# Analytical Methods

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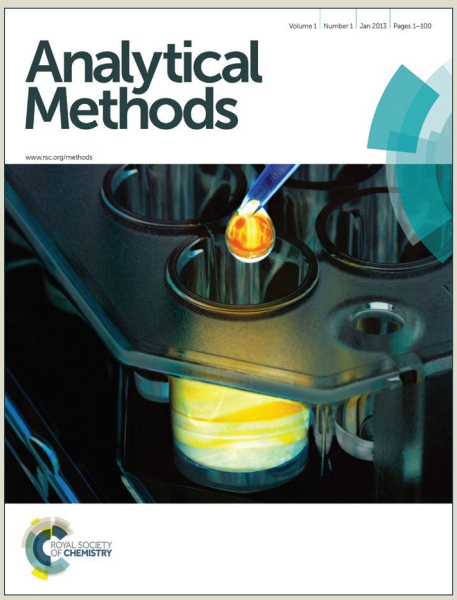


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ARTICLE

# A low-cost forward and reverse blood typing device—A blood sample is all you need to perform an assay

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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For all user-operated blood typing devices in today's market, including those designed by us in our previous research, a buffer-activation or buffer-washing step is required. The buffer-activation step, as is employed in some commercial blood typing devices, involves dissolving the antibodies deposited in the assaying zones of the device before the introduction of a blood sample for an assay. The buffer-washing step involves washing the blood sample in the assay zone in the end of the assay for result reporting. While all these devices work well, the activation or washing step does reduce the adaptability of those devices to resource-poor areas and under emergent circumstances. In this study, we designed a new device to perform forward and reverse blood typing assays without the buffer-activation or buffer-washing. Low-cost plastic slides were patterned to form channels containing dried grouping antibodies. Blood typing assays can be performed by simply placing a few micro Litres of a blood sample into the channels and then tilting the slide. The sample flows along the channel under gravity, dissolving dried antibody and then spreading into a film, unveiling the reaction of red blood cells (RBCs) and antibodies. This device enables easy visual identification of the agglutinated and non-agglutinated RBCs in typically 1 minute. Both forward and reverse blood typing assays can be performed using this device. To optimize the device design, antibody dissolution profile, assay sensitivity, and the device longevity were investigated in this work.

## Introduction

In today's world there is an increasing need for affordable healthcare devices which would enable many conventional diagnostic assays to be carried out from home. The home-based and patient-operated assays, if made reliable and rapid, can significantly alleviate the pressure on hospitals and pathological laboratories in developed countries [1, 2]. At the same time, these technologies also carry the hope to minimize the impact of disease outbreaks and to increase drinking water safety in impoverished areas [3-7]. This is because the centralised laboratories and hospitals taken for granted in the cities of the developed world are absent in remote and impoverished areas. In the past few years, novel diagnostic devices built on low-cost substrates such as paper, thread, and plastic and glass slides have demonstrated the possibility for such a hope to become reality [8-21]. These innovations showed the potential impact of low-cost analytical technologies on future human health and environmental care. Among those innovations, a series of blood typing diagnostics based on paper and thread platforms have been developed [19, 22-28]. The paper- and thread-based devices have significant advantages over the current laboratory- and hospital-based technologies due to their high adaptability to unsupported field conditions, user-friendliness and assaying speed.

Blood typing is a routine clinical test, but also a test of paramount importance for avoiding fatal haemolytic transfusion reactions (HTRs) during surgeries, clinical emergencies and blood transfusions. Equally important, since the world annual blood donations are around 75 million units [1], routine and rigorous sorting of blood types must to be performed in large numbers and speedily. These healthcare and clinical requirements urge the continuous development of accurate, user-friendly and low-cost blood typing technologies [29-31].

For ABO and Rh blood groups most clinical techniques are based on the visual observation of heamagglutination reactions; although advanced gene-sequencing technology providing precise determination of blood type through DNA analysis is now available [32]. Upon the contact of RBCs with an antibody, the absence of RBC agglutination indicates that there is no heamagglutination reaction; this observation confirms that there are no corresponding antigens on the RBC surface to the grouping antibody. Conversely, if agglutination is observed, it

confirms that corresponding antigens to the antibody are present on RBC surface.

Furthermore, for the ABO blood system the Landsteiner's rule applies [31], which states that, for an individual, if an antigen is present on the surface of his RBCs, the corresponding antibody will be absent from his blood plasma. Instead, the reciprocal antibody will be present in the plasma or serum. For example, an individual of blood type A has A antigen on his RBCs and antibody B in his serum. The normal blood typing assay, also known as the forward blood typing assay, uses blood grouping antibodies to identify the specific antigens on RBCs. Conversely, there is another blood typing assay which determines the antibodies in the serum by using the reagent RBCs with known antigens. This blood typing assay is known as reverse blood typing. Since a reversed blood typing assay determines the interactions between the reagent RBCs and the antibodies in a patient's serum, it also relies on observation of RBC agglutination. Details to explain the forward and reverse blood typing can be found in Figure S1 and S2 in the ESI. In many countries, both forward and reverse blood typing are required to confirm the patient's blood type before a blood transfusion or transplantation is allowed to proceed. The laws that determine blood types by forward and reverse blood typing are shown in Table 1.

