Electronic Supplementary Material (ESI) for Analyst.

Supporting Information: Wash-free Paper Diagnostics for the Rapid Detection of Blood Type Antibodies

Michael J. Hertaeg,[†] Vidhishri Kesarwani,[†,‡,¶] Heather Mcliesh,[†] Julia Walker,[†] Simon R. Corrie,[†] and Gil Garnier[*,†]

[†]*BioPRIA, The Department of Chemical Engineering, Monash University, Clayton, VIC 3800 Australia*

[‡]*ARC Centre of Excellence in Convergent BioNano Science and Technology*

[¶]*Centre to Impact AMR, Monash University, Clayton, VIC 3800 Australia*

E-mail: gil.garnier@monash.edu

Additional methods for SARS-COV-2 antibody tests

Peptide synthesis

Peptides were synthesised as outlined by Alves et al.[S1] Three peptide sequences/elements from the same SARS-CoV-2 spike protein were chosen. These were selected based off results from earlier database mining studies.[S2] Peptides were synthesized via a microwave assisted solid-phase synthesis using a Rink amide resin (0.05 mmol scale, 100-200 mesh, 1% DVB, ChemPep, Inc.). Syntheses were performed in an automated microwave synthesizer (Liberty Blue, CEM Corporation, North Carolina). Resin was swelled at room temperature for 5 min with N,N-dimethylformamide (DMF) before deprotection with 20%

4-methylpiperidine in DMF (v/v) for 30 s at 75 °C and 90 s at 90 °C. Subsequently, Fmoc amino acids (0.25 mmol, ChemPep, Inc.) were coupled with a 1:1:1:2 ratio of amino acid/1-hydroxybenzotriazole hydrate (HOBT, AK Scientific, Inc.)/HCTU (AK Scientific, Inc.)/N,N-diisopropylethylamine (DIPEA) in 4 mL of DMF at 70 °C for 5 min prior to deprotection with 20% 4-methylpiperidine in DMF (v/v) for 5 min at 75 °C. Fluorescein was included in the N-terminal region by coupling of 5,6- carboxyfluoroscein (0.25 mmol) to the final amino acid in the sequence, after Fmoc deprotection, with a 1:1:1:2 ratio of amino acid/ HOBT/HCTU/DIPEA in 4 mL of DMF at 70 °C. The fluorescein coupling step was performed two times to ensure complete labelling of the synthesized peptide sequences. After solid-phase synthesis, the resultant dried Rink amide resin was transferred to a 25 mL solid-phase peptide synthesis vessel (CG1866, Chemglass) and treated with 10 mL of trifluoroacetic acid (TFA)/phenol/water/triisopropylsilane (88/5/5/2) cleavage cocktail for 2 hours while bubbling with nitrogen at room temperature. The TFA cleavage solution was collected by filtering through the fritted glass into a 25 mL round-bottom flask. The remaining resin was further rinsed twice with 5 mL of fresh TFA cleavage cocktail to collect any residual peptide. The cleavage solution was combined and precipitated into cold diethyl ether. Upon centrifugation, the precipitate was collected and reconstituted in HPLC grade water/ acetonitrile (MeCN) with 0.1% TFA. Peptides were purified by preparative HPLC using a linear gradient of MeCN and water: (1) 10% MeCN, 0.1-2.1 min; (2) 10-95% MeCN, 2.1-23.1.1 min; (3) 95% MeCN, 23.1-26.1 min. Purified peptides were lyophilized to yield off-white powder and characterized by electrospray ionization mass spectrometry (ESI-MS).

Bioconjugation of peptide and anti-D

Bioconjugation reactions were performed according to the protocol described by Alves et al.[S1] Briefly, approx. 3 mg/mL of anti-D-IgG was reacted with NHS-(PEG)2-maleimide at 50-fold molar excess and incubated for 2 h at 4 °C. This mixture was then desalted using zebaspin columns to remove excess PEG, before reacting with peptide. Thiolated

peptide was added to the PEGylated anti-D in 15-20-fold molar excess, incubated at room temperature for 1 h. The bioconjugates were purified using zebaspin columns to remove unreacted peptide. Anti-D peptide conjugates were stored in 1× PBS stocks at 4 °C and were stable for at least several weeks. Concentrations of bioconjugates were evaluated using a Nanodrop spectrometer.

