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(54) **Title:** DEVICE AND METHOD FOR IDENTIFYING AND REPORTING BLOOD SAMPLE TEST RESULTS

FIGURE 3(a)

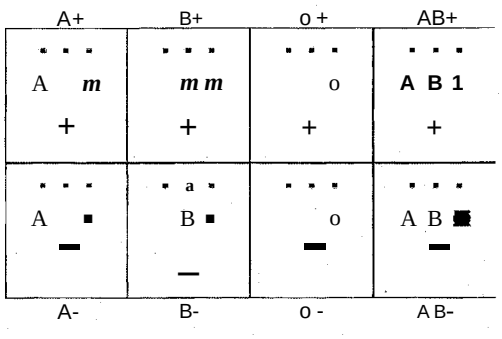
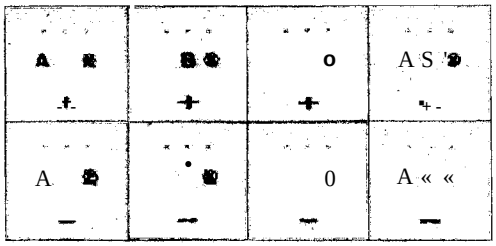


FIGURE 3(a)



(57) **Abstract: A** testing device for identifying a blood sample, including: at least one substrate having at least one testing zone on which the blood sample is delivered; the at least one testing zone having a plurality of portions, wherein a first portion has a permanent marking and at least one antibody is deposited on a second portion; and wherein the permanent marking on the first portion and the at least one antibody reacting with the blood sample on the second portion thereby results in a visual indication within the testing zone.

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DEVICE AND METHOD FOR IDENTIFYING AND REPORTING BLOOD SAMPLE TEST RESULTS

FIELD OF THE INVENTION

[0001] The present invention is directed to a device and method for identifying a blood sample and the reporting of those results. While the invention will be described with specific reference to its use in determining a person's blood type, it is to be appreciated that other applications of the invention are also envisaged.

BACKGROUND TO THE INVENTION

[0002] The use of patterned bioactive paper as a tool for biochemical analysis has become a platform for making low-cost and user-operated devices for diagnosis, point of care (POC), pathogen and biomarker detection, food and drinking water quality testing, etc. Recent developments in patterned bioactive paper research have attracted much attention from researchers and industries because of its potential to provide affordable, equipment-free, sensitive, specific, and user-friendly diagnostic sensors for disease screening, healthcare and drinking water quality evaluation to the developing countries. Whilst biochemistry know-how for diagnoses of most major diseases is available in the developed world, challenges of building these diagnostic chemistries into low-cost sensors and ensuring that they function under conditions of remote and developing regions still remain, since even the basic medical facilities may not be available.

[0003] From the end of the last decade, there has been an explosion of research in bioactive-paper-based low-cost sensor fabrication, electronic transmission of colorimetric assays for real-time diagnosis, as well as new diagnostic and environmental applications using low-cost sensors. This research has presented new concepts and possibilities of using colorimetric and electrochemistry means to perform qualitative and semi-quantitative tests for healthcare, POC and environmental applications. Colorimetric and

electrochemical methods are the preferred analytical approaches for bioactive-paper-based sensors; this is because these methods are proven to be effective in qualitative and semi-quantitative sensing and can be easily adapted onto paper. Paper is white and can be conveniently used as a low-cost substrate for colorimetric reporting of the diagnostic results. Recent research also has found that paper does not present significant interference to electrochemical measurements and therefore is a suitable substrate to support electrochemical analysis. However, those studies also revealed certain limitations of these new concepts in their practical sensing applications. Whilst a colorimetric or voltametric signal can report the testing results, in most situations such results need to be interpreted by trained personnel. This is particularly true if an assay has multiple outcomes and requires careful examination in order for the results to be concluded. Therefore, among the many challenges in low-cost diagnostics, unambiguous reporting of the testing results by the sensors to the users is critical. Sensors can be fabricated that are robust enough to function under an unsupported field condition, for use in developing regions for large-scale disease screening. However, misinterpretation of the assay results, by inexperienced personnel may still be a significant factor that can compromise the value of low-cost diagnostics.

[0004] There are other means by which bioactive paper sensors for diagnostic results can be designed and implemented.

[0005] POC devices of moderate costs ($10 -$50) that can report test results in text are commercially available now, but current text-reporting interfaces are built for electronic displays, with the best-known examples being glucose monitoring devices and single-use pregnancy testing devices. Equipment-free bioactivepaper-based diagnostic sensors capable of reporting multiple conditions in written text from a single test are not commercially available.

[0006] It is an object of the present invention to provide a low-cost bioactive paper-based blood-typing device that is capable of reporting ABO RhD blood types rapidly and in written text.

[0007] 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

[0008] According to a first aspect of the present invention there is provided, a testing device for identifying a blood sample, including: at least one substrate having at least one testing zone on which the blood sample is delivered; the at least one testing zone having a plurality of portions, wherein a first portion has a permanent marking and at least one antibody is deposited on a second portion; and wherein the permanent marking on the first portion and the at least one antibody reacting with the blood sample on the second portion thereby results in a visual indication within the testing zone.

[0009] Preferably the visual indication of the test result in the above device is text.

[0010] The permanent marking may be formed by non-bioactive water insoluble ink. Further, the permanent marking may be formed by printing of the non-bioactive water insoluble ink.

[0011] The at least one antibody is preferably selected from one or more of the following: A, B, or D antibodies for determining blood type.

[0012] The substrate of the testing device is preferably formed from paper or other fibrous material, more preferably paper towel.

[0013] According to a second aspect of the present invention there is provided a method for identifying a blood sample using a testing device as described above.

[0014] According to a third aspect of the present invention there is provided a method for identifying a blood sample including the steps of: printing the permanent marking using non-bioactive water insoluble ink on the testing zone; printing the at least one antibody on the testing zone; depositing the blood sample on the testing zone; washing the testing zone with saline; and identifying the blood sample by the resultant visual indication within the testing zone.

[0015] Preferably the method for identifying a blood sample as described above uses a testing device as described above.

[0016] The method for identifying a blood sample as described above may further include detecting blood type or illness from the visual indication.

[0017] A method for identifying a blood sample according as described above further including transmitting through a wireless communication means the visual indicator which identifies the blood sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[001 8] It will be convenient to further describe the invention with respect to the 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:

[0019] Figures 1(a) and 1(b) show schematics of the composite text symbols design for reporting blood test results in text of a preferred embodiment of the testing device of the present invention.

[0020] Figure 2 shows fabrication and testing procedures of text-reporting blood-typing devices, according to a preferred embodiment of the present invention.

[0021] Figure 3(a) shows a schematic of the expected reports of all eight blood types by the testing device, according to a preferred embodiment of the present invention; Figure 3(b) shows a photograph of the actual blood type test results of all eight blood types.

[0022] Figure 4 shows schematics of text symbols for reporting diagnostics for minor blood group typing.

[0023] Figure 5 shows a photograph of the actual blood-type test results of the minor blood groups using a device according to another embodiment of the present invention.

[0024] Figure 6(a) shows a text-reporting blood-typing device, according to another embodiment of the present invention; Figure 6(b) shows a photograph of the actual blood type test results using the device shown in Figure 6(a).

[0025] Figures 7(a) and 7(b) show a text-reporting blood-typing device according to another embodiment of the present invention.

[0026] Figures 8(a) and 8(b) show a photograph in which Figure 8(a) shows an haemagglutination reaction has occurred and Figure 8(b) shows that no haemagglutination reaction has occurred.

[0027] Figure 9 shows a fabrication procedure of porosity contrast on a porous substrate.

[0028] Figure 10 shows another text-reporting blood-typing device according to an embodiment of the present invention. Specifically, Figure 10(a) shows the

fabrication of the text-reporting blood-typing device for reverse blood typing; Figure 10(b) shows the procedure and diagnostic results of the text-reporting blood-typing device; and Figure 10(c) shows a further text-reporting blood-typing device according to another embodiment of the present invention.

