



(43) International Publication Date
28 June 2012 (28.06.2012)

(10) International Publication Number
WO 2012/083361 A1

(51) International Patent Classification:
G01N 33/80 (2006.01)

(21) International Application Number:
PCT/AU2011/001650

(22) International Filing Date:
21 December 2011 (21.12.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2010905684 24 December 2010 (24.12.2010) AU
2011903413 25 August 2011 (25.08.2011) AU

(71) Applicant (for all designated States except US): **MON-ASH UNIVERSITY** [AU/AU]; Wellington Road, Clayton, Victoria 3168 (AU).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SHEN, Wei** [AU/AU]; 1 Landen Avenue, Glen Waverley, Victoria 3150 (AU). **LI, Xu** [CN/AU]; 49 Townsend Street, Glen Waverley, Victoria 3150 (AU). **BALLERINI, David Robert** [AU/AU]; 23 Ward Avenue, Oakleigh South, Victoria 3167 (AU).

(74) Agent: **WATERMARK PATENT & TRADE MARKS ATTORNEYS**; Level 2, 302 Burwood Road, Hawthorn, Victoria 3122 (AU).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: METHOD AND SYSTEM FOR DETECTION OF BLOOD TYPES

(57) Abstract: A system for identifying a blood group type of a blood sample, including: at least one thread treated with an antibody; a delivery zone on which the blood sample is delivered to the thread; and a testing zone through which capillary wicking of the blood sample can occur; wherein visual indications of the blood group type can be provided on the thread due to interaction of the antibody with the blood sample. A method for identifying a blood group type of a blood sample using a system as described above. A blood sampling tool including a needle eye shaped aperture for sampling a quantity of blood.



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METHOD AND SYSTEM FOR DETECTION OF BLOOD TYPES

FIELD OF THE INVENTION

The present invention is directed to a method and system for the detection and identification of blood types.

5 BACKGROUND TO THE INVENTION

Correct grouping of human blood is imperative for numerous reasons; among them transfusion compatibility is paramount. The term "blood group" is a classification of an individual's blood based upon the inherent presence or absence of antigens on the surface of their red blood cells (RBCs). In the blood
10 plasma antibodies exist which are formed against foreign antigens to protect the body from perceived threats. This reciprocal relationship, Landsteiner's Law, means that individuals who lack a RBC antigen (A, B or D for example) will possess the corresponding serum antibody, whilst those who possess the antigen must not. There are 30 discrete blood type systems recognized by the ISBT
15 Committee on Terminology for Red Cell Surface Antigens. Among these the ABO and Rh systems are of primary importance when transfusing blood, as incompatibility may lead to an acute haemolytic reaction with catastrophic results such as shock and renal failure leading to death. Without ABO compatibility testing, around one-third of unscreened blood transfusions would be expected to
20 cause a haemolytic reaction. For this reason the ability to quickly and cheaply identify blood type is highly valued, and may lead to the saving of many lives.

The vast majority of techniques for ABO and Rh grouping of blood to date have been based upon the principle of haemagglutination reactions between RBCs and serum antibodies. The absence of agglutination indicates no
25 haemagglutination reaction. An exception to this is the technique of gene-sequencing, which analyses DNA to precisely determine blood type, albeit at considerable cost.

Commonly used methods include the slide test, tube test, micro plate method, and the column agglutination system. These methods require specific
30 antiserum addition during testing or well-trained personnel to perform centrifugation, and are therefore difficult to perform away from a laboratory setting. Disposable systems such as lateral flow devices and bedside test cards,

though rapid and without the need of expensive equipment, always require the pre-treatment of blood samples or reconstitution of antibody.

All of the techniques mentioned suffer from shortcomings such as (i) high cost (ii) necessity of trained personnel (iii) sample pre-treatment (iv) additional reagent handling or (v) large sample volume requirements. This limits use in developing regions and emergency situations where medical facilities are unavailable. A recent study performed blood typing on paper strips coated with antibody eliminating the pre-treatment of blood samples, but with the sample volume required for each test being 20 μ L, venipuncture is necessary.

The restrictive requirements of existing techniques render them less suitable for point-of-care monitoring and treatment, especially for use in under-developed areas, emergency situations, and general field use.

It is therefore an object of the present invention to provide a testing platform utilising the porous nature of thread which avoids these limitations whilst being simple, rapid and inexpensive with low sample volume requirements.

A further object of the present invention is to demonstrate a simple recyclable blood sample device. It is therefore desirable that the present invention allows the required volume of blood sample to be extracted from a finger pricked with a conventional lancet device safely and adequately for testing, increasing the ease of the procedure.

Discussion or mention of any piece of prior art in this specification is not to be taken as an admission that the prior art is part of the common general knowledge of the skilled addressee of the specification in Australia or any other country.

SUMMARY OF THE INVENTION

The present invention uses thread as a flexible and low-cost substrate for the rapid grouping of blood. In particular, a capillary substrate such as thread is used for blood grouping utilising the sensitivity of the flow resistance of large particles in narrow capillary channels to separate agglutinated red blood cells (RBCs) from plasma. Large and discrete particles formed in a continuous liquid phase do not provide a capillary wicking driving force and fall behind the capillary wicking front, leading to their separation from the wicking liquid. The capillary substrate therefore provides a very promising but different mechanism for the

separation of the RBCs and the blood serum phase compared to most existing blood grouping methods. The principle of chromatographic separation is also exploited in this study via the use of suitable dyes to enhance the visual detection of the agglutinated RBCs and the serum phase; surprising and encouraging outcomes are obtained. Using a thread-based device, the ABO and Rh groups can be successfully determined with only 2 μ L of whole blood from a pricked finger tip within 1 minute and without pre-treatment of the blood sample. It is hoped that a new, inexpensive, rapid and simple method may provide an easy-to-use blood grouping platform well suited to those in developing or remote regions of the world.

According to an aspect of the present invention there is provided, a system for identifying a blood group type of a blood sample, including: at least one thread treated with an antibody; a delivery zone on which the blood sample is delivered to the thread; and a testing zone through which capillary wicking of the blood sample can occur; wherein visual indications of the blood group type can be provided on the thread due to interaction of the antibody with the blood sample.

One visual indication may be due to the separation of agglutinated red blood cells from the serum of the blood sample. Another visual indication may be due to the non separation of the red blood cells from the serum of the blood sample.

