

A Red Blood Cell Fragility Meter for Facilitating Blood Triage

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Introduction:

Red Blood Cell (RBC) transfusion is a common medical procedure, which most often involves stored RBC units. During storage, RBC quality degrades due to various factors (“storage lesion”). This decreases cell viability *in-vivo* and thus often compromises transfusion efficacy. The degradation of any given unit of RBC solution varies according to several factors besides time (e.g., variability among donors’ average RBC age, post-collection storage/handling conditions), rendering the current metric of time alone a poor proxy by which to estimate degradation. There is not yet a clinically available or feasibly adoptable test for measuring actual degradation in any individual unit of blood product. Current FDA regulations do call for 75% of RBC to survive post-transfusion, but even compliance with this standard cannot be reliably assessed.

Blaze Medical Devices, LLC – a Michigan startup company of which the author is a co-founder – is presently in the process of developing a Red Blood Cell Fragility Meter (RBC-FM) device to measure the degradation (and thus prospective efficacy) of each unit of stored blood product at or near the point-of-use. It is determined via the marker of the progressively increasing “fragility” of the cell membrane during storage. Pending clinical validation, the expected correlation is predicated on existing research indicating that RBC fragility is a strong marker for prospective transfusion efficacy due to 1) increased propensity for hemolysis *in vivo*, 2) decreased oxygen-delivering capacity for the surviving cells, and 3) various indirect/systemic impacts on tissue oxygenation. Knowing the respective degradation states of particular units will allow hospital blood banks and clinicians to match units to patient groups based on relative efficacy needs. Moreover, it will enable optimization of inventory routing and distribution decisions throughout the supply chain. This paper describes the work under development by the founders and collaborators of Blaze Medical Devices, LLC.

Background:

RBCs (erythrocytes) are highly specialized cells responsible for delivery of oxygen to, and removal of carbon dioxide from, metabolically-active cells via the capillary network. They are shaped as biconcave discs and average about 8-10 microns in diameter. The membrane is very flexible so as to allow the cell to travel capillaries with diameters of only 4-5 microns. At the same time, the membrane must be strong enough to withstand significant and repeated flow-induced stresses while avoiding tears or fragmentation. An erythrocyte with normal membrane stability and plasticity is able to circulate effectively and without damage, whereas a cell with lower-deformability and higher-fragility/rigidity is likelier to suffer damage even under normal circulatory stresses. During storage, erythrocytes experience an array of morphological and biochemical changes associated with lower deformability and increased fragility [1].

RBC deformability loss has been measured by a variety of techniques: micropore filtration, optical tweezers, micropipette techniques, resistive pulse spectroscopy, and laser-assisted diffractometry (ektacytometry). Note that although in most cases the results are presented and discussed nominally in terms of RBC “deformability,” the underlying cell properties that are actually being measured by these various tests are not necessary all the same. For example, the ability to deform (analogous to material ductility) is different from the ability to do so repeatedly (plasticity) as is required *in vivo*. Additionally, some techniques perform the measurements on all RBC in a sample, while others derive results from a single-cell measurement.

About 14 million units of Red Blood Cells (RBC) and Whole Blood (WB) are used annually on over 4.5 million patients, with RBC being the most common in the US. While the best way to store blood is said to be “within the donors,” unfortunately the use of the freshly-collected blood product is generally not practical. So, a variety of anticoagulant and preservative (A-P) solutions have been developed to allow for long-term storage. RBC units in a liquid state are stored at 1-6°C. The maximum storage interval (shelf life) is 21, 35, or 42 days, depending on the A-P solution used.

A recent study shows the average age of transfused RBC to be 21 days [2], by which point there can be a wide range of quality among units. The unknowable degradation status of particular units has been dealt with in different ways: a “restrictive protocol” seeks to minimize transfusions wherever possible, whereas a “liberal protocol” utilizes additional units in a transfusion to increase the chance of getting sufficient viable RBC. Both approaches have their costs and complications. The highly-cited TRICC Trial [3] compared these approaches for critically ill patients, and found that a restrictive approach is generally equivalent if not superior to a liberal approach. Considerable clinical data suggest adverse effects from the liberal protocol on critical-care patient outcomes, as reviewed by Marik and Corwin [4]. However in some cases massive transfusions remain a necessary evil; as reported by Spinella, certain segments of trauma patients received a mean of 7.7 RBC units per patient [5], which can lead to various complications and their associated costs – while wasting units that may have been helpful to non-critical patients.