Cell preparation

Functionalisation of R2R2 reagent red cells (RRBCs) (Haemokenesis Pty Ltd) was performed as described by Alves et al.,[S1] with minor modifications. Briefly, R2R2 cells were washed with PBS and concentrated to 30% via centrifugation. To this, anti-D peptide conjugates (anti-D P1, anti-D P2 and anti-D P5) were added to achieve a biconjugate/D-antigen ratio of 2:1 and then incubated at room temperature for 20 minutes. Cells were washed four times in Celpresol solution and stored at 4°C till use. Cell functionalisation with anti-D peptide conjugates was confirmed via flow cytometry.

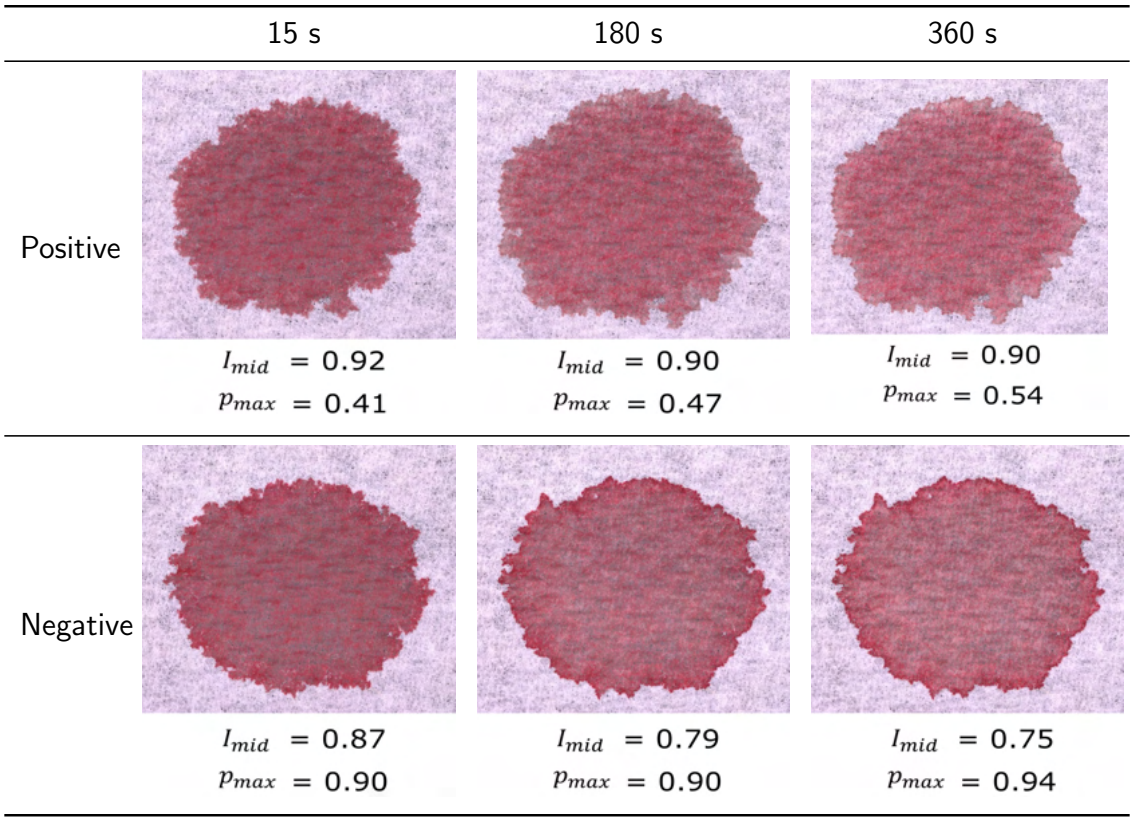


Figure S1. Evolution of stains and image processing parameters with time for positive and negative tests on paper towel.