Preferably the antibody is selected from one of the following groups consisting of: A, B, or D antibodies.

The thread may initially be given a plasma treatment to remove surface contaminants before being soaked in an antibody solution. The antibody may be mixed with a water soluble dye solution prior to treatment of the thread.

The system described above may include a plurality of threads, each thread being treated with a different antibody.

The system may further include a testing platform, preferably formed from a polymer film, upon which the threads are affixed.

In the system, the threads may be affixed in a generally star shaped pattern and may be in contact with one another to thereby form a central delivery zone for the threads.

According to another aspect of the present invention there is provided, a method for identifying a blood group type of a blood sample using a system as described above.

According to a further aspect of the present invention there is provided, a
5 blood sampling tool including a needle eye shaped aperture for sampling a quantity of blood. The blood sampling tool aperture preferably has a dimension of about 0.6mm x 4.1mm.

BRIEF DESCRIPTION OF THE DRAWINGS

It will be convenient to further describe the invention with respect to the
10 accompanying drawings. Other embodiments of the invention are possible, and consequently, the particularity of the accompanying drawings is not to be understood as superseding the generality of the preceding description of the invention. In the drawings:

Figure 1 shows samples of whole blood of type A+, B+ and O- on antibody
15 treated polyester threads. Columns show results on threads A, B and D from left to right, whilst rows show the results for the different blood types as labelled on the left.

Figure 2 is a graph showing separation distance due to agglutination for
20 positive reactions with the different antibody solutions: anti-A, anti-B and anti-D, including cyan and magenta ink jet ink modified anti-D solutions.

Figure 3 shows blood testing on threads CD, MD and D (for comparison).

Figure 3a shows an A+ blood sample applied to thread CD.

Figure 3b shows an O- blood sample applied to thread CD.

Figure 3c shows an A+ blood sample applied to thread MD.

25 Figure 3d shows an O- sample applied to thread MD.

Figure 3e shows a positive result on unmodified thread D.

Figure 3f a negative result on unmodified thread D.

Figure 4 is a graph showing blood sample penetration length on thread for
micro-pipette and needle eye.

30 Figure 5 shows a single step blood grouping testing device, composed of thread A, B and D wedged in a square of polymer film and sharing an intersecting sample delivery zone.

Figure 5a shows an unused device with a sample delivery zone indicated within a circle

Figure 5b shows an enlargement of the right-hand side of the device illustrated in Figure 5a after whole blood has been introduced into the delivery zone in the centre via needle eye. The separation occurring on thread A and D indicates that the blood is of type A+.

Figure 6 shows a method for detecting the blood type of a sample.

DESCRIPTION OF PREFERRED EMBODIMENT

Recent research into the use of thread as a substrate for biomedical and environmental diagnostics has shown the material to be excellent for use in microfluidic devices due to its “*ready-made*” channel structure. Thread is able to wick liquids through capillary motion due to the capillary structure formed by spaces between fibres comprising the thread. Cotton and polyester threads are inexpensive and globally ubiquitous. They have excellent colour display properties, physical strength and wettability. By impregnating thread with serum antibodies, agglutination of RBCs will occur upon the addition of whole blood to the dried thread. This reaction occurring between RBC surface antigens and serum antibodies results in separation of the RBCs and plasma, resulting in three distinct, visible regions when the test is positive. When no interaction occurs the test is negative and no separation is visible.

The chromatographic separation principle, when used for blood typing with a porous substrate such as thread, offers an unexpected advantage for the identification of haemagglutination. It was found that adding suitable dyes to antibodies significantly enhanced the clarity of results. Having low affinity to cellulose fibre surfaces, the dyes are eluted by the serum phase remaining at the wicking front, generating a visible gap between the dye and agglutinated RBCs.

Another preferred aspect of the present invention is to provide a simple, recyclable blood sample handling tool. This tool allows the required volume of blood sample be extracted from a finger pricked with a conventional lancet device safely and adequately for testing, increasing the ease of the procedure.

EXAMPLE:**Preparation of antibody impregnated thread**

Polyester thread was obtained from the School of Fashion and Textiles, RMIT University, Melbourne, Australia. Polyester thread was used as the substrate because it has excellent colour display properties, which aid in the identification of the separation of sample components in capillary channels. The thread used was given an initial plasma treatment to remove surface contaminants which greatly increased its ability to be wet. EpicloneTM anti-A, anti-B and anti-D monoclonal grouping reagents were sourced from CSL Australia. Anti-A and anti-B are a transparent cyan and a yellow solution respectively, whilst anti-D is a clear solution. Threads were treated with antibody via soaking in the grouping reagent followed by blotting with standard blotting paper (Drink Coaster Blotting, 280 gm⁻²) to remove any surplus of antibody solution from the thread. After drying under a fume hood for 10 minutes, coated threads were ready to be used for ABO and Rh blood typing. In this work, "thread A", "thread B" and "thread D" are used as abbreviations for the three types of thread, treated with anti-A, anti-B and anti-D/Rh respectively.

Modification of antibodies for enhanced detection

The addition of strongly water-soluble dyes to antibody solutions was found to significantly assist in the visual detection of the agglutination of RBCs. Water-soluble dyes were added to the clear anti-D solution to achieve this enhancement to result interpretation. Ink solutions (cyan and magenta) for visual indication of successful separation were prepared by diluting commercial Canon ink jet inks (CLI Y-M-C-BK, PGBK (<http://www.canon.com.au>) with Millipore filtered water. Diluted inks were then mixed with antibody solution in the ratio of 1:25 (V:V) ink to antibody solution. Thread was soaked in the coloured anti-D solutions and then dried under a fume hood. Abbreviations of "thread MD" and "thread CD" were adopted for threads treated with magenta and cyan modified anti-D solutions.

Blood sample tests using the thread-based platform

Blood samples (A+, B+ and O-) were sourced from donors of known blood type, with samples being drawn by a trained nurse, and stored in Vacutainer® test tubes containing lithium-heparin anticoagulant. All blood samples were stored at 4°C, and used within 5 days of withdrawal. For the primary investigation of the feasibility of using antibody treated thread as a blood typing system, three treated threads (thread A, B, D) were immobilised in folded polypropylene films with lodging slits to aid testing, and a micropipette (Eppendorf research® 0.1–2.5 µL) was used to dose a 1 µL blood sample onto each thread. Threads MD and CD were tested in a similar fashion to evaluate whether coloured anti-D enhanced the visibility of separation. For positive results showing separation of agglutinated RBCs and serum, the separation distance between the edge of RBCs and serum was measured using a vernier calliper.