DESCRIPTION OF PREFERRED EMBODIMENT

[0029] Correct typing of human blood is extremely important in blood transfusions and in events of medical emergency. The blood type of an individual is determined by the presence or absence of certain antigens on the surface of a red blood cell (RBC). On the other hand, antibodies that exist in blood serum protect the body from incompatible and hostile antigens. According to Landsteiner's Law, when an RBC possesses certain antigens on its surface, the corresponding antibody is absent in the blood plasma and vice versa. Therefore the vast majority of techniques for ABO and RhD typing of blood to date have been based upon the principle of haemagglutination reactions between RBCs and antibodies. The absence of agglutination indicates no haemagglutination reaction. Recently low-cost bioactive paper and bioactive thread microfluidic sensors have been reported for human blood grouping applications. These devices are also based on the principle of haemagglutination reactions. Through observing the differences in wicking distances of the agglutinated red blood cells and the blood serum, an indication of haemagglutination reaction can be identified. However, paper-and thread-based technologies still require trained nurses or users who have the blood typing knowledge to interpret the result. In order for the blood typing devices to have even wider applications in developing regions, they will need to report results unambiguously to users who may not have the knowledge to interpret the results based on the first principle.

EXAMPLES:

Materials, reagents and blood samples

[0030] The paper substrate employed in this study was Kleenex paper tower (Optimum, Kimberly Clark). Kleenex paper tower is made with primarily softwood fibres; it has large interfibre pore sizes compared to filter paper. Experimentation

has confirmed that non-agglutinated RBCs can be more easily washed out of a more open paper sheet than a dense paper sheet such as filter paper.

[0031] Alkyl ketene dimer (AKD) was received from BASF as a papermaking ingredient. AKD is a cellulose reactive chemical reagent. It is used to hydrophobize cellulose fibre and paper. AR grade n-heptane was obtained from Aldrich; it was used to formulate an ink-jet-printable solution for patterning text on paper by forming a strong hydrophilic-hydrophobic contrast.

[0032] Blood samples were sourced from a pathological laboratory in Melbourne, Australia, and were stored at 4°C for use within five days of withdrawal. The blood types of all samples were determined by the pathological laboratory using the current mainstream blood typing technology. The blood type information was used for the purpose of comparison with the results obtained in this experiment following the ethical protocol.

[0033] Non-commercial grouping antibodies, Anti-A, clone 10090; Anti-B, clone 10091 and Anti-D, clone 20093, were received from Lateral Grifols. They were used as received. A saline solution containing 0.9% (w/v) and NaCl was prepared with MilliQ water and AR grade NaCl (Univar); it was used as the washing solution to remove only the non-agglutinated RBCs from the text patterns, but not the agglutinated RBCs (see below).

Device fabrication and operation

[0034] A digital pattern of the device was generated electronically. A reconstructed Canon ink jet printer (Pixma iP3600) was used to print the AKD-heplane solution onto a Kleenex paper sheet followed by a heat treatment to cure the hydrophobic effect. After the formation of the text patterns on paper, 2.5 µL of antibody solutions were introduced into the corresponding text patterns with a micropipette (Eppendorf research®, 0.1-2.5 pL) to complete the device fabrication. The pattern design for the device used for this example can be seen in the Figures.

[0035] Three microlitres of a blood sample were introduced into the text patterns defined by AKD printing and treated by the addition of antibody solutions with a micropipette. Twenty seconds were allowed for the antibodies to react with the antigens carried by the RBCs inside the text patterns. After 20 seconds, two 50 µL aliquots of saline solution were introduced into patterns to wash out the non-agglutinated RBCs. After washing, the text which identifies the blood type can be seen from the device.

Device design

[0036] The text-reporting blood grouping devices identified and described in the examples are partly based on the use of haemagglutination reactions. The fabrication of these devices involves the use of strong hydrophobic-hydrophilic contrast to form text patterns and to use these text patterns as sites for RBC and antibody interactions. Such a hydrophobic-hydrophilic contrast is necessary, as it will ensure unambiguous legibility of the text pattern of agglutinated blood to be displayed. In the design used in this example the text patterns are made hydrophilic and antibody solutions are introduced into the corresponding text patterns (i.e. Anti-A into text pattern "A", etc.). To clearly identify the occurrence of haemagglutination reactions inside the hydrophilic text patterns, a saline-washing step is employed. If the antibody in a text pattern is not the corresponding antibody to the antigens carried by RBCs, there will be no haemagglutination reaction; the non-agglutinated RBCs can be easily washed out of the text pattern with the saline solution. Contrary to this, if the RBCs have a haemagglutination reaction through the antibody-antigen interaction, agglutinated RBCs will form inside the fibre matrix of the paper and cannot be washed out by the saline solution. Those text patterns occupied by the agglutinate RBCs therefore have unambiguous legibility with high resolution and contrast.

Logic in displaying blood type results using text

In devices for ABO RhD blood typing, three antibodies (Anti-A, Anti-B and Anti-D) can be used to display blood typing results by text. The interaction of each antibody with RBCs can have two possible outcomes, i.e. "Agglutination" and "No agglutination". The total number of blood types determinable by the three

antibodies will be $2^3 = 8$. Taking an A-positive blood sample for example, the interaction of the A-positive blood sample with the three antibodies can be expressed as A $\bar{B}$D i.e. **A** (agglutination with antibody A occurs) $\bar{B}$ (No agglutination with antibody B) D (agglutination with antibody A occurs). Table 1 below shows the blood types that can be reported in text by specific antigen-antibody agglutination reactions before and after implementing the experimental design solutions of the testing device. Following this notation, table 1 shows all different blood types (column iii) determinable by these antibodies through the presence and absence of RBC agglutination (column ii). Column (i) in table 1 identifies the blood type test number; column (ii) identifies with which antibodies agglutination occurs (a line above a letter indicates that no agglutination occurred with that antibody); column (iii) identifies the blood type determined by RBC agglutination; column (iv) identifies RBC antigens which cannot be expressed in text by RBC agglutination and the reasons in brackets why they cannot be expressed; column (v) identifies RBC antigens that cannot be expressed in text after implementation of design solution 1, that is for Rh- blood samples; column (vi) identifies those states that can be expressed in text after implementation of both design solutions 1 and 2.

TABLE 1

i	ii	iii	iv	v	vi
no.	Agglutination or non-agglutination observations	Blood types determined by RBC agglutination	RBC antigens CANNOT be expressed by text by RBC agglutination (and reasons)	RBC antigens CANNOT be expressed in text after implementing design solution 1	States CAN be expressed in text after implementing design solutions 1 & 2
1	$AB\bar{D}$	AB+			✓
2	$A\bar{B}\bar{D}$	A+			✓
3	$AB\bar{D}$	AB-	× $(\bar{D})$		✓
4	$A\bar{B}\bar{D}$	A-	× $(\bar{D})$		✓
5	$\bar{A}B\bar{D}$	B+			✓
6	$\bar{A}\bar{B}\bar{D}$	O+	× $(\bar{A}, \bar{B},)$	× $(\bar{A}, \bar{B},)$	✓
7	$\bar{A}B\bar{D}$	B-	× $(\bar{D})$		✓
8	$\bar{A}\bar{B}\bar{D}$	O-	× $(\bar{D}, \bar{A}, \bar{B},)$	× $(\bar{A}, \bar{B},)$	✓

[0037] If text reporting is made only by the occurrence of haemagglutination reactions, many blood types cannot be unambiguously reported by text. This situation includes two circumstances where blood types have no haemagglutination reactions with the grouping antigens; therefore no visually perceivable text patterns can be formed to make the text report the identified blood type. The first circumstance involves all Rh- blood types blood samples; their interactions with Anti-D result in no haemagglutination reaction (i.e.$\bar{D}$); these interactions therefore cannot form visually identifiable text patterns. The second circumstance involves O-type blood samples. Since red cells of O-type blood do not carry A and B antigens, they do not have haemagglutination reactions with either Anti-A or Anti-B (i.e., they can be denoted as $(\bar{A}\bar{B})$. O-type blood cannot be identified or reported in written text formed by a haemagglutination reaction only. Column (iv) in table 1 lists all five blood types that are affected by these two circumstances. The testing device therefore had to be designed further to overcome the aforementioned disadvantages.

Design solution 1 - Text reporting for Rh- blood samples

[0038] A design of a composite text symbol that can report both D and $\bar{D}$ unambiguously was developed. This composite text symbol take the form of "+"; it consists of a permanent non-bioactive "-" printed using a water-insoluble ink and a bioactive "-" printed using Anti-D (Figure 1 (a)). In a blood test when a sample is introduced into "I", the sample is mixed with Anti-D inside "I". If the sample carries RhD antigen, then haemagglutination reaction will occur inside "I" and saline washing will not be able to remove the agglutinated RBCs out of pattern "I". The composite text symbol will report "+". On the other hand, if the sample does not carry RhD antigen, then no haemagglutination reaction will occur. After saline washing, no agglutinated blood sample will be perceivable in "I"; the composite text symbol will report "-" (Figure 1 (a)). This design fulfils the requirement of using one composite pattern to unambiguously report Rh+ and Rh- in text.

