A biochip based medical device for point-of-care ABO compatibility: towards a smart transfusion line

Karine Charrière[1], Alain Rouleau[2], Pascal Morel[3], Véronique Bourcier[4], Christian Pieralli[2], Wilfrid Boireau[2], Lionel Pazart[1] and Bruno Wacogne[1,2*]

[1]INSERM CIC 1431, Besançon University Hospital, 2 place St Jacques, 25030 Besançon cedex, France

[2]FEMTO-ST Institute, Univ. Bourgogne Franche-Comté, CNRS, 15B avenue des Montboucons, 25030 Besançon, cedex, France
[3]Etablissement Français du Sang Bourgogne/Franche-Comté, 8 rue du Docteur Jean François Xavier Girod, Hauts de Chazal, Temis Santé, BP 1937, 25020 Besançon cedex, France

[4]Hemovigilance Service, Besançon University Hospital, 1 Bd Alexandre Fleming, 25030 Besançon cedex, France

* Corresponding author:

Bruno Wacogne
FEMTO-ST institute, Univ. Bourgogne Franche-Comté, CNRS, 15B avenue des Montboucons, 25030 Besançon, cedex, France Emali : bruno.wacogne@univ-fcomte.fr
Tel: +33 (0) 381666388

Abstract:
ABO mismatch between donor and patient's blood is still the cause of accident which are sometimes lethal. The main causes of mis-assignment are human errors and wrong identification of patients or blood product. Only a final compatibility test at the patient's bedside can avoid these errors. In some countries, this test is performed using manual procedures. This does not prevent from human manipulation and interpretation errors. In this paper, we present a prototype able to automatically perform a final ABO compatibility test. It relies on the use of disposable antibodies grafted biochips inserted into a mobile

reader/actuator. Red blood cells are selectively captured on biochips grafted with antibodies complementary of antigens present on the cells surface. Detection of capture cells is based on optical absorption techniques. So far, our device achieved blood compatibility test with 99.3 % sensitivity and 97.9 % specificity.

Keywords:

Biosensor, surface plasmon resonance, human red blood cells, automated ABO compatibility test, optical detection, opto-fluidic prototype

1 Introduction

In all countries before blood transfusion, a concordance verification test between patient's data and red cells to be transfused is performed at the patient's bedside. In most countries, a cross-match test is performed in a biology laboratory before the concordance test. However, it becomes useless when an error occurs after the delivery (the wrong blood bag to the wrong patient, the most frequent case). In very few countries (in France for example), a biologic compatibility test is performed at the patient's bedside.

In countries for which the hemo-vigilance is reliable and where a unique test is performed, the ratio of adverse effects due to ABO incompatibility approaches 1/40000 red cell concentrate (RCC). This was the case in France before 2003 when only ABO compatibility was tested. After this date, the use of both concordance and ABO tests at the patient's bedside reduced the adverse effects to about 1/600000. However, in most countries, only the concordance test is considered.

There is a need for a second test at the patient's bedside in order to reduce the number of ABO errors. There exist ABO test cards, but they rely on delicate manual operation and require a long and specific training. Furthermore, the compatibility card requires a human interpretation of the agglutination test. So, this method is a source of various errors: manipulation, reading and interpretation difficulties. Also, medical staff are exposed to blood when sampling patient's blood. Concerning the interpretation difficulty, iso-group compatibility is relatively straightforward but non iso-group compatibility still leads to interpretation errors or stresfull situations.

For all these reasons, a point-of-care device able to automatically perform an ultimate compatibility test with minimum manipulation and without human interpretation would be

nice to have, in particular when considering the increase of blood product distributed during the last decade. For example in France an increase of the RCC delivery of almost 24% has been observed between 2000 and 2011 (EFS, 2012). In 2011, more than 2.3 million of RCC where distributed (ANSM, 2012).

Several methods have been proposed for blood typing. They are mainly based on gel agglutination (Cid, 2006 – Langston, 1999). SPR (Malomgre, 2009 – Quinn, 2000 – Quinn, 1997) and Surface Plasmon Resonance imaging (SPRi) (Berthier, 2011 – Boozer, 2006 – Campbell, 2007 – Mansuy-Schlick, 2006) techniques can also be used. However, these studies demonstrate the possibility to detect captured cells with commercial laboratory apparatuses. Therefore, a direct translation to the patient's bedside may be difficult because the entire device used should be re-thought for point-of-care use.