Confirmation of donor blood type using the glass slide method

Donor blood types were confirmed using the conventional glass slide technique to provide a control for the thread-based platform. The testing procedure was provided by CSL, Australia. This established method was also used to confirm that the dyes added to anti-D solutions did not affect the results by inhibiting agglutination (in the positive case where the blood contains the corresponding antigen) or by causing agglutination when none should occur (by mixing ink solution and blood with no antibody present).

Separation of agglutinated RBCs from blood serum on thread

Identifying the agglutination of RBCs using porous media has a unique advantage: large and discrete particles in a suspension system undergo a phase separation from the continuous liquid phase in the capillary channels. As the whole-blood sample wicks through the inter-fibre gaps of the antibody treated threads, blood serum will dissolve the antibody molecules deposited on the surface of the fibres. Therefore, if RBC agglutination occurs as a result of a haemagglutination reaction it is expected to take place at the blood wicking front first. Haemagglutination reactions occur when antibodies (immunoglobulin molecules, IgG and IgM) bond to the specific binding sites on the antigens of

adjacent RBCs. The aggregation of the RBCs by the antibody molecules leads to the formation of significantly larger particles that cannot be stably suspended in the serum phase. As the sample wicks along the thread, the size of the agglutinated particles increases. Since the agglutinated RBCs form discrete particles, they cannot contribute to the capillary driving force required for the blood sample to continue wicking forward. Instead, the agglutinated RBC particles can only be carried by the serum to move forward. In this situation agglutinated particles will be gradually left behind from the serum wicking front. Furthermore, the agglutinated particles in the inter-fibre channels may act as a "filter" which permits serum to pass, but prevents other agglutinated RBC particles from passing. This causes the separation of the agglutinated RBCs from the serum phase. This reaction is visually identifiable by the appearance of a pale pink coloured band (identified between two dashed lines) between the RBCs and dry antibody residue, as seen in Figure 1(a, c, e, f). In the case where the deposited antibody does not react with the antigens of the RBCs, no separation is visually detectable (as seen for type O- blood in Figures 1g- i).

Figure 2 displays collected data quantifying the average length of separation distance for different types of antibody treated thread. Error bars represent one standard deviation from the arithmetic mean (10 repetitions). Although there is a small difference in separation distance between differing antibodies, a distance of approximately 3mm was typical and easily visible by the human eye, being of similar length to a standard "stitch" found on a shirt cuff. Typically, results became easily visible within 1 minute of sample dosing, making the test quite rapid compared to existing techniques.

Identifying a person's ABO and Rh blood type requires three tests, two for the A and B antigens to determine ABO grouping, and a further test for the D antigen to determine Rh grouping. A results-matrix is shown in Table 1 which aids in the interpretation of results. Due to the serious consequences of blood transfusion incompatibility, it is recommended that a serum cross check be performed to ensure a safe transfusion, as is standard with all existing blood grouping methods.

Table 1 shows a results-matrix for aiding interpretation. Ticks indicate a positive result (separation has occurred) whilst crosses indicate a negative (no

separation). The user simply identifies which column matches their result to find their blood type

TABLE 1:

Anti – A	✓	✓	✓	✓	x	x	x	x
Anti – B	✓	✓	x	x	✓	✓	x	x
Anti – D	✓	x	✓	x	✓	x	✓	x
	AB+	AB–	A+	A–	B+	B–	O+	O–

5 Effect of adding colouring agents to antibody solutions

As the detection of separation is performed visually, it is more difficult to identify the separation of the blood components on thread B and D compared to thread A, due to the similar colour of the anti-B and anti-D solutions and the blood plasma on thread. The addition of dyes to the antibody solutions can assist interpretation of such results. The antibodies purchased are coloured for the purpose of identification, with anti-A being cyan coloured, anti-B a light yellow whilst anti-D/Rh is colourless. It is from this colouring that the enhancement effect was first noticed on thread, with positive results for thread A being much easier to interpret due to the presence of a light coloured band formed between the agglutinated RBCs and the cyan antibody solution. This band is caused by the migration of the blood plasma.

The cyan and yellow dyes in the commercial Anti-A and Anti-B solutions are patent blue V and tartrazine, respectively. Patent blue V is an acid dye with two sulphonic groups; it dissociates in aqueous solutions and is negatively charged. This dye therefore has a weak affinity towards cellulose and polyester fibre surfaces, which also carry a weak negative charge in aqueous solutions. Tartrazine is an azo dye with three dissociable acidic groups - two sulphonic and one carboxylic. Its negatively charged character in aqueous solutions indicates that it also has weak affinity towards cellulose and polyester. When these dyes are deposited onto thread together with antibodies, they can be readily dissolved and carried forward along the thread by the wicking blood sample. When Anti-A binds the RBCs and causes their agglutination, the agglutinated RBCs will be left further and further behind the wicking front of the plasma. The dissolved dye

molecules, in contrast to RBCs, will be carried by the plasma and remain in the wicking front of the plasma phase until it stops. The different migration behaviour of the agglutinated RBCs and these dyes allows a gap to develop between the RBCs and the dye as the plasma wicks along the antibody-treated thread. This gap results in the formation of a band of very light colour in contrast to the agglutinated RBCs and the colour of the dye in the plasma wicking front. The length of the band is comparable to the separation length previously mentioned for unmodified anti D (Figure 2), but the contrasting colours make separation more easily visible, enhancing the ability of the user to detect agglutination. This effect is minimal for the Tartrazine doped Anti-B due to its light yellow colouring which is similar to that of plasma. In situations where agglutination does not occur, this band does not develop.

Diluted ink jet printing inks were used as colouring agents to modify antibody solutions. Despite detailed information about the dyes used in the ink formulations being unavailable, it is expected that the dyes must have excellent solubility in aqueous solutions (a basic requirement for ink-formulation dyes). Since all dyes for ink jet ink formulation carry negative charges for reasons of safety (positively charged dyes may possess mutagenic properties), ink jet ink dyes also have a poor affinity with the fibres used. Although this poor affinity has a well known and unwanted effect upon ink jet printing, it makes these ink solutions well suited for the doping of antibody solutions.

