# Test device for Blood transfusion safety
## *How acoustics can help preventing any red cells incompatibility*

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Abstract: During red cells concentrates transfusion, red cells incompatibilities still occur despite the laboratory controls based on immuno-hematologic techniques. Red cells incompatibilities appear when patient's antibodies bind to red cells to be transfused. Up to now, all pre-transfusion testing are addressed using techniques based on immunology. This is time consuming, expensive and some incompatibility situations cannot be addressed at the patient's bedside. In this position paper, we propose a completely novel paradigm. Our hypothesis is that red blood cells sensitized by the patient's antibodies see their deformability greatly reduced. This induces changes of the rheological properties of the "red cells concentrate /patient's blood" mixture. Studies described in this position paper aim at characterizing these modifications by measuring the characteristics of acoustic waves propagating in the mixture and to produce a mobile and automated acousto-micro-fluidic device which would allow detecting any incompatibility at the patient's bed side.

## 1 INTRODUCTION

In most countries, a crossmatch (a compatibility test between blood for transfusion and the receiver's blood) is carried out in a laboratory prior to transfusion, but is of no use when an error occurs after the blood has been transfused. The current techniques for carrying out a crossmatch are either manual, with blood reagents and samples being mixed in tubes or being placed on gel columns before centrifuging (e.g. Across Gel® Cross Match, from Dia Pro or ID-Card 50531 from Bio-Rad), or automated (e.g. the Qwalys analysers from Diagast). As far as these automated systems are concerned, the analysers may only be used in the laboratory and are difficult to adapt for use at the patient's bedside, in particular because of the need to treat the blood samples before they are analysed.

Many countries are thus looking at solutions at the patient's bedside in order to reduce the rate of transfusional accidents related to avoidable immunological incompatibilities. Currently, the final pre-transfusional test at the patient's bedside consists exclusively of an identity check (identity of the blood pouch and identity of the patient). This method cannot guarantee that there will be no transfusional accident because 50% of the reported adverse effects are due to human error (SHOT, 2011). Therefore, in spite of increasingly effective safety systems, it is currently impossible to eliminate entirely the risks due to human error, both in the laboratory and at the time of the transfusion.

A few countries, including France, carry out a second ABO compatibility test at the patient's bedside immediately before the blood transfusion, using control charts requiring several handling procedures. These tests makes allows us to limit fatal transfusional accidents in France, but does not completely prevent human error, which is the main cause of transfusional error (Linden, 2000 - Myhre, 2000 - Sazama, 2003). These errors can occur during the test procedure at the patient's bedside, in

particular when the hemagglutination reaction is read and interpreted (Henneman, 2007 - Myhre, 2000).

In this position paper, we propose a conceptual and technical innovative approach which will address any red cells incompatibility in a mobile and easy to use medical device.

The scientific hypotheses can be summarized as follows. Red cells are nucleus-free cells with an average diameter of 7.2 µm. Their cytoplasm is rich in haemoglobin. Their membrane is extremely flexible. This makes them deformable enough to propagate in the much smaller blood capillary network (Kim, 2015 – Tomaiuolo, 2014 – Mohandas, 2008). Physiological aging or various pathological situations jeopardize this deformability (Franco, 2013 – Mourao, 2016 – Chien, 1987). Among the factors, deformability is reduced when red cells are covered in antibodies. More importantly, a loss in red cell deformability results in a significant increase of blood viscosity (Kim, 2015).

When the red cells concentrate is compatible with patient's blood, antigens at the surface of the red cells to be transfused are not complementary to antibodies present in the patient's blood. No antigen/antibody reaction occurs and deformability of the red cells to be transfused is not modified. Transfusion is then allowed.

On the contrary, when the red cells concentrate is incompatible, antigens at the surface of the red cells to be transfused are recognized by the patient's antibodies. This induces an immune reaction whose consequences are more or less critical (alloimmunization, haemolytic reaction, patient's death). Our hypothesis is that fixation of antibodies on the red cells to be transfused reduces their deformability and alters the rheological properties of the "red cells concentrate (blood bag)/patient's blood" mixture. This alteration can be measured using acousto-fluidic interactions. In this case, no supplementary reagent is required and any immune incompatibility situation can be detected.

