**Flow cytometric comparison of platelets from a whole blood and finger-prick sample: impact of 24 hours storage**

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**ABSTRACT**

In this study, we investigate the validity and laboratory utility of flow cytometry when analyzing platelet activation by studying CD41, CD42b, CD62P and CD63. We compare flow cytometry results from citrated whole blood and finger-prick samples directly after collection and also after storing both a finger-prick and whole blood sample for 24 hours. Citrated whole blood and finger-prick samples were taken from three healthy individuals on two occasions, and a total of 60 000 cells were analyzed for each of the 4 phycoerythrin-labelled monoclonal antibodies. Half of each sample was analyzed immediately after sampling while the other half was kept in the fridge at 6°C for 24 hours before analysis. No significant difference was found between the sampling methods or the period of time before analysis. Results therefore suggest that an appropriately prepared finger-prick sample can be used for platelet function analysis, and samples can be stored for 24 hours in the fridge at 6°C before analysis.

**Keywords:** Flow Cytometry; Platelets; Whole blood; Finger-prick, Storage

## INTRODUCTION

Flow cytometry is a powerful technique, and its importance in research and clinical investigation has long been established.[1] Markers used in flow cytometry may give us valuable information regarding cellular activity and since 1989 it has been deemed an essential tool for the investigation of platelets.[2] Flow cytometry thus provides a numerical technique, which is both objective and quantitative, to assess platelet function.[3]

Several surface glycoproteins (GP) are found on the membrane of platelets and flow cytometry has been used to a great extent in the immunophenotyping of these entities. The study of platelet function, physiology and their interaction with other cells has been advanced by investigating the recognition of these surface glycoproteins by specific monoclonal antibodies (MoAbs). Not only can the glycoproteins on the surface of the platelet membrane be detected by MoAbs, but molecules transferred to the surface from the internal platelet granules can be identified.[3] This is of particular interest in the study of platelet activation.

The application of a panel of MoAbs is preferred for flow cytometric analysis of platelets, since different flow cytometric probes reveal different characteristics of platelet function.[5] CD41 as well as CD42b are frequently used as platelet identifiers, since they are only present on platelets and not any other circulating blood cell.[2, 6, 7] Alterations in antigenic determinants can also be investigated and MoAbs are used to label epitopes specifically expressed on the platelet membrane. Studies of extracorporeal circulation have mainly used CD62P and CD63 to investigate platelet activation.[8] Therefore, flow cytometry can be employed to establish the amount of activated and non-activated platelets.[3]

Whole blood can be obtained from either blood drawn in blood tubes with added anti-coagulants (e.g. citrate tubes) or by sampling blood from a finger-prick. These methods have been used previously by Wall and co-workers to investigate platelet dense granule release and uptake. Both whole blood collected in sodium citrate tubes and 20 μl of blood from a “fingerstick”, as they referred to it, without added anticoagulant were used. After collection, the samples were immediately diluted with Hanks balanced salt solution (HBSS) and a fluorescent marker, mepacrine, was added.[18]

In research where a sample population needs to be followed over a prolonged period of time, or over consecutive days, it is not practical to frequently draw blood. It is much more acceptable for the participants to donate a finger-prick sample. The question also arose whether storage times would influence results. Therefore, in the current manuscript we investigate the repeatability of flow cytometry results when using blood drawn in citrate tubes and samples from finger-pricks - either used immediately, or after samples were stored for 24 hours.

## METHODS

### Blood collection

Blood was collected from 3 healthy, control male individuals. The participants were non-smokers, did not use any chronic medication and did not have a history of thrombotic disease. None of the participants were using aspirin or aspirin analogues. 5ml of blood was drawn into a citrate tube (0,5 ml of Sodium Citrate (3,8%) for 4,5ml of blood) and 20µl of blood was collected from finger-pricks. For each concentration of citrate analyzed, a separate finger-prick was taken to ensure

that any possible platelet activation after the first sampling was eliminated. Procedures were done in duplicate. The flow cytometer was calibrated with flow cytometric beads before each procedure.

