Background and objectives
ABO-incompatible red blood cell transfusions still represent an important hazard in transfusion medicine. Therefore, some countries have introduced a systematic bedside ABO agglutination test checking that the right blood is given to the right patient. However, this strategy requires an extremely time-consuming learning programme and relies on a subjective interpretation of ABO test cards agglutination. We developed a prototype of a fully automated device performing the bedside agglutination test that could be completed by reading of a barcoded wristband. This POCT checks the ABO compatibility between the patient and the blood bag.

Materials and methods
Proof of concept and analytical validation of the prototype has been completed on 451 blood samples: 238 donor packed red blood cells, 137 consecutive unselected patients for whom a blood group determination had been ordered and on 76 patient samples selected with pathology that could possibly interfere with or impair performances of the assay.

Results
We observed 100% concordance for ABO blood groups between the POCT and the laboratory instrument.

Conclusion
These preliminary results demonstrate the feasibility of ABO determination with a simple POCT device eliminating manipulation and subjective interpretation responsible for transfusion errors. This device should be linked to the blood bank system allowing all cross-check of the results.

Key words: ABO incompatible, agglutination test, blood transfusion haemovigilance, transfusion safety.

Introduction
The Serious Hazards of Transfusion scheme (SHOT) in the UK between 1996 and 2004 analysed 2630 reports of adverse reactions and events associated with transfusion of labile blood components [1]. Among the 1832 events classified as incorrect blood component transfused (IBCT), 249 ABO-incompatible transfusions were reported.

During this 8-year period, taking into account the 27 million blood components issued by the UK blood services, the risk of IBCT can be estimated at 1:15 000 blood components issued, an ABO-incompatible transfusion at 1:100 000 and the risk of death as a result of an IBCT at 1:1 500 000 [1]. In 2012, almost 10 years after, the annual SHOT report revealed that there were 10 ABO-incompatible red cell transfusions corresponding to a risk of 1:214 678 red cells issued, all resulting from clinical errors with 4 inducing major morbidity from haemolysis [2].

In 2008, the Food and Drug Administration (FDA) reported that 7% of transfusion-related deaths were attributable to ABO-associated haemolytic reactions [3]. Linden et al. [4] observed that one error-related ABO-incompatible transfusion occurred every 38 000 RBC units transfused in New York State.

The Haemovigilance network in France showed similar findings from 1994 to 1998 and estimated the risk of
death due to ABO-mismatched transfusion at 1:1 800 000 allogeneic red cell units transfused [5]. Since 1985, the French authorities have introduced a systematic bedside ABO agglutination test for checking that the right blood is given to the right patient. Consequently, the incidence of ABO-incompatible transfusions has decreased to 1:235 000 [6]. Their experience underlines the efficiency of bedside verification of ABO group on both patient and donated blood. However, this strategy requires an extremely time-consuming learning programme and still relies on a human interpretation of bedside card ABO agglutination test.

To our knowledge, there is currently no automated bedside agglutination assay allowing an easier verification of ABO group just before transfusion. We therefore decided to evaluate the feasibility of a device able to perform an agglutination test that could be fully automatizable to be used at the bedside. It should be associated with a patient identification system using barcoded wristbands. This point-of-care testing (POCT) will deliver the result of the ABO compatibility between the patient and the blood bag without any human intervention either to perform the test or to interpret the results. The objective of this work is the proof of concept of a simple device for bedside blood group determination. This should lead to the development of a fully automated device based on the same principle that will be fully validated in clinical settings.

Materials and methods

Sample selection

The study protocol was carried out according to the principles of the Declaration of Helsinki.

Samples for which a blood group determination had been ordered to the laboratory were tested using the prototype within the 48 h following sampling. Overall, 451 samples were tested in three successive series of validation 238 from donors and 213 from patients.

The first validation was performed on samples from 238 blood units stored in two different conservative solutions: the AS-3 and SAGM solutions. The selected blood units were from 100 O, 80 A, 38 B and 20 AB blood groups (Table 1).

The second validation phase was performed on 137 random EDTA blood samples collected from consecutive unselected patients of our institution for whom a blood group had been requested, and the blood group distribution was 60 O, 45 A, 22 B and 10 AB (Table 1).

The third validation was performed on 76 EDTA blood pathological samples submitted to our haematology laboratory for routine testing. All tested patients had already a known blood group in the laboratory information system (LIS) of the hospital. The pathological samples included 20 high MCV (85–135 fL), 16 low MCV (67–80 fL), 10 anaemic Hb ranging from 6.8 to 12.7 g/dl, 10 samples from homozygote sickle cell disease patients, eight samples from polytransfused patients, four low HCT with negative direct antiglobulin test (DAT), seven samples with a normal HCT and a positive DAT (with anti-IgG or IgG and C3d) and one sample with cold agglutinins.

