LOW-COST AUTOMATED PLASMA SPLITTING FROM WHOLE BLOOD USING GILSON'S PIPETMAX®

TECHNICAL NOTE TN232

TECHNICAL FEATURES

- MAX4x1200 Pipette Head
- Precise tip control with LLF
- Custom racks for 18 mm spaced blood tubes

TECHNICAL BENEFITS

- Uses the full range of the D1200 for single head installation
- Allows for the pipetting of blood plasma

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INTRODUCTION

Blood plasma is the major constituent of blood and makes up around 55% of the total blood volume. It is a clear, straw-coloured liquid that carries the platelets, red, and white blood cells. When separated from the red blood cells, plasma can be used in many clinical tests in the laboratory. Such tests include the monitoring of therapeutic drugs, the detection of HIV, Hepatitis, steroid profiling, and an array of other common clinical tests. To separate the plasma from the whole blood sample, the sample is first centrifuged to force the red blood cells to the bottom of the tube (Figure 1). This then allows the plasma to be removed as the top layer, usually by pipetting.

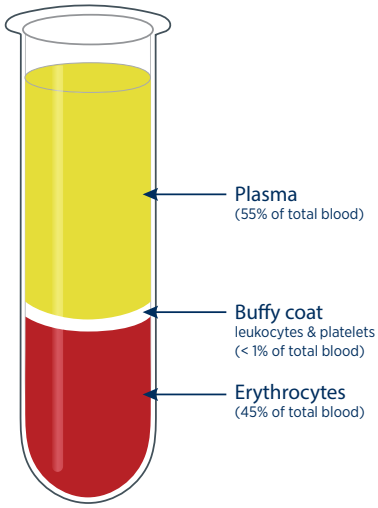

Figure 1

Typical composition of whole blood after centrifugation

In this technical note, the use of a PIPETMAX® automated liquid handling system is described for the pipetting of the plasma from the whole blood sample. PIPETMAX can be used to aspirate a precise volume of the plasma and transfer this to a suitable analysis or storage tube. If the specific laboratory workflow will benefit from it, then diluent can also be added to the analysis tubes and the plasma can be mixed into the diluent as part of the same method.

MATERIALS AND METHODS

The plasma splitting was performed on a Gilson PIPETMAX liquid handler. PIPETMAX utilizes air displacement pipetting in the same way as typical manual pipettes do. This makes its performance and results like existing laboratory norms while removing some of the user errors that can occur during pipetting. These can occur due to bad habits/practices, lack of training, rushing due to heavy workloads, etc.

PIPETMAX:

A PIPETMAX with a standard cover (Figure 2) was used in this application, along with a single pipette head and a standard tray for use with 96-well plates. TRILUTION® micro v3.0 software was used for running of the protocols provided.

Figure 2

Gilson PIPETMAX Liquid Handler

Pipetting Head:

A 4x1200 pipetting head was chosen for this application. Having the larger volume tips allows for dispensing of diluent to the destination tubes in an efficient manner while still being capable of transferring the plasma from the sample tubes accurately at the volume levels requested.

The 4x1200 pipetting head utilizes Gilson PIPETMAN® DIAMOND D1200 tips. (It can also use the DF1200ST sterile filter tips if these are a requirement for the laboratory). In this configuration, the tips are mounted at a spacing of 18 mm, which is ideal for typical sample tubes used in blood plasma testing. For example, a typical vacutainer blood tube has an outer diameter of 16 mm.

This is more effective than using one of the 8-channel options, where the spacing is too narrow at 9 mm spacing.

A second pipetting head was not required for this application, so the left side of the PIPETMAX head carriage was fitted with a ‘blank’ head.

Tray and Racks:

A standard PIPETMAX tray (See Figure 3 for tray layout) was used because this is a lower cost option than the one that is required for working with 384-well plates. The PIPETMAX tray has nine SBS standard positions. One of these positions was dedicated to a tip waste bin. Another was fitted with the D1200 tips; one rack of 96. In the third position, a reservoir plate was placed to provide access to the diluent, and the final six positions were taken up by the sample tubes and destination tubes: three positions each.