This communication is organized as follows. In section 2, we present a schematic representation of the foreseen medical device. In part 3, we present preliminary results obtained concerning acoustic mixing, activation and detection of liquid rheological modifications. In part 4, and in line with the scope of a position paper, we present scientific and socio-economic impacts such a device could address.

# 2 DESCRIPTION OF THE FORESEEN DEVICE

The device under development includes studies on microfluidics, acoustic mixing, acoustic activation and acoustic detection. Examples of acoustic manipulation of liquids will be presented in section 3. In this section, we detail the biological and technological studies currently under investigation.

At the cellular level, deformability of red cells is investigated using conventional techniques: Atomic Force Microscopy, possibly optical tweezers and quantitative phase imaging (Kim, 2015). Experiments are conducted in condition as close as possible to real clinical conditions. For example, we are setting up experiments with compatibility or incompatibility situations induced by natural and/or unexpected antibodies against red cell antigens. Variations of the rheological properties of the bag's cell/patient's blood mixture will be investigated using a device such as the one described in figure 1 in the case of an incompatibility.

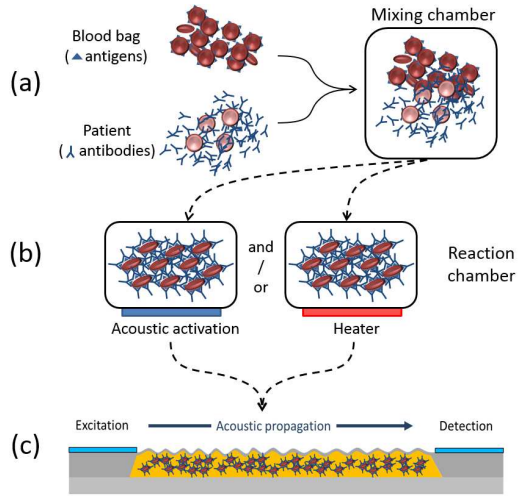


Figure 1: Schematic representation of the foreseen device. (a) Driving the samples to the mixing chamber. (b) Reaction activation either by acoustic activation or heating. (c) Acoustic detection of incompatibilities.

Patient's blood and bag's red cell will be transferred to a mixing chamber (part (a) in figure 1). The goal is to make the mixture as homogeneous as possible in order to optimize recognition of the red cell antigens by antibodies from the patient. Mixing by diffusion would be too long for a practical use at the patient's bed side. Therefore, acoustic mixing comparable to what is presented in section 3 will be employed.

The blood mixture will be transferred in a reaction chamber as illustrated in part (b) of the figure. In order to reduce the time required for the antigen/antibody reaction, this chamber may have to be temperature controlled and/or equipped with an acoustic activation device as presented in the next section.

Once the antigen/antibody reaction is completed, the mixture is then transferred into a test chamber equipped with acoustic transducers (part (c) in the figure). The transducer generates acoustic waves (Lamb waves in this case) which propagate through the mixture and are detected by the acoustic detector. This detector will detect variations of the rheological properties of the mixture through measurement of both amplitude and phase by means of an integrated network analyzer. In this case, measurement obtained with the acoustic detector will have to be compared to values obtained in the case of compatibility. This may become an issue since referenced or calibrated measurement are difficult to perform in an automated way at the patient's bed side. Also, Lamb waves only interact with liquid in the vicinity of the membrane. A more bulky architecture may be required in order to enhance the sensor's sensitivity.

A possible simpler architecture is describe in figure 2. Here only one chamber and only one transducer is used. Red cells to be transfused and patient's blood are injected in the chamber and the piezoelectric transducer is driven with frequencies and amplitudes suitable for either fluid mixing, activation or reaction sensing. The acoustic transducer may be patterned so that several acoustic modes can be generated. Natures of the most suitable acoustic modes will have to be defined for the aimed function. Next, the same transducer is used to monitor the time-dependent evolution of the acousto-fluidic properties of the mixture during the antigen/antibody reaction. This make the method potentially reference or calibration free.