**Table 1. Blood type confirmation by forward and reverse blood typing methods**.

| Reaction of Antibodies with Blood | | Forward Blood Type | Reaction of reagent cells with serum | | Reverse Blood Type |
|---|---|---|---|---|---|
| Anti-A | Anti-B | | $A_1$ Cells | B Cells | |
| + | − | A | − | + | A |
| − | + | B | + | − | B |
| + | + | AB | − | − | AB |
| − | − | O | + | + | O |

Technical designs of blood typing assays therefore focus on using a variety of different methods to differentiate the agglutinated RBCs from the non-agglutinated RBCs. Common methods include the tube test [33], slide test [34], and column agglutination system (e.g. Gel Card) [35, 36]. These methods rely on the different sedimentation velocities of agglutinated RBC lumps and non-agglutinated RBCs in a serum suspension, or different migration rates of agglutinated RBC lumps and non-agglutinated RBCs in a gel column of uniform pore size [31]. The recently reported paper- and thread-based blood typing devices function as a size-based filtration device. Since within these devices the interactions of RBCs and antibodies occur inside the fibre networks of paper and thread, the fibre networks of paper and thread restrict the movement of agglutinated RBC lumps, but do not restrict non-agglutinated RBCs from moving with the serum phase or a carrier phase of buffer. Paper- and thread-based assays allow a short incubation of RBCs with the grouping antibody (typically 20 seconds for ABO and RhD tests), followed with a saline wash [19, 25-26]. The inability of agglutinated RBC lumps to move in a paper fibre network during saline washing makes them easily differentiated from the non-agglutinated RBCs, which can be readily washed out of the fibre network.

In order to further improve the adaptability of paper-based devices to non-laboratory condition, future designs will need to explore new concepts that can significantly reduce the effort of the user required to perform the blood typing assay. In this work we present a new concept of a blood typing device which does not require the user to apply the saline buffer for activating or washing the device in order to perform an assay. This concept relies on the dissolution of blood grouping antibodies deposited on a non-absorbing substrate by a blood sample, and the subsequent thinning of the sample into a film for blood typing result identification. In the fabrication of the device, channels are formed to guide the blood sample flow. Furthermore, we investigated two factors that affect the performance and sensitivity of the device: the thickness of the blood sample film and the antibody dissolution behaviour. A chromatographic elution method was designed to provide a semi quantitative estimation of the antibody dissolution profile from the device surface. The antibody longevity on the plastic substrate was studied for one month under ambient laboratory conditions. Apart from performing the general forward blood typing assays, we have also demonstrated the use of our new device to perform reverse blood typing. Presently, reverse blood typing can only be performed in central laboratories and hospitals. This study is the first one to demonstrate reverse blood typing using a low-cost device. We believe that our device concept will allow forward and reverse blood typing to be combined into one user-friendly device.

## Experimental

### Materials

Plastic slides were purchased from 3M (3M Visual Systems Division, USA). Blood samples were sourced from Red Cross Australia, Sydney. They were stored at 4°C and used within 7 days of collection. All the antibodies were purchased from ALBA Bioscience, Edinburgh, UK.

The red blood cells required for reverse blood typing, including 15% A1 cells, 15% B cells and 3% C1 cells, were obtained from CSL, Australia; they were concentrated to 45% hematocrit level (the average human whole blood) by centrifugation, stored at 4°C and used within 30 days. The 0.9% (w/v) NaCl saline solution and the phosphate-buffered saline (PBS) were prepared with AR grade NaCl (Univar) and Phosphate (Aldrich), using MilliQ water. Glycerol and Tween 20 were purchased from Aldrich. Surface treatment of the plastic slides was carried out using a plasma reactor (K1050X plasma asher (QuorumEmitech, UK)).

### Methods

**Blood typing procedure.** A blood typing device for use in impoverished regions must allow for direct visual identification of test results; the device should function with a minimum effort from the user and without the need of any equipment. Following these requirements, we explored a new device design concept which provides direct and rapid visual identification of the test result, while minimizing the effort from the user to perform the test. It focuses on eliminating the saline washing step. Figure 1 describes the advantage of forming a blood sample film on an antibody treated plastic slide for blood typing assays and the device design. Figure 1 (a) and (c) show the result of adding a drop of blood sample into a drop of antibody solution on a plastic slide. Although agglutination of RBCs by the corresponding antibodies had occurred, it could not be visually observed. However, if the slide is tilted to allow the drop to flow under gravity and form a thin film, the user can immediately identify the agglutination of the RBCs by an antibody. Figure 1 (b) and (d) show the flowing blood sample and the grouping antibodies on a tilted plastic slide, clearly showing agglutinated and non-agglutinated RBCs, respectively. In order to reduce the users' efforts to perform the assay, blood grouping antibodies were coated onto the plastic slide so that users are not required to administer antibodies for forward blood typing. This device design principle requires the rapid dissolution of a sufficient quantity of grouping antibodies by the blood sample; therefore the antibody dissolution profile must be verified experimentally.