**Sample preparation**

*Whole blood preparation*

Experiment 1

From the 5ml of citrated blood, 20µl was transferred into 1ml aliquots of sheath fluid. The 20µl of blood was chosen as to be able to compare the whole blood sample to that of the 20µl of blood collected from the finger-prick. 100µl of the mixture was placed into four flow tubes. The remainder of blood mixture was placed in the fridge at 6°C . Each tube was stained with 20µl of one of the following probes separately: CD41-PE, CD42b-PE, CD62P-PE and CD63-PE (from Beckman Coulter). The flow tubes containing CD62P-PE and CD63-PE were also stained with CD41-FITC.

Experiment 2

20µl of the 5ml whole blood drawn in the citrate tube was placed into 1ml aliquots of sheath fluid. 100µl of the mixture was placed into four separate flow tubes. Each tube was stained with 20µl of CD41-FITC and 20µl of one of the following probes: CD41-PE, CD42b-PE, CD62P-PE and CD63-PE (from Beckman Coulter).

24 hours after sampling, the remaining blood mixture, placed in the fridge at 6°C overnight, was prepared in the same manner as described above.

Experiment 3

From the 5ml of citrated blood, 20µl was transferred into 1ml aliquots of sheath fluid containing various concentrations of thrombin namely i) 5µl thrombin, ii) 10µl thrombin and iii) 20µl thrombin. This volume of blood (20µl) was chosen as to be able to compare the whole blood sample to that of the 20µl of blood collected from the finger-prick. 100µl of the mixture was placed into four separate flow tubes. Each tube was stained with 20µl of CD41-FITC and 20µl of each of the following probes separately: CD41-PE, CD42b-PE, CD62P-PE and CD63-PE (from Beckman Coulter).

*Finger-prick preparation*

Experiment 1

For the finger-prick sample collection, three separate finger-pricks were done with a lancet. 20µl of blood was collected from each finger-prick and immediately placed in 1ml aliquots of sheath fluid containing various volumes of citrate (3,8% Sodium Citrate) namely: i) No citrate, ii) 5µl of citrate and iii) 10 µl of citrate. 100µl of the blood, sheath fluid and citrate combination was transferred to four individual flow tubes from each sample. The remaining blood, sheath fluid and citrate combination was placed in the fridge at 6°C . 20µl of the following probes were added to each tube separately: CD41-PE, CD42b-PE, CD62P-PE and CD63-PE (from Beckman Coulter). The flow tubes containing CD62P-PE and CD63-PE were also stained with CD41-FITC.

Experiment 2

The 20µl samples of blood collected from three separate finger-pricks were immediately placed in 1ml aliquots of sheath fluid containing various volumes of citrate (3,8% Sodium Citrate) namely: i) No citrate, ii) 5µl of citrate and iii) 10µl of citrate . 100µl of the blood, sheath fluid and citrate combination was transferred to four individual flow tubes. Each tube was stained with 20µl of CD41-FITC and 20µl of one of the following probes: CD41-PE, CD42b-PE, CD62P-PE and CD63-PE (from Beckman Coulter).

24 hours after sampling, the remainder of the blood, sheath fluid and citrate mixture, placed in the fridge at 6°C overnight, was prepared in an identical manner as described above.

**Flow cytometric analysis**

Samples stained with the different probes, were incubated at room temperature in the dark for 20 minutes before analyzed by a flow cytometer (FC 500, Beckman Coulter). Forward scatter and 90º side scatter were displayed on logarithmic scales. Two platelet gates were set. The first gate was set according to the morphological characteristics of platelets while the second gate was set according to CD41-FITC fluorescence, a platelet specific marker. For the duplicate procedures 180 000 platelets were counted and analyzed per phycoerythrin-labelled monoclonal antibody (including CD41-PE, CD42b-PE, CD62P-PE and CD63-PE). The fluorescence of the different antibodies was plotted on 256-channel log histograms. The acquired Listmode data was analyzed with CyflogicTM software, CyFlo Ltd, Finland. The results were expressed in arbitrary units as mean channel fluorescence intensity (MCFI).