Recently, long-range surface plasmon-polaritons to detect red blood cells (RBC) selectively captured by the surface chemistry was demonstrated (Krupin, 2014). However, because packed RBC must be diluted in a buffer of controlled refractive index, translation of the device to clinical use is still challenging. Techniques based on image processing on plate test have been reported (Ferraz, 2010 – Ferraz, 2013). In this case, image processing is used to objectively observe and interpret red cell agglutination obtained manually. Issues concerning blood and antibodies manipulation still exist. Spectroscopic methods have also been reported (Ramasubramanian, 2008 and 2009). However, the use of an optical spectrometer to measure absorption of diluted red cells may be difficult in clinical practice. In fact, although these new devices are able to realize blood typing by objectively reading agglutination, they still require hard translational research work before to be installed in the patient's room.

In this paper, we present a mobile device meant to address the above mentioned issues. The main idea is to replace the four reaction zones of the current manual compatibility card with four IgMs grafted biochips inspired from Surface Plasmon Resonance (SPR) and SPRi biochips. Hemagglutination is therefore replaced by red cell capture. The detection of capture red cells rely on a simple optical absorption technique. Biochips are inserted in a mobile reader/actuator which drives the fluids (blood, red cell concentrate (RCC) and physiological serum) and performs the optical reading and final interpretation. This concept is currently protected by two patents (Pazart, 2011-1 and 2011-2) and was describe in a previous paper (Charriere 2015).

Research actions to set-up this device include four main steps. The first series of test consisted in studying the IgMs grafting and red cell capture using SPR and SPRi methods with

homemade biochips. This has been previously reported (Charrière, 2011 and 2012). The second set of experiments consisted in translating the SPR biochip to biochips inserted into cartridges and to detect the capture of red cells in these half-bulk conditions together with the correlation between the number of captured cells and optical reading (Charrière 2015). **The third part of the experiments is the subject of the current publication.** It consists in using a large number of whole blood (WB) and RCC samples to test the automated fluid flow control, optical reading and software interpretation of the ABO compatibility result. The goal is to determine sensitivity and specificity of the device together with the blood group concordance between what is expected and what the device reads. We also studied the performance of the device according to the age of RCC. The last part of the work consist in experimenting the use of the venous return to drive patient's blood into the device with reduce risk of exposure to blood for medical staff (Pazart, 2011-1).

2 Materials and methods

2.1 *Biochip fabrication*

The fabrication and testing of biochips using SPR and SPRi techniques has been previously reported (Charrière 2011 and 2012). The antibodies used were IgM anti-A or IgM anti-B (DIAGAST, France). The running buffer was saline physiological serum (NaCl 0.9%).

2.2 Description of the device

A schematic view as well as pictures of the device is shown in figure 1.

The heart of the device consists of two cartridges, one used to test the patient's blood, the other for the RCC. Both of them contain two IgMs grafted biochips, one with anti-A, the other with anti-B antibodies. When blood (either WB or RCC) is applied to the biochips, antigen-antibody recognition occurs.

These microfluidic cartridges are placed into an optical clamp that consists of blue LEDs and photodetectors. Each biochip can then be interrogated with its own LED/Detector pair. Red cells trapped onto the biochip absorb light. The detection principle consists in measuring the transmission before red cells are driven onto the chip, when physiological serum fills the circuitry, (reference measurement) and after red cell/surface interaction followed by washing with physiological serum (final measurement). The optical reading is therefore an absorbance measurement given by:

Absorbance= (reference-final)/reference (1)

In what follows, positive chips are defined as chips that have captured red cells, regardless of the blood group. Conversely, negative chips correspond to chip with no capture.

Fluids (blood, RCC and physiological serum) are driven by means of automated syringes controlled via dedicated software. This software also drives the optical measurement, human-machine interface and USB connection to a PC for data recording and processing.

2.3 Surface analysis

After experiments, cartridges were removed from the laboratory prototype and observed with a macroscope (Leica MSV266; soft-ware Leica Application Suite V3.7.0). The percentage of RBC trapped on surfaces was estimated with ImageJ for each biochip by aver-aging the percentage of RBC on surface of 5 random macroscopic fields.

2.4 Statistical analysis

Statistical comparisons were made with the Kruskal–Wallis test followed by Dunn's multiple comparison tests using GraphPad prism 5.

2.5 First validation of the use of the venous return

Experiments were conducted using an intravenous trainer arm (Adam,Rouilly, AR251). A Baxter Y-type transfuser (RMC 5849) was modified. 2 ways valves were inserted in the patient's end of the transfuser. They are used to drive the red cell concentrate to the patient, to drive patient's blood to the device. They are also used to isolate the patient during the test and to protect the medical staff from contact with the patient's blood. The tested fluids were physiological serum and coloured solution to represent blood. Fluidic behaviour of the device was assessed visually.

3 **Results**

3.1 Device's testing

The device was tested using 148 blood aliquots. This represents 296 biochips and therefore 148 cartridges. Blood comes from both RCC and WB. Samples were provided by the

Etablissement Français du Sang in accordance with the ethical rules and with informed consent obtained from donors.