Two custom racks were designed for the specific workflow required by the laboratory. One type of custom rack was created to hold the blood tubes. These are BD Microtainer® MAP microtubes and the rack was created to hold 24 tubes. A second custom rack was created to hold the destination tubes, in this case these are screw cap cryovials and the rack was designed to also hold 24 tubes. This provides a simple 1-1 ratio allowing for error-free placement of sample and destination tubes.

With six positions remaining on the tray for sample tubes and destination tubes, this allows for three of each. On PIPETMAX this is two full rows, so three sample tube racks were placed on the middle row and three cryovial tube racks were placed on the front row of the tray. As each rack holds 24 tubes, this means that a total of 72 samples can be processed per run.

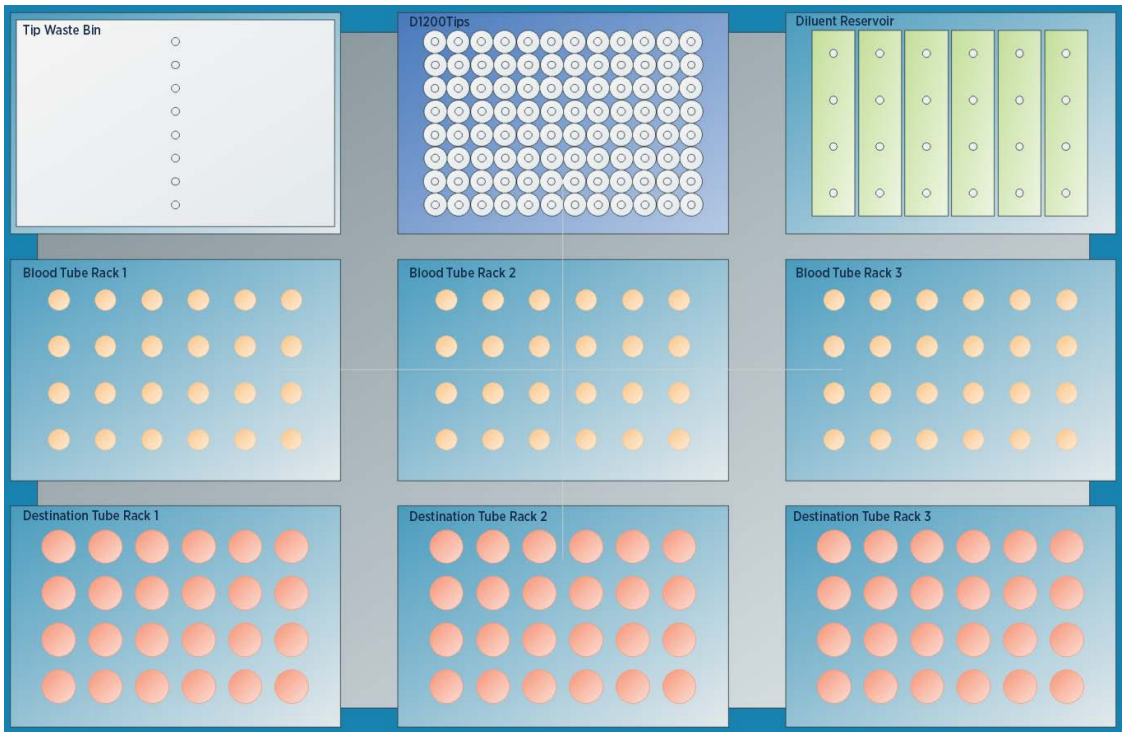

Figure 3
PIPETMAX Tray Layout

Software:

PIPETMAX was controlled using Gilson's TRILUTION micro software v3.0, and the protocol being run was written using Gilson Protocol Builder v2.1.

To make optimization of the pipetting parameters as easy as possible, the protocol was written initially with several variables. This allows these chosen settings to be adjusted on the TRILUTION micro software at run time, rather than requiring new protocol versions to be created each time. This benefits the speed of optimization of the protocol, allowing the user to adjust the parameters 'on the fly' in the lab and from run-to-run with no programming actions.