Prototype

The evaluated device was a prototype developed in our laboratory including six assay positions enabling it to determine simultaneously the ABO group of the blood donor and the recipient. Each test unit consists of two superimposed chambers separated by a fixed membrane with a porosity selected to be permeable to free erythrocytes and impermeable to haemagglutinated erythrocytes. A reagent is disposed on the surface of each membrane.

The first test unit contains 50 µl anti-A blood group reagent, the second 50 µl anti-B blood group reagent and the third 50 µl of a mixture saline–albumin–EDTA.

Fifty microlitres of patients' blood is layered in the superior chamber of the first three test units and 50 µl of the blood to be transfused on the next three test units. If the corresponding antigen is present on the red cells, the specific antigen–antibody will result in a direct haemagglutination on the surface of the membrane. ABO blood grouping on the prototype was determined with anti-A and anti-B antibodies blood grouping reagents NOVA-CLONE from Immucor Gamma.

After a short centrifugation of 3 min at 220 g, free erythrocytes pass through the membrane in the second cavity on the bottom of the test-tube, or in the case of haemagglutination, red cells stay at the surface of the membrane. The presence of free erythrocytes collected in the bottom of the test unit is detected by infrared (IR) absorption measurements.

The phototransistor (ELD-810-525 EPIGAP optoelectronic GmbH) detects the infrared beam, and an electric signal is sent to the processor which converts the result from an analog to a digital value. The results are stored in the computer, and a home-made programme translates them into a graph (Fig. 1). In case of haemagglutination, a low signal is send to the process corresponding to a low peak on the graph. If free erythrocytes pass through the membrane, the IR beam is stopped and the high signal corresponds to a high peak on the graphic. The performances of the prototype were validated in comparison with the routinely used haemagglutination test using a...
gel microcolumn assay (GMA) according to the manufacturer’s instructions (Bio-Rad, Nazareth Eke, Belgium).

Statistics

Data were analysed using GraphPad Prism version 5.0 (GraphPad Software Inc: La Jolla, CA, USA). The measured data allowed a clear discrimination between ‘agglutination’ and ‘non-agglutination’ for each test unit corresponding to the positions 1–6 in the device.

A Receiver operator characteristic (ROC) curve was plotted to determine the optimal cut-off values. Cut-off values were individually determined for each analytical position. For each of them, we defined a ‘security interval’ of 20% around the cut-off value to minimize the risk of wrong results. All results falling into this interval were

<table>
<thead>
<tr>
<th>Results from prototype</th>
<th>Donor blood O</th>
<th>Donor blood A</th>
<th>Donor blood B</th>
<th>Donor group AB</th>
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<tr>
<td>O</td>
<td>100</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>A</td>
<td>0</td>
<td>80</td>
<td>0</td>
<td>0</td>
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<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>38</td>
<td>0</td>
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<tr>
<td>AB</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
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<tr>
<th>Results from prototype</th>
<th>Blood group O</th>
<th>Blood group A</th>
<th>Blood group B</th>
<th>Blood group AB</th>
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<tr>
<td>0</td>
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<td>0</td>
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<tr>
<td>A</td>
<td>0</td>
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<td>B</td>
<td>0</td>
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</table>

Fig. 1 Haemagglutination results and graph representation of the ABO blood group determination using the prototype. Each panel depicts the situation obtained with a patient of the indicated blood group. Each figure shows from the top to the bottom: the cupule, the bottom of the tube, the graph of optical signal and the value of the area under the curve measured by the prototype.
considered as ‘not interpretable’ and should lead to an overall interpretation as ‘do not transfuse’.

Results

Analytical performances were assessed on 451 blood samples (238 samples from packed red blood cells, 137 from random patients and 76 from pathological patients).

(1) The results for ABO typing of all 238 blood donor samples were in accordance with the results obtained with the prototype. The concordance rates were 100% for all blood groups (Table 1). The cut-off values were defined for each position. Positions 1–2–3 represent one blood group test (for the patient) with anti-A in position 1, anti-B in position 2 and NaCl–albumin–EDTA in position 3. The next positions 4–5–6 represent the other blood group test (for the blood unit) with anti-A in position 4, anti-B in position 5 and NaCl–albumin–EDTA in position 6. The results were divided into two groups defined as ‘agglutination’ and ‘no agglutination’. The ABO blood group was derived from the presence or absence of agglutination in the presence of anti-A and anti-B. The third condition was a negative control in which an agglutination should lead to a ‘not interpretable’ result. The ABO results derived from the POCT were compared to the ABO group obtained in the laboratory using the gel agglutination automated assay. All normal blood donor samples were correctly classified with none in the interval of the cut-off value ±20% (Fig. 2).