The enhanced visual identification effect can be achieved for threads B and D by adding ink to the antibody solution before thread treatment. Figure 3 illustrates the effect, showing threads (CD and MD) which have been treated with anti-D solution modified to include cyan and magenta inkjet inks. When compared to earlier results for thread D, one can see that the separation of plasma and RBCs (illustrated by the dashed lines) is much clearer with the modified antibody.

Figure 3a shows an A+ blood sample applied to thread CD, a light band can be seen between the RBCs and cyan antibody solution indicating separation of plasma and RBCs. Figure 3b shows an O- blood sample applied to thread CD, no separation is visible. Figure 3c shows an A+ blood sample applied to thread MD; a positive result is indicated by a light band. Figure 3d shows an O- sample

applied to thread MD, no separation is visible. Figure 3e shows a positive result on unmodified thread D. Figure 3f a negative result on unmodified thread D.

Figure 2 shows the mean separation distance between agglutinated RBCs and serum on threads CD and MD from ten repeated measurements to be 4.2 mm and 3.6 mm respectively. It is expected that the use of suitable dyes to enhance visual identification of blood typing results is applicable for other porous substrates.

Low-cost dosing device

Blood samples of 1 μL were deposited by micro-pipette for the examples described above. However, no necessity exists for maintaining the sample volume at exactly 1 μL to achieve accurate results. Blood samples (A+) of different volume (0.6, 1, 2 and 4 μL) were deposited onto portions of thread A (six repetitions per volume) to validate this. All threads showed positive results, with separation of agglutinated RBCs and plasma occurring. This result indicates that the use of a simple and low-cost dosing tool to deliver blood samples is feasible if sample volumes fall within the range of 0.6 μL to 4 μL . Various tools for dosing blood into the thread-based system were investigated, with cost, consistency of sample volume delivered and disposability/recyclability being of primary importance. These tools remove the requirement for more precise and expensive tools (for example, micropipettes) which are not universally available in under-developed areas or for general users.

Among these tools the use of tool having a sewing needle eye shaped aperture for sample dosing was exemplary as it was (i) able to deliver relatively consistent whole blood sample volumes (ii) easily cleaned and sterilised using a flame (iii) inexpensive to obtain (iv) easily transported and extremely portable and (v) commonplace globally. The procedure simply involves dipping the needle eye shaped aperture into a drop of whole blood from a pricked finger. Blood is drawn into the aperture and can then be transferred by pressing the aperture to the thread, which has finer capillary channels and therefore stronger liquid absorption ability.

The repeatability of this technique was assessed by measuring whole blood penetration length into untreated thread. If the thread is considered

relatively uniform then penetration distance is proportional to delivered sample volume. Samples were dosed using a tool having a needle eye shaped aperture (of aperture dimensions 0.6mm x 4.1mm) yielding an average penetration of 15.1mm with a standard deviation (s.d.) of 1.1mm from six repetitions. In
5 comparison the micropipette yielded 16.0 ± 0.5 mm (average $\pm$ s.d.) for 1 μ L blood sample and 14.6 ± 0.5 mm for 0.6 μ L (see Figure 4). This result suggests that although the needle eye method does not achieve consistency of the same degree as the expensive micropipette, it is still capable of delivering samples within the range necessary for the test. Although the metal needles used are
10 recyclable and easily sanitised, a disposable plastic device could be made which operates upon the same principal, albeit at lower cost.

Single-step method

In order to further simplify the testing process a single-step system and method was investigated which can perform the three different antibody tests
15 simultaneously. By immobilising a sample of threads A, B and D in a small square of polymer film in a criss-cross arrangement, it is possible to determine a person's ABO and Rh blood groups with a single dose of whole-blood. A sample of 2 μ L was proven to be sufficient for the test, and could easily be delivered using the needle eye technique previously described (albeit with a larger needle eye) to the
20 centre of the device where the threads intersect. As the sample wicks along each thread, antibody is chromatographically eluted along the length of the threads and away from their intersection much quicker than between different threads; hence any migration of antibody between threads is greatly reduced preventing interference to correct grouping. Figure 5 displays one possible embodiment of a
25 single step testing method. Figure 5 shows a single step blood grouping testing device, composed of thread A, B and D wedged in a square of polymer film and sharing an intersecting sample delivery zone.

In Figure 5, 51 is the anti-B treated thread, 52 is the anti-A treated thread and 53 is the anti-D treated thread. Figure 5a shows an unused device with a
30 sample delivery zone indicated within a circle.

The sample delivery zone 50 has a sample of blood applied to it. Figure 5b shows an enlargement of the right-hand side of the device illustrated in Figure

5a after whole blood has been introduced into the delivery zone in the centre via needle eye. This blood then reacts with the treated threads and forms a separation profile as shown in Figure 5b. Figure 5b is an enlarged image of the right half of the system, the separation achieved (illustrated by dashed lines) indicates the sample is of type A+. The system shown in Figure 5a was constructed simply by wedging the threads in small slits in the polymer film, but it should be noted that a sturdier device could easily be constructed by sewing or gluing the threads to the film. This device is simple to use and produce, compact and portable. A generous cost estimate of only \$0.009 USD per device suggests suitability for use in regions which are economically under-developed.

Figure 6 shows the steps involved in the use of a single step thread based blood testing device. Firstly blood is obtained from the patient. It is then collected on a delivery device, in this case the eye of a needle. The sample of blood is then placed on a delivery zone. Once the blood has interacted with the treated thread, the separation profile can be obtained and compared to known separation profiles for determining the blood type of the patient.

Longevity

The efficacy of the thread-based platform after a 4 week storage period was investigated. Two sets of samples were stored in micro-tubes wrapped in foil; one at 4°C the other at ambient temperature. Storage testing gave good results for all antibody treated thread types, suggesting that the devices could be transported and stored for a moderate period of time without degrading if sealed in airtight and light-proof packaging.

Conclusion

The results show that the use of thread as a porous substrate for ABO and Rh blood typing is viable and reduces the volume of blood required (to ~2µL) compared to conventional methods. The technique is rapid, requires only whole blood and eliminates the need for the end user to either handle other testing reagents or perform sample dilutions. The addition of dye to the antibody solution chromatographically enhances the visibility of the separation of agglutinated RBCs and plasma, making it easier for the user to identify a positive reaction. The

simple and recyclable (or disposable) metering device used enhances the simplicity of the test and further reduces the need for complicated or expensive equipment.