Figure 4 shows the variables available in the development version of the protocol which included a dilution and mixing step as well as the transfer of the upper layer of plasma from the sample tubes. Here the aspirate, dispense, and purge flow rates have been made available for adjustment. The samples to run is also provided as a variable. For the mixing step of the process, the number of mix cycles and the volume to be pipetted during the mix cycle are adjustable as run time variables.

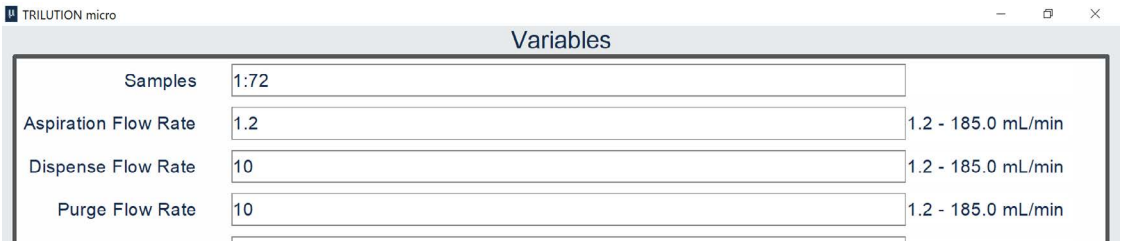

Figure 4

Optimization Variables

Pipetting Protocol:

With PIPETMAX, it is possible to have many different protocols available to run. These can also include both complete workflows and parts of workflows, saved as separate protocols. The full pipetting protocol for this application begins by aspirating 1260 µL of diluent from a reservoir and dispensing this diluent to the specified cryovials. This is done by using the Protocol Builder task 'Transfer, Deliver from Pool'. The parameters for the task are set such that PIPETMAX will retain the same tips for the task and deliver diluent to all Cryovials before ejecting the tips. Although the requested volume is above the maximum for the pipetting head/tip combination, PIPETMAX intelligently handles this request and delivers the volume in two even aliquots.

Following the delivery of the diluent to the vials, a second task then transfers 140 µL of each of the samples from the Sample Tubes to the Cryovials. This task is also a transfer task but used in 'stamp transfer' mode and has parameters set to force PIPETMAX to change the tip between each sample. This is done to avoid any possibility of cross-contamination from sample to sample. At the end of the transfer task, the mix option is selected, this is so that PIPETMAX will mix the sample into the diluent immediately after dispensing using the tip used for the sample transfer. As well as mixing the sample into the diluent, this also ensures that any sample droplets remaining in the tip after the sample dispensing are washed out and end up in the cryovial, leading to a greater level of accuracy and precision.

The protocol steps can be seen in Figure 5 below.

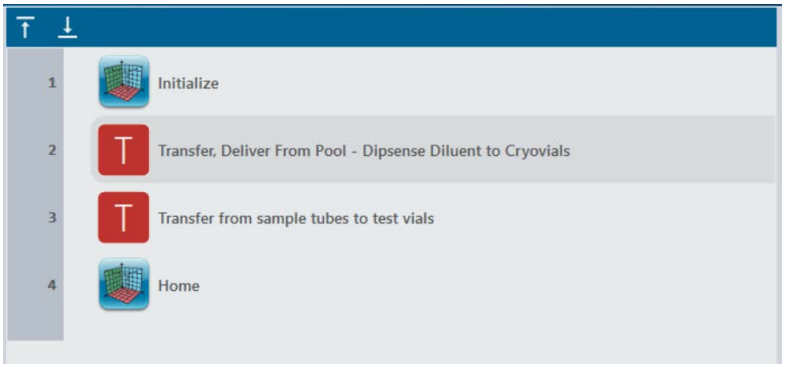

Figure 5

Pipetting Protocol

Sample Selection:

One of the difficulties of pipetting plasma from blood samples is the sample inconsistency. The composition of the blood samples will vary depending on the individual that the blood is taken from. If a patient is anaemic, then there will be lower levels of red blood cells in relation to the sample volume. Other patient-based variabilities can arise from the patient's state of health, such as hydration level, fitness, gender, age, recent ingestion of drugs or alcohol, and exposure to toxins or viruses.