(2) The second validation step was performed on 137 random EDTA blood samples collected from patients for whom a blood group was requested without any specific selection. The ABO typing of all 137 blood donor samples was in accordance with the results obtained with our device. The concordance rates were 100% for all blood groups (Table 1). All the samples were correctly classified into the two groups ‘agglutination’ or ‘no agglutination’ with none giving values into the grey interval of the cut-off value ±20%.

(3) Of the 76 EDTA blood pathological samples submitted to our haematology laboratory for routine testing, 72 samples were correlated with the LIS results. The samples collected include 20 high MCV (85–118 fl), 16 low MCV (67–80 fl), 10 Hb ranging from 6.8 to 12.7 g/dl, 10 samples with sickle cells, eight samples from polytransfused patients, four low HCT with negative direct antiglobulin test (DAT), seven samples with a normal HCT and a positive DAT (with anti-IgG or IgG and C3d) and one sample with cold agglutinins (Table 2). Three results of the 76 tested samples were reported by the device as undetermined (Fig. 2a).

The first sample giving an uninterpretable result was from an AB+ 1-year-old boy mainly transfused with irradiated packed red blood cells from A and O blood groups.

![Fig. 2](https://example.com/fig2.png)

Fig. 2 (a) Agglutination data obtained for three samples giving uninterpretable results with the prototype. The points corresponding to the donors’ samples are black colored and the points corresponding to the patients’ samples are colored • Patient 1; ◆ Patient 2; ♦ Patient 3. (b) Agglutination data obtained for the three same samples giving uninterpretable results with the gel technique.
Table 2 Pathological samples

<table>
<thead>
<tr>
<th>Pathological parameters</th>
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<tr>
<td>MCV ↑ (85–118 fl)</td>
<td>20</td>
</tr>
<tr>
<td>MCV ↓ (67–80 fl)</td>
<td>16</td>
</tr>
<tr>
<td>Hb ↓ (6–12.7 g/dl)</td>
<td>10</td>
</tr>
<tr>
<td>Sickle cells</td>
<td>10</td>
</tr>
<tr>
<td>Poly-transfused patients</td>
<td>8</td>
</tr>
<tr>
<td>HCT ↓ (17 6–35 8%) with DAT-</td>
<td>4</td>
</tr>
<tr>
<td>DAT+ with normal HCT</td>
<td>7</td>
</tr>
<tr>
<td>DAT+ with cold agglutinins</td>
<td>1</td>
</tr>
</tbody>
</table>

DAT, Direct antiglobulin test; HCT, Haematocrit; MCV, Mean corpuscular volume; Hb, Haemoglobin.

The ABO POCT delivered a valuable result for the internal control and an AUC result below the threshold value for the position with anti-A reagent corresponding to a haemagglutination reaction due to the presence of A antigen. For the test with anti-B reagent, the device delivered a result into the interval of the cut-off value finding an uninterpretable result.

The gel routine laboratory method was also uninterpretable for the blood group because of the presence of mixed field reaction in the gel column with anti-B reagent (Fig. 2b).

Our device and the gel automated method both gave uninterpretable results due to the presence of different red cell populations in the peripheral circulation of this patient.

The second patient had an haemoglobin concentration of 5.3 g/dl, and the haptoglobin had collapsed. The DAT was strongly positive (C3d with 4+ reactivity) with cold agglutinins interfering with the gel agglutination blood group delivering an uninterpretable result because of the positivity of the internal control (Fig. 2b). The ABO POCT also delivered an uninterpretable result with a positive internal control caused by the IgM cold agglutinins (Fig. 2a).

The third case corresponds to the blood group realized for a neonate with an A blood group at birth. The ABO blood group realized in the laboratory was O- and the haptoglobin had collapsed. The DAT was negative (with cold agglutinins 1+), with normal HCT 7 g/dl) (17 6–35 8%) with DAT- 4, DAT+ with normal HCT 7, DAT+ with cold agglutinins 1.

The ABO POCT delivered the same result (Fig. 2a).

Connected to the LIS, the POCT will deliver an alarm because of the discordance with the LIS blood group.

This neonate suffered from anaemia at birth due to maternal red-cell alloimmunization and required partial exchange transfusions with O packed red cells. Both, the intralaboratory gel method and the POCT method, detected the donor O red cells because of the large exchanged blood volumes relative to the neonate circulatory volume.

Discussion

This study, focusing on the validation of the prototype performances, was first conducted on samples from 238 blood units stored in two different conservative solutions, the AS-3 and SAGM solutions, and 137 random EDTA blood samples collected from patients. We found a 100% concordance rate between the ABO typing obtained with the prototype and, respectively, the ABO blood group of the blood unit and the GMA method.