Notably, a number of studies suggest that RBC transfusions often fail to deliver their desired clinical benefit, and in some cases even may be harmful to critical-care patients [6, 7]. Controversy remains as to appropriate transfusion thresholds, and on how transfusions could be individualized based on patients’ clinical circumstances. The precise reason(s) for the observed adverse effects also remain controversial, with RBC storage lesion a suggested contributor and/or marker [8]. Studies diverge on whether “age” of blood product is correlated with transfusion inefficacy [1], with various hypotheses being proposed to explain this inconsistency – such as different stored units degrading at varying rates, as well as there existing a substantial distribution of average RBC ages among donors at time of collection.

Device and Testing Procedure:

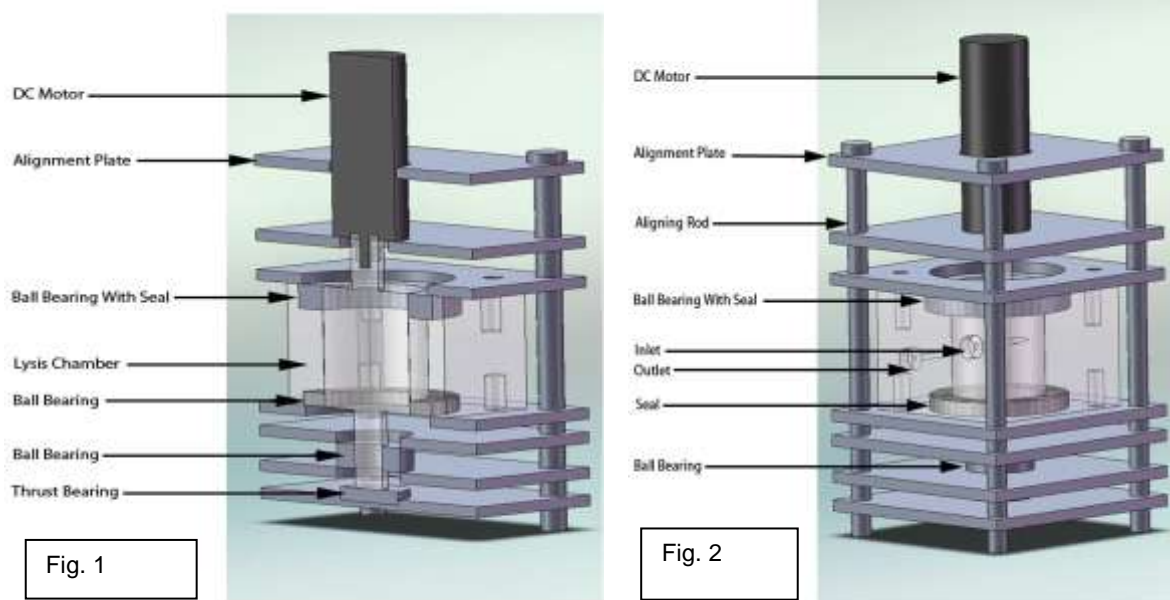
The testing method under development by Blaze Medical Devices involves the following major steps being taken on a sample from any given unit of stored blood/RBC product: 1) the select application of mechanical (shear) stress of variably-controlled intensities and durations/iterations, followed by 2) optical/spectrophotometric determination of the fraction of hemolysis in the sample, and 3) computational processing of the results via statistical modeling to quantify the prospective efficacy/suitability of the sampled unit for specific patient groups.