We already mentioned that the age of red cells influences their membrane deformability (Franco, 2013). If needed, micro-filtration units will be added to the device in order to remove aged red cells. These units will be inserted in the circuitry employed to transfer the red cells to be transfused and the patient's blood to the mixing chamber (part (a) in figure 1). However, a device similar to the one we propose for blood incompatibility could be used in order to qualify the age of red cells contained in transfusion bags. This constitutes one of the scientific impacts we describe in section 4.

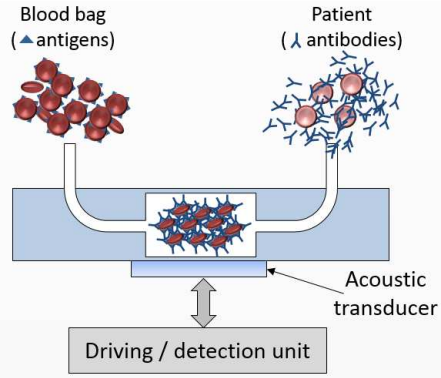


Figure 2: Simplified architecture with one sensor/actuator.

# 3 ACOUSTIC INTERACTIONS WITH FLUIDS

In section 3, we mentioned that acoustic manipulation is used for fluid mixing, antigen/antibody reaction activation and rheological properties monitoring. In what follows, we present preliminary results concerning acoustic fluids manipulations which can be directly adapted for blood compatibility assessment.

## 3.1 Acoustic mixing

We demonstrated acoustic mixing using patterned acoustic transducers (Kardous, 2014). This structure allowed exciting different acoustic modes in a liquid droplet as illustrated in figure 3.

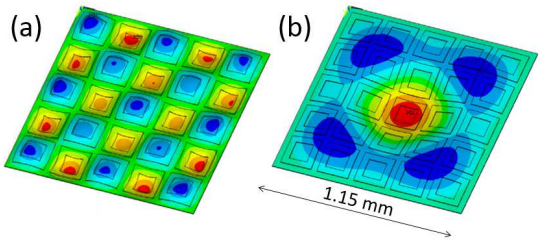


Figure 3: (a): Generation of acoustic mode (5.5). (b) Generation of a degenerated mode (1,3)+(3,1). Figure adapted from (Kardous, 2014).

Mixing various viscous fluids have been tested. Liquids to be mixed are rhodamine in water and 40% glycerol in water. Fluorescence of rhodamine is used to visualize the mixing of these two liquids. The result is shown in figure 4. In this figure, negative times indicate that no acoustic activation is applied. For positive times, acoustic mixing is applied. It can be noted that the two liquid phases are completely mixed after about 3-5 min.

Acoustic mixing was also demonstrated in order to homogenize blood red cells in a physiological serum droplet. This is illustrated in figure 5. Note that acoustic manipulation can also be used for reversible concentration/dispersion of micro-particles in liquids. This is not shown here because it is out the scope of this communication.

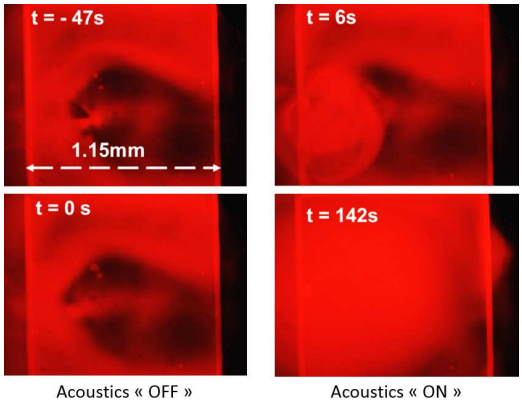


Figure 4: Acoustic mixing of water and glycerol solution. Rhodamine is used to visualize mixing using fluorescence. Figure adapted from (Kardous, 2014).