[0039] Figure 1 shows the schematics of the designs of the composite text symbols for reporting blood test results in text. Figure 1(a) shows the composite text symbol for reporting the presence of RhD takes the form of "+"; it consists of a permanent V printed using water-insoluble ink and a "I" printed using Anti-D. Figure 1(b) shows the composite text symbol for reporting "0" and "non-O" types of blood samples takes the form of "®"; it consists of a permanent letter "0" printed using non-bioactive water-insoluble ink and a "X" printed using an equal-volume mixture of Anti-A and Anti-B.

Design solution 2 - Text reporting for O-type blood samples

[0040] Since O type blood samples do not have A and B antigens, they correspond to $(\bar{A}\,\bar{B})$, (i.e. they do not interact with the A- and B-antibodies). This second design again uses another composite text symbol to report both "O" and "non-O" types of blood samples. This composite text symbol takes the form of "®"; it consists of a permanent letter "O" printed using water-insoluble ink and a "X" printed using an equal-volume mixture of Anti-A and Anti-B (see Figure 1(b)). In a blood test when a sample is introduced into "X", the sample is mixed with Anti-A and Anti-B inside "X". If the sample carries A-antigen or B-antigen, or both A- and B-antigens, then haemagglutination reaction will occur inside "X" and

saline washing will not be able to remove the agglutinated RBCs out of the pattern. The composite text symbol will report "®". On the other hand, if the sample does not carry A-antigen and B-antigen, then no haemagglutination reaction will occur. After saline washing, no agglutinated RBCs will be perceivable in "X"; the composite text symbol will report "0" (Figure 1(b)). This design fulfils the requirement of using one composite pattern to unambiguously report "O" and "non-O" types of blood samples in text.

Device fabrication procedure

[0041] A negative text patterns consisting of letters "A", "B" of font size 20 and two text symbols, "X" and "I", were created electronically. A reconstructed Canon ink jet printer was used to print a 2% (w/v) AKD-heptane solution on to the paper sample, forming the negative patterns of letters and symbols. AKD is a cellulose reactive reagent used in papermaking industry; it has a 4-member lactone ring connected to two saturated hydrocarbon chains of C_{16} to C_{20}. When AKD is introduced onto cellulose fibres, its four-member lactone ring reacts with an OH group, imparting strong hydrophobic effect to the cellulose fibre surface with its two long hydrocarbon chains. Printing of negative patterns of letters and symbols ensures that these patterns remain hydrophilic and are surrounded by hydrophobic areas as shown in Figure 2(a). The hydrophilic patterns are, however, invisible. After printing of the hydrophilic patterns, letter "0" was printed using a water insoluble ink over the hydrophilic and invisible "X" symbol; symbol "-" was also printed with a water insoluble ink over the hydrophilic and invisible "I" symbol as shown in Figure 2(c).

[0042] Figure 2 shows the fabrication and testing procedures of the text-reporting blood-typing devices. Figure 2(a) shows the negative digital patterns of letters and symbols that are printed using an AKD-heptane solution; letters and symbols remain hydrophilic and are surrounded by hydrophobic areas. Figure 2(b) shows the step when anti-A and anti-B are introduced into the corresponding text pattern. An equal-volume mixture of Anti-A and Anti-B is introduced into "X", and Anti-D is introduced into "I". Figure 2(c) shows that the letter "0" and symbol "-" are printed over "X" and "-", respectively. Figure 2(d) shows what occurs when

a blood sample is introduced in the testing device for determining the blood type of the sample. Figure 2(e) shows the result after washing each pattern with 50 µL of saline solution. As can be seen, the blood type result is reported by the device in text.

[0043] Three microlitres of Anti-A, clone 10090; Anti-B, clone 10091 and Anti-D, clone 20093 were introduced into the hydrophilic patterns "A", "B" and "i" respectively by printing with the ink jet printer or writing with a pen (Figure 2(b)). Three microlitres of an equal-volume mixture of Anti-A and Anti-B was introduced into the pattern "X" (Figure 2(c)). Since the hydrophilic letter and symbol patterns were surrounded by the AKD-hydrophobized area, antibody solutions are restricted inside the corresponding patterns only. The strong hydrophilic-hydrophobic contrast ensures a high resolution of the text patterns formed by antibody solutions. After drying under an ambient condition the device is ready for use.

Sample introduction and result reporting

[0044] Three microlitres of blood sample was introduced into each of the hydrophilic patterns (i.e. A, B, X and I) with a micropipette. Twenty seconds were allowed for the antibodies in the letter and symbol patterns to react with the antigens carried by the RBCs. After 20 seconds, a 50 µl_ aliquot of saline solution was introduced into each of the letter and symbol patterns. In cases where haemagglutination reactions occurred in certain text or symbol patterns, the agglutinated blood sample could not be washed out of those patterns. The agglutinated RBCs formed legible text patterns that report the occurrence of the specific haemagglutination reaction. On the other hand, if the haemagglutination reaction does not occur, the non-agglutinated blood can be readily washed out of the patterns through the vertical flow, leaving almost no visible trace of the blood sample. The legible letter and symbol patterns, in combination, form the text which identifies and reports the blood type of the sample. Figure 3(a) shows the schematic of the expected reports for the eight blood types by the device. The corresponding blood types are given to assist the correlation by the reader.

Figure 3(b) shows a photograph of the results of the actual blood type tests of all eight ABO RhD blood types. No other text assistance is given.

[0045] Forty blood samples were tested using the device described in the examples above; all tests identified the blood types unambiguously without exception and thus provided accurate text reports for each blood type. It is expected that an expansion of text-reporting bioactive paper sensors beyond the ABO RhD blood typing application may be possible.

[0046] To design equipment-free devices to report blood grouping results in written text is an easy way to bridge the gap with analysing, interpreting and reporting test results, in particular a person's blood type. The present invention obtains a test result based on first principles, and then reports the result in written text to the user. Furthermore, the present invention is a low-cost diagnostic device which can report test results in written text. A preferred embodiment is a bioactive-paper-based blood typing device. By combining the functional printing and paper-based microfluidics techniques, a device with a simple design that can report ABO and RhD blood groups rapidly and unambiguously in written text has been designed. A preferred embodiment of the present invention, being a bioactive-paper-based device capable of reporting test results in text will significantly enhance the capability of low-cost diagnostics through reducing incidents of misinterpretation of the test results.

Use of composite symbols containing bioactive and non-bioactive reactions to report minor groups

[0047] There are 30 blood types identified in human blood. Apart from ABO and RhD groups, pathological tests also routinely test D^{VI}, C, E, c, e, K, k, Fya, Fyb, Jka, Jkb, M, N, S, s, P1, Lea, Leb, Lua, Lub. These blood types are referred to as minor groups. Paper-based assays can be fabricated to perform assays of the minor groups. Figure 4 shows the text-reporting diagnostics for minor grouping.

[004δ] Similar to the text-reporting diagnostics for RhD groups, the text-reporting blood-typing device for minor groups is also partly based on the principle of haemagglutination reactions. In order to unambiguously report minor blood groups, a composite text symbol "+" is used. This symbol consists of a permanent "-" printed using a non-bioactive water-insoluble ink and a hydrophilic "I" encircled with a hydrophobic barrier which is printed using AKD. Then, an antibody solution of minor group was added into "|" to makes the pattern bioactive for testing corresponding minor blood group. A label was printed using inks above the text symbol "+" to indicate the minor blood types to be tested. In a blood test for minor groups, a sample is introduced into "|". If the sample carries corresponding antigen, haemagglutination reaction will occur inside "|" and saline washing will not be able to remove the agglutinated RBCs out of pattern ")". The composite text symbol will report "+". On the other hand, if the sample does not carry the corresponding antigen, then no haemagglutination reaction will occur. After saline washing, no agglutinated blood sample will be perceivable in "|"; the composite text symbol will report "–". This device design fulfils the requirement of using one composite pattern to unambiguously report whether the red blood cells in a sample contain (+) or do not contain (-) certain minor group antigen. Figure 5 shows the actual testing results of a series of minor blood groups for one blood sample using the paper-based text-reporting device described. Testing showed that this blood sample is positive for c, e, k, Jka, Jkb, N, S, P1, Lea, but is negative for C, E, K, Fya, M, Leb and Lua. This test result is consistent with an independent test of the same blood sample by the Australian Red Cross laboratory using the mainstream blood typing technology.