Among these 296 chips, 4 are not taken into account because errors occurred while assembling the cartridges.

Therefore, only 292 biochips were tested. Remember that 2 biochips are required to test 1 sample. For two samples, inversions of the anti-A and anti-B biochips were made. Although the biochips behave correctly and are taken into account for biochip testing, the corresponding samples were not taken into account for compatibility testing. At the end, 142 samples were tested for compatibility.

The repartition of samples in terms of RCC, WB and blood group is given in table 1.

3.2 *Biochip efficiency*

Here, 292 biochips were tested. Figure 2 shows the absorbance measured as a function of positive and negative biochips for both RCC and WB. Positive and negative biochips correspond to the 4 blood group.

There is a strong difference between positive and negative biochips. No statistical variation of the absorbance was observed in negative biochips (0.003 ± 0.001 for RCC neg and 0.007 ± 0.02 for WB neg). Conversely, significant difference is observed in positive biochips between RCC and WB (0.36 ± 0.006 for RCC pos and 0.18 ± 0.008 for WB pos). This result may be related to the large difference of erythrocytes number in samples ($4.3x10^9 \pm 10^8$ RBC/mL for RCC and 10^9 C/mL for WB).

The best absorbance threshold to discriminate between positive and negative biochip was set to 0.05 (minimization of mis-assignments). In this way, only 4 errors occurred. One biochip represents a false negative. For it, not enough red cells were captured although the biochip should have been positive. Indeed, red cell capture is not homogenous on the surface, maybe due to an antibodies graft problem. This means that 1 patient of group AB was detected as A. Three other biochips were false positive. For them a strong non-specific retention of red cells was recorded due to washing problem. This means that 1 patient of group O was detected as A and 2 patients B detected as AB.

From these results and differentiating between A and B biochips allows calculating sensitivity and specificity of the device. This is summarized in table 2. Almost all sensitivities are 100%, except for anti-B biochips (97%) used with WB (false negative described earlier). It is the

same for specificities: all biochips are 100% specific, except the anti-A biochips used with WB (3 false positives described previously). Table 3 presents the same parameters regardless of the blood type and for the entire device. At the end, specificity of the device is 99.3% and specificity is 97.9%. Improving fabrication of the cartridges would probably resolve these mis-assignments and improved sensitivity and specificity.

Biochips performance was also tested as a function of the age of the blood donation. In this case, only RCC were considered because WB is meant to be fresh. For this test we used 30 positive biochips with 6 to 8 days old donations, 31 negative biochips with 6 to 8 days old, 29 positive with 43 to 44 days old and 40 negative with 43 to 44 days old. Figure 3 shows the absorbance obtained in terms of positivity/negativity. No difference was observed between all kinds of positive biochips: 0.35 ± 0.011 for 6 to 8 days old RCC and 0.37 ± 0.01 for 43 to 4 days old RCC. The same is observed between negative biochips: 0.001 ± 0.002 for 6 to 8 days old RCC and 0.005 ± 0.002 for 43 to 44 days old RCC. It is quite clear that the age of the blood donation does not impact biochips performances.

For compatibility interpretation, 74 tests were performed. In all cases the software delivered the right compatibility information, perfectly coherent with what happened at the biochip surface. Of course, we mentioned cases where mis-assignments occurred. However, this part of the test concerns the fact that the device delivers the right information from the result of the optical measurement. For example, with sample SO11 (WB of group O), a strong non-specific RCC retention has been observed on the anti-A biochip, with an absorbance of 0.06 corresponding to a percentage of red cells of 21% on the biochip surface. This "patient" was considered A group. When testing the compatibility with B group RCC, the device concluded that the transfusion should not be allowed. Therefore the optical reading and the interpretation software work properly.

Experiments to test the ability of the device to correctly identify blood groups have been conducted. Among 142 concordance tests performed 4 mis-assignments occurred. The concordance performance is therefore 97% as detailed in table 4. Mis-assignments reported here correspond to those already mentioned above.

The next experiment concerned the percentage of red blood cells on the biochips surfaces as a function of the positive or negative nature of the biochips. The results is given in figure 4. While the threshold in terms of absorbance was set to 0.05, the threshold in terms of percentage of the surface covered with red blood cells is about 17.5%.

3.3 *Venous return*

The last experiment concerned the validation of the use of the venous return to sample patient's blood. For this, preliminary fluidic tests were performed using an intravenous trainer arm. This is illustrated in figure 5 which shows frames of a video filmed during the experiments. Preliminary tests show that a slightly modified conventional transfuser can be used to correctly drive fluids the device. As seen in figure 5(a), the coloured solution to represent blood rises in the catheter when the catheter is placed. When the 3 ways valve is correctly positioned, the patient's blood is correctly driven toward the device (figure 5(b)). In figure 5(c), the patient's is correctly isolated from the physiological serum used to rinse the tubes. Tubes and device connection are correctly rinsed (figure 5(d)).