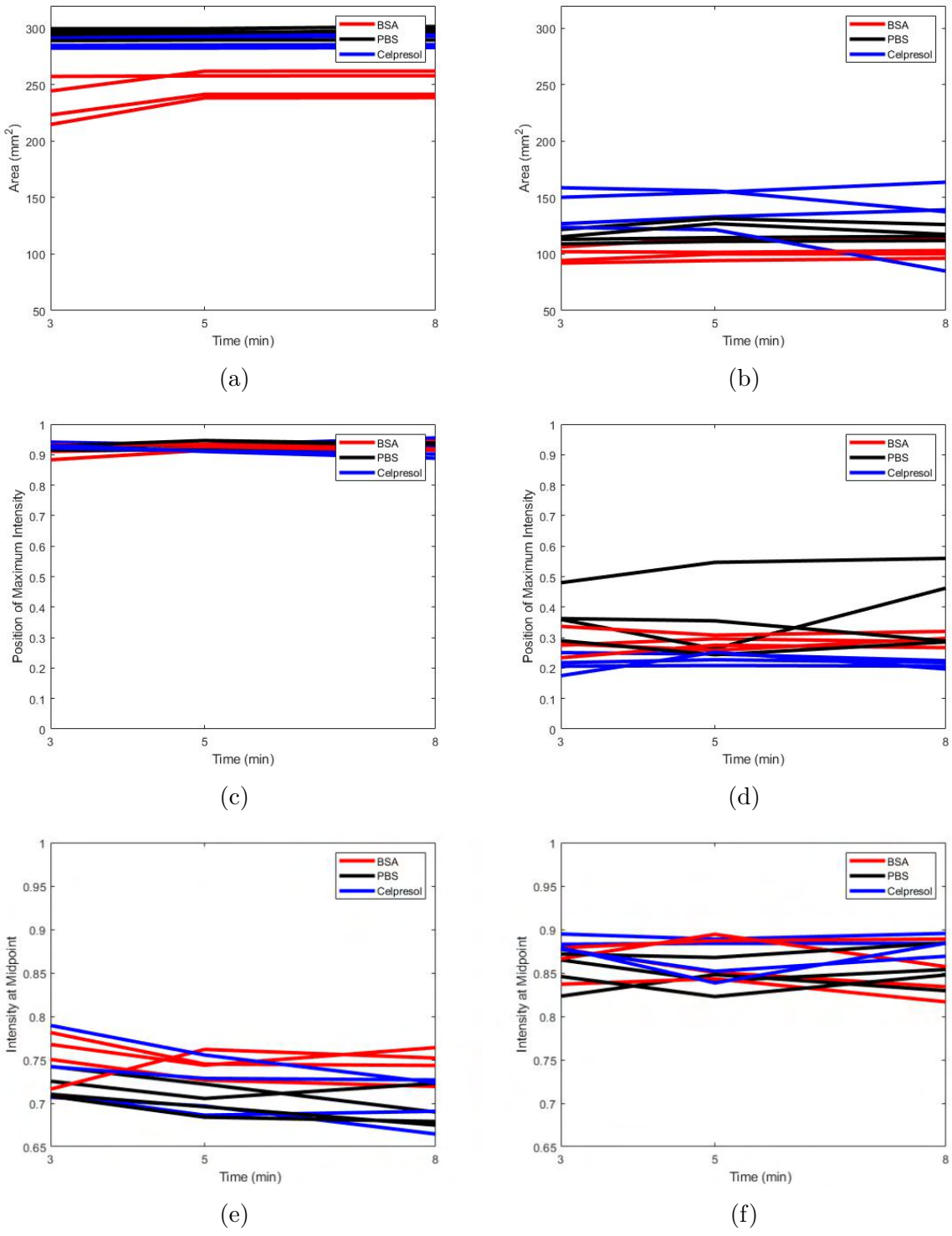


Figure S2. Comparison of different diluents and wicking time for blood typing tests. Each figure contains four lines using each diluent, two of these were incubated at 4 ° and the other two at 23 °. Results from the two temperatures were indistinguishable and are therefore plotted together. a) Stain area on handsheet, negative tests. b) Stain area on handsheet, positive tests. c) Position of maximum intensity on paper towel, negative tests. d) Position of maximum intensity on paper towel, positive tests. e) Intensity at midpoint on paper towel, negative tests. d) Intensity at Midpoint on paper towel, positive tests.

