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Digital Microfluidic Hemagglutination Assays for Blood Typing, Donor Compatibility Testing, and Hematocrit Analysis

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Alexandros A Sklavounos, Julian Lamanna, Dimpy Modi, Sidharth Gupta, Alex Mariakakis, Jeannie Callum, Aaron R Wheeler
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Guest: Alexandros Sklavounos is a doctoral candidate at the Department of Chemistry at the University of Toronto in Toronto, Ontario.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett. Blood typing, donor compatibility testing, and hematocrit measurements are performed to ensure the compatibility of the donor red blood cells, plasma or platelets with the transfusion recipient and to determine the need for transfusion. These tests are routinely performed in a number of settings, most frequently prior to blood transfusions.

These tests are typically performed in centralized laboratories with sample batching, but the time taken to transport the blood specimen to the laboratory may lead to adverse outcomes especially for critical care patients. A paper describing a new digital microfluidic blood analysis technique appears in the December 2021 issue of *Clinical Chemistry* and provides a step towards providing rapid results at the bedside or trauma unit.

Our guest in this podcast is Alexandros Sklavounos, the lead author for that study. He is a doctoral candidate at the Department of Chemistry at the University of Toronto in Toronto, Ontario and a senior member from the laboratory of Professor Aaron Wheeler, also an author of the study.

First of all, Alex, for our listeners who might not be from laboratory backgrounds, what are blood groups and hematocrit?

Alexandros Sklavounos: So to start with, both blood grouping and hematocrit are blood tests. Speaking of blood, blood comprises red blood cells, white blood cells and platelets and are all suspended in a liquid called plasma. Blood grouping, in particular, describes the process of categorizing a blood sample based on protein molecules found on the surface of red blood cells. These proteins are also called antigens. An individual's blood type depends on which genes they inherited from their parents. And according to the International Society of Blood Transfusion, there are currently 43 recognized blood group systems that are

genetically determined by 48 genes and many antigens found on red blood cells.

Blood groups are used to determine pre-transfusion blood compatibility between a donor and a recipient. As for hematocrit, it is a term that comes from the Greek words hema- which means blood and krites which means judge and defines the percentage of red blood cells in whole blood. Since we are measuring by volume, the measurement depends both on the number and the size of red blood cells.

Bob Barrett: So why are these tests important and in what clinical settings are they key?

Alexandros Sklavounos: Blood grouping tests are important because they are used to determine compatibility prior to transfusion. To put it in perspective, every two seconds someone in the US needs a blood transfusion. Also, blood groups are used to determine compatibility for organ transplants as well. Among blood groups, ABO is the most well-known and this is because antibodies against the ABO antigens are formed naturally. The ABO group is determined by the presence or absence of two antigens, antigen A and antigen B or the combination of the two resulting in four blood types known as Type A, Type B, Type AB and Type O.

Naturally occurring antibodies means that their production is triggered when the immune system encounters the missing ABO antigens. In the environment for example in food or microorganisms, these happens at an early age because sugars that are identical to or very similar to the ABO antigens are found throughout nature and although most individuals will never be exposed to red blood cells of a different ABO group, they already have antibodies against these antigens in their plasma that are ready to neutralize the foreign threat. As a result, the receiving blood from the wrong ABO group may result in an acute immune response that can be life-threatening.

Another well-known blood group is the Rhesus group. It is the second most important blood group system after the ABO. The Rhesus group system consists of several blood group antigens including antigens D, C, and many more. However, the terms Rhesus positive, Rhesus negative and Rhesus factor often refer to the Rhesus D antigen only. Although antibodies against the Rhesus antigens are not naturally occurring like the ABO, they still pose a risk for hemolytic transfusion reactions and are very important during pregnancy as they can be responsible for the hemolytic disease of the fetus and new

born as it is called a condition that develops in the fetus when antibodies passed from the mother through the placenta during pregnancy. Together, the ABO and the Rhesus groups make up eight blood types that most people know as A positive, A negative, B positive, B negative and so on.

Today, blood components are often transfused separately, making O negative the universal blood sample and AB positive the universal plasma sample, meaning that they can be safely given to patients with any blood type. As a result, these blood types are the ones in the highest demand but blood banks have definite amount of such blood types. Also bear in mind that only 7% of the population has O negative blood and about 3% has AB positive blood. Hence, when in need of transfusion, the sooner the blood type of the recipient is determined, then less of these precious blood samples will be used.

As for hematocrit, it is a routine test often performed as part of blood count test. Since the purpose of red blood cells is to transfer oxygen from the lungs to blood tissues, the hematocrit can be used to estimate the amount of oxygen delivered. For males, the hematocrit ranges between 41% and 50%, while for females it is a bit lower ranging between 36% and 44%. Abnormal hematocrit levels that are either too high or too low can indicate a blood disorder or a medical condition such as dehydration. A hematocrit level below the normal range is also called anemic as it indicates there are too few red blood cells in the blood. To tie this back to transfusion, a hematocrit level of 38% is also considered the minimum needed for donating blood.

Bob Barrett: We hear a lot about microfluidics these days but what exactly is digital microfluidics?