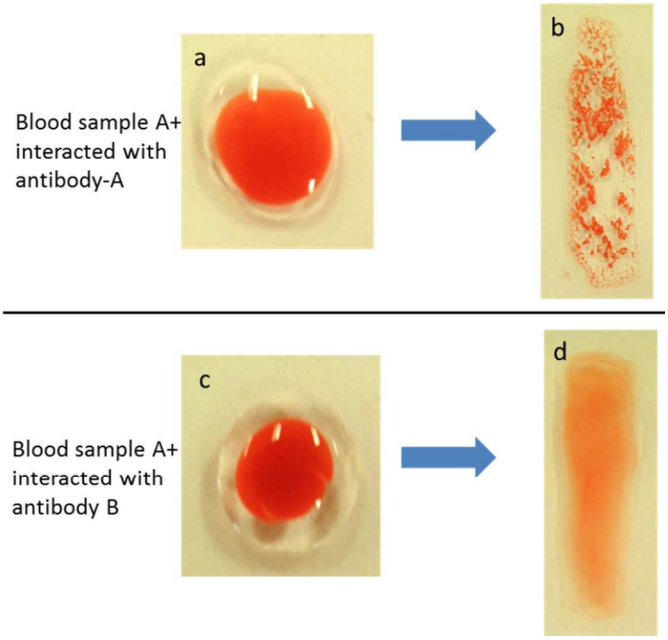


Figure 1. Demonstration of a blood typing assay of an A+ blood sample on a plastic slide substrate by: a) placing a drop of blood sample on a drop of anti-A; b) tilting the substrate to allow the blood sample and anti-A solution to spread into a film, immediately revealing the positive assay result; c) a drop of A+ blood sample placed on a drop of anti-B; d) tilting the substrate to reveal the negative assay result.

Plastic slides were first treated with plasma at an intensity of 50 W for 1 minute; a water-resistant pen was then used to draw the boundaries to demarcate the sample flow channels. Three micro Litres of antibodies (anti-A, anti-B and anti-D) were dropped respectively on top of the three channels designated to these antibodies, and slide was tilted to allow antibodies to flow through the channel under gravity. After the antibodies covered the entire length of the channels and were completely dried, 3 μL of blood sample was dropped from the top of each channel, following the same procedure. Typically, it takes 30 seconds for the blood sample to flow through the entire channel. Antibody-specific agglutination of RBCs can be identified immediately as the blood sample flows through the channels treated with antibodies and forms films. A schematic protocol of the test is shown in Figure 2.

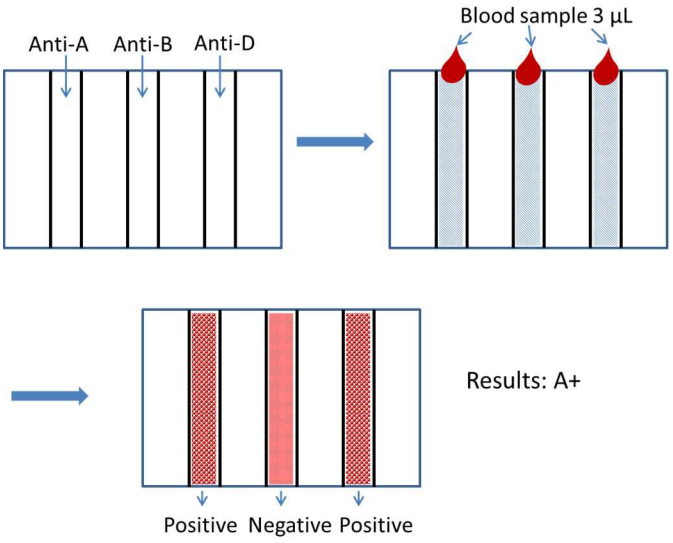


Figure 2. The designed procedure for blood typing on a plastic slide.

**Quantification of antibody dissolution rates from plastic slides.** Since the working principle of the plastic slide device relies on the rapid dissolution of the antibodies deposited on the slide, it is necessary to characterize the dissolution rates of all three antibodies employed for the device design. To do this, PBS buffer was used to perform a controlled dissolution study of all antibodies from the plastic slide surface. Figure 3 shows the experimental procedure: Channels on a plastic substrate were pre-treated with antibody and allowed to dry under ambient condition. A 10 µL PBS was used to flow through each channel to dissolve the antibody; the contact time was controlled at 30 s. The flow-through PBS solutions containing the dissolved antibodies were collected using Kleenex paper towel; the solutions wetted the paper towel and formed circular wetting zones as shown in Figure 3.