A simplified sample introduction method for blood typing (major and minor groups)

[0049] Blood samples used for blood typing for ABO and RhD groups need to be introduced into hydrophilic and antibody-loaded patterns of letters and symbols, i.e. "A", "B", "X" and "I". To complete the sample introduction, the user needs to perform four sample transfers into the above four different patterns.

[0050] The following example, shown in Figures 6(a) and 6(b), describes a simplified method that enables the user to fill more than one hydrophilic pattern (zones) with a single blood sample dose. Figure 6(a) shows a text-reporting blood-typing device that uses only a single blood sample dose. The grey area on the device is hydrophobic, and the white area is hydrophilic. The white circular area is part of the testing zone on which a blood sample is delivered, and the six white bars are six hydrophilic channels containing different antibodies forming another part of the testing zone. The darker bars, perpendicular to the white bars, are printed using water insoluble inks. In this device, letters are also printed using water insoluble inks to indicate the corresponding antibodies inside the channels adjacent to the letters (i.e. Anti-A in channel adjacent to letter "A"; Anti-B in channel adjacent to letter "B"; Anti-D in channel adjacent to letter "D", etc.). The substrate, in this example paper, is folded to ensure that the circular hydrophilic zone is in contact with all six hydrophilic channels (see the right hand side of Figure 6(a)). When a drop of a blood sample is added onto the testing zone, also referred to as a sample introduction zone (circle), it penetrates through the circle, then when the substrate is folded, the blood sample flows intb the hydrophilic channels in contact with the circle. If the red blood cells of the blood sample carry a corresponding antigen to an antibody in the channel, haemagglutination reaction will occur inside the channel and saline washing will not be able to remove the agglutinated RBCs out of channel. A visible symbol "+"will be seen. This visible symbol, together with the letter adjacent to it, reports the presence of the antigen on the red blood cell of the sample. On the other hand, if the haemagglutination reaction does not occur inside the channel, a visible symbol of "-" will be seen. The “−”, together with the letter adjacent to it, reports the absence of the antigen on the red blood cell of the sample. Figure 6(b) shows a photograph of an actual blood-typing test using this device.

Text-reporting blood-typing device with rotatable sample delivery zone

[0051] Figure 7 shows another text-reporting blood-typing device. This device includes a rotatable sample delivery zone. It can fill more than one zone with a single blood sample introduced into the testing zone. As shown in Figure 7(a), the testing zone can rotate. Figure 7(a) shows the device with the sample

delivery zone in a rotated, unaligned position. Thus this device can store the sample and not initiate a reaction or commence testing until the rotatable zone is aligned in the device (as shown in Figure 7(b)) allowing the blood or other sample to pass into the testing zone and initiate a reaction. Therefore it can deliver the blood sample for testing, as and when required.

Alternative material for making text-reporting device

[0052] There are many kinds of !ow-cost materials such as paper, film, cloth, fabric etc., which can be used as the substrates for fabricating text-reporting devices. Figure 8 shows that haemagglutination reaction can occur on a non-woven fabric substrate and that saline washing cannot remove the agglutinated RBCs from the substrate. However, the non-agglutinated RBCs can be easily washed off. Based on the significant visual difference, these substrates can be used to fabricate different kinds of blood-typing devices for reporting both major and minor blood groups in text. The text patterns can be fabricated on the materials by using either hydrophobic-hydrophilic contrast, or by using the porosity contrast. Treatments such as solvent printing and hot die stamping, but not limited to these methods, can be used to create porosity contrast on fibrous substrates.

[0053] One method of using printing technology to print fibre mat can generate porosity contrast of fibre mat (as shown in Figure 9). In a solvent-printed area, the fibres are dissolved by the solvent and form a less porous or non-porous area. The non-printed area remains porous. In this way, antibody solution can be absorbed into the non-printed area to form a device for blood typing assay.

[0054] Another method uses a heated metal die with engraved pattern on the surface of the die. When the heated die is put in contact with a polymer fibre mat, the high temperature on die surface will melt the fibre, making it non-porous. However, because an engraved pattern cannot contact the fibre mat, the fibres under the engraved pattern will not be melted and remain porous. In this way, a

contrast of porosity can be generated to absorb liquid into patterns. Text patterns so created can therefore report blood-typing assay in text.

Text-reporting diagnostics for reverse blood typing

[0055] The concept of text-reporting can be used not only for forward blood-typing to identify the types of antigens on the red blood cell surface, but also for reverse blood-typing to test the types of antibodies in the blood serum. The above described blood-typing assay is forward blood typing assay, in which known antibodies are used to detect the presence and the absence of the antigens on the red blood cell surfaces. Different from the forward blood-typing, a reverse blood-typing uses the purified red blood cells that carry known antigens as reagents to detect the presence of antibodies in the serum of blood sample. The serum of A-type blood contains antibody B; the serum of B-type blood contains antibody A; the serum of O-type blood contains both antibody A and B; and the serum of A, B blood contains neither antibody A nor B. In a reverse typing of blood types A, B and A, B and 0 , purified red blood cells carrying antigens A_1 and B are used as the reagents to detect the presence or absence of the corresponding antibodies in the serum. For the ABO group system, the identification the types of antibodies in the serum, the antigens on the red blood cells can be confirmed. For reverse typing of a type-O blood, purified RBCs reagents are used to detect the presence of both antibodies A and B in the serum. Figure 10 shows the design and the use of a text-reporting device for reverse blood-typing. This device includes a printed letter (i.e. A or B) using water-resistant ink. The letters are printed inside hydrophilic circles. When a red blood cell suspension is introduced into the circular testing zone, it will penetrate into the area within the circle and cover the whole testing area. Then, the device is ready to determine the correspondent antibody in the serum (Figure 10(a)). In a reverse blood typing, a sample (serum) is introduced and spreads over the whole testing zone so that it mixes with the red blood cells reagent. A saline wash will follow to confirm if haemagglutination reaction has occurred. If the sample carries the antibody corresponding to the antigen on the surface of the red blood cell, haemagglutination reaction will occur inside the test zone (circle) and saline washing will not be able to remove the agglutinated red blood cells. The printed

text/letter (i.e. A or B), therefore, cannot be visually seen. On the other hand, if the sample does not carry the corresponding antigen, then no haemagglutination reaction will occur. After saline washing, the printed text/letter symbol will reappear on the testing zone (Figure 10(b)).

[0056] The interpretation of the result follows the logic illustrated in Figure 10(a). When red blood cells carrying antigen A_1 is used, and if no agglutination occurs, then the blood serum sample does not have antigen A. If antibody A is absent from the serum, then the red blood cell surface must have antigen A. Therefore the reappearance of letter "A" on the paper confirms that the blood sample is an A-type.

[0057] The forward blood-typing device and reverse blood-typing device can be engineered on one substrate. An example of the combined device can be seen in Figure 10(c). Reverse blood-typing is only required for the ABO system, not for the RhD system.

[0058] Bioactive paper-based text-reporting devices should meet the basic requirements of text legibility and the capability of report all possible outcomes of a test. Several design considerations are taken to fabricate the bioactive paper-based text-reporting device for blood typing tests to meet these requirements. First, a design using the paper-based vertical microfluidic concept was adopted to facilitate a rapid and thorough saline washing step. In this example the saline solution penetrated through the paper sheet, instead of wicking laterally along the paper sheet. With another sheet of absorptive paper placed underneath the text pattern, saline washing can be carried out rapidly. Another advantage of using vertical microfluidic design on paper, instead of lateral microfluidic design is that the non-agglutinated RBCs can be removed from the device onto the absorptive sheet underneath the device. This gives the device a brighter background to report the test result in text. It is more difficult for the lateral microfluidic design to provide a bright background, because non-agglutinated RBCs will remain on the device surface. Liquid penetration into and through a paper sheet has been a subject of numerous studies; the vertical penetration of liquid through a sheet of

paper is always accompanied by a degree of lateral penetration. The design solution adopted in this example was to use AKD to generate patterns, which facilitate vertical liquid penetration by significantly suppressing the lateral penetration. Such a treatment has been shown to allow the vertical transportation of liquid with very little lateral spreading. This design ensures a high degree of legibility of text patterns formed for result reporting.