Hence the device/system consists of a hemolysis (lysis) component that provides variable shear stress intensity and duration combinations up to those necessary to lyse 100% of all RBC in a given sample, a spectrophotometric unit for collecting the relevant spectra, and data processing algorithms for synthesizing and analyzing the proportions of hemolysis occurring at each respective shear stress level – thereby calculating a complete fragility profile matrix for each RBC sample tested. The implications of this profile matrix are also the subject of specialized mathematical processing, as noted below.

Note that the stress involved in “fragility” or “plasticity loss” testing is of higher intensity than that involved in “deformability loss” testing; the former repeatedly stresses cells until lysis occurs, whereas the latter stretches the membrane of individual cells to measure their ability to be reshaped. Though related, fragility likely better resembles the stresses that transfused RBC experience *in vivo*, and no existing high-accuracy test for this is clinically-adoptable. Deformability measurements such as ektacytometry can actually be both accurate and clinically-adoptable, but so far these have been pursued only in diagnostics – where the measured properties are likely more relevant.

The overall device and utilization methods are the subject of a recently filed provisional patent application by Michael Tarasev and Kenneth Alfano on behalf of Blaze Medical Devices, LLC. A particularly advantageous means of accomplishing the optical step is described in a published utility patent application, “Blood Hemolysis Analyzer” by Michael Tarasev [Publication No. US2007/0259436 A1] also on behalf of Blaze Medical Devices; this provides a way to rapidly and accurately determine cellular and cell-free hemoglobin concentrations, and thus the proportion of hemolysis of RBC, either in WB or RBC solution.

For preliminary testing and validation, off-the-shelf equipment can be used for the optical and computational steps (i.e., spectrophotometer, centrifuge, laptop PC); however a customized prototype is still needed for the lysis unit. A current prototype (developed cooperatively via recent sponsorship of a student team course project) is a concentric cylinder device in which lysis occurs as a result of RBC solution flowing through a 0.1 mm gap between a stationary outer and rotating (up to 10,000 rpm) inner cylinder. The system also incorporates a blood reservoir, a peristaltic pump, and some peristaltic tubing to transport the test volume of blood. Figures 1 and 2 illustrate the configuration of this unit, which is currently pending a redesign/refabrication with a contract engineering firm in order to reduce the sample volume from approximately 15ml to 2ml (which will allow for using the external test strips rather than entering the bag for a sample). This will in turn enable more significant experimentation than has been feasible to date.



The pump enables a consistent flow rate from a syringe – through the inlet of the lysis chamber, into the gap between the inner cylinder and outer cylinder (where cells are subjected to a shear stress due to the rotation of the inner cylinder), and finally out through the lysis chamber outlet – allowing for either a continuous spectrophotometric analysis of the sample, or for a collection of individual sub-samples for discrete step-wise measurement. As one subsample exits the device through the outlet port, another subsample is pumped into the gap space. The RPMs on the motor are increased (and/or sample residence time decreased, via slower pumping), and each subsample is subjected to progressively increasing shear stress intensity and/or duration.

Initial testing has demonstrated that the designed system provides generated shear stress varying from 0% (no additional lysis beyond in-bag/autolysis) to 100% (total RBC lysis). A model of a 2-D fragility curve to be enabled by this device is shown in Figure 3.