On top of the variability of the sample composition, it is also possible that the volume provided to the lab can change. When blood samples are taken, it is by vacutainer tube, where the process involves the use of a vacuum to draw blood from the body into the receiving tube. Vacuum draws are not a good way of obtaining a consistent volume of sample. So, on top of sample composition variability, there is also likely to be a degree of volume variability too.

This variability can result in some samples that are not suitable for automated plasma sampling by PIPETMAX. Being a low-cost solution, PIPETMAX does not have liquid level detection capability and needs to be programmed to start aspirating the plasma from a defined height within the sample tube. This means that there needs to be at least X volume of total sample within all the sample tubes, so that there is plasma to aspirate into the PIPETMAX tips at the programmed height. Once aspiration has started, PIPETMAX is programmed to follow down with the liquid level by means of a software algorithm that is part of the standard software package. The value of X in this case will be determined by the needs of the laboratory based on the samples being received.

The other part of the variability that should be considered is whether there is sufficient plasma between the tip starting point and the red blood cell layer for the aspiration volume requested. This can be better considered by working on the basis of the red blood cells needing to be below a volume that can be defined as volume Y.

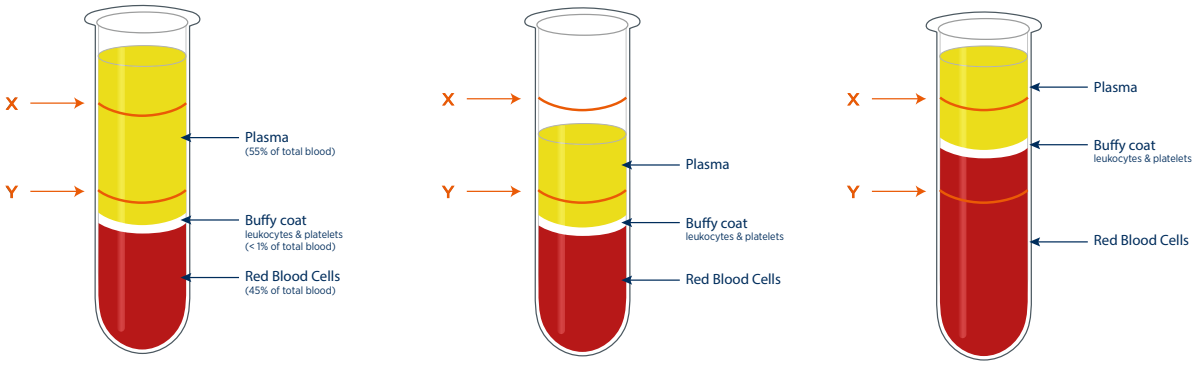

Figure 6
Acceptance Criteria for Samples

In summary, if the volume of red blood cells is less than volume Y and the total volume of sample is greater than volume X then the sample is suitable for pipetting using PIPETMAX (see figure 6). Because blood samples come in well-defined blood tubes that are consistently made, it is reasonable to estimate these volumes based on height within the tube rather than needing to measure the volume. This height within the tube can be estimated by eye and a simple 3D printed holder can be used to assist in this visualization.

For best workflow optimization, the visual selection of suitable samples should be done at the point that the sample tubes are removed from the centrifuge after centrifugation to separate the red blood cells from the plasma. Any samples that are picked out as unsuitable need to be considered individually. It is possible to have some samples where the red blood cell composition is high and the total volume is low, this may result in a situation where there is not enough plasma in the sample to obtain the required volume. In this case, no method of pipetting will solve the issue and the analysis will need to be done on a smaller sample volume or a request for more sample will need to be made. Some sample tubes may have excessive amounts of sample leading to sufficient plasma but with a larger red blood cell volume than acceptable. These can be put aside and worked manually, or if there is enough of these samples, then an alternative method can be run on PIPETMAX, which uses a different starting position for aspiration.