## Statistical analysis

Results from the flow cytometric analysis were compared by using the paired two-sided Student's t-test. MCFI results are represented as mean ± standard deviation (SD). A p-value of ≤0.05 was considered significant.

Since 3 similar participants were chosen, the MCFI observations for the 3 participants can be regarded as being independent and identically distributed random variables for each of the different blood-preparation techniques. Furthermore, since the MCFI for each participant was calculated as the mean fluorescence of a large sample of platelets (10 000 platelets per individual per sample), the well-known Central Limit Theorem assures us that the Normal distribution is a close approximation for the distribution of the MCFIs. This allows us to make use of the paired two-sided Student's t-test to compare the results from the flow cytometric analysis at a 5% level of significance.

## RESULTS

*Gating strategies*

Two platelet gates were set, according to the specific markers utilized. For experiment 1, the platelet gate was set according to the forward scatter (FS) and side scatter (SS) properties of platelets. Platelet rich plasma (PRP) was stained with CD41-PE analysed on a dot-plot with FS on the y-axis and SS on the x-axis and the gate was set as seen in Figure 1.

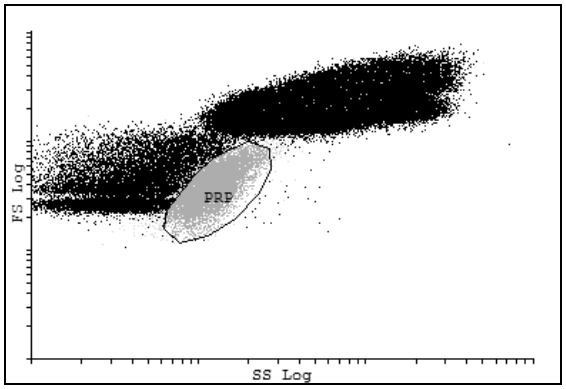


**Figure 1.** PRP-gate. Platelet gate set according to forward scatter (FS) and side scatter (SS) of platelet rich plasma (PRP).

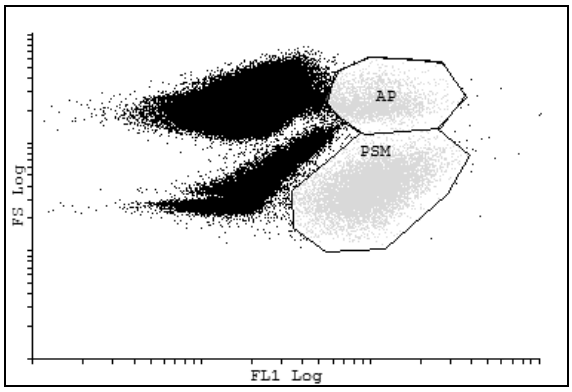


**Figure 2.** PSM gate. Platelet gate set according to FL1 of CD41-FITC platelet specific marker (PSM). Activated platelets (AP) are also incorporated in the PSM gate.

Figure 2 shows the platelet gate set for experiment 2 and 3 according to CD41-FITC fluorescence, a platelet specific marker on a dot-plot displaying FS on the y-axis and FL1 fluorescence on the x-axis. CD41 is used as a reliable platelet-specific marker. It enables the differentiation of platelets in a whole blood sample.[2] The CD41-FITC marker was used in conjunction with the mentioned phycoerythrin-labelled monoclonal antibodies (CD41-PE, CD42b-PE, CD62P-PE and CD63-PE). For the platelet specific marker (PSM) gate, a clear distinction can be made between the

unactived platelets (to the lower right of the diagram) and the activated platelets (to the upper right part of the diagram).