4 **Discussion**

In this paper, we presented a new device able to semi-automatically perform an ultimate ABO compatibility test. It is based on biochips grafted with anti-A and anti-B antibodies. They are inserted into disposable cartridges and placed into a mobile and re-usable reader/actuator. The latter includes embarked software that drives and controls the fluid flows, performs the optical detection of captured red cells and interpret the result in terms of ABO compatibility. 292 biochips were tested. The device exhibits sensitivity and specificity equal to 99.3% and 97.9% respectively. At this stage of the project, different aspects can be discussed.

Concerning the automatic fluid driving. Cartridges proved to be efficient in driving the fluids onto the biochip with minimum non-specific interactions. Taking into account the very strong affinity-avidity of the antigen-antibody couple, the test duration do not exceed a few minutes, which is perfectly compatible with clinical constraints.

The optical absorption detection method also proved to be efficient. We demonstrated efficient detection with low haematocrit levels (down to about 10%).

We still need to fully understand why 4 mis-assignments occurred during the tests. However, for the 3 false positives, washing was imperfect, probably due to a slight motor dysfunction. For false negative biochips, IgMs were probably not optimally grafted which may explain the non-uniform red cells capture. Optical reading and software interpretation are not to be blamed. However, just after these 4 false results have been observed, the same samples used with new biochip were re-tested. This time, everything worked correctly and no mis-

assignment was observed. In fact, these problems will probably be solved when transferring the device for industrial development according to the quality policy of the involved company.

More generally, the immune-opto-fluidic cartridge we present here proved to be able to detect red cell capture even when only a few red cells are trapped. Therefore, a large number of application can now be envisaged like anti-D detection for example.

5 Conclusion

To conclude, we believe that the concept described here may help enhancing blood transfusion safety, not only in countries where a double ultimate test is already performed, but especially in countries where only one test is considered. Furthermore, such a device is meant to drastically reduce ABO compatibility accidents in countries where the whole transfusion process (blood donation, conservation, delivery and transfusion) is not yet fully satisfactory.

6 Acknowledgements

This work was partly supported by the EFS (grant DECO-13-0128), the INSERM-CNRS (patent file CNRS/REF:02682-V), OSEO and the University of Franche-Comté (grant A1105005I). This work is developed in the frame of the French RENATECH network and the Biom'@x transversal axis at FEMTO-ST.

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Figure caption:

Figure 1: Views of the device. A.B. There is 1 cartridge for the patient and another one for the red cell concentrates. C. Each cartridge includes 2 biochips grafted with anti-A and anti-B antibodies. D. Optical camps are used to detect capture of red cells at the chip's surface. E. Fluids are driven using syringes controlled by the internal micro-processor. F. The human-machine interface allows setting the opto-fluic parameters and displaying compatibility test results.

Figure 2: Absorbance versus positive or negative biochips. Box plot: 5-95 percentile. Kruskal-Wallis test followed by Dunn's multiple comparison tests. *** $p < 0.001$. Negative values are due to a slight drift of the electronics. Red diamond shows cases of mis-assignment when the threshold is set to 0.05.

Figure 3: Absorbance versus the age of the red cell concentrate in terms of positive and negative biochips. . Box plot: 5-95 percentile.

Figure 4: Percentage of the biochip's surface covered with red cells in terms of positive and negative chips. RCC: red cell concentrate. WB: whole blood. Box plot: 5-95 percentile.

Figure 5: Frame captures of a video filmed while testing the efficiency of the blood venous return.

Table caption:

Table 1: Number of samples used in this study.

Table 2: System performance in terms of biochips.

Table 3: Performance in terms of antibodies and for the entire device.

Table 4: Detail of the concordance test (m-a: mis-assignment).

Tables

Group	A	B	AB	O
RCC	19	25	14	20
WB	17	13	20	14

Table 1: Number of samples used in this study.

	RCC		**WB**	
	Anti-A	**Anti-B**	**Anti-A**	**Anti-B**
Number of Biochips	82	78	68	64
Expected positives	36	39	39	33
Recorded positives	36	39	39	32
Sensitivity	**100%**	**100%**	**100%**	**97%**
Expected negatives	46	39	29	31
Recorded negatives	46	39	26	31
Specificity	**100%**	**100%**	**89.7%**	**100%**

Table 2: System performance in terms of biochips.