[0059] Second, composite text patterns are designed to ensure that the device is capable of reporting all testing outcomes of the ABO RhD blood typing. As discussed above, the use of haemagglutination reaction is unable to report negative results where there is no haemagglutination reaction. Thus, from the experiment described above, suitable device designs using combinations of permanent and bioreactive patterns can solve this problem.

[0060] The examples explained above focus on identifying blood type or blood group; however this testing device could also be used to identify if a blood sample is infected with HIV or other illnesses.

[0061] 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 spirit and scope of the invention. Modifications and variations as would be deemed obvious to the person skilled in the art are included within the ambit of the present invention as claimed in the appended claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A testing device for identifying a blood sample, including:

at least one substrate having at least one testing zone on which the blood sample is delivered;

the at least one testing zone having a plurality of portions, wherein a first portion has a permanent marking and at least one antibody is deposited on a second portion; and

wherein the permanent marking on the first portion and the at least one antibody reacting with the blood sample on the second portion thereby results in a visual indication within the testing zone.

2. A testing device for identifying a blood sample, according to claim 1 wherein the visual indication is text.

3. A testing device for identifying a blood sample, according to claim 1 or claim 2 wherein the permanent marking is formed by non-bioactive water insoluble ink.

4. A testing device for identifying a blood sample, according to claim 3 wherein the permanent marking is formed by printing of the hon-bioactive water insoluble ink.

5. A testing device for identifying a blood sample, according to any one of the preceding claims wherein the at least one antibody is selected from one or more of the following: A, B, or D antibodies.

6. A testing device for identifying a blood sample, according to any one of the preceding claims wherein the substrate is formed from paper or other fibrous material, preferably paper towel.

7. A method for identifying a blood sample using a testing device according to any one of the previous claims.

8. A method for identifying a blood sample including the steps of:

printing the permanent marking using non-bioactive water insoluble ink on the testing zone;

printing the at least one antibody on the testing zone; depositing the blood sample on the testing zone;

washing the testing zone with saline; and identifying the blood sample by the resultant visual indication within the testing zone.

9. A method for identifying a blood sample according to claim 8 using a testing device according to any one of claims 1-6.

10. A method for identifying a blood sample according to any one of claims 8 or 9 further including detecting blood type from the visual indication.

11. A method for identifying a blood sample according to any one of claims 8 or 9 further including detecting illness from the visual indication.

12. A method for identifying a blood sample according to any one of claims 8 to 11 further including transmitting through a wireless communication means the visual indicator which identifies the blood sample.

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FIGURE 1

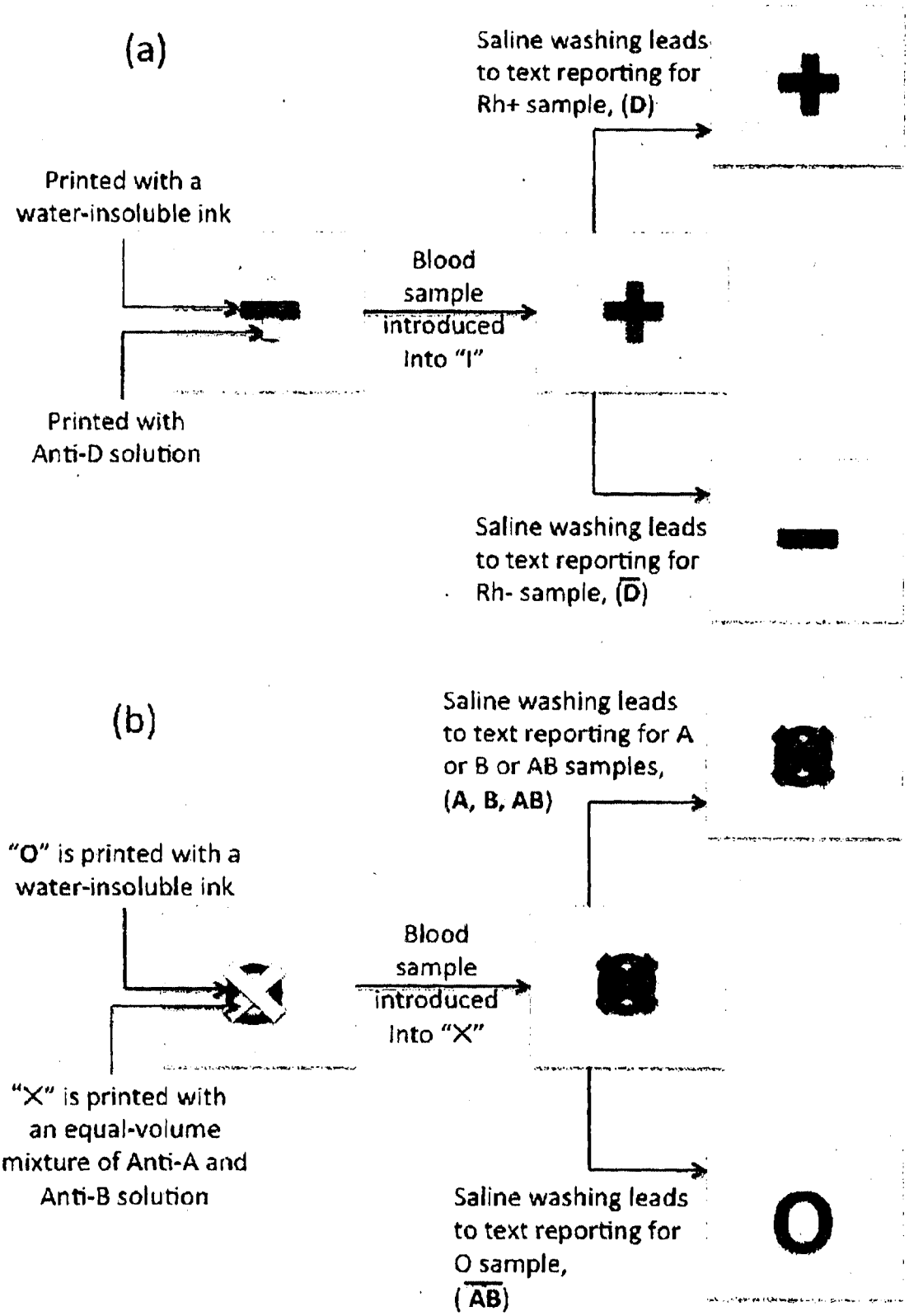

(a)
Saline washing leads to text reporting for Rh+ sample, (D)
Printed with a water-insoluble ink
Blood sample introduced into "I"
Printed with Anti-D solution
Saline washing leads to text reporting for Rh- sample, (D̄)
(b)
Saline washing leads to text reporting for A or B or AB samples, (A, B, AB)
"O" is printed with a water-insoluble ink
Blood sample introduced into "X"
"X" is printed with an equal-volume mixture of Anti-A and Anti-B solution
Saline washing leads to text reporting for O sample, (AB̄)
O