An overlay of the two platelet gates is shown in Figure 3. In Figure 3 A it is shown that the PRP gate only corresponds to the non-activated platelets in the population while Figure 3 B shows the activated platelets are incorporated in the PSM gate but not the PRP gate. Therefore, from Figure 3 we can deduce that the gating strategy is very important if all parameters are to be fully evaluated.

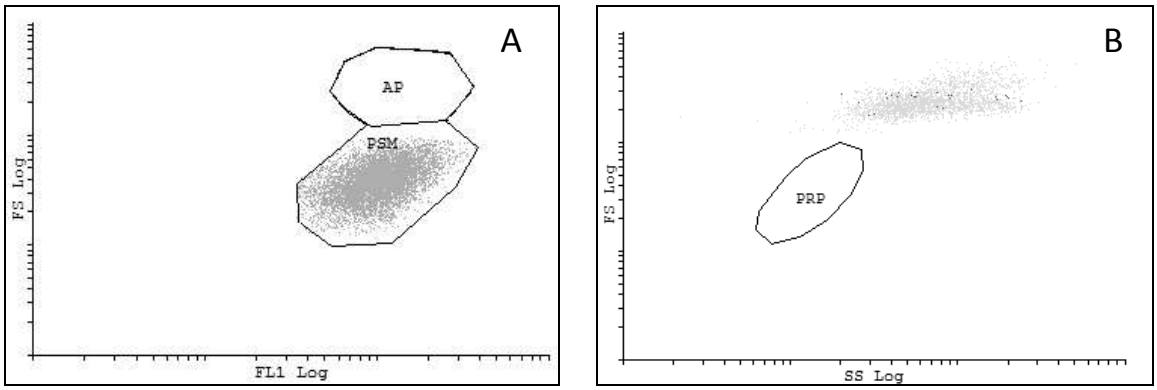


**Figure 3.** Overlay of Platelet gates. A = PRP only corresponds to the non-activated platelets of the PSM-gate. B = The population of cells shown to the upper right of the PRP-gate is the activated platelets (AP) not incorporated in the PRP-gate.

Both gating strategies were used for analysis of the different phycoerythrin-labelled monoclonal antibodies used. The platelet rich plasma (PRP) gate showed a significantly lower MCFI value compared to the platelet specific marker (PSM) gate for all instances.

*Comparisons*

To determine the integrity of the different sampling methods, whole blood samples were compared to finger-prick samples containing various volumes of citrate

immediately after sampling. The volumes of citrated added to the sheath fluid before sampling was: i) no citrate, ii) 5µl of citrate and iii) 10µl citrate. The comparison of whole blood samples with various volumes of thrombin added directly after sampling was done to determine the activation of platelets. Thrombin volumes added to whole blood samples before analysis was: i) 5 µl, ii) 10 µl and iii) 20 µl. The influence of storage time was compared for whole blood and finger-prick samples analyzed 24 hours after sampling.

The results from Experiment 1 and Experiment 2 were pooled and each of the mentioned phycoerythrin-labelled markers were used to analyse specific characteristic of platelets gated by CD41-FITC. For each marker, 10 000 platelets were counted and analyzed per person adding up to a total of 60 000 platelets for the duplicate procedures.

The following results, obtained immediately after sampling, were compared to determine any significant changes: Table I displays results from whole blood sample containing no thrombin (non-activated whole blood sample) compared to finger-pricks containing various concentrations of citrate. Table II displays results from whole blood samples containing various concentrations of thrombin.

Table III shows the results of immediate analysis compared to results of analysis after 24 hours: Whole blood analyzed immediately was compared to the whole blood sample stored for 24 hours. The finger-prick containing no citrate analyzed immediately was compared to the same sample stored for 24 hours. The same procedures were performed for the other finger-prick samples, containing 5µl and 10µl citrate respectively, immediately analyzed after collection and the aliquot stored for 24 hours.