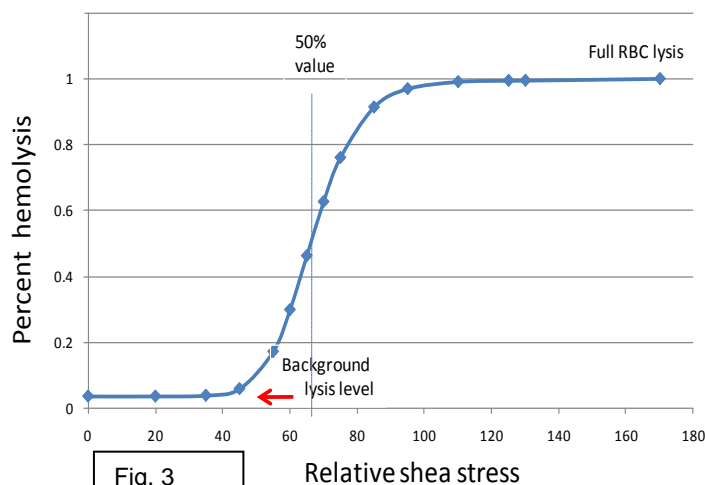


Fig. 3

Note that various anticipated changes can occur to this curve as fragility increases – the statistical analyses of the multivariate components of which are a critical part of the computational step generating the ultimate output for predicting a given unit’s efficacy/suitability for specific patient groups. A vital aspect of the testing system is the recursive accumulation of copious output and other associated clinical data and the mathematical analyses thereof to optimize algorithms by which to characterize subsequent test output as meaningfully as possible.

Ultimately, marketed versions of the device will be integrated systems with benchtop units and disposable cartridges or chips for each test. This will incorporate proprietary optical and computational elements providing fast and easy operation. The disposable portion is where the blood sample will be deposited, and from which the readings will be taken by the bench-top device. Figure 4 is a conceptual CAD (SolidWorks®) rendering of the disposable component alone (left), and integrated with the bench-top device (right).

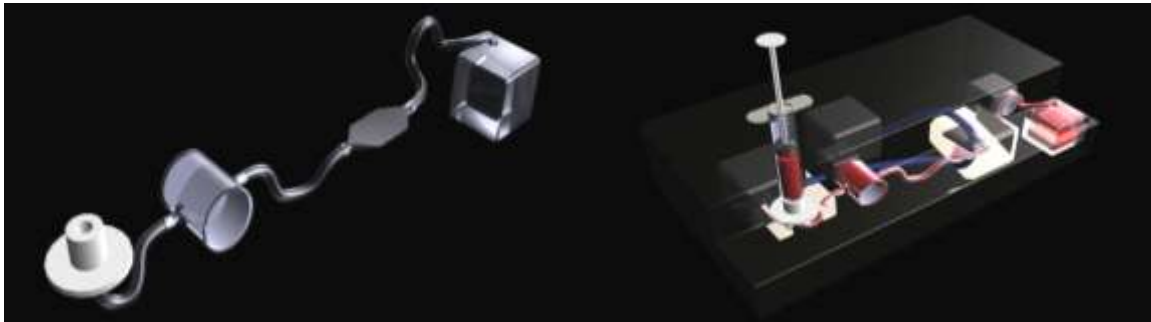


Fig. 4 – The syringe doc leads to the lysis chamber, which in turn proceeds to an optical cuvette.

Validation Steps:

Two major hurdles are anticipated to achieving clinical validation (and thus widespread clinical acceptance) for this testing. First, trials must be performed to conclusively confirm the existence and extent of variability in degradation states among blood units of comparable “age” or storage time. Blaze is presently working in cooperation with the University of Michigan Hospital Blood Bank to plan such a study. Secondly, and more challengingly, trials must be performed to actually link the results of the testing with patient outcomes. The latter will be notably more difficult to establish, as there is not yet any accepted “gold standard” for transfusion efficacy.

The initial regulatory hurdles are minimal, because the RBC-FM device is likely to be a 510K device requiring only pre-market notification rather than pre-market approval (PMA). This is because there is no patient contact involved, and it does not need to involve any patient receiving any blood which would not already be FDA approved under current standards. (In the future it is possible that the 42-day shelf life could be entirely supplanted by a viability test such as this, but for now the testing merely re-routes or re-allocates blood that was simply being randomly selected anyway.)

Expected Implementation:

The main initial users of Blaze’s blood testing system are the technicians employed in hospital blood banks, who would simply add this test to the battery of other tests periodically performed. The results of this test, however, would be interpreted by clinicians so as to facilitate triaging of blood based on patient groups’ known efficacy needs.

It would likely constitute a relatively small additional cost to all the testing already performed on the blood supply, and any costs will be more than recovered in savings from the noted complications of the status quo. And far from further straining supply – as quality-control measures often can – this testing could be deployed throughout the supply chain so as to optimize inventory management based on real-time data; this will increase the proportion of blood reaching its destination in the best possible state.