Although only ABO and Rh/D blood groups were tested, it is expected that
5 the platform could easily be extended to identify other groups of interest that follow similar antibody/antigen interactions.

The thread-based blood grouping system and method is simple and robust enough to be employed by the end user without assistance. It is portable, disposable, can be easily preserved and maintains a low cost of construction,
10 making it attractive for diagnostics and point of care testing especially in remote and developing regions.

Although this new platform is not expected to replace current methods practiced in the developed world, it is hoped that it may find use in remote and developing regions where access to laboratories or hospitals is unavailable.

15 As the present invention may be embodied in several forms without departing from the essential characteristics of the invention, it should be understood that the above described embodiment should not be considered to limit the present invention but rather should be construed broadly. Various modifications and equivalent arrangements are intended to be included within the
20 spirit and scope of the invention.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A system for identifying a blood group type of a blood sample, including:
at least one thread treated with an antibody;
a delivery zone on which the blood sample is delivered to the thread; and
a testing zone through which capillary wicking of the blood sample can occur;
wherein visual indications of the blood group type can be provided on the thread due to interaction of the antibody with the blood sample.
2. A system according to claim 1, wherein one said visual indication is due to the separation of agglutinated red blood cells from the serum of the blood sample.
3. A system according to claim 1 or 2, wherein one said visual indication is due to the non separation of the red blood cells from the serum of the blood sample.
4. A system according to claim 1, 2 or 3, wherein the antibody is selected from one of the following groups consisting of: A, B, or D antibodies.
5. A system according to claim 4, wherein the antibody is mixed with a water soluble dye solution prior to treatment of the thread.
6. A system according to any one of the preceding claims, including a plurality of threads, each thread being treated with a different antibody.
7. A system according to claim 6, further including a testing platform upon which the threads are affixed.
8. A system according to claim 7, wherein the testing platform is formed from a polymer film.

9. A system according to claim 6 or 7, when the threads are affixed in a generally star shaped pattern and are in contact with one another to thereby form a central delivery zone for the threads.
10. A system according to any one of the preceding claims, wherein the thread is initially given a plasma treatment to remove surface contaminants before being soaked in an antibody solution.
11. A method for identifying a blood group type of a blood sample using a system according to any one of the previous claims.
12. A blood sampling tool including a needle eye shaped aperture for sampling a quantity of blood.
13. A blood sampling tool according to claim 12, wherein the aperture has a dimension of about 0.6mm x 4.1 mm.

