H077	0	0	+	+	0	0	+	0	O,Rh+,CCee
H078	A	+	+	+	0	+	0	A,Rh+,Ccee	H078	+	0	+	+	+	0	+	0	A,Rh+,Ccee
H079	AB	+	+	+	+	+	0	AB,Rh+,CcEe	H079	+	+	+	+	+	+	+	0	AB,Rh+,CcEe
H080	A	+	+	0	0	+	0	A,Rh+,CCee	H080	+	0	+	+	0	0	+	0	A,Rh+,CCee
H081	O	+	+	0	+	+	0	O,Rh+,CCEe	H081	0	0	+	+	0	+	+	0	O,Rh+,CCEe
H082	O	+	+	+	0	+	0	O,Rh+,Ccee	H082	0	0	+	+	+	0	+	0	O,Rh+,Ccee
H083	O	+	+	0	0	+	0	O,Rh+,CcEe	H083	0	0	+	+	0	0	+	0	O,Rh+,CcEe

No.	Result from TUH (references method)							Result	No.	DROP&READ								Result
	ABO	Rhesus					Mi ^a			ABO		Rhesus					Mi ^a	
		Rh(D)	C	c	E	e				A	B	D	C	c	E	e		
H112	O	+	+	0	0	+	0	O,Rh+,CCee	H112	0	0	+	+	0	0	+	0	O,Rh+,CCee
H113	B	+	+	+	+	+	0	B,Rh+,CcEe	H113	0	+	+	+	+	+	+	0	B,Rh+,CcEe
H114	B	+	+	+	+	+	0	B,Rh+,CeEe	H114	0	+	+	+	+	+	+	0	B,Rh+,CeEe
H115	B	+	+	+	+	+	0	B,Rh+,CeEe	H115	0	+	+	+	+	+	+	0	B,Rh+,CeEe
H116	AB	+	+	0	0	+	0	AB,Rh+,CCee	H116	+	+	+	+	0	0	+	0	AB,Rh+,CCee
H117	AB	+	+	+	+	+	0	AB,Rh+,CcEe	H117	+	+	+	+	+	+	+	0	AB,Rh+,CcEe
H118	O	+	+	0	0	+	0	O,Rh+,CCee	H118	0	0	+	+	0	0	+	0	O,Rh+,CCee
H119	O	+	+	0	0	+	0	O,Rh+,CCee	H119	0	0	+	+	0	0	+	0	O,Rh+,CCee
H120	B	+	+	0	0	+	+	B,Rh+,CCee,Mia	H120	0	+	+	+	0	0	+	+	B,Rh+,CCee,Mia
H121	O	+	+	+	+	+	0	O,Rh+,CcEe	H121	0	0	+	+	+	+	+	0	O,Rh+,CcEe
H122	O	+	+	0	0	+	0	O,Rh+,CCee	H122	0	0	+	+	0	0	+	0	O,Rh+,CCee
H123	O	+	+	0	0	+	0	O,Rh+,CCee	H123	0	0	+	+	0	0	+	0	O,Rh+,CCee
H124	A	+	+	+	+	+	0	O,Rh+,CcEe	H124	A	0	+	+	+	+	+	0	O,Rh+,CcEe
H125	O	+	+	0	0	+	0	O,Rh+,CCee	H125	0	0	+	+	0	0	+	0	O,Rh+,CCee
H126	A	+	+	0	0	+	+	A,Rh+,CCee,Mia	H126	+	0	+	+	+	0	+	+	A,Rh+,CCee,Mia
H127	O	+	+	+	0	+	0	O,Rh+,Ccee	H127	0	0	+	+	+	0	+	0	O,Rh+,Ccee
H128	B	+	+	+	+	+	0	B,Rh+,CcEe	H128	0	+	+	+	+	+	+	0	B,Rh+,CcEe
H129	O	+	+	0	0	+	0	O,Rh+,CCee	H129	0	0	+	+	0	0	+	0	O,Rh+,CCee
H130	B	+	+	0	0	+	0	B,Rh+,CCee	H130	0	+	+	+	0	0	+	0	B,Rh+,CCee
H131	A	+	+	0	0	+	0	A,Rh+,CCee	H131	+	0	+	+	0	0	+	0	A,Rh+,CCee
H132	A	+	+	+	+	+	0	A,Rh+,CcEe	H132	+	0	+	+	+	+	+	0	A,Rh+,CcEe
H133	O	+	+	0	0	+	0	O,Rh+,CCee	H133	0	0	+	+	0	0	+	0	O,Rh+,CCee
H134	O	+	+	0	0	+	0	O,Rh+,CCee	H134	0	0	+	+	0	0	+	0	O,Rh+,CCee
H135	A	+	+	0	0	+	0	A,Rh+,CCee	H135	+	0	+	+	0	0	+	0	A,Rh+,CCee
H136	A	+	+	0	0	+	0	A,Rh+,CCee	H136	+	0	+	+	0	0	+	0	A,Rh+,CCee
H137	B	+	+	0	0	+	0	B,Rh+,CCee	H137	0	+	+	+	0	0	+	0	B,Rh+,CCee
H138	B	+	+	+	+	+	+	B,Rh+,CcEe,Mia	H138	0	+	+	+	+	+	+	+	B,Rh+,CcEe,Mia
H139	B	+	+	+	0	+	0	B,Rh+,Ccee	H139	0	+	+	+	+	0	+	0	B,Rh+,Ccee