FIGURE 2

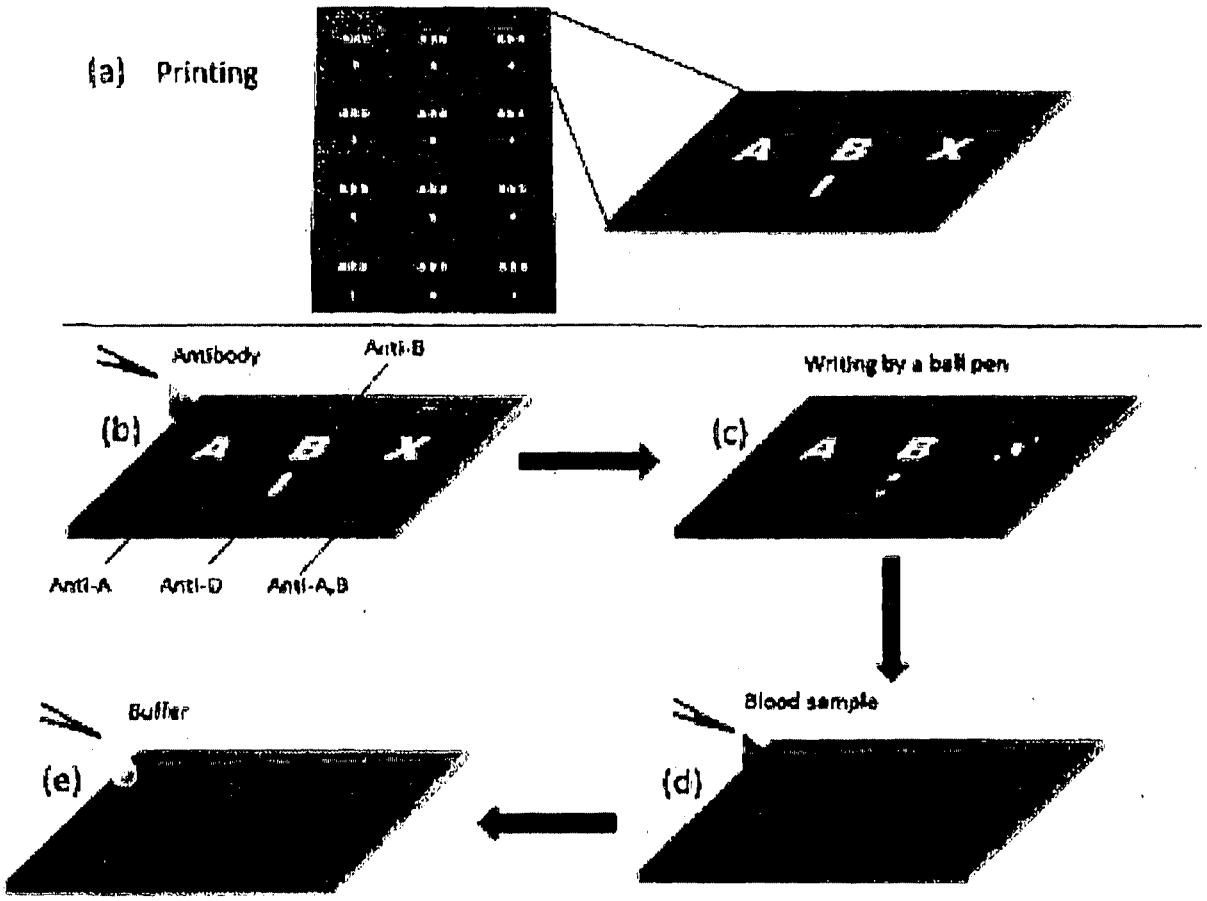

(a) Printing
A
B
X
Antibody
Anti-B
(b)
Anti-A
Anti-D
Anti-A,B
Writing by a ball pen
(c)
Blood sample
(d)
Buffer
(e)

FIGURE 3(a)

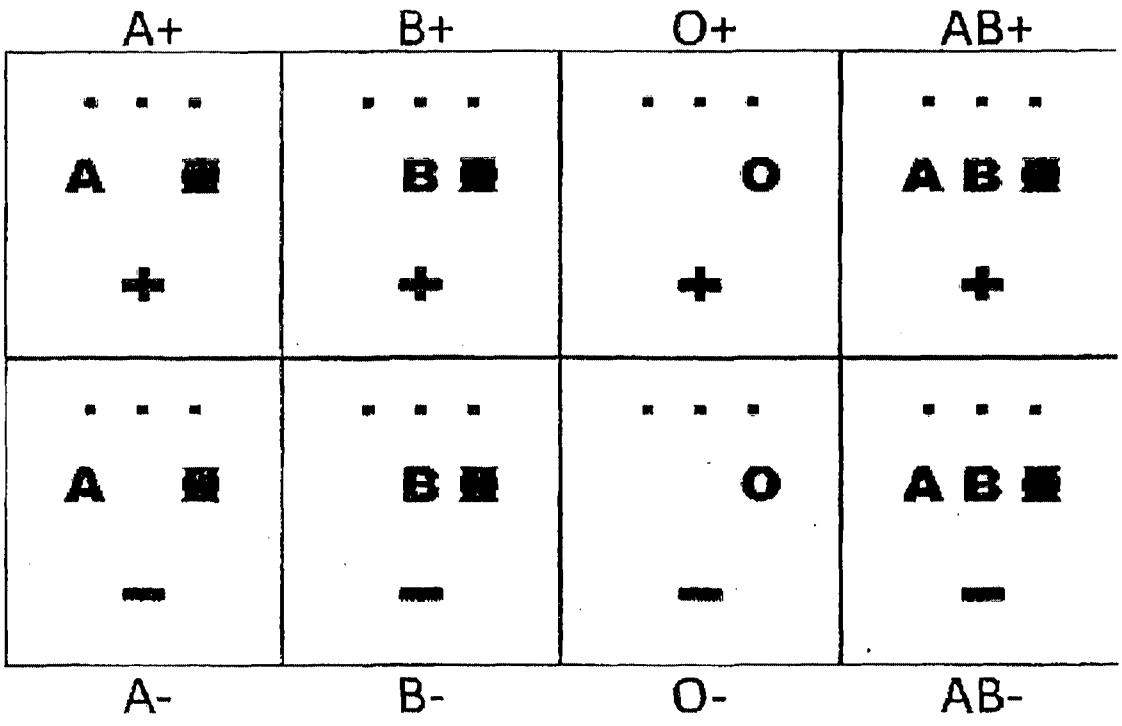
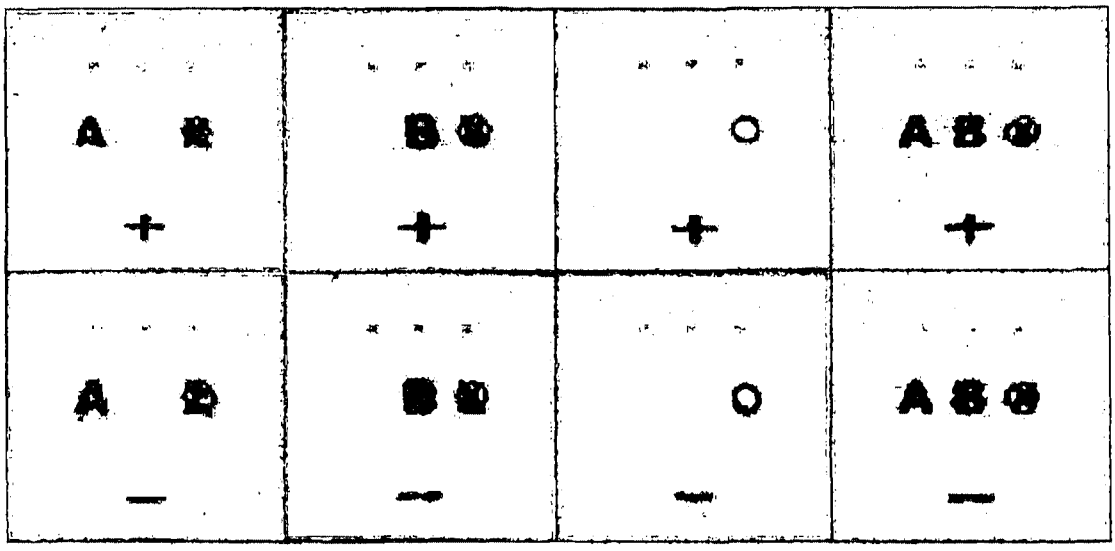


FIGURE 3(a)