Liquid Handling Optimization:

To obtain the best results from the pipetting procedure, PIPETMAX liquid handling parameters were adjusted. The key liquid handling parameters that were found to be necessary were as follows.

Liquid Level Following: This feature of PIPETMAX needs to be used. If liquid level following is not used, then the tip position must be set lower in the tube for the entire aspiration, and this is found more likely to result in aspirated red cells.

Aspiration Flow Rate: The speed of aspiration for the pipetting was set to the minimum speed for the 4x1200 head used. This allowed for aspiration of the plasma without disturbing the red blood cell layer. This parameter was not fully studied in the optimization, and it is possible that faster flow rates would also be suitable but at this flow rate the total processing time was already short enough to be acceptable.

Dispense Flow Rate: The dispense flow rate was set to 10 mL/min which allows for good release of the plasma from the pipette tip while not creating any splashing which was seen when much faster flow rates were used.

Purge Flow Rate: The purge flow rate was also set at 10 mL/min and the purge tip position was set to a position at the top of the receiving tube but offset such that the tip was touching the side of the tube. This side dispensing technique maximizes the sample transfer reproducibility. In a method that just transfers the sample, this purge occurs directly after the sample transfer, whereas in a method that also includes dispensing of diluent and mixing, the purge occurs after the mixing has been completed. The mixing is done using the same tip that has been used for the transfer of the sample.

Number of Mixes: The PIPETMAX mixing process involves aspiration from the bottom of the tube and then dispensing from a height above. This process of aspirating low and dispensing high allows for an optimal liquid movement to ensure proper mixing and three cycles of this procedure was found to be sufficient. Depending on the diluent used and the relative volumes of diluent to sample, the number of cycles may need to be adjusted appropriately.

Mixing Volume: Mixing volume for automated liquid handling should usually be around ¾ of the volume to be mixed. This is sufficient to allow good mixing without risking aspirating air and creating unwanted bubbles. In this application, the total volume in the final tube was 1.4 mL and a mixing volume of 1 mL was therefore around the ¾ mark recommended; this worked well as expected.

Tip Changes: Each blood sample is a different patient sample and so must always be pipetted using a new clean tip. This is to ensure that cross-contamination of samples is avoided. To further ensure zero contamination, PIPETMAX can be used with filter tips, providing a further in-tip barrier. The dispensing of the diluent to the receiving tubes was done as a batch process before the processing of the samples, in this way one set of four tips could be used for the entire diluent dispensing.

Equilibration Time: Throughout the protocol an equilibration time of 1 second was used. This allows for the 'settling' of the liquid after any pipetting action before the PIPETMAX head moves to its next position.

RESULTS AND CONCLUSIONS

When large quantities of blood samples need processing for specific trials or projects, handling the workload manually is a daunting task. In this study, it has been shown that PIPETMAX can absorb this workload, freeing up lab staff time for other purposes.

The availability of a 4-channel head for PIPETMAX suits this kind of tube-to-tube processing very well. With 4-channel heads in 20, 200, and 1200 µL volumes, PIPETMAX can deal with a large range of volume needs in this application. Where many other automated liquid handlers have only 1 or 8 channel capability, restricting them to using 1 channel at a time for tube access, PIPETMAX can use all four channels at a time. This makes the processing 4x faster than it would otherwise be.

The ability of the 4x1200 head to pipette volumes as low as 60 µL accurately also means that in this protocol only one pipetting head is needed, leading to a lower cost specification, making the purchase of PIPETMAX cost-effective for most laboratories with such a need.

Dispensing diluent in a batch at the beginning of the protocol to all the tubes allows for minimal usage of the pipette tips, and does not require 'conductive', 'special' or 'automation specific' tips, which leads to a much lower overall running cost of the system from year-to-year. A full batch of 72 samples, diluent dispense, and sample transfer with mixing, is completed with 76 pipette tips used in total.