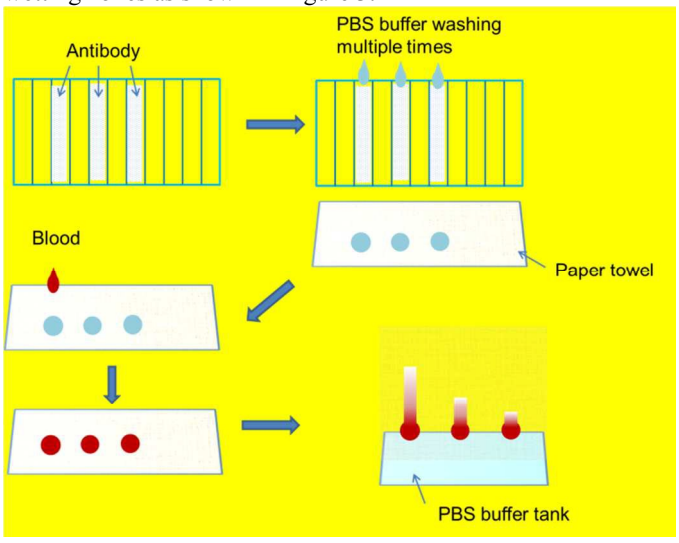


Figure 3. Schematics of the procedure for studying the antibody dissolution behaviour from the plastic slide. This procedure involves an antibody dissolution step by PBS, followed by quantifying the concentration of the antibodies washed off the plastic slide.

In order to quantify the dissolved antibodies, antibody concentrations of the collected PBS washing solution were analyzed. We designed the quantification step as follows: A blood sample carrying the corresponding antigen to an antibody was introduced into the zone of the collected PBS washing solution on paper towel. After 30 seconds of incubation time under ambient condition, the paper was immersed into a chromatography tank containing PBS for elution (Figure 3), following the method we reported previously [24]. If the antibody concentration is high enough, the RBCs of the blood sample will agglutinate, forming a blood stain with a strong colour which cannot be eluted away by ~~BPS~~PBS. The elution pattern was scanned into a computer and then converted to a monocolour mode (Grey scale mode) with the ImageJ software. The optical density of the grey scale image of the blood spot was determined using the software. The gray scale is digitized into 256 steps, which represent different tones from dark to bright in ascending manner, with 0 being the darkest and 255 being the brightest tone. The optical density of the blood spot therefore provides a simple and semi-quantitative method for determining the antibody dissolution behaviour. An antibody dilution standard calibration curve can be established by determining the optical densities of agglutinated blood spots by a series of step dilutions of an antibody.

## Results and Discussions

There are in total eight different blood types in the ABO and RhD blood groups; all of them can be clearly identified by the plastic slide method (Figure 4). After a blood sample was introduced into the channels, it takes typically up to 1 minute for the 3 µL of blood sample to flow through the entire length of the channel. During this process, antibody-specific agglutination of RBCs form large lumps, which become clearly identifiable when the blood sample flows halfway through the channels, making the assay time with the plastic slide shorter than 1 minute.

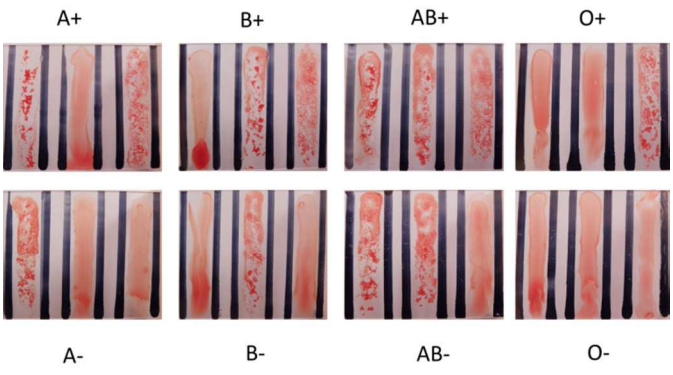


Figure 4. Assay results reported by the plastic slide device for all 8 ABO and RhD blood groups

