Designing an automated blood fractionation system

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Background
UK Biobank will be collecting blood samples from a cohort of 500,000 volunteers and it is expected that the rate of collection will peak at ~3000 blood collection tubes per day. These samples need to be prepared for long-term storage. It is not considered practical to manually process this quantity of samples so an automated blood fractionation system is required.

Methods
Principles of industrial automation were applied to the blood fractionation process leading to the requirement of developing a vision system to identify the blood fractions within the blood collection tube so that the fractions can be accurately aspirated and dispensed into micro-tubes. A prototype was manufactured and tested on a range of human blood samples collected in different tube types.

Results
A specially designed vision system was capable of accurately measuring the position of the plasma meniscus, plasma/buffy coat interface and the red cells/buffy coat interface within a vacutainer. A rack of 24 vacutainers could be processed in <5 min.

Conclusion
An automated, high throughput blood fractionation system offers a solution to the problem of processing human blood samples collected in vacutainers in a consistent manner and provides a means of ensuring data and sample integrity.

Keyword Automated blood fractionation

Introduction
Blood fractionation involves the centrifugation of whole blood followed by the determination of the positions of the interfaces between the different layers so that aliquots of the fractions of interest can be aspirated from the blood collection tube. The positions and volumes of the interfaces and the turbidity of the fractions can vary markedly from sample to sample, and fraction separation normally relies on a skilled operator visually determining the positions of interfaces and separating them with manual pipetting. This can lead to variation in the way in which the fractions are recovered from the blood collection tube, particularly with the buffy coat, which only forms a thin layer.

UK Biobank will be processing and storing blood from a large number of people. This presents a huge logistical challenge so UK Biobank commissioned a feasibility study to investigate an automated, high throughput blood fractionation system (Elliott P, Peakman TC. unpublished results). This article describes the work undertaken, as part of this study, to develop a fast, reliable and consistent method of identifying the positions of the interfaces between layers and using the data to automatically generate liquid handling protocols to aspirate the correct fractions and dispense them into micro-tubes ready for long-term storage.

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Methods

Principles of industrial automation were applied to the blood fractionation process leading to the choice of industrial robots, proprietary laboratory automation equipment and RTS Sprint software to form the basis of an automated system. However, no suitable proprietary equipment was found for the detection of the interfaces between the different layers of the fractionated blood. After exploring a range of detection techniques, vision technology was selected as the most robust and accurate without disturbing the sample. We decided to develop a vision system based on RTS vision technology capable of detecting all three fractions.

A critical area for the performance of an automated system is the choice of labware so this was carefully selected in conjunction with UK Biobank. Ten-millilitre plastic BD vacutainers with Hemogard™ caps were selected for blood collection.

Development of vision system

Development work started with mock-ups of blood in vacutainers to come up with a concept for a vision system for detecting the three blood fractions. To create a high throughput system, we decided that vacutainers should be processed in racks capable of being handled by a robot—holding up to 24 vacutainers each. Initial work concentrated on experimenting with different lighting and camera arrangements to take account of the shape and optical properties of the vacutainers.

A test rig consisting of a light-proof box, camera and lighting was constructed and a range of blood samples were placed into it. Images were captured using different settings. These images were then used to refine the lighting and lens arrangement. The test rig was modified and a further range of blood samples were used to optimize the lighting and camera configuration. The images captured during these tests were then used to develop the software algorithms for processing the images and determining the positions of the blood fractions.

The final stage of development involved the construction of a fully working vision system for testing.

Testing of vision system

Venous blood was drawn from six healthy volunteers into $3 \times 10$ ml plastic BD vacutainers with Hemogard™ caps (EDTA, SST™ and PST™). The SST and PST tubes are gel separator tubes for isolating serum and plasma, respectively. The EDTA and PST™ tubes were centrifuged (10 min, 2500 r.p.m.) immediately after collection. The SST™ tubes were allowed to stand for 30 min prior to centrifugation to allow clotting. Once fractionated the height of the interfaces from the base of the tube were measured manually using digital callipers. The interface heights were measured using the RTS vision system (International Patent Application pending—WO2006/037941). In order to assess the repeatability of the vision system the interfaces of each tube were measured a minimum of six times from different locations within the rack.

Results

As a result of extensive trials, we chose to adopt an approach using two images of each vacutainer (Figure 1). This allows a cross-check between images and provides a more robust solution.