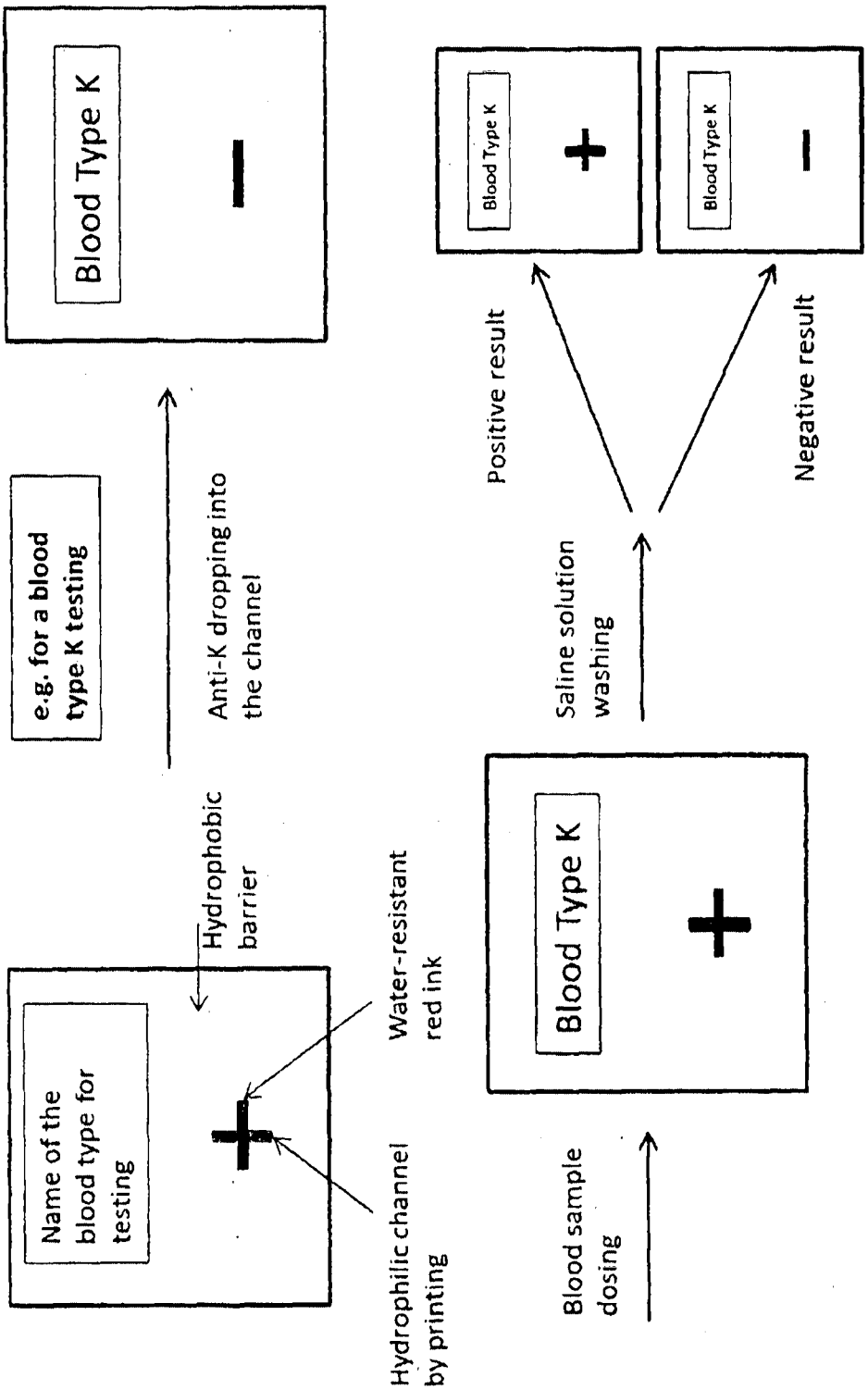

Name of the blood type for testing
Hydrophobic barrier
Water-resistant red ink
Hydrophilic channel by printing
Blood sample dosing
Blood Type K
Saline solution washing
Positive result
Negative result
Blood Type K
Blood Type K
e.g. for a blood type K testing
Anti-K dropping into the channel
Blood Type K

FIGURE 4

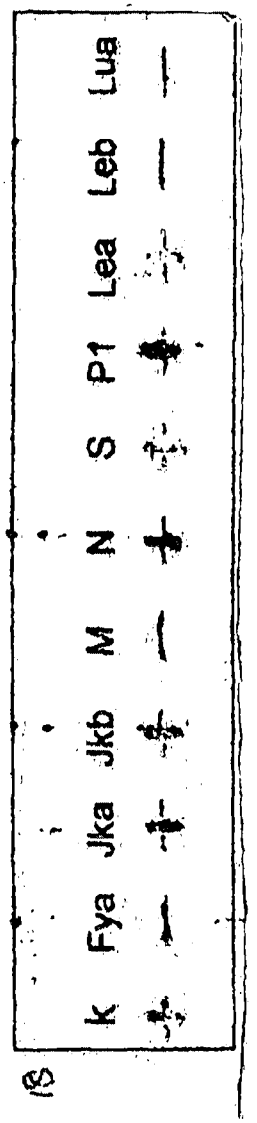
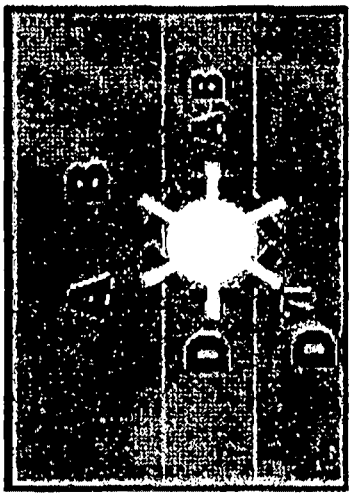


FIGURE 5



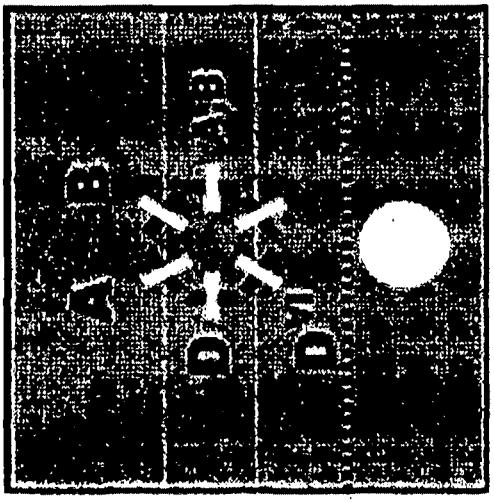


FIGURE 6(a)

FIGURE 6(b)

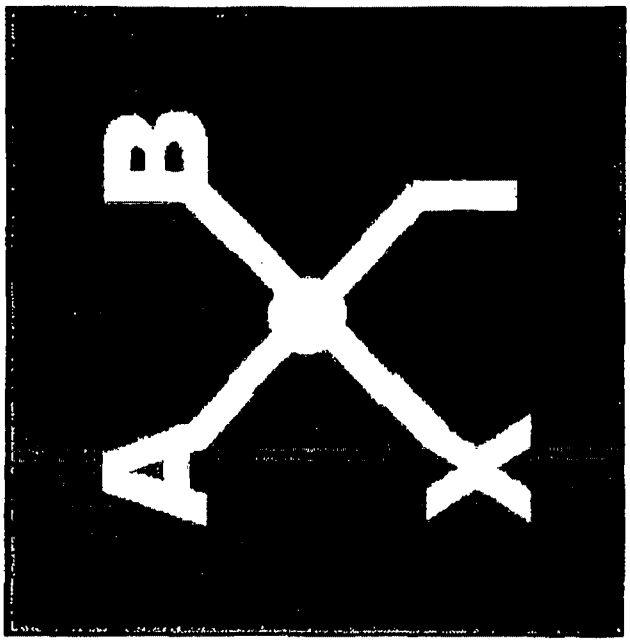


FIGURE 7(b)

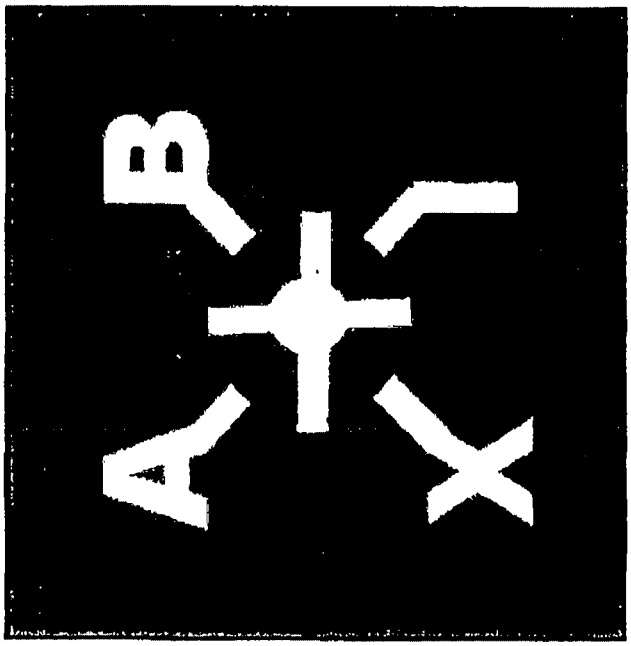


FIGURE 7(a)

FIGURE 8(b)

FIGURE 8(a)