After the necessary validation, the first major target market will be larger (500+ bed) hospitals – whose blood banks account for about 30% of all transfusions. Figure 5 gives a rough breakdown of blood use by hospital size. Also notable is that larger hospitals tend to receive older blood (closer to its outdating), because the less-trafficked hospitals have longer and less-predictable intervals between transfusions.

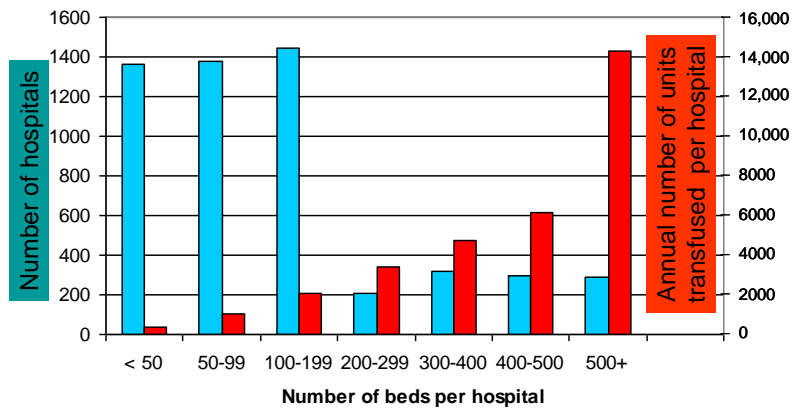


Fig. 5

Another aspect of bringing this testing to the healthcare market will be to target the types of anemias by priority of their needs for prospective efficacy (and thus need for knowledge of quality/degradation level), while considering the size of these respective categories as well. (Some aspects of the validation process may also become specific to anemia condition.) Table 1 gives a breakdown of anemia by patient group.

Breakdown of Annual Transfusions Attributable to Specific Anemia Types				
<i>Anemia</i>	<i>Number of Cases</i>	<i>Number of Units Transfused</i>	<i>Total Transfusions</i>	<i>Units Transfused</i>
Chronic				
Sickle Cell Anemia	70,000	25	1,750,000	1,750,000
Thalassemia	65,000	25	1,625,000	1,625,000
Myelodysplastic Syndromes	100,000	25	2,500,000	2,500,000
Gastrointestinal Bleeding	140,000	4	560,000	560,000
Chronic Inflammation	40,000	2.6	104,000	104,000
Treatment-Induced				
Chemotherapy	160,000	4	640,000	640,000
Surgery and Trauma				
Non-Critical Care	120,000	2.6	312,000	312,000
Crit. Care (Non-Neonatal)	1,200,000	4.6	5,520,000	5,520,000
Neonatal		(split units are used)		
Neonatal (All)	300,000	1.8	2,700,000	270000
Other			1,000,000	1,000,000
<i>Total Transfused:</i>			<i>16,711,000</i>	<i>14,281,000</i>

Table 1

Finally, while the initial focus of the RBC-FM device is targeted toward applications in blood quality control, the core technology of clinically-conductive quantification of RBC fragility via stress-induced hemolysis and subsequent optical and computational analysis could indeed have broader application – such as in disease diagnosis. However, in these applications, fragility testing (high-stress) would be competing with deformability testing (low-stress), which is already under development for such markets.

Acknowledgments:

The author of this paper is one of two co-inventors of the described testing procedure and device; Michael Tarasev is the other co-inventor, and co-founder of Blaze Medical Devices, LLC, as well as the sole inventor of the noted proprietary optical component which will ultimately be used in marketed versions of the device.

The founders greatly appreciate the continued advice and support of Dr. Davenport, Medical Director of University of Michigan Hospital Blood Bank, and Dr. Napolitano, Division Chief of Acute-Care Surgery for University of Michigan Hospital.

The CAD rendering for Figure 4 was provided by Gene Parunak on a freelance basis; Figures 1 and 2 were produced by Chris Williams and Chris Panjwani (members of a student team) as part of Blaze's sponsorship of a project for a senior design course.

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