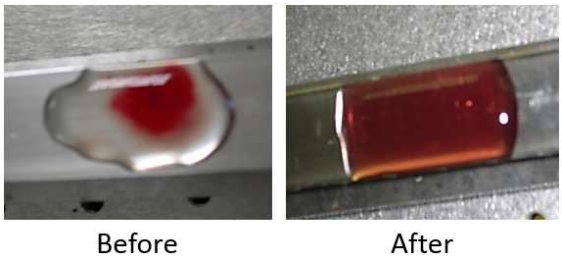


Figure 5: Acoustic homogenization of red cells in a physiological serum droplet.

## 3.2 Acoustic activation

Antigen/antibody recognition can be enhanced using acoustic activation during antibodies immobilization step (Kardous, 2011). Monoclonal antibodies A9H12 were deposited in droplets of 400 nL at the surface of membrane compatible with Surface Plasmon Resonance imaging (SPRi) experiments. Five antibodies spots were deposited. The corresponding antigen was LAG-3 proteins. Figure 6 shows SPR images of the biochip were (a) corresponds to the capture of LAG-3 without acoustic activation and (b) corresponds to the same spots when acoustic activation is used. Amplification of the bio-recognition ranges from 1.5 to 3. This is illustrated in figure 6(c) and it highlights the efficiency of acoustic mixing regarding antigen/antibody reaction.

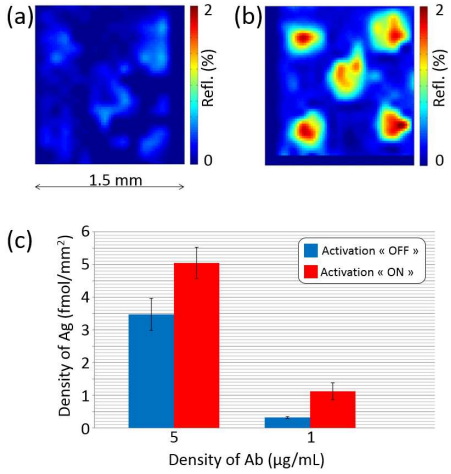


Figure 6: Acoustic activation of antigen/antibody recognition. (a) LAG-3 antigens captured by A9H12 antibodies on a SPRi biochip without acoustic activation. (b) The same experiment using acoustic activation. (c) Amplification rate obtained for 2 values of the antibodies concentrations. Figure adapted from (Kardous, 2010).

## 3.3 Acoustic sensing

Acoustic sensing of a fluid may be achieved using the Lamb waves. Various Lamb waves exist such as symmetric modes noted $S_i$ and the anti-symmetric modes noted $A_i$. $A_0$ and $S_0$ are shown in figure 7(a).

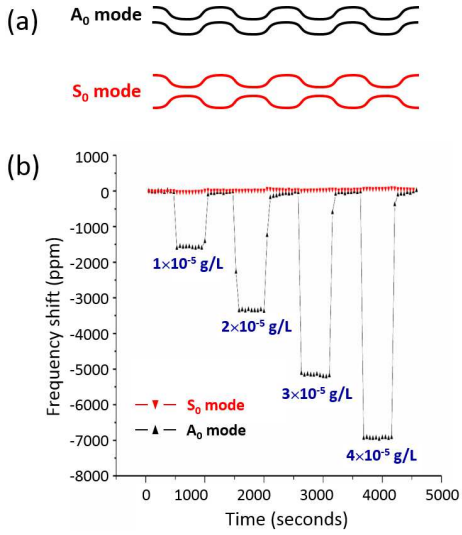


Figure 7: Acoustic sensing. (a) Anti-symmetric and symmetric Lamb waves modes. (b) Sensing solutions of varying concentrations in sodium chloride.