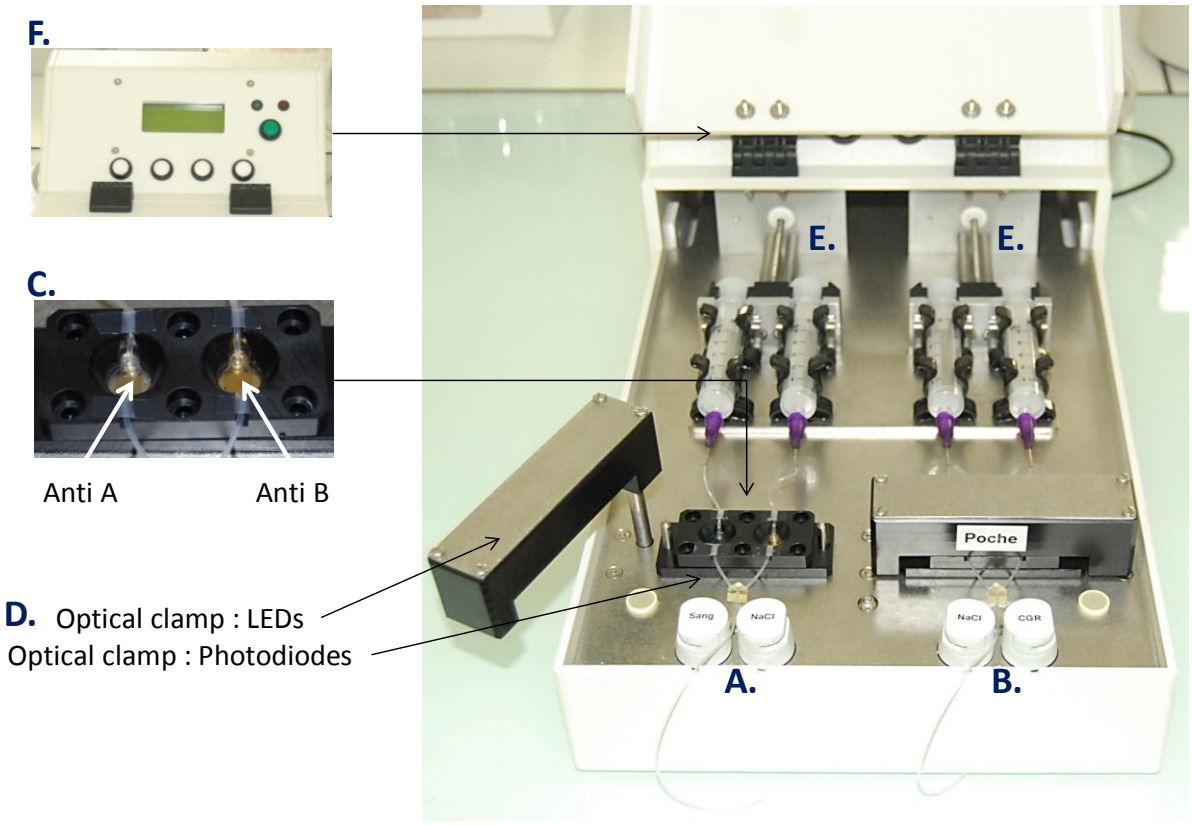
	RCC + WB		**Entire device**
	Anti-A	**Anti-B**	
Number of Biochips	150	146	292
Expected positives	75	72	147
Recorded positives	75	71	146
Sensitivity	**100%**	**98.6%**	**99.3%**
Expected negatives	75	70	145
Recorded negatives	72	70	142
Specificity	**96%**	**100%**	**97.9%**

Table 3: Performance in terms of antibodies and for the entire device.

Group		A	B	AB	O
RCC	N° of tests	19	25	14	20
	Concordance %	**100**	**100**	**100**	**100**
WB	N° of tests	17	13	20	14
	Concordance %	**100**	**84.6** 2 m-a	**95** 1 m-a	**94.4** 1 m-a
		All groups			
RCC + WB	N° of test	142			
	Concordance %	**97** 4 m-a			

Table 4: Detail of the concordance test (m-a: mis-assignment).