**Table I:** Analysis immediately after sampling: whole blood (WB) sample and finger-pricks (FP) containing various concentrations of citrate. Results represented as mean ± SD of MCFI. (n = 60 000 platelets analysed for each of the sampling procedures)

| MoAb | WB $_{\text{No thrombin}}$ | FP $_{\text{No citrate}}$ | FP $_{\text{5µl citrate}}$ | FP $_{\text{10µl citrate}}$ |
|---|---|---|---|---|
| **PRP gate** | | | | |
| CD41-PE | 24.04 ± 2.71 | 17.74 ± 0.38 | 23.02 ± 3.12 | 24.36 ± 4.27 |
| CD42b-PE | 29.68 ± 3.37 | 28.17 ± 1.32 | 30.81 ± 2.85 | 32.54 ± 3.17 |
| CD62P-PE | 79.94 ± 2.15 | 78.16 ± 4.61 | 77.20 ± 3.32 | 76.99 ± 2.16 |
| CD63-PE | 49.54 ± 2.09 | 47.01 ± 0.72 | 48.61 ± 1.09 | 45.88 ± 4.88 |
| **PSM gate** | | | | |
| CD41-PE | 47.72 ± 6.04 | 70.51 ± 1.24 | 64.13 ± 2.52 | 52.82 ± 9.45 |
| CD42b-PE | 35.07 ± 3.97 | 47.58 ± 2.35 | 40.39 ± 1.74 | 36.44 ± 3.55 |
| CD62P-PE | 115.49 ± 5.95 | 100.97 ± 3.24 | 99.15 ± 0.47 | 107.55 ± 4.86 |
| CD63-PE | 63.52 ± 2.53 | 54.86 ± 7.26 | 49.12 ± 6.35 | 57.31 ± 5.94 |

**Table II:** Analysis immediately after sampling: whole blood (WB) samples containing various concentrations of thrombin to evaluate activation. Results represented as mean ± SD of MCFI. (n = 60 000 platelets analysed for each of the sampling procedures)

| MoAb | $WB_{No\ thrombin}$ | $WB_{5\mu l\ thrombin}$ | $WB_{10\mu l\ thrombin}$ | $WB_{20\mu l\ thrombin}$ |
|---|---|---|---|---|
| **PSM gate** | | | | |
| CD41 | 47.72 ± 6.04 | 54.46 ± 18.28 | 53.90 ± 18.70 | 71.24 ± 5.72 |
| CD42b | 35.07 ± 3.97 | 37.75 ± 6.66 | 39.26 ± 6.82 | 40.11 ± 6.48 |
| CD62P | 115.49 ± 5.95 | 148.57 ± 19.97 | 155.92 ± 25.96 | 174.92 ± 9.40 |
| CD63 | 63.52 ± 2.53 | 76.07 ±13.55 | 72.73 ± 9.00 | 84.13 ± 12.49 |

**Table III:** Analysis 24 hours after sampling. Results of the whole blood (WB), first finger-prick containing no citrate, the second finger-prick containing 5μl of citrate and the third finger-prick containing 10μl of citrate analysed 24 hours after the sample was taken. Results are represented as mean ± SD of MCFI. (n = 60 000 platelets analysed for each of the sampling procedures)

| MoAb | $WB_{No\ thrombin}$ | $FP_{No\ citrate}$ | $FP_{5\mu l\ citrate}$ | $FP_{10\mu l\ citrate}$ |
|---|---|---|---|---|
| **PRP gate** | | | | |
| CD41-PE | 25.99 ± 1.84 | 46.32 ± 0.44 | 48.93 ± 8.39 | 28.07 ± 1.64 |
| CD42b-PE | 37.88 ± 6.60 | 38.79 ± 0.38 | 36.40 ± 7.59 | 39.76 ± 0.81 |
| CD62P-PE | 81.32 ± 0.84 | 75.98 ± 4.76 | 72.87 ± 0.82 | 73.53 ± 2.28 |
| CD63-PE | 45.63 ± 1.95 | 42.84 ± 6.05 | 46.63 ± 0.50 | 47.25 ± 1.51 |
| **PSM gate** | | | | |
| CD41-PE | 51.41 ± 1.58 | 70.51 ± 1.24 | 64.13 ± 2.52 | 60.82 ± 3.41 |
| CD42b-PE | 32.01 ± 0.31 | 47.58 ± 2.35 | 40.39 ± 1.74 | 39.13 ± 2.62 |
| CD62P-PE | 118.69 ± 4.61 | 100.97 ± 3.24 | 99.15 ± 0.47 | 104.59 ± 4.12 |
| CD63-PE | 62.86 ± 1.17 | 54.86 ± 7.26 | 49.12 ± 6.35 | 53.98 ± 7.42 |