Table S1. Paper properties

Paper	O.D Weight ($g/m^2 \pm SD$)	Wet Weight ($g/m^2 \pm SD$)	Dry Thickness ($\mu m \pm SD$)	Wet Thickness ($\mu m \pm SD$)	Dry Density (kg/m^3)	Water Capacity (g/m^2)
Towel	25.6 ± 0.3^{S3}	55.4 ± 1.8^{S3}	98 ± 2^{S3}	76 ± 2^{S3}	261.0^{S4}	29.8^{S4}
Handsheet	27.6 ± 0.3	51.5 ± 1.5	79 ± 1	72 ± 2	350	23.9

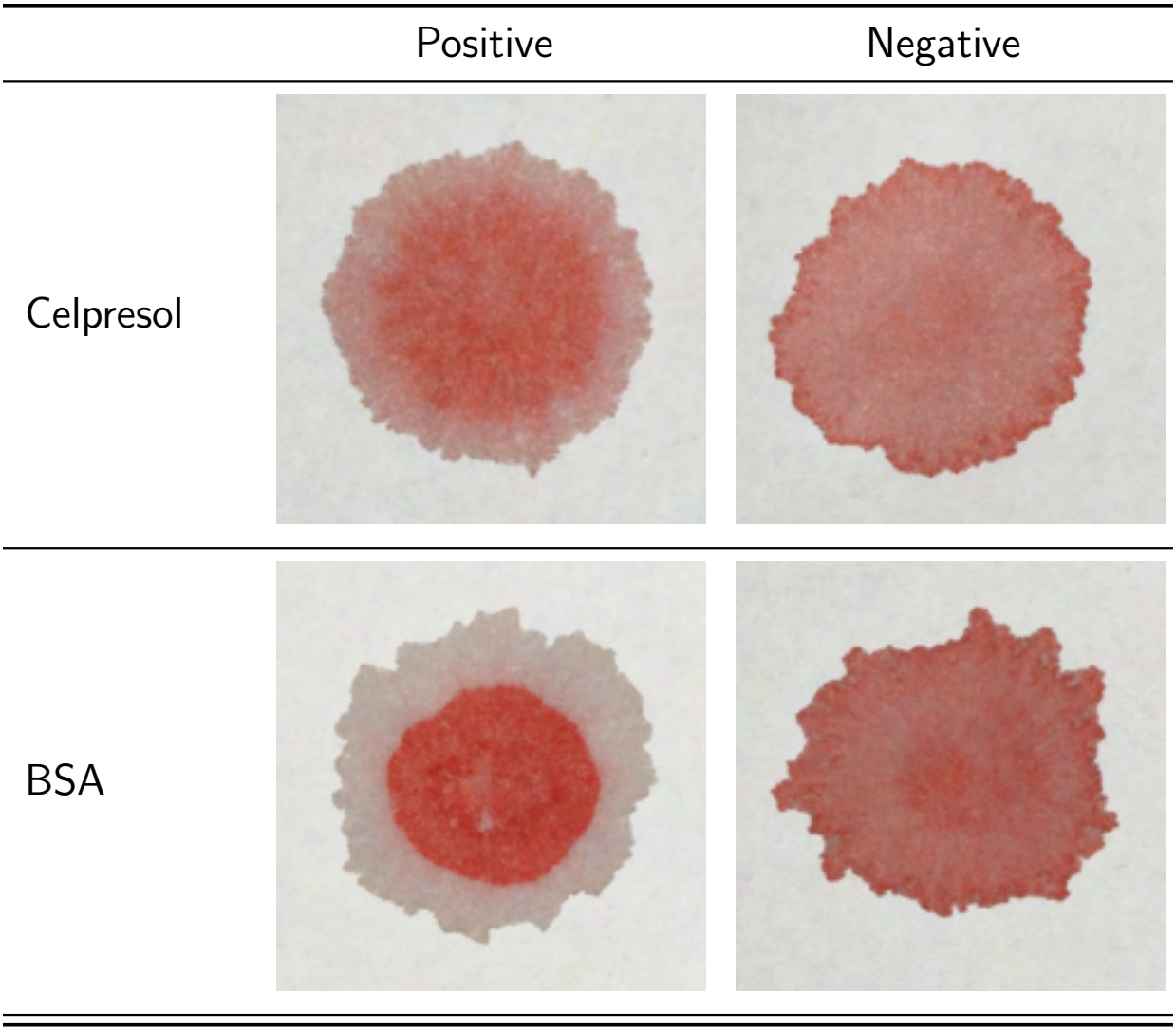


Figure S3. Stain appearance 3 minutes of blood typing tests after droplet deposition on handsheet paper for Celpresol and BSA diluents.