No.	Result from TUH (references method)							Result	No.	DROP&READ								Result
	ABO	Rhesus					Mi ^a			ABO		Rhesus					Mi ^a	
		Rh(D)	C	c	E	e				A	B	D	C	c	E	e		
H140	B	+	+	0	0	+	0	B,Rh+,CCee	H140	0	+	+	+	0	0	+	0	B,Rh+,CCee
H141	O	+	0	+	+	0	0	O,Rh+,ccEE	H141	0	0	+	0	+	+	0	0	O,Rh+,ccEE
H142	O	+	+	+	+	+	0	O,Rh+,CcEe	H142	0	0	+	+	+	+	+	0	O,Rh+,CcEe
H143	O	+	+	0	0	+	0	O,Rh+,CCee	H143	0	0	+	+	0	0	+	0	O,Rh+,CCee
H144	O	+	+	0	0	+	0	O,Rh+,CCee	H144	0	0	+	+	0	0	+	0	O,Rh+,CCee
H145	O	+	+	0	0	+	0	O,Rh+,CCee	H145	0	0	+	+	0	0	+	0	O,Rh+,CCee
H146	O	+	+	0	0	+	0	O,Rh+,CCee	H146	0	0	+	+	0	0	+	0	O,Rh+,CCee
H147	A	+	+	0	0	+	0	A,Rh+,CCee	H147	+	0	+	+	0	0	+	0	A,Rh+,CCee
H148	A	+	0	+	+	+	0	A,Rh+,ccEe	H148	+	0	+	0	+	+	+	0	A,Rh+,ccEe
H149	A	+	+	+	+	+	0	A,Rh+,CcEe	H149	+	0	+	+	+	+	+	0	A,Rh+,CcEe
H150	AB	+	+	0	0	+	0	AB,Rh+,CCee	H150	+	+	+	+	0	0	+	0	AB,Rh+,CCee
H151	O	+	0	+	+	0	0	O,Rh+,ccEE	H151	0	0	+	0	+	+	0	0	O,Rh+,ccEE
H152	O	+	+	0	0	+	0	O,Rh+,CCee	H152	0	0	+	+	0	0	+	0	O,Rh+,CCee
H153	B	+	+	0	0	+	0	B,Rh+,CCee	H153	0	+	+	+	0	0	+	0	B,Rh+,CCee

Figure S2. Example result obtained from blood donor, 014-018 (recently blood transfusion samples) and 019-021 (DAT positive sample). Gel card was performed as references method for ABO, Rh (C, c, E, e) and Mi^a phenotyping. For our result, blood group phenotyping was read and represented as a captured screen.

Recently Blood Transfusion patient

Sample	ABO-Rh(D) Tube test	C,c,E,e,Mi ^a Gel card	ABO-Rh(D) DROP& READ	C,c,E,e,Mi ^a DROP& READ
 014	B, Rh(D)+			
 015	O, Rh(D)+			
 016	B, Rh(D)+			
 017	B, Rh(D)+			
 018	Misinterpretation			
 019*	B, Rh(D)+			
 020*	O, Rh(D)+			
 021*	AB, Rh(D)+	