*Whole blood vs. Finger-prick*

There were no significant difference between the whole blood samples and the finger-prick samples containing 10μl of citrate (p-values >0.05). However, significant changes were shown for the other finger-prick samples containing no citrate and 5μl of citrate separately (p-values <0.05).

Both gating strategies were used for analysis of the different phycoerythrin-labelled monoclonal antibodies used to evaluate the different sampling methods. The platelet rich plasma (PRP) gate showed a significantly lower MCFI value compared to the platelet specific marker (PSM) gate for all instances.

*Non-activated vs. Activated*

Whole blood samples were activated by adding different volumes of thrombin to separate samples. The whole blood sample containing no thrombin (thus, the non-activated sample) was compared to whole blood samples containing 5µl, 10µl and 20µl of thrombin. A significant difference was found between the non-activated whole blood sample and the activated whole blood samples ($p$-value $<0.05$). This was also true when the activated whole samples were compared to the finger-prick sample containing 10µl citrate. The MCFI for all phycoerythrin-labelled monoclonal antibodies (CD41-PE, CD42b-PE, CD62P-PE and CD63-PE) increased with the increase in thrombin added to the whole blood samples.

*Immediate analysis vs. 24 hours*

No significant difference was found for the MCFI of whole blood analyzed immediately compared to whole blood analyzed 24hours after sampling ($p$-value $>0.05$). This was also true for the finger-prick samples with 10µl of citrated added before sampling. The immediate analysis of the finger-prick samples with no citrate and 5µl of citrate added separately, showed significant differences from the same samples analyzed 24 hours after sampling ($p$-value $<0.05$).

## DISCUSSION

The current investigation confirms that both a whole blood sample and a finger-prick can be used interchangeably for flow cytometric analysis of platelets. Storage time of up to 24 hours in a fridge at 6°C also doesn't influence the platelet activation in the blood sample. Aspects such as the strategy employed for gating the sample, the specific flow cytometer instrument used, the preparation of the samples, the specific probes used as well as the storage time will be discussed in greater detail.

*Gating strategies*

Flow cytometry enables the researcher to detect several parameters from a single sample. Different probes or markers can simultaneously be analysed by using multicolor flow cytometry.[17]

The function of "gating" or isolating particular cell clusters in flow cytometry enables classification and investigation of platelets in the mixed populations of cells like found in whole blood.[3] Two parameters are employed to facilitate platelet gating namely 1) forward light scatter (FSC) which arranges cells according to size, and 2) the use of a platelet-specific antibody like CD41 or CD42b.[3]

Multicolour flow cytometry has the advantage of gathering more information from a single sample therefore minimizing the sample size and decreasing preparation effort. This technique also decreases variation since fewer sample preparation is required and is an effortless method to study platelet population.[17]

The reliability of the population of platelets in a whole blood sample is determined by the gating strategy employed.[11, 19, 20] Van Velzen and colleagues have recently investigated the effect of different platelet gating strategies. They stated that the

changes in scatter characteristics after platelet activation will differ from the morphology of non-activated platelets and therefore a pure platelet population will not be selected. They asserted that utilizing a platelet antigen like CD41 or CD42b, which is commonly present on the platelet membrane, is preferred above the use of the forward scatter/side scatter strategy. By using a platelet specific marker not only eliminate coincidence but also excludes complexes platelets form with monocytes and decreases possible contamination.[17]

This research of van Velzen et al was done for a fixated whole blood sample. In this investigation similar results were found for an unfixed whole blood sample. Since the platelet rich plasma (PRP) gate only shows the unactived platelets in the absence of CD41-FITC, it is important to firstly stain the sample with CD41-FITC and secondly to set the platelet gate according to the FL1 information obtained from CD41-FITC i.e. the platelet specific marker (PSM) gate. This indicates the importance of multicolor gating for optimal analysis of platelets.

