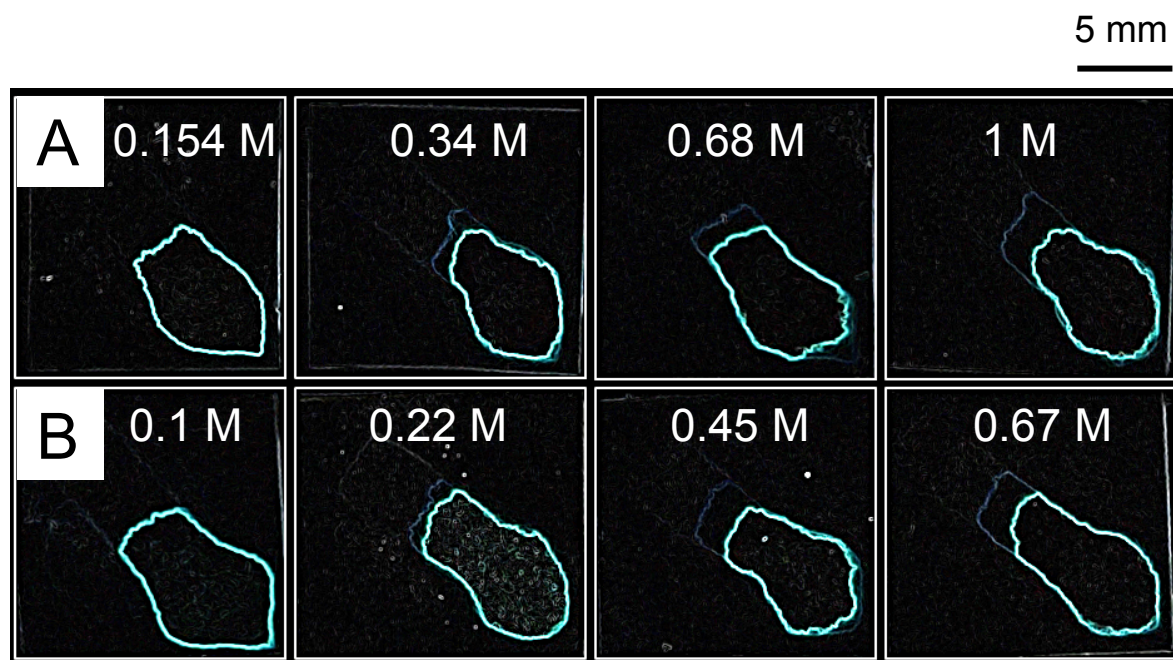


## Appendix A.

### Supplementary Information

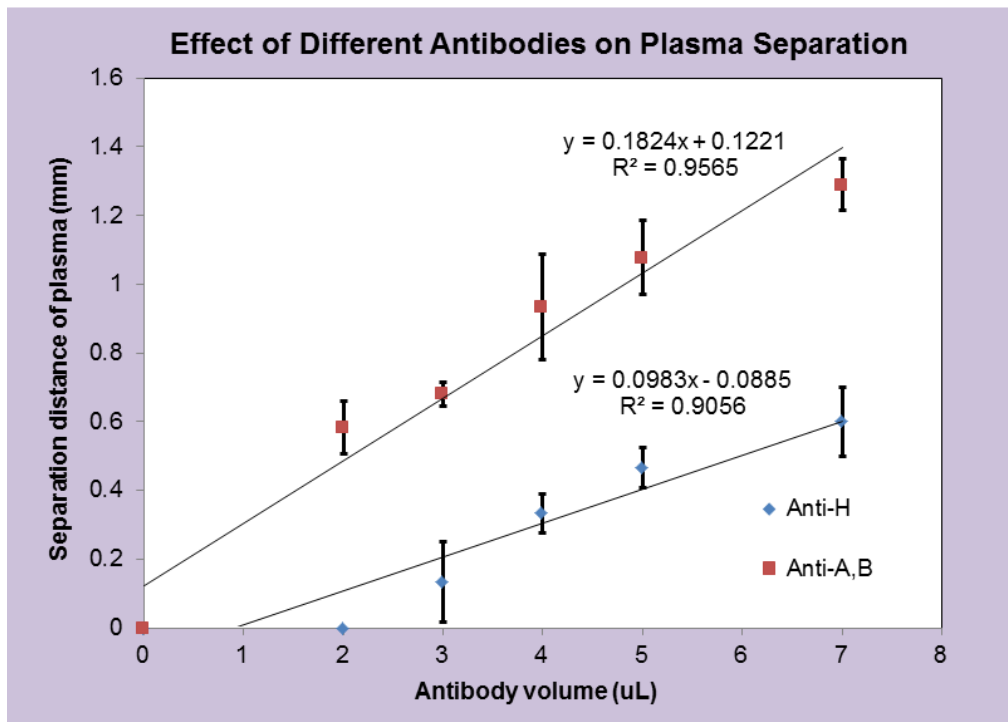


**Fig. 1S:** Effect of different salt solutions on plasma separation. The inlet points of the devices were functionalized using 0.5  $\mu\text{L}$  of (A) sodium chloride and (B) magnesium chloride solutions, before adding 5  $\mu\text{L}$  of whole blood sample. The images transferred to ImageJ for further analysis and measuring the separation distance of plasma (See Figure 2).