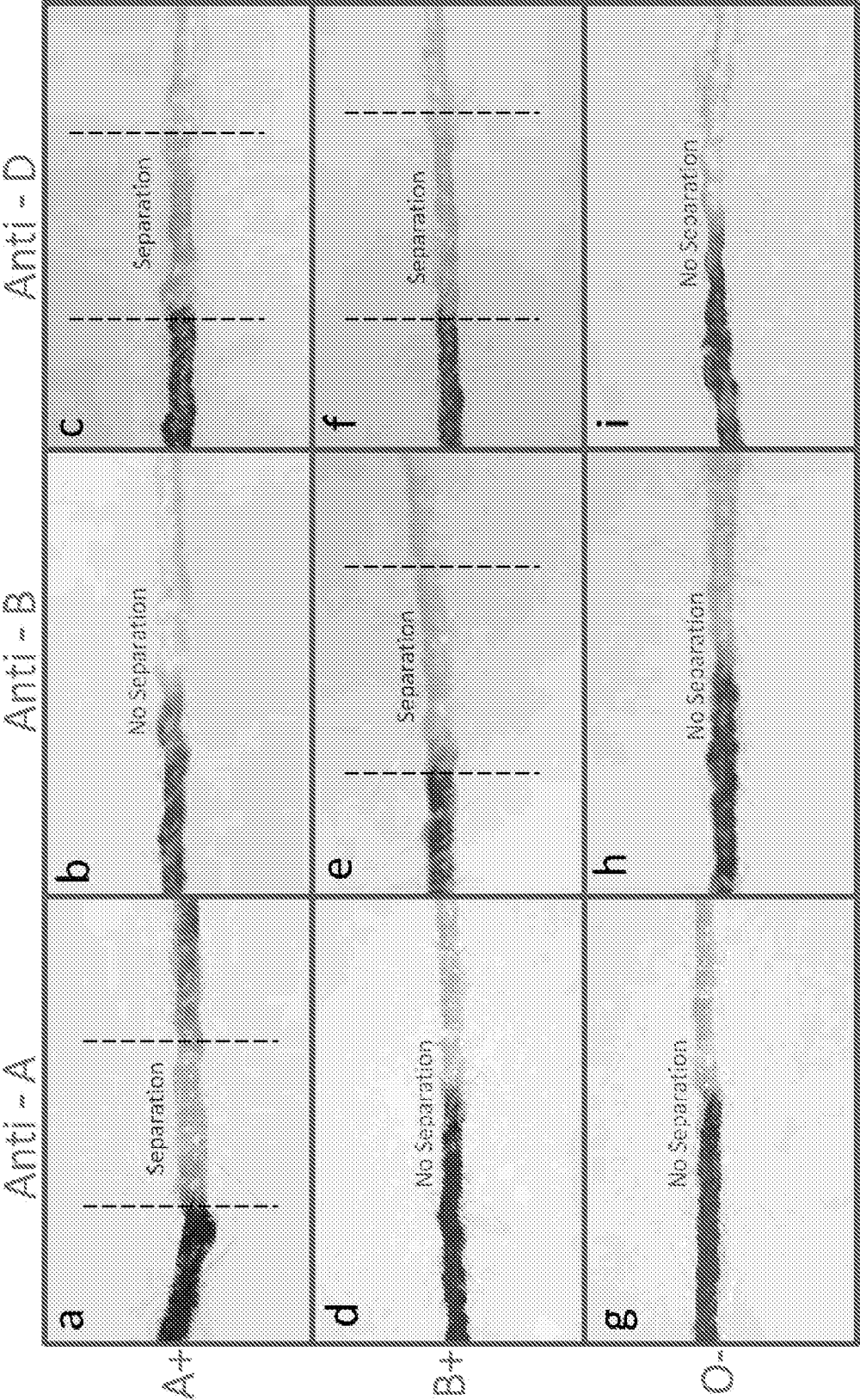


FIGURE 1

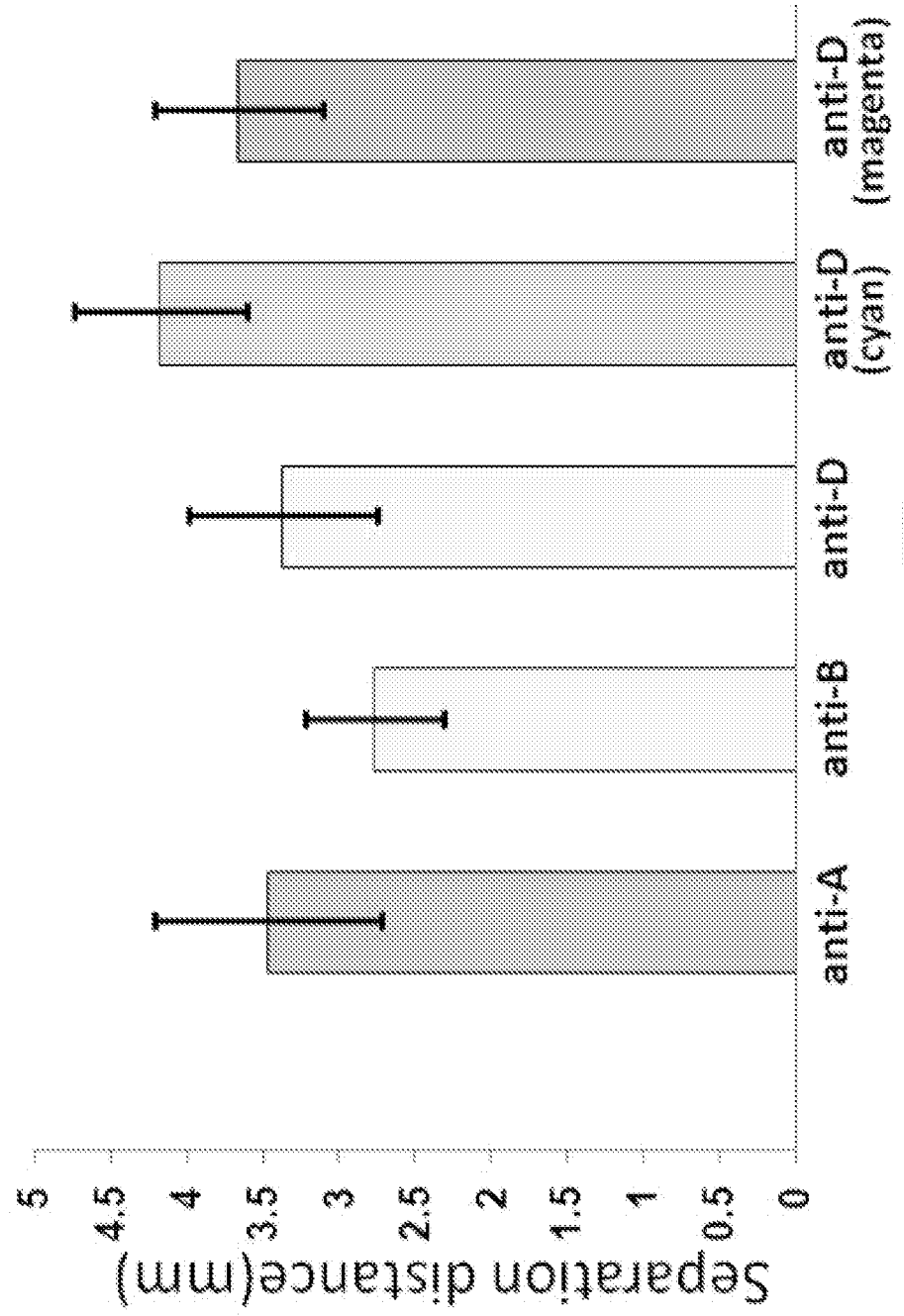


FIGURE 2

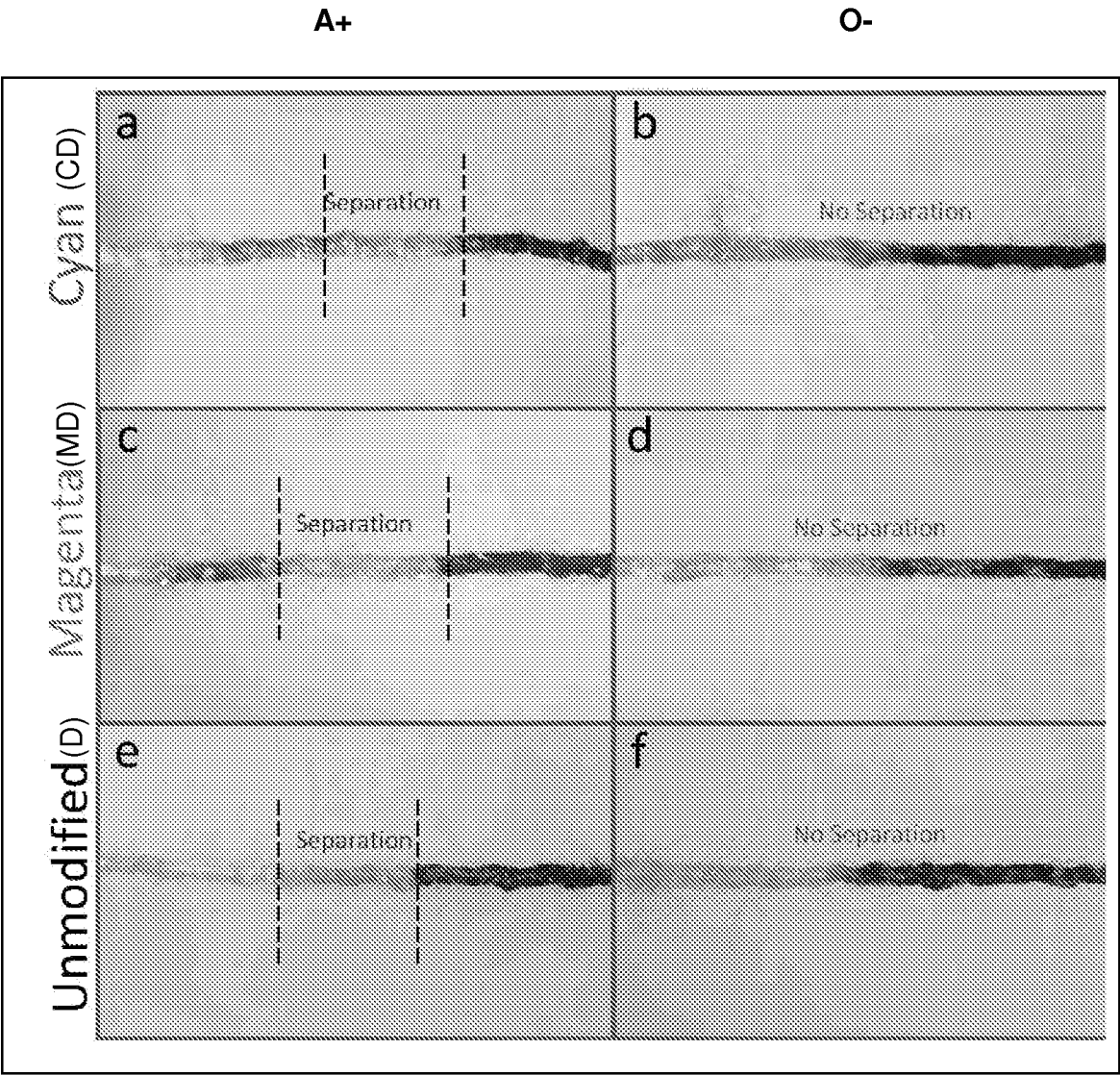


FIGURE 3

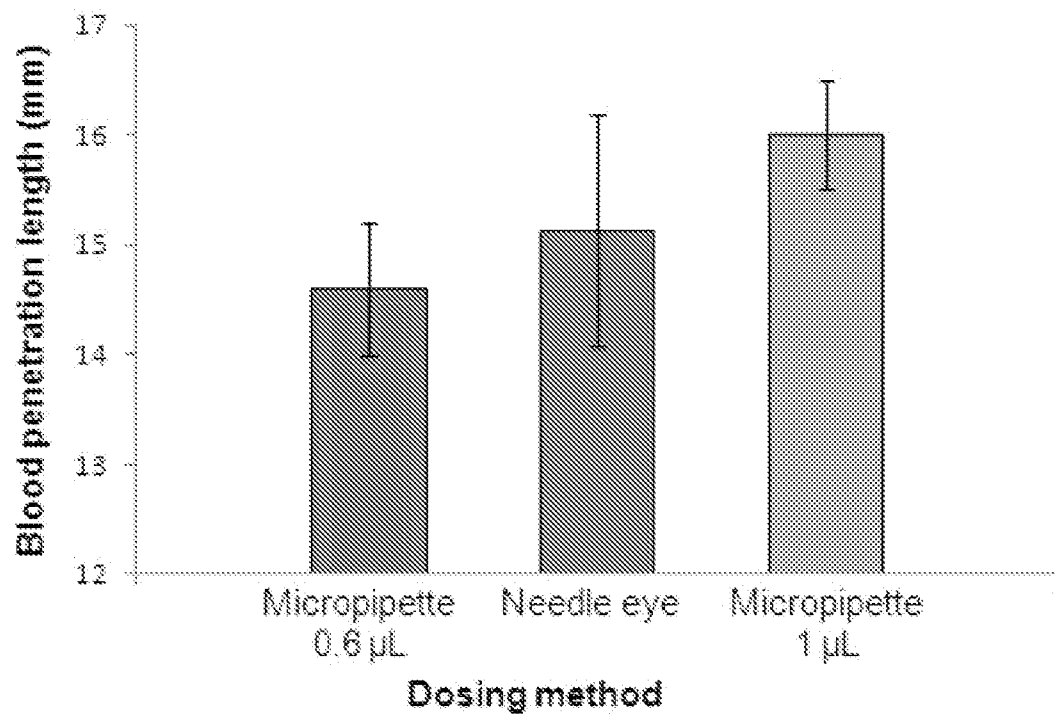


FIGURE 4

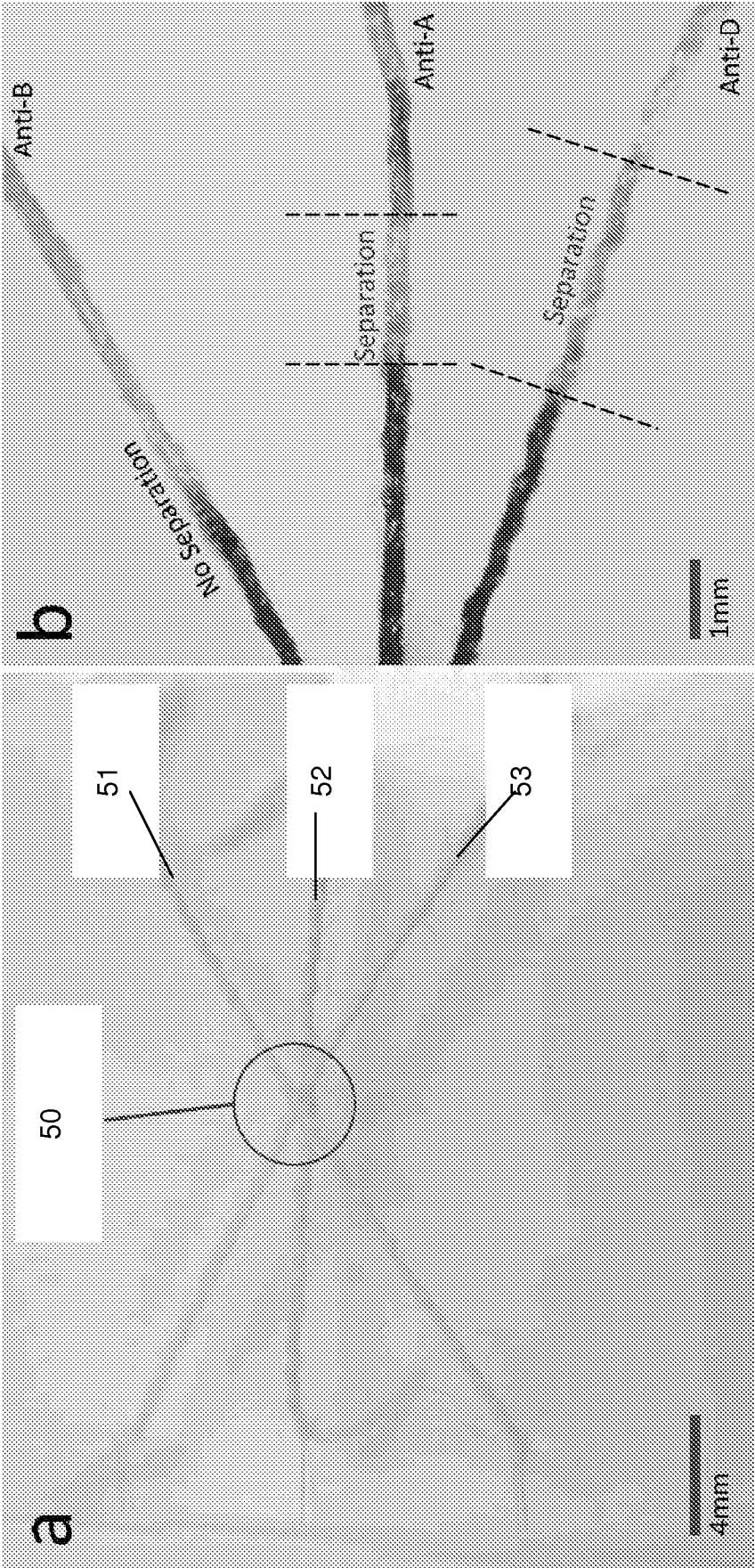


FIGURE 5

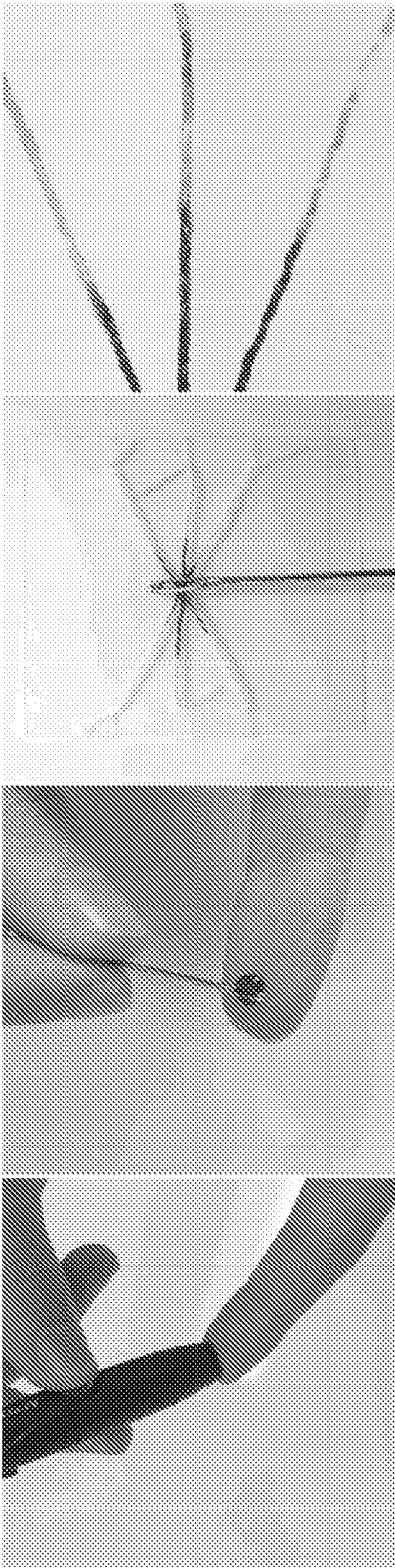


FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2011/001650

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

G01N 33/80 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPOQUE – EPODOC, MEDLINE and WPI (keywords: blood, group, type, anti, immunoglobulin, thread, porous and the like)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2000-111555 A (WAKO PURE CHEM IND LTD) 21 April 2000 See para. [0009]-[0011], [0015], [0022], [0036] and Drawings 5-11.	1-4, 6-8, 11
X	JP 2001-056341 A (WAKO PURE CHEM IND LTD) 27 February 2001 See para. [0014], [0022], [0025], [0026], [0031] and Drawings 5-10	1-4, 6-8, 11



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
27 February 2012Date of mailing of the international search report
15 March 2012Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustalia.gov.au
Facsimile No. +61 2 6283 7999Authorized officer
BRADLEY MAN
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No : +61 2 6283 2034

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2011/001650

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