Of the 76 EDTA blood pathological samples, the ABO blood group of 73 samples was concordant with the GMA. Three samples gave an uninterpretable result with both our POCT and the laboratory gel routine assay explained by the presence of cold agglutinins. As for the laboratory automate, the POCT will deliver an alarm that will be sent to the blood bank.

These preliminary results demonstrate that this new technique is feasible and correlated with the automated assay used in our laboratory. These results should be confirmed by a more complete validation with more difficult specimens. More abnormal samples such as haemolysed blood, weak subgroups of A and samples with cold agglutinins must still be tested.

Though this study only describes the analytic performances of the prototype, this device should allow the ABO bedside check process to detect patient errors and prevent an ABO mismatch. The next step of this project is to produce a final device that can be easily used by the medical and nursing staffs, which is not yet possible with the current version. To perform a test, a small drop of patient blood obtained by finger prick will be placed on a specific disposable test cartridge and a segment of tubing from the blood bag will be introduced in another disposable test cartridge. Both cartridges are placed in the POCT to perform the test exactly as it is described with the actual prototype.

The ABO compatibility result is delivered in 3 min on a digital display. The electric signal depending on the presence or absence of red cells in the bottom of the cartridge is sent to the processor and converts to a digital value indicating an haemagglutination or non-haemagglutination result. The haemagglutination results obtained for the three parts of the test (anti-A, anti-B and negative control) determined the ABO blood groups of both patient and blood bag. An algorithm based on the ABO blood group compatibility rules calculates the compatibility between the patient and the blood bag.
Process mapping of the task of prescribing, supplying and administering blood components is a very complex chain with possible errors at several critical points. The SHOT analysis of the IBCT events revealed that in approximately 50% of events, there was more than 1 error in the process, that approximately 70% of errors occurred in clinical areas and that failure in the final patient identification check at the bedside remained the most frequent error [1]. The contributory factors are slips and lapses, taking short cuts, distractions and omissions of essential steps [7]. These human factors are similar in all areas of medical practice where there is a human intervention.

France which is one of the few countries that implemented an ABO agglutination bedside test linked with a complete identification patient check [6] has shown its incidence of ABO-incompatible transfusions to decrease more than in other countries.

At this point, the important limitation of the existing manual bedside ABO agglutination test that must be taken into account is its poor reliability in terms of user performances and defective techniques.

In a study conducted by Ingrand et al. [8], the reliability of the pretransfusion bedside compatibility test was evaluated with 48 nurses who performed agglutination tests, interpreted compatibility and decided whether to transfuse red cells for 12 randomly and blindly selected donor and recipient blood sample pairs. They made erroneous decisions in 18-2% of 576 tests, including 12 decisions to transfuse incompatible blood. The authors concluded that the bedside pretransfusion compatibility determination should not be considered a reliable supplemental safety procedure in the hands of inexperienced operators [8].

Therefore, several electronic systems were developed to improve the safety of blood transfusions.

They generally use an identification bracelet which is a barcoded wristband and a hand-held portable computer that allows the identification of the patient and the blood bag by scanning [9–12]. However, barcode systems have their disadvantages. They were found in some studies not to have improved performance, and a recent study estimated that more than 1 in 84 000 barcode scanning events generated an incorrect patient identifier [13, 14]. Moreover, these systems are unable to detect errors occurring inside the blood banks, especially in case of emergency.

These results support the need to link the ABO agglutination test to patient and blood checks within the bedside procedure as recommended by Daurat [6].

Based on all these reasons, we have concluded that transfusion safety could be improved by a POCT system performing both ABO agglutination bedside tests of the patient and the blood bag in addition to the electronic checks but without any human interpretation.

This simply hand-held device will provide the verification by coupling the ABO blood group compatibility between the donor and the recipient with the bedside checks using a barcode identification of the patient and the blood unit. The results stored to the LIS will be validated and registered. The blood bank will thereby be able to follow the transfusion checks and will be instantly alerted in case of ABO incompatibility. The device in his final version will be validated in a pilot study in collaboration with blood banks in three different hospital centres.

Therefore, the participation of the blood bank in implementing such a system is of high importance and will strengthen the collaboration between the blood banks and the clinical area.

However, as a blood banker, we must agree, as written by Dzik, that new technology alone will not improve transfusion safety [15] but will, if supported, by adequate user training.

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Authorship contributions

H.E.K. and FC conceptualized and designed research, acquired and analysed data and wrote the manuscript. The authors revised the manuscript and approved the final version.

Patent

The device is patented by the Université Libre de Bruxelles (Brussels, Belgium): international patent PCT/EP2012/074481 (WIPO publication number WO/2013/083619).

Conflict of interest disclosures

The authors declare no competing financial interests.
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