**Table 1S:** The effect of blood grouping antibodies on the separation distance of plasma (defined as the wicking distance of separated plasma from the nearest RBC front). Different volumes of blood typing antibodies (anti-H, and anti-A,B without dilution) have been deposited on Whatman No.4 filter paper and left to dry for 10 min at room temperature. A 5  $\mu$ L of whole blood samples, types of O+ and A+, have been placed onto anti-H, and anti-A,B treated zones, respectively. The experiment was repeated 3 times with 6 different blood samples. The images transferred to ImageJ for further analysis and measuring the separation distance of plasma. As shown in the table, anti-H could not efficiently separate plasma from the whole blood sample in diagnostic tests.

8 mm

<b>Effect of Blood Typing Antibodies on Plasma Separation</b>					
Volume of deposited antibody ( $\mu$ L)	2	3	4	5	7
Anti-A,B					
Anti-H					

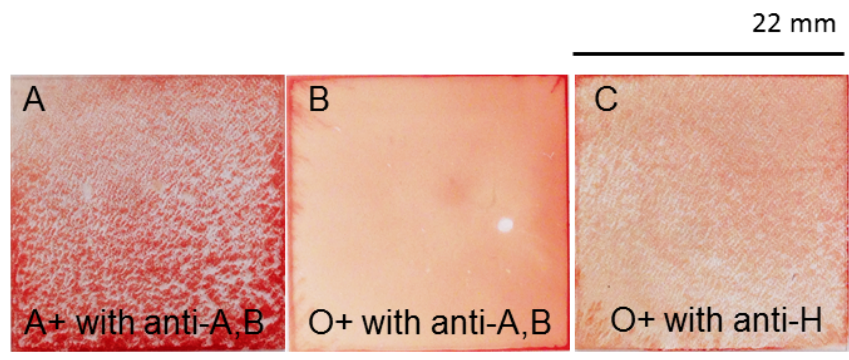


**Fig 2S:** The data has been extracted from Table 1 and transferred into Microsoft Excel for further analysis. As shown, anti-H was not able to provide efficient plasma separation on paper for diagnostic applications.

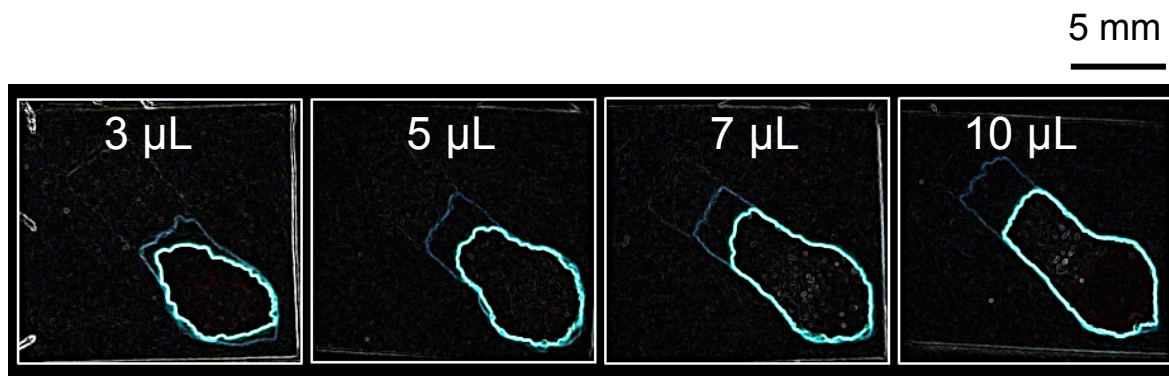
### **Effects of anti-H on plasma separation:**

A weak antibody-antigen interaction could be related to low concentration of antibody in an assay, but it could also be related to the weak antigen on the red blood cell surface. Although H-antigen is present on red cells of 99.9% human population, it is well known that H antigen is the precursor of antigens A and B. H antigen does not express strongly on A1 and B cells, since it converts into A1 and B antigens.<sup>1</sup> The weakest H antigen expression is on group AB cells, followed A1 and B group cells.<sup>2</sup>

Therefore, Anti-H has weak interactions with red blood cells of AB, A1 and B types and forming small agglutinated lumps with red blood cells of those types. For this reason, there has been no report in the literature showing that anti-H can separate red cells from plasma on paper. The real reason is not the antibody concentration, but the weak anti-H and cell interactions.



**Fig 3S:** A 5 $\mu$ L of blood grouping antibody (used as received without dilution) was introduced onto each glass slide before adding the same amount of whole blood sample. As shown, the size of RBC aggregates is different in each experiment.



**Fig 4S:** The effect of initial blood sample volume on plasma separation. The sample inlet point of the devices were functionalized using 0.5  $\mu\text{L}$  of 0.68 M saline solution before adding 3, 5, 7 and 10  $\mu\text{L}$  of whole blood sample in each device. The images captured and analyzed using ImageJ software (See Figure 4).

1. G. Daniels and M. Bromilow, *Essential Guide to Blood Groups*, Blackwell Publishing, 2007, pp. 27.
2. L. Dean, *Blood Groups and Red Cell Antigens*, Bethesda (MD): National Center for Biotechnology Information (US); 2005, Chapter 6. Online access: <http://www.ncbi.nlm.nih.gov/books/NBK2261/>.