See supplemental box.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-11

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2011/001650

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KHAN, M. S. et al., "Paper Diagnostic for Instantaneous Blood Typing.", Analytical Chemistry, 2010, Vol. 82, No. 10, pages 4158-4164. See page 4159, 'Blood Chromatographic Separation', 'Materials', 'Transport of the Blood / Antibody Solution on Paper', 'Results'; Page 4160, Figure 2; Page 4163, 'Paper Diagnostic for Blood Typing'; Page 4164, Figure 8.	1-11
Y	RECHES, M. et al., "Thread as a Matrix for Biomedical Assays." Applied Materials & Interfaces, 2010, Vol. 2, No. 6, pages 1722-1728. See page 1722, 'Introduction'; Page 1723, 'Choice of Thread'	1-11
P, X	BALLERINI, D. R. et al., "An inexpensive thread-based system for simple and rapid blood grouping." Analytical and Bioanalytical Chemistry, 2011, Vol. 399, pages 1869-1875. Whole document	1-11
P, X P, Y	WO 2011/035385 A1 (MONASH UNIVERSITY) 31 March 2011 Page 4, line 11-12; Page 10, Example 4; Figure 7 Page 4, line 11-12; Page 10, Example 4; Figure 7	1-6, 9-11 7, 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2011/001650

Supplemental Box

(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box No: III

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- (i) Invention 1: Claims 1-11 are directed towards a system for identifying blood group.
- (ii) Invention 2: Claims 12 and 13 are directed towards a blood sampling tool.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

The invention is defined by two independent claims, claims 1 and 12, wherein the first independent claim defines a process for identifying blood groups while the second independent claim defines an apparatus for sampling blood. An invention comprising different categories of claims, in this instance a process claim (claim 1) and an apparatus claim (claim 12), share a common special technical feature when the apparatus is specifically designed for carrying out the claimed process. However, the apparatus defined by claim 12 is not specifically directed towards the process of claim 1, but instead for the sampling of blood in general. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

It is also considered that search and examination for the second invention will require more than negligible additional search and examination effort over that for the first invention, and therefore an additional search fee is warranted.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2011/001650

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member
WO	2011035385	NONE
JP	2000111555	NONE
JP	2001056341	NONE
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.		
END OF ANNEX		