**Antibody dissolution from the plastic slides**

Figure 5 (a) shows the chromatographs of agglutinated type A blood sample by a serially diluted anti-A; the dilution was made from 1 (original anti-A) to 512 folds. The serial dilution data show that the anti-A retained its activity after being diluted 128 folds. Further dilution, however, weakens the antibody activity, causing weak RBC agglutination. Figure 5 (b) presents the anti-A dilution curve of the blood spot colour density against the dilution factor. Since the concentration of the commercial antibody was unknown, the dilution factor was used as the relative antibody concentration. Figure 5 (c) shows the result of a serial dissolution of anti-A from the plastic slide; the anti-A standard solution was gradually diluted into a series of concentrations and dropped onto the paper towel, followed by the introducing of reagent red blood cell A onto each antibody spot. Then the chromatographic elution method was applied to the paper towel and the colour intensity of each blood spot was tested for building the standard curve of anti-A dilution behaviour, as is shown in Figure 5 (a) and 5 (b). The standard curve in Figure 5 (b) shows that a significant loss of anti-A activity to A antigen on the RBC surface by visual evaluation occurred only when it was diluted to 1/128 of its original concentration. This result suggests that the dissolving rate of anti-A is slow, and the following phenomenon provided reasoning. The standard curve in Figure 5 (b) can be fitted to a logarithmic formula (Formula (1)) to establish the relationship of the colour density and the relative concentration of the antibody. This standard curve provides a way to quantify the antibody that was washed off the plastic substrate by the saline solution. Since the precise original antibody concentrations were unknown, we could assume that they were $C_A$, $C_B$ and $C_D$, and measure the concentration changes caused by the saline dissolution [37]. Through measuring the colour density of each blood spot in Figure 5 (c), the relative concentration of anti-A released from the substrate after each saline wash can be calculated; the results are shown in Table 2. Anti-A deposited on the plastic substrate dissolved only 11.1% by the first wash, the remaining anti-A on the substrate still retained sufficient bioactivity for blood typing after another three such washes.

$$\text{Optical Density A} = 26.5 - 14.6 \times \ln(f_A - 5.42 \times 10^{-4});\ f_A = \frac{C'_A}{C_A} \quad (1)$$

Where $f_A$ is the dilution factor of anti-A; $C'_A$ is the concentration of anti-A of each dilution.

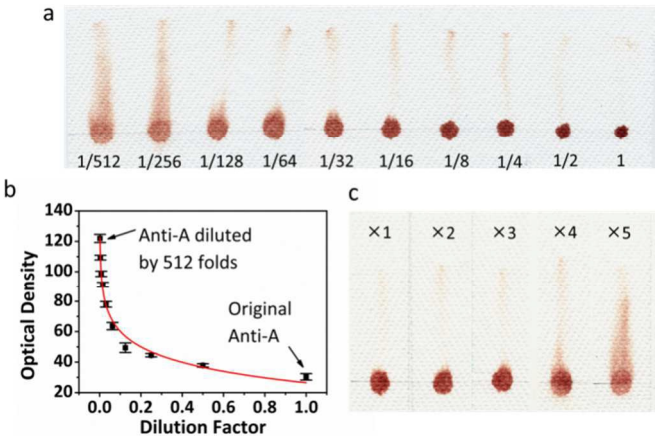


Figure 5. Antibody dissolution behaviour study for anti-A: (a) Anti-A activity as a function of a series of dissolutions with PBS; antibody activity can be determined by the reflective optical density of the agglutinated A1 reagent red cell (concentrated from the commercial reagent to a hematocrit level of 45%) on paper. (b) The calibration curve of the A1 cell optical density as a function of anti-A dilution factor; the red curve is the fitting curve of a natural logarithm function (formula 1); (c) anti-A dissolution behaviour of five consecutive PBS dissolution rinses collected from the plastic substrate.

Following the same procedure for quantifying anti-A, the dissolution behaviour of antibodies B and D have also been quantified. The calibration curve of anti-B dilution showed that anti-B lost its activity after a dilution of 1/16, indicating a weaker activity compared with anti-A (See Figure S3a in the supported information). The calibration curve was fitted with formula (2) (Figure S3b), which quantitatively showed that the concentration of anti-B in the first saline wash was 23.1% of its original concentration $C_B$ (Table 2), The more efficient dissolution of anti-B by saline solution than of anti-A confirms that anti-B can be dissolved more easily from the plastic slide, therefore anti-B weakened more rapidly than anti-A with the number of washes by saline (Figure S3c). As shown in Figure S1c, anti-B could sustain three washes and the residual anti-B on the plastic substrate still had sufficient activities for unambiguous blood typing. However, as for anti-D, the dissolution was even more efficient (Figure S4a and S4b), our measurement showed that 92.0% of its original concentration was dissolved (Formula (3) and Table 2) and removed from the plastic substrate in the first saline wash; anti-D lost its activity at 1/8 dilution (Figure S4c).

$$Optical\ Density\ B = 41.4 - 17.2 \times ln(f_B - 0.02);\ f_B = \frac{C'_B}{C_B} \quad (2)$$

$$Optical\ Density\ D = 81.1 - 10.7 \times ln(f_D - 0.03);\ f_D = \frac{C'_D}{C_D} \quad (3)$$

Where $f_B$ and $f_D$ are the dilution factors of anti-B and anti-D; $C'_B$ and $C'_D$ are the concentrations of anti-B and anti-D after each dilution.