Each mode exhibits its own resonant frequency and penetration depth in the liquid under investigation. An example of acoustic sensing is

given in figure 7(b). Here, resonant frequencies of the modes are about a few MHz. Lamb waves were used to monitor the concentration in sodium chloride. Addition of sodium chloride increases the density of water. This results in shifts of the resonant frequency of the acoustic mode. The figure clearly shows that acoustic sensing allows measuring the sodium chloride concentration (*i.e.* the viscosity) when $A_0$ mode is excited. $S_0$ mode does not experience frequency shifts due to its inadequate penetration depth.

# 4 SCIENTIFIC AND SOCIO-ECONOMICAL IMPACTS

As mentioned above and in the scope of a position paper, we present the scientific and socio-economic impacts such a device potentially produce in the next section.

The new paradigm which constitutes the acoustic detection of immuno-incompatibilities potentially allows detecting any cause of incompatibility. Given the increasing exchanges of populations and the increasing number of multiple transfusions, some unexpected antibodies against red cell antigens are not currently detected or identified. The acoustic technique we propose here would detect incompatibility without the need of prior identification.

Outside the field of transfusion safety, studies are ongoing concerning the effect of the age of the red cells concentrates on the transfusion efficiency (Vallion, 2015 – Lacroix, 2015 – Lapierre, 2007 – Desmarets, 2016). In 2008, Luten *et al.* showed that about 30% of bag's red cell are eliminated by the organism within 24 hours after transfusion, probably because of the loss in membrane deformability (Luten, 2008). Therefore, a device like the one we propose could be adapted in order to perform a selection of the red cells based on the detection of the less deformable cells at the moment of blood donation. This potentially would improve blood transfusion efficiency.

By changing the principle of incompatibility detection, this acousto-fluidic device will be a technological advance because no expensive antibodies will be used (human IgGs). The foreseen cost of the disposable part of the device is estimated to about 5$.

Technology development related to this device will considerably simplify ultimate compatibility controls by proposing a mobile device which can be used by non-trained staff at the patient's bed side. In most countries, organization of care will benefit from this technological and conceptual breakthrough. In countries where the transfusion chain (from donation to transfusion) is well organized, this device will contribute to the harmonization of pre-transfusion controls as advocated by the World Health Organization. In countries where the transfusion chain is not or partially organized, such a device will offer a cost-effective solution to enhance blood transfusion safety. In the future, this device could be proposed for every red cell concentrates transfusion which means at least 17 million tests in Europe, 20 million in the US and 140 million worldwide.

In the world, only 62% of countries have a legislation and care organization concerning the quality and the security of blood transfusions. In 2016, 40 countries admit that no qualification of the blood donation is performed due to the lack in qualified staff and economic issues (WHO factsheets).

The World Health Organization highlights the need for international standardization of safety processes, in particular for what concerns blood incompatibilities. Simplifying the blood transfusion safety controls would improve the access to safe and cost-effective blood transfusions in more countries than it is today and to the benefit of a rapidly increasing population.

# 5 CONCLUSION

In this position paper, we have described how immuno-erythrocytic incompatibilities can have severe or lethal consequences. We pointed out that there exists no method to address all the incompatibility situations.

Here, we propose a new paradigm based on the use of acoustic sensing to detect incompatibilities. The hypothesis is that when red cells from the red cells concentrate are covered in antibodies issued from the patient's blood, the deformability of their membrane is strongly reduced. As a consequence, rheological properties of the mixture red cell concentrate/patient's blood are modified and these modifications can be acoustically detected. Furthermore, this acoustic detection is independent of the immunologic origin of a possible incompatibility. Examples of already demonstrated acoustic mixing, activation and sensing using other fluids have been presented. The preliminary results demonstrate the feasibility of the method we

propose. Experimental studies are currently ongoing using whole blood samples issued from donators and red cell concentrates.

When completed, these studies will lead to a global solution able to address any incompatibility situation which can be used by non-specialized staff directly at the patient's bed side. This incompatibility detection has to be coupled to an identification of the antibody / antigen responsible of the immunological reaction. This technology will reinforce transfusion safety in countries where the transfusion chain is already organized. At the same time, such a device will offer an affordable solution to enhance blood transfusion safety in other countries.

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