Figure 1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C.
Anti A
Anti B
D. Optical clamp : LEDs
Optical clamp : Photodiodes
E.
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Poche
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NaCl
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CGR
A.
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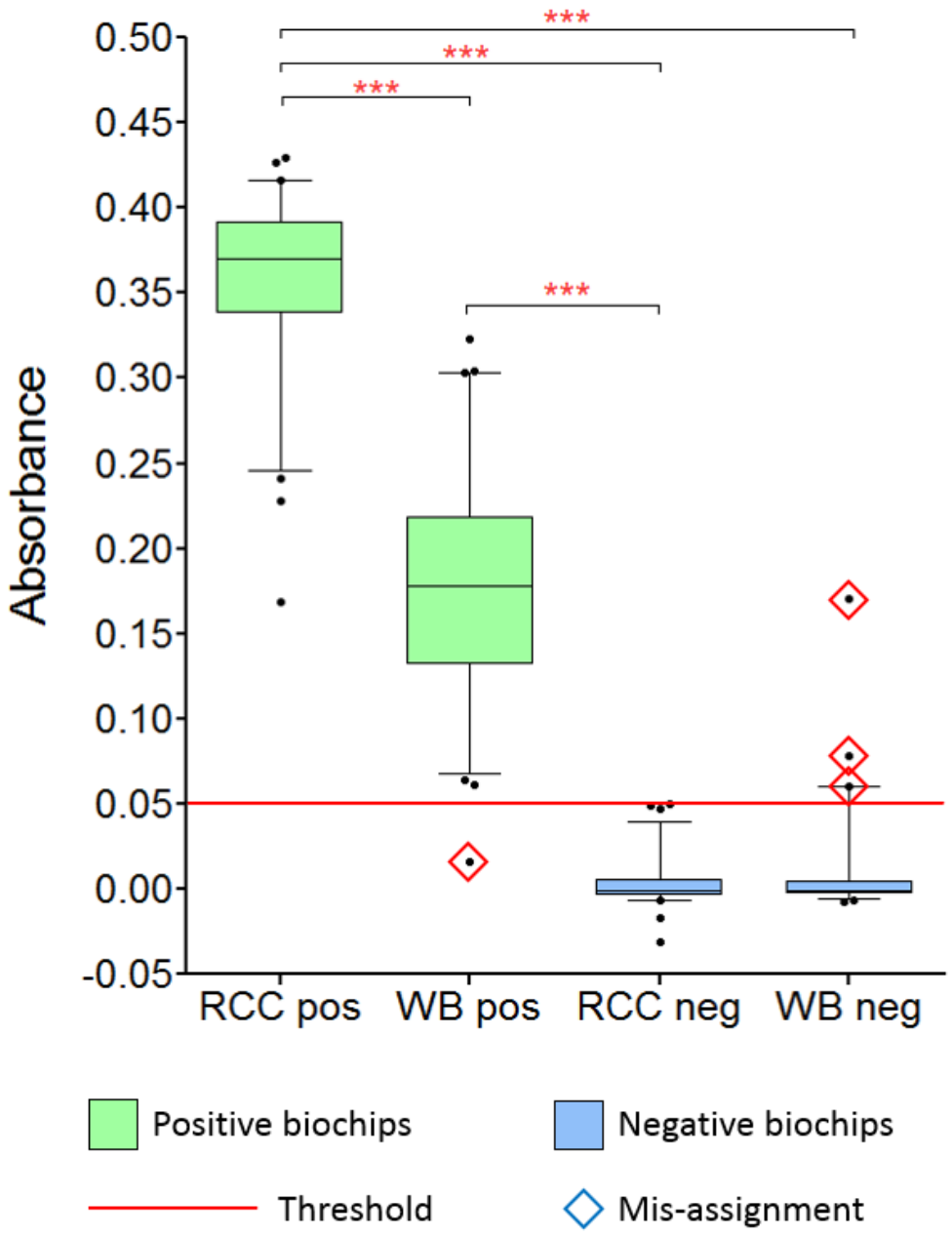
Figure 2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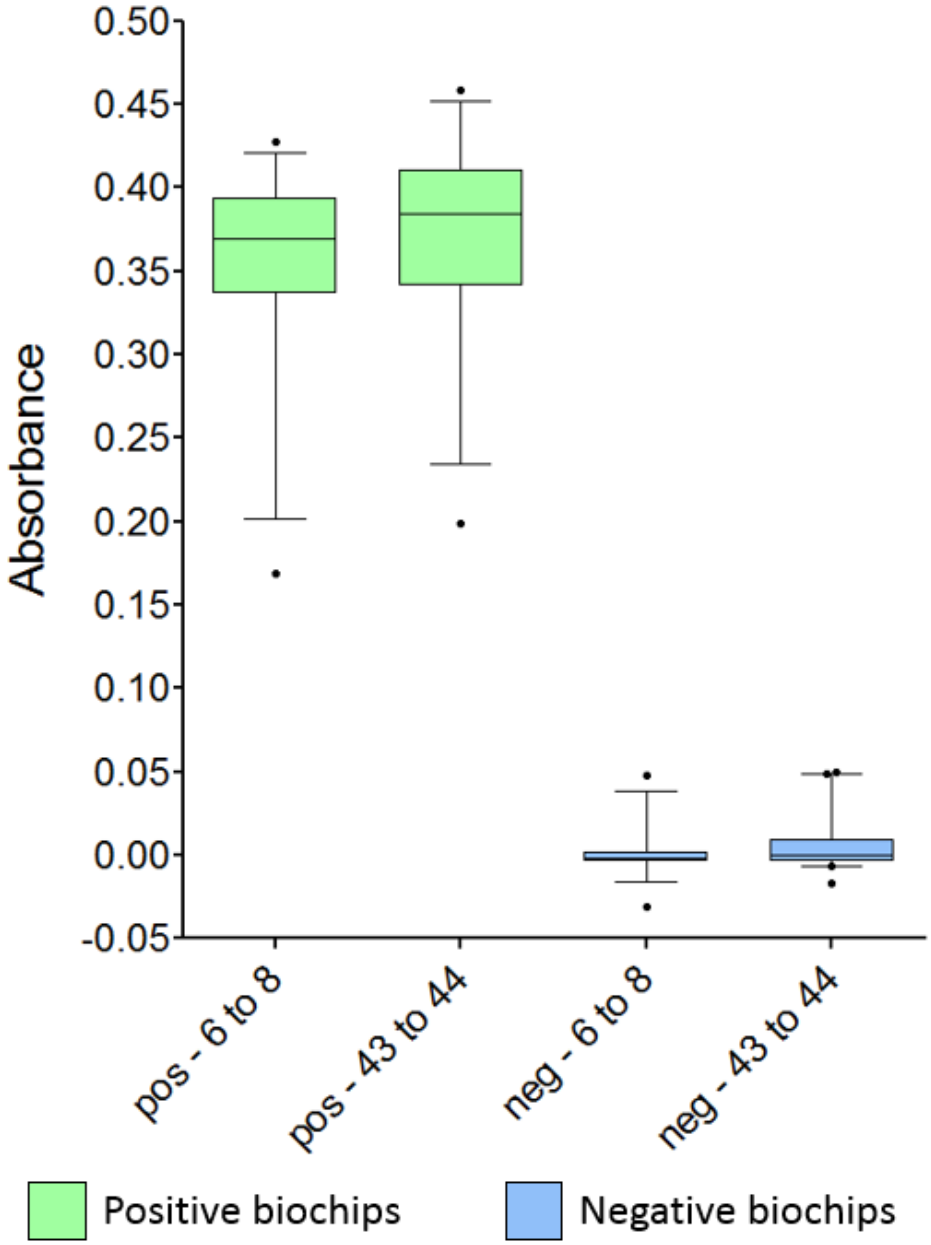
Absorbance
RCC pos
WB pos
RCC neg
WB neg
Positive biochips
Negative biochips
Threshold
Mis-assignment