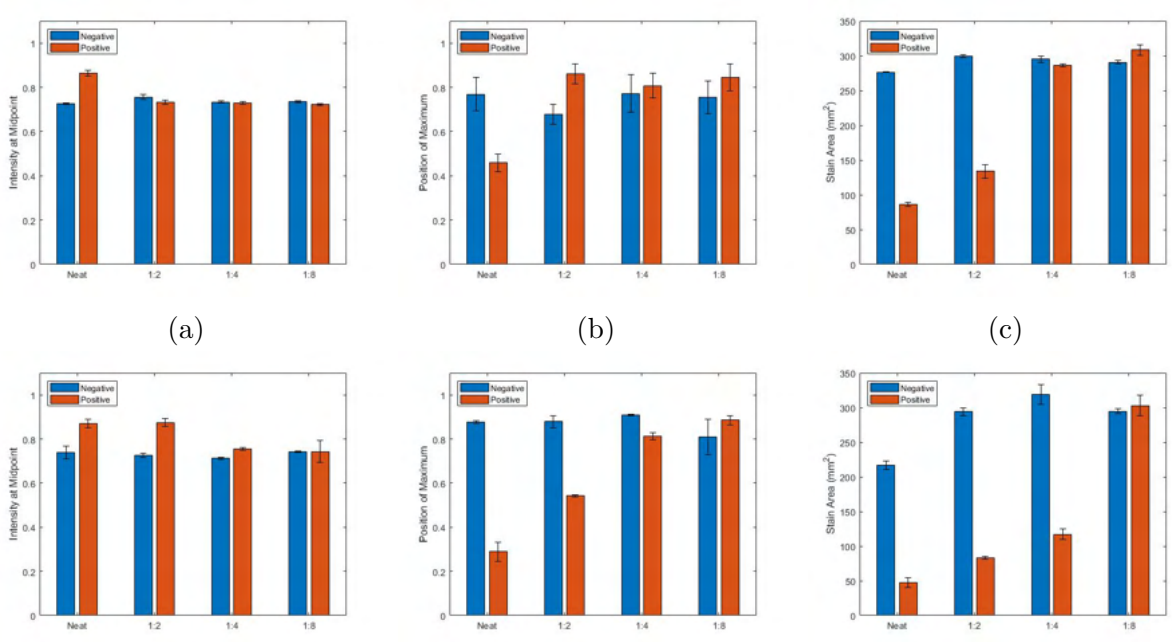


Figure S4. Effect of dilution on sensitivity for 23% RBC suspensions on blood typing tests. a) Intensity at midpoint on paper towel, A2 plasma. b) Position of maximum on paper towel, A2 plasma. c) Stain size on handsheet, A2 plasma. d) Intensity at midpoint on paper towel, B1 plasma. e) Position of maximum on paper towel, B1 plasma. f) Stain size on handsheet, B1 plasma.