*Instrumentation*

Earlier flow cytometric analysis has been performed on FACScan cytometer (Becton Dickinson). Platelet microparticles [21] and magnetic labeling of platelets [22] have been studied on the FC500 (Beckman Coulter). Erythrocytes [23], dysfunctional T regulatory cells [24] and stem cells [25] amongst others has also been analysed on the FC500 (Beckman Coulter). This is the first study conducted on the FC500 (Beckman Coulter) to investigate platelets in an unfixated whole blood and finger-prick samples as well as the effect of storage time on the platelets.

*Blood preparation for flow cytometry*

Whole blood was drawn in citrate tubes (0,5 ml of Sodium Citrate (3,8%) for 4,5ml of blood). For the finger-pricks, citrate had to be added to the sheath fluid to prevent coagulation of the blood sample. Weil stated that, if blood is mixed with a sodium citrate solution in appropriate portions, the blood will not clot since the calcium salts are not accessible for coagulation.[26] Weil added that blood mixed with sodium citrate can be kept in the fridge at 6°C for several days, and only slight changes in cell structure can be observed after one week of storage.[26] The results indicate that a volume of 10µl of citrate added to the sheath fluid aliquot is adequate to prevent coagulation of a finger-prick sample.

Typically, platelets have been studied after preparing platelet rich plasma or washing the platelets.[9, 10] These separation procedures lead to *in vitro* activation of platelets due to the formation of artifacts. The pioneer use of whole blood for flow cytometric studies by Shattil and colleagues [11] was just the beginning for important improvements for the relevance of flow cytometry for clinical application.[5] The flow cytometric analysis of whole blood holds many advantages for the study of platelet activation. This method has been used for over 20 years to investigate platelet function. Ault et al. used the MoAbs specific for GPIb (CD42b) and GMP140 (CD62P) to measure platelet aggregation and release reaction in a whole blood sample.[12] Activation-dependent variations in multiple surface receptors can be determined in a whole blood sample. In vitro platelet activation is also prevented, since there is minimal manipulation of the samples and it will decrease the possible loss of platelet subpopulations.[5]

*Probes*

A Pubmed search revealed few references in recent literature that discuss the use of CD41-PE, CD42b-PE, CD62P-PE, CD63-PE and CD41-FITC in whole blood and finger-prick samples as well as the particular storage methods employed in this study. Current literature showed that flow cytometry has been employed to study platelet function testing in apheresis products, where all the above-mentioned probes were used in conjunction with secondary antibodies.[8] Other studies include flow cytometric analysis of platelet count [13], platelet reactivity [14], platelet function in children [15] and the importance of the sampling site in patient about to undergo surgery [16]; these studies used only one or two of the above-mentioned probes. . All the mentioned studies were performed on a FACScan cytometer (Becton Dickinson).

The most recent investigation of platelet activation was done by van Velzen et al in 2012. They incorporated all 4 the mentioned antibodies; however, the conjugated fluorochromes differed from the probes used in this study and the samples were fixated before analysis. [17]

*Sampling method*

No significant difference was found between the whole blood sample and the finger-pricks containing 10µl of citrate. There was a significant difference between the whole blood sample and the finger-pricks containing no citrate and with 5µl of citrate added. This indicated that the method of sampling, both the whole blood drawn in the citrate tube as well as the finger-prick samples, was adequate. It also shows that 10µl of citrate is the preferred concentration to be added to the sheath fluid before adding blood from a finger-prick. The whole blood sample and each of the finger-

prick samples were done on separate days. This was done to eliminate the possible effect of platelet activation after the first sample.