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**Table 2. The blood spot optical density data and the calculated concentrations of three antibodies of each dissolution from the plastic substrate.**

| Antibody | | Number of dissolution washes | | | | |
|---|---|---|---|---|---|---|
| | | X1 | X2 | X3 | X4 | X5 |
| Anti-A | Colour Density | 58.7±2.3 | 62.0±2.8 | 66.3±2.4 | 75.8±1.8 | 99.6±2.6 |
| | Relative concentration | (11.1±1.7)%$C_A$ | (8.9±1.7)%$C_A$ | (6.6±1.1)%$C_A$ | (3.5±0.4)%$C_A$ | (0.7±0.1)%$C_A$ |
| Anti-B | Colour Density | 68.3±1.9 | 76.1±1.6 | 86.8±2.5 | 100.5±2.8 | |
| | Relative concentration | (23.1±2.4)% $C_B$ | (15.6±1.3)% $C_B$ | (9.4±1.1)% $C_B$ | (5.5±0.5)% $C_B$ | |
| Anti-D | Colour Density | 82.4±1.7 | 110.6±3.2 | 143.9±2.2 | | |
| | Relative concentration | (92.0±14.3)% $C_D$ | (9.5±1.9)% $C_D$ | (3.2±0.1)% $C_D$ | | |

According to the definition of reflective optical density used in the printing industry [40], the reflective optical density is defined as a logarithmic ratio of the reflected radiation from a printed grey tone on paper to the reflected radiation from the unprinted paper. This is usually presented in the form of logarithm based to 10 [40], but can be easily converted to the form of natural logarithm:

$$D = -ln\frac{I}{I_0} \quad (4)$$

Where D is the reflective optical density, usually measured with a reflective densitometer in the printing industry, $I$ and $I_0$ are the reflective radiation intensity from a printed grey tone and from unprinted paper, respectively. In this study grey tones generated by the agglutinated blood spot on paper loaded with different amount of antibodies, creates a similar concept for the tones to be quantified by reflective optical density.

Since blood spot optical density data (Figure 5, S3b and S3c) can also be correlated with concentrations of corresponding antibody (or dilution) data by logarithm functions, it suggests that optical density data can be correlated to the concentration ratios of the dissolved antibodies. Such correlations have been experimentally given in equations (1) – (3) and are expected to provide semi-quantitative results for antibody dissolution evaluation.

**Sensitivity**

The sensitivity of any blood typing device must be investigated for typing blood samples with low concentrations of RBCs. This requirement is essential, as clinically the RBC concentration from blood samples of anaemia patients could be more than 50% lower than those from a healthy patient. Since almost all commonly used blood typing assays rely on the development of large agglutinated RBC lumps, those method can be less sensitive to samples with low RBC concentrations.

The sensitivity study of the plastic slide method was conducted by identifying the agglutination patterns of serially diluted reagent RBCs that carry known antigens. The original RBC samples used for testing were the red cells A1, B and C1. To prepare samples with low RBC concentrations, the suspension media of the reagent red cells was first removed by centrifugation and then the red cells were diluted to haematocrit of 45% with PBS; this RBC concentration simulates the blood RBC concentration of a healthy individual. Low RBC concentration samples were prepared by diluting this sample by factors of 75%, 50% and 25% with PBS. The diluted blood samples were then used for the sensitivity tests of the device and results are shown in Figure 6. All positive tests of diluted blood samples A, B and D can still be clearly identified via RBC agglutination, even though they were diluted to 25%. These results confirm that the plastic slide blood typing device is able to deliver the equivalent sensitivity of a high performance blood typing device. The film forming process of

the blood sample provides a simple way to enhance the sensitivity of blood typing using the plastic slide method.

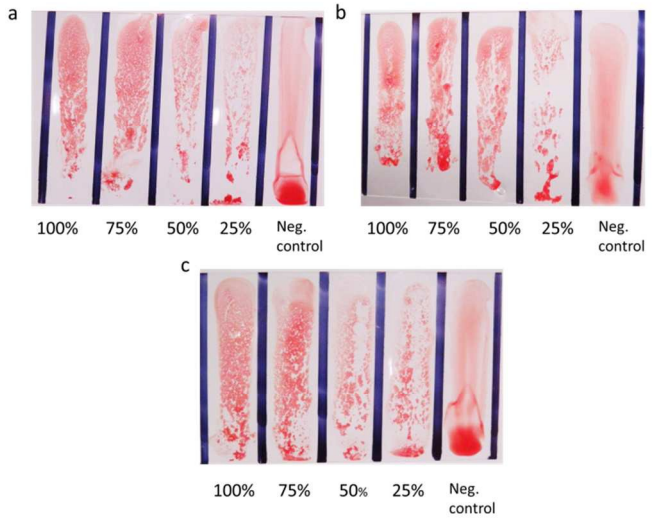


Figure 6. Sensitivity tests of the plastic slide method by dilution of reagent red blood cell carrying known antigens: a) A1 cells in the anti-A treated channel; b) B cells in the anti-B treated channel; c) C1 cell in the anti-D treated channel. The negative control channel was treated with BSA only.