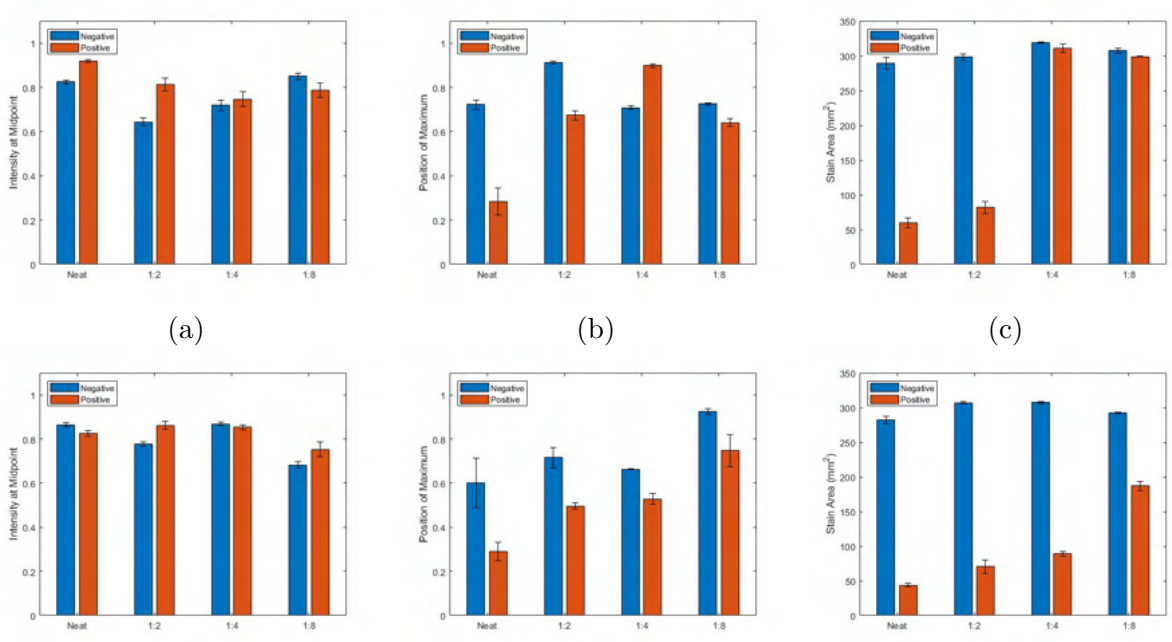
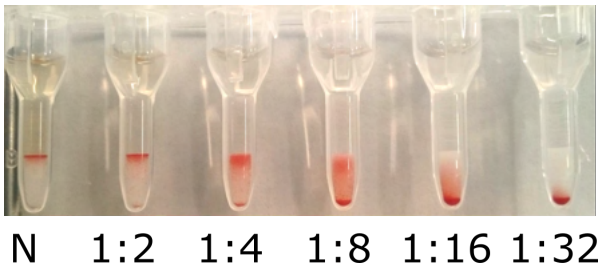
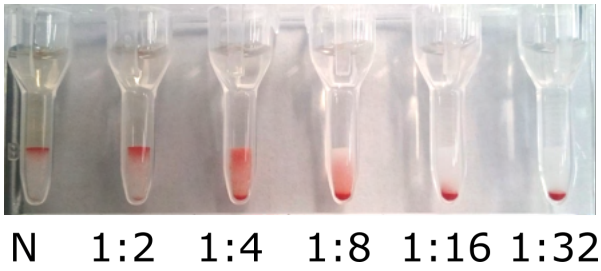


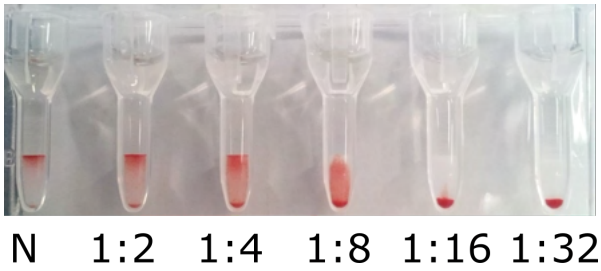
Figure S5. Effect of dilution on sensitivity for 15% RBC suspensions on blood typing tests. a) Intensity at midpoint on paper towel, A2 plasma. b) Position of maximum on paper towel, A2 plasma. c) Stain size on handsheet, A2 plasma. d) Intensity at midpoint on paper towel, B1 plasma. e) Position of maximum on paper towel, B1 plasma. f) Stain size on handsheet, B1 plasma.



(a)



(b)



(c)

Figure S6. Column agglutination tests with patient plasma diluted in PBS. a) A1 plasma. b) A2 plasma. c) B1 plasma.

References

(S1) Alves, D.; Curvello, R.; Henderson, E.; Kesarwani, V.; Walker, J. A.; Leguizamon, S. C.; McLiesh, H.; Raghuwanshi, V. S.; Samadian, H.; Wood, E. M.; McQuilten, Z. K.; Graham, M.; Wieringa, M.; Korman, T. M.; Scott, T. F.; Banaszak Holl, M. M.; Garnier, G.; Corrie, S. R. Rapid Gel Card Agglutination Assays for Serological Analysis Following SARS-CoV-2 Infection in Humans. *ACS sensors* **2020**,

(S2) Wang, H.; Hou, X.; Wu, X.; Liang, T.; Zhang, X.; Wang, D.; Teng, F.; Dai, J.; Duan, H.;

Guo, S.; Li, Y.; Yu, X. SARS-CoV-2 proteome microarray for mapping COVID-19 antibody interactions at amino acid resolution. *bioRxiv* **2020**,

(S3) Hertaeg, M. J.; Tabor, R. F.; Garnier, G. Effect of protein adsorption on the radial wicking of blood droplets in paper. *Journal of Colloid And Interface Science* **2018**, *528*, 116–123.

(S4) Hertaeg, M. J.; McLiesh, H.; Tabor, R. F.; Garnier, G. A Rapid Paper-Based Blood Typing Method from Droplet Wicking. *Analyst* **2020**,