Figure 3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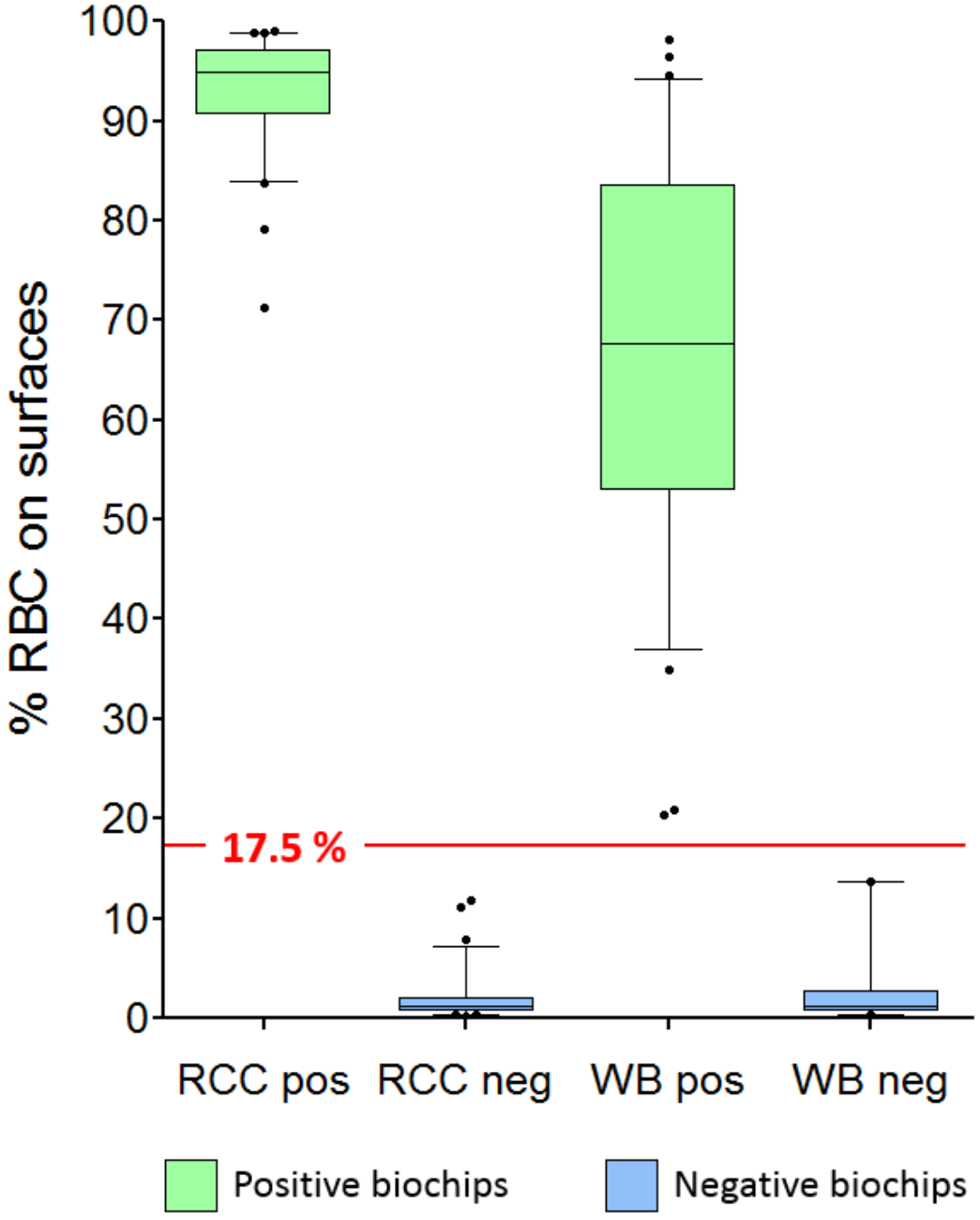
Absorbance
pos - 6 to 8
pos - 43 to 44
neg - 6 to 8
neg - 43 to 44
Positive biochips
Negative biochips