Having captured the two images, advanced software algorithms determine the positions of the blood fractions. The vision system works by lifting two vacutainers out of their rack into a box where the lighting can be carefully controlled. The vacutainers are rotated, so that the barcode label does not obscure the camera and then the images are captured. Once processed, the vacutainers are returned to the rack and another pair is lifted out for processing.

The three heights are stored in a database against each vacutainer record. The vision system was designed to be self-calibrating. Vacutainers are positioned against a datum. A calibration scale is mounted between the two vacutainers and is fixed relative to the datum. The calibration scale appears on every image and the software calculates the fraction heights relative to its position. Another piece of software reads these heights from the database and generates a series of liquid handling protocols. The protocol generator software is configurable, but it was initially designed to maximize the amount of buffy coat collected by developing pipetting protocols corresponding to tube heights 1 mm.

Figure 1 Principles of operation of the vision system
Figure 2 Results of vision system tests using 6 separate tubes of whole blood
above and below the reference point for buffy coat. In this way, greater than 80% of buffy coat is recovered from all samples.

The plasma is aspirated first, leaving a small amount behind so that the buffy coat is not disturbed. The buffy coat is aspirated next. The entire buffy coat is aspirated including a small amount of the red cell layer to ensure that the entire buffy coat has been recovered. Lastly the red cell fraction is aspirated, if required.

When imaging gel separator tubes the RTS vision system outputs heights of the base of the gel plug and the meniscus of the serum/plasma. The repeated measurement of the base of the gel plug and the serum/plasma meniscus was seen to be consistent with a <3% coefficient of variation (CV) and a <0.4% CV, respectively. Graphs 1 and 2 in Figure 2 show that the automated measurement of the gel height and plasma/serum meniscus is comparable to the heights measured manually.

Blood fractionated by centrifugation in EDTA tubes separates into three layers: plasma, buffy coat and red blood cells. Thus when imaging EDTA tubes the vision system outputs the height of the plasma meniscus, the plasma/buffy coat interface and the buffycoat/red blood cell interface. The CV of the automated measurements of the three interfaces were <0.3, <0.8 and <0.3%, respectively with the height of the buffycoat/plasma interface measured by the vision system averaging 1.0 mm higher than the manual measurement (see graphs 3 and 4 in Figure 2).

The vision system was capable of processing a rack of 24 vacutainers in <5 min. This throughput, installed on each of UK Biobank’s three blood processing platforms, is an important performance target to ensure labile blood fractions are processed within 24 h of collection.

**Discussion**

These data show that the RTS vision system is capable of accurately identifying the interfaces between the layers of fractionated blood in a reproducible manner. The high determination of the interface between the plasma and buffy coat compared with the manual measurement is a function of the software algorithm used to interpret the images taken by the vision system. However, it is possible to adjust the algorithm in order to optimize the identification of the buffy/plasma interface to suit the type of samples expected.

One of the considerations of the study was the area of data tracking. It is obviously crucial to the success of the operations of UK Biobank that all samples stored can be traced back to their source. To aid the tracking, all vacutainers and storage tubes are uniquely identified. Vacutainers have a barcode label and the storage tubes have a data matrix on the bottom. At the highest level, the system only tracks vacutainers—it does not know anything about the donors—the link between vacutainers and donors is maintained in a separate UK Biobank database.

All the records relating to the samples are stored in a central database. Each blood fractionation system updates the database in real-time and the completion of a rack of storage tubes triggers the generation of a file. This file is written to a specified folder where it can be imported by the Laboratory Information Management System.

The data mapping is achieved in stages (Figure 3). A special reader is used to read the data matrix code on the bottom of each tube and to map its position within the rack. This information is then stored in the database. The vision system reads the barcode on each vacutainer and maps the vacutainer position within
the rack. This information is also stored in the database together with the heights of the fractions. Finally, when a rack of vacutainers is delivered to a liquid handling system, the protocol generator reads the vacutainer records from the database and determines the number of storage tubes required for the liquid transfers. It then generates liquid handling protocols to aspirate from vacutainers and dispense into the storage tubes. The link between the source vacutainers and the destination tubes is then established in the database. The volume and fraction type are recorded against the tube records in the database.

In conclusion, an automated, high throughput blood fractionation system offers a solution to the problem of processing human blood samples collected in vacutainers in a consistent manner and provides a means of ensuring data and sample integrity and high sample yield.

**Conflict of interest:** None declared.