**Antibody longevity on the plastic slide**

It was found that the original antibodies gradually lost their activity within 10 days of being deposited on plastic slides and allowed to dry. This is because the dried antibodies dehydrated after long exposure to air. As a result, the surface of the channel became more hydrophobic and this significantly reduces the speed of blood flow in the channel. Figure 7 shows the hydrophobic development of the slide surface with time. To solve this problem, glycerol and Tween 20 were chosen as additives to prevent antibody dehydration and to increase the channel surface wettability. Glycerol has been used as a traditional additive for protecting biomolecules from denaturing [38]. Its high humectant effect attracts water molecules and prevents biomolecules from dehydration; such a protective effect is most likely related to the presence of 3 hydroxyl groups in the glycerol molecule and its small molecular size. Apart from attracting water molecules, glycerol may also provide direct hydrogen-bonding, like many sugar molecules, to stabilize the biomolecule [39]. The addition of Tween 20 as a biologically compatible surfactant is intended to enhance the wettability of the deposited antibody layer on the plastic slide after ageing. Additives with a series of different proportions of glycerol and Tween 20 were mixed with the antibodies to form solutions to treat the channels of the device; the wettability of the channels was tested at different storage times by measurement of the flow length of blood samples in the channels in 30 seconds; results are presented in Table 3. Our results show that antibody solutions containing 20% glycerol, or 10% Glycerol and 0.1% Tween 20 enhanced the channel wettability; testing after 30 days of storage showed that the device's wettability was unchanged and all antibodies still retained their activity. We chose the 10% Glycerol and 0.1% Tween 20 as the preferred additive formulation to perform further experiment. Figure 10 shows the blood test results obtained after 45 days of storage under ambient temperature and open to air; all the antibodies were still active and gave accurate blood typing within 30 seconds (Figure 8).

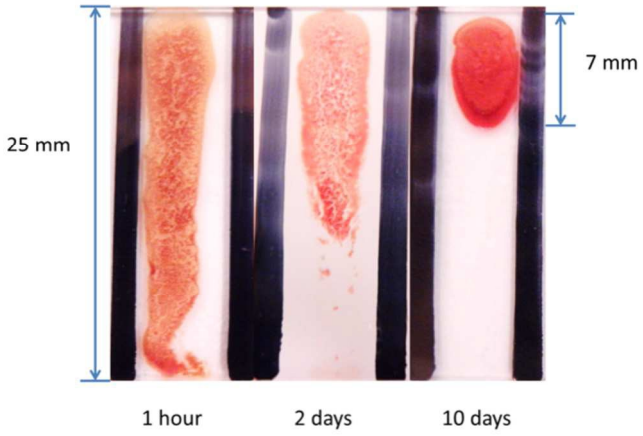


Figure 7. Lifetime detection for blood group A interaction with anti-A treated device staying for different periods.

**Table 3. Additives affect on the surface hydrophilicity for blood flowing with the increase of time**

| Additive | Blood sample flow distance (mm) | | | |
|---|---|---|---|---|
| | 1 hour | 2 days | 10 days | 30 days |
| Pure antibody | 21 | 13 | 8 | 4 |
| Tween 20 (0.1%) | 25 | 14 | 3 | 3 |
| Tween 20 (0.5%) | 25 | 12 | 3 | 3 |
| Tween 20 (1%) | 20 | 8 | 3 | 3 |
| Glycerol (1%) | 25 | 15 | 8 | 4 |
| Glycerol (5%) | 25 | 17 | 12 | 6 |
| Glycerol (10%) | 25 | 25 | 22 | 20 |
| Glycerol 10%+ Tween 20 0.1% | 25 | 25 | 25 | 25 |
| Glycerol (20%) | 25 | 25 | 25 | 25 |

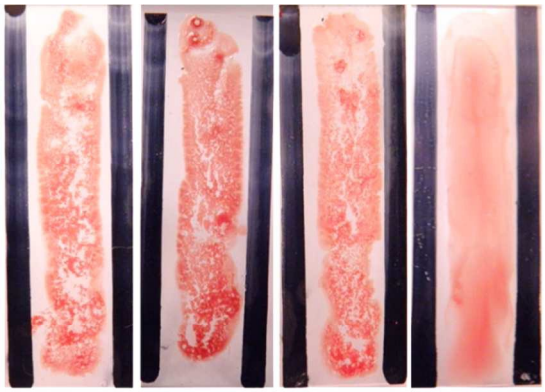


Figure 8. Antibody activity for its corresponding antigen after being deposited for 45 days on the device surface coated with an additive mixture of 10% of glycerol and 0.1% of Tween 20. Test was conducted under an ambient laboratory condition.