Alexandros Sklavounos: Digital microfluidics are a relatively new type of microfluidics. It is also referred as the DMF in short so I might be using that term in the future. So DMF is the fluid handling the link used to control small volumes of liquids. Using devices of about the size of a credit card, discreet droplets are manipulated on an array of electrodes using electrostatic forces. Droplets can be dispensed, moved, merged, mixed, and split, enabling a wide range of reaction protocols to be implemented on a single device.

In addition to this, in the past two or so decades, since it is an introduction to microfluidics community, DMF has been used in a variety of chemical and biological applications. One great advantage of DMF is that it can effortlessly handle both homogenous and heterogenous liquids whereas other microfluidic technologies can be

limited in manipulating the latter mainly because of clogging. In the vein of working with heterogenous samples, DMF is often paired with magnets and magnetic particles to automate the sample cleanup and preconcentration of analytes.

On a different -- DMF has also been shown to be useful for automating and miniaturizing cell culture and analysis as well. In general terms, DMF is a fluid processing technique. However, DMF has been paired with a plethora of detector modalities as part of the concept of performing both the sample processing and generating the readout using a single instrument. For example, DMF has been successfully coupled with many in-line analysis sensors including optical sensors, mass spectrometry, nuclear magnetic resonance spectroscopy and various electrochemical sensors.

Bob Barrett: So why did your group in Toronto choose to use digital microfluidics for hemagglutination assays?

Alexandros Sklavounos: That's a great question. Today, hemagglutination assays are performed either manually by a lab technician or automated using a large batch analyzer. Both cases however suffer from some drawbacks with the most important being poor reliability for those manual assays and long turnaround times for the batch analyzers. In the meantime, the microfluidics community has offered numerous solutions to the aforementioned issues, but none of them seem to have succeeded in addressing them both. This seem like a great opportunity for our DMF technology that has been previously used to automate several assay protocols that involve whole blood.

With DMF we can process blood samples without any dilution because of the open nature of the devices. This does not post any risk for clogging which is a major issue that several microfluidic systems have struggled with. I also like to use the slide agglutination test and manual test as an example to describe how our system works. Imagine that you have a microscope slide, a small droplet of agglutinating cells, and a cover slip at top. Once the two slides are fixed, the droplet is pushed down and it cannot be moved but this is enough to visualize any agglutinates large or small in the droplet.

Now, what if you could move that droplet between the two slides in any direction and in a very controlled fashion? This is exactly what DMF allows us to do. One thing we realized early on was that the gentle mixing that DMF offers allow the formation of large agglutinates in short times, while the architecture of our devices allow for easy visualization of the agglutinates even by eye. Since the

result of the agglutination assays was so easy to see, we decided to take our system a step further and automate the agglutination detection.

Using a low-cost webcam and computer vision, we developed a method that we called droplet agglutination assessment on digital microfluidics, or DAAD, as we like to call it in the lab. DAAD relies on an open-source image analysis toolset and is a robust algorithm with minimal requirements that was used to identify agglutination in droplets on a DMF device. DAAD allowed us to eliminate the subjective interpretation of the result and to fully automate the process from start to finish which can be completed in under six minutes.

During the development of the blood grouping assays, we noticed another thing. The agglutinates separated very easily from the plasma. Hence, we decided to use a chemical agglutination agent and modified our DAAD algorithm which allowed us to determine the ratio between the red blood cells and the sample and the surrounding plasma regardless of the blood type of the patient. By doing so, we were able to determine the hematocrit of the patient as well. And in addition, without any further modifications, we could swap the blood grouping antibodies with plasma or blood from the potential donor and perform a compatibility test between the recipient and the donor within the same time scale.

Bob Barrett: Well, finally Alex, what are the further clinical applications of such assays and is there any potential in commercializing this technology?

Alexandros Sklavounos: In this study, we demonstrated that using hemagglutination assays we could determine the blood group of the patient, perform a compatibility test between the donor and the recipient, and determine the hematocrit of the patient all using the same multi-purpose DMF device. We also asked technician Dimpy Modi at Sunnybrook Hospital to run some of these assays to showcase how simple to use our system is by non-expert users. In essence, once a sample is loaded and the user starts the protocol, the system processes the sample automatically and returns the result within a few minutes without any other requirements from the user.

All three tests are performed routinely in hospitals all over the globe. However, for example, when a patient is admitted to a hospital, it can take from 30 minutes to several hours to determine their blood type. So for a scheduled surgery, this is not a need. But in case of trauma, when a patient bleeds excessively and is in need of transfusion stat, every minute counts. We believe that

our DMF platform can be deployed right by the bedside and expedite all those tests providing reliable and automated results sooner. And remember, a specimen for blood typing, the sooner the patient is grouped, the less universal blood will be wasted as well preserving it for those cases when it is really needed.

As for commercializing the technology, we do see great value in the system and it is an option that we would most definitely like to explore in the future.

Bob Barrett: That was Alex Sklavounos from the Department of Chemistry at the University of Toronto in Toronto, and the lead author for a study that developed a portable digital microfluidic system to carry out hemagglutination assays. That paper appears in the December 2021 issue *of Clinical Chemistry.*

I'm Bob Barrett. Thanks for listening.