Figure 4


% RBC on surfaces
100
90
80
70
60
50
40
30
20
10
0
17.5 %
RCC pos
RCC neg
WB pos
WB neg
Positive biochips
Negative biochips

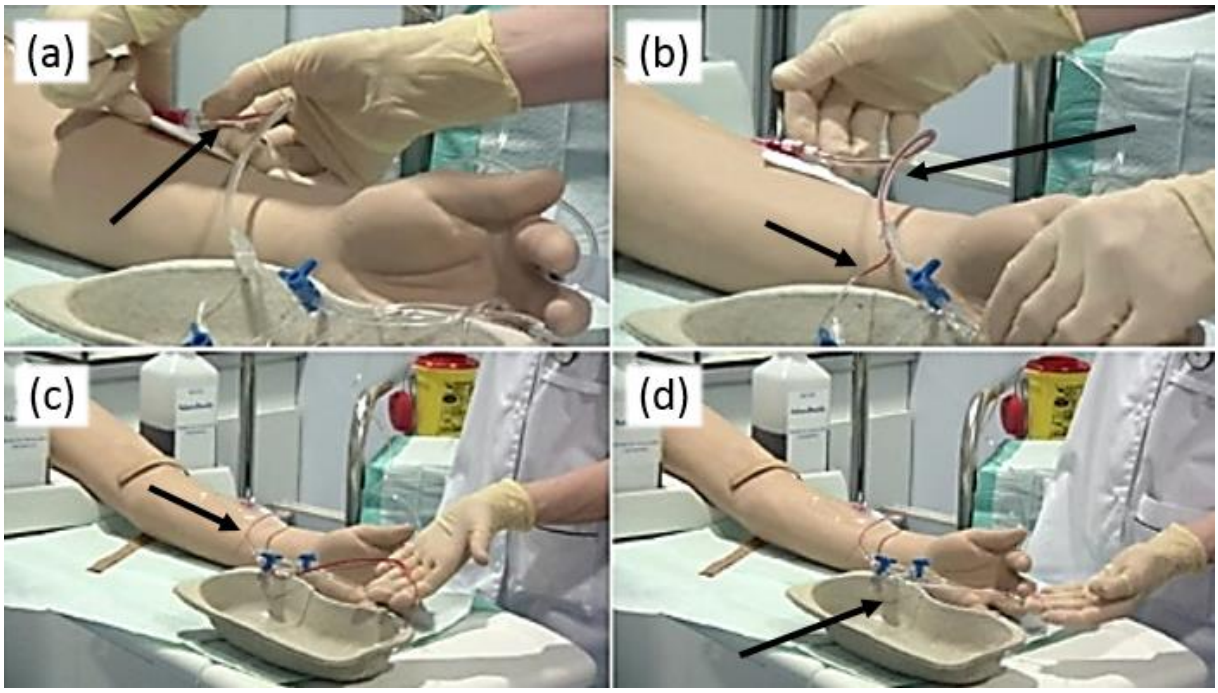



Figure 5