**Reverse blood typing using the plastic slide assay**

The reverse blood typing assays were also performed using the plastic slide method. Patients' blood serum was first separated from the whole blood sample, which can be ~~by~~prepared by the traditional centrifugation or the new low-cost POC methods on membrane[41, 42]or paper [43]. ~~and t~~Then the serum samples were dropped into the channels on the plastic slide and allowed to spread throughout the channel. Reagent red blood cells A and B were then pipetted into separate serum-coated channels. By allowing the reagent RBCs to spread in the channel and form a film, the agglutination RBCs can be clearly identified by the naked eye without any aid (Figure 9).

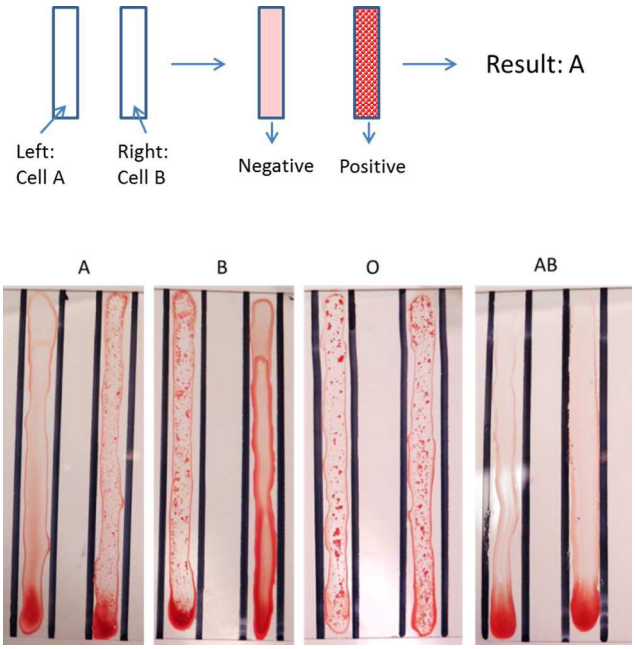


Figure 9. The reverse blood typing results for identifying ABO blood groups.

**Proposed mechanism of RBC agglutination on the plastic slide**

The use of film formation as a sensitive method for blood typing takes advantage of the following two processes: Firstly, the spreading of blood sample over the antibody coated channel surface provides a large contact area between the blood sample and antibody. The large contact area promotes interactions between the RBCs in the blood sample and the antibodies on the plastic slide; agglutination of RBCs by their corresponding antibodies can therefore form quickly. Secondly, the relatively slow spreading of blood samples in an antibody treated channel is likely to give sufficient time for blood sample and antibody to interact and incubate. This, combined with the small thickness of the blood sample film, tends to lead to the formation of sheet-like agglutination lumps, which are very easy to visually identify. The plastic slide device thus offers a sensitive means for rapidly performing both forward and reverse blood typing assays.

## Conclusions

In this work we designed and demonstrated a new user-friendly blood typing device. The device was fabricated by patterning the plastic slide with channels treated with different blood grouping antibodies; this design requires the user to simply introduce the blood sample into the channels to complete the blood typing assay, without the need of buffer washing or stirring operations. This device functions by letting the blood sample spread under gravity over a surface treated with blood grouping antibodies. The agglutinated RBC lumps can be rapidly and clearly differentiated from the non-agglutinated RBCs, providing unambiguous visual identification of the positive and negative blood typing assays. This method provides a user-friendly design concept that requires minimum effort from the user to perform an assay to determine the blood type of a patient within 1 minute.

The film-forming principle of this method provides a high level of sensitivity to identify blood types of samples with low RBC concentrations. Preliminary investigation of the longevity of the device was also conducted; a mixture of glycerol and Tween 20 was chosen as an effective additive mixture to maintain the bioactivity of the antibodies on the device for the testing period of 30 days.

## Acknowledgements

This work is supported by the Australian Research Council (ARC). Funding received from ARC through grant numbers DP1094179 and LP110200973 is gratefully acknowledged. The authors thank Haemokinesis for its support through ARC Linkage Project, and Mr Hansen Shen for proof-reading the

manuscript. Ms. Miaosi Li also thanks the Monash University Research and Graduate School and the Faculty of Engineering for postgraduate research scholarships.

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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# A low-cost forward and reverse blood typing device—A blood sample is all you need to perform an assay

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A user-friendly device is designed to perform forward and reverse blood typing assays within one minute but without the buffer-activation or buffer-washing of the sensor.