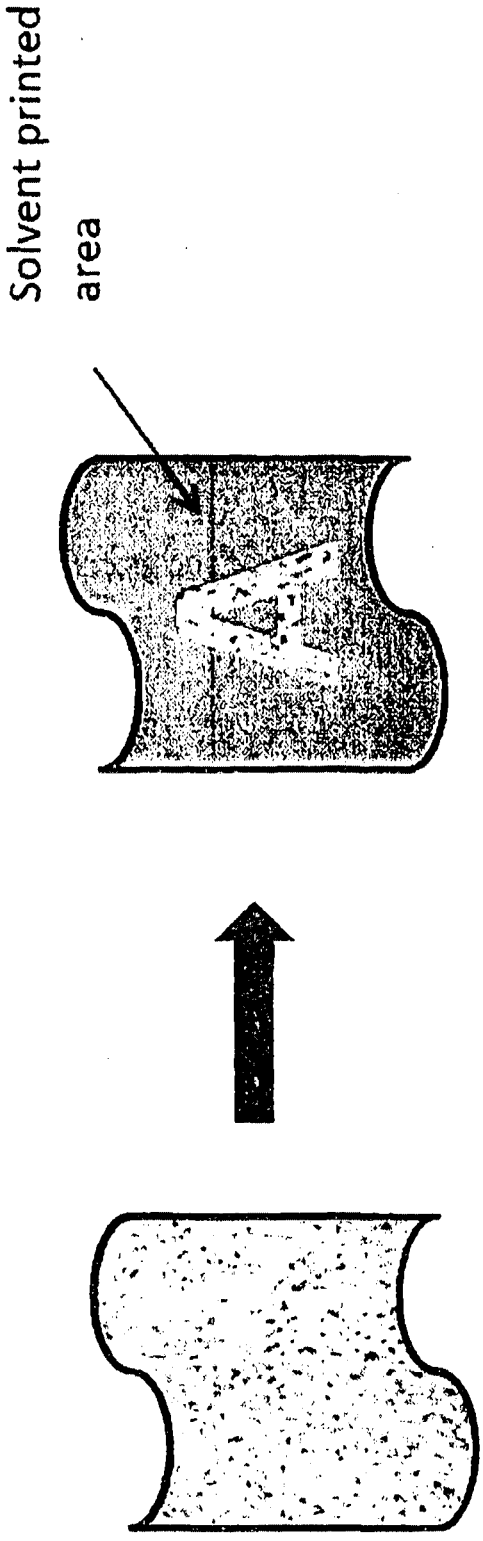


FIGURE 9

FIGURE 10(a)

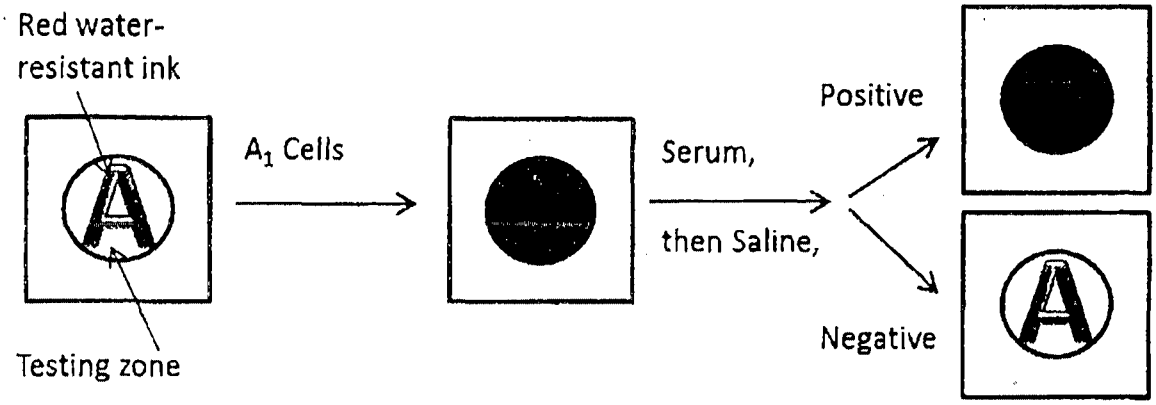


FIGURE 10(b)

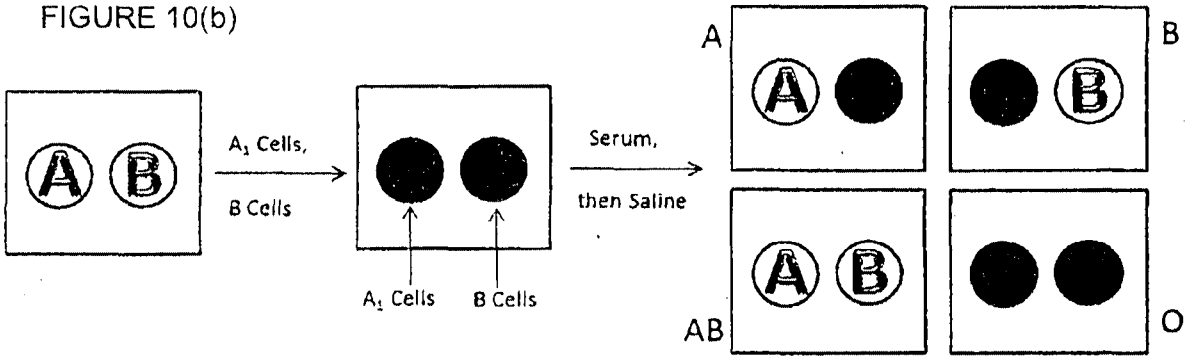
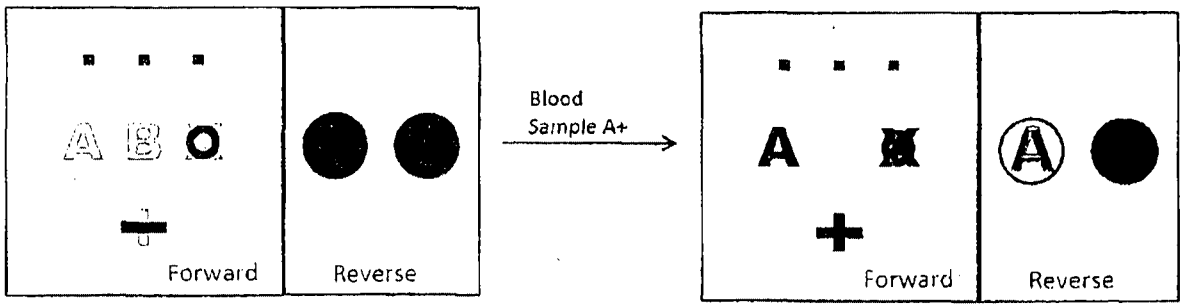


FIGURE 10(c)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2012/001523

A. CLASSIFICATION OF SUBJECT MATTER

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)

INTERNET (GOOGLE SEARCH), INSPEC, WPI and ESPACENET; BLOOD, SERUM, PLASMA, RED_CELLS; TEXT, WRITTEN, LETTER, MARKING, INDICIA, SYMBOL, MARK, PATTERN, PLUS_SIGN, MINUS_SIGN, LABEL, RECOGNIZABLE; ANTIBODY, ANTIGEN, ANTISERUM, REACT, IMMUNO; ASSAY, DIAGNOSE, TEST, INDICATE, DETECT, DETERMINE; PAPER, FIBROUS, SUBSTRATE, CARD, FILM, SHEET, STRIP, CELLULOSE, BIBULOUS, ABSORBENT; PRINT, INK; TYPE, ILLNESS, ABO, HIV, DISEASE, VIRUS, PATHOGEN, GROUP, RHESUS; MOBILE, TRANSMIT, WIRELESS ; RAPID, QUICK, SIMPLE, EASY, REPORT, INTERPRET and similar keywords

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] **Further documents are listed in the continuation of Box C** [X] **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

"E" earlier application or patent but published on or after the international filing date

"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)

"0" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 **February** 2013	20 **February** 2013
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE **PO BOX** 200, **WODEN** ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au Facsimile No.: +61 2 6283 7999	Eva De Kool AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262832477

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2012/001523**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	NL 1010900 C2 (Stichting Ziekunhuizen Noord-Limburg te Venlo) 27 June 2000 entire document but in particular see Abstract, pages 3-4 and Claims 1, 4, 6-8 and 10 entire document but in particular see Abstract, pages 3-4 and Claims 1, 4, 6-8 and 10	1-5, 7-1 1 6, 12
Y	"Paper-based diagnostics", Rajendrani Mukhopadhyay, article from "Chemistry World", November 2010, pages 50-53, available from the Internet from several websites, see for instance the following website: http://www.mediabistro.com/portfolios/samples _files/979567 _JRfeQtETcZmNfNe _Co XE7WN_C.pdf title and pages 51-53	6, 12

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2012/001523**

This Annex lists known 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/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
NL 1010900 C2	27 Jun 2000	None	

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.