*Activation of platelets*

The activated whole blood samples showed significantly higher MCFI values when compared to the non-activated whole blood sample and the finger-prick sample containing 10µl of citrate. We can therefore deduce that the whole blood sampling containing no thrombin, as well as the finger-prick with 10µl of citrate added, did not contain an activated platelet population.

Once receptor activation occurs, the internal platelet granules are secreted and the cytoskeleton will be rearranged leading to signal transduction.[27, 28]

Upon activation, platelets exhibit elevated levels of specific activation markers on the platelet surface for example CD62P [29] and CD63 [30]. Both these markers are dominant immunologic indicator of platelet activation.[30, 31]

CD62P, also referred to as P-selectin, is an activation-dependent MoAb most extensively used in the study of platelet granule membrane proteins.[32] It is a constituent of a resting platelet's α-granule membrane.[31] It will only be expressed on the surface membrane once the contents of the α-granule are secreted.[33-35] For this reason, the MoAb specific for CD62P will not bind to resting platelets, only to degranulated, activated platelets.[5] Once activated, platelets also rapidly transfer CD63 from the lysosome-like granules to the platelet surface through the surface canalicular system.[2]

Van Velzen et al. also found the expression of CD62P and CD63 to be increased when platelets were exposed to thrombin activation in a fixated sample.[17]

*Period between sampling and analysis*

CD41 is a calcium-dependent compound of GPIIb/IIIa. The interactions between cells and the cells with the matrix are mediated by GPIIb/IIIa.[36, 37] CD42b is a receptor for von Willebrand factor, which plays a critical role in the adhesion of platelets to the wall of injured blood vessels.[38]

CD41 and CD42b are reliable platelet-specific markers. These MoAbs are used to differentiate between platelets and “debris” or fragments.[2]

Since the expression of CD41 and CD42b in the whole blood samples were similar for immediate analysis of the sample and analysis 24 hours after sampling we can infer that these platelets identified by CD41 and CD42b expression were not activated after sampling and remained inactive for at least 24 hours. Michelson and associates indicated on several occasions that the binding of CD42b to resting platelets is noticeably increased compared to its binding with activated platelets.[39-41] The decreased expression on the surface of activated platelets possibly results from the translocation of this complex to the surface-connected canalicular system membranes.[41, 42] This shows that CD42b could be a sensitive marker of in vivo activation of platelets.

Although platelet storage has been associated with increased expression of CD62 in the past [3] our results indicate that both an unfixated whole blood and a finger-prick sample can be stored in the fridge at 6°C if prepared appropriately. The time of analysis, whether it is immediately after the sample was take or 24hours after sampling, appears to not have an influence on the expression of CD41 and CD42b. However, since the participants were all young and healthy individuals, the effect of age and disease will have to be investigated for this method.

## CONCLUSION

Firstly, the importance of gating strategy for a unfixated sample was established. If all platelets, activated and non-activated, are to be taken into consideration, then the platelet specific marker (PSM) gate should be employed and all samples should be stained with CD41-FITC and not only with the specific phycoerythrin-labelled monoclonal antibodies (including CD41-PE, CD42b-PE, CD62P-PE and CD63-PE).

Secondly, we can conclude that an unfixated whole blood and a finger-prick sample are identical with regards to platelet function. Both sampling methods showed little activation when compared to activated whole blood samples. Therefore, a whole blood sample and a finger-prick sample can be used interchangeably for flow cytometric analysis of platelets. This is advantageous for research where a sample population needs to be examined over consecutive days or an extending time interval. In these cases, when it is not practical to repeatedly draw blood, a finger-prick will be sufficient.

And lastly, samples can be stored for 24 hours after sampling. Sheath fluid is a sufficient medium for storing unfixated whole blood and finger-prick samples in the fridge at 6°C for 24 hours, provided that a sufficient amount of citrate is added to the sheath fluid for a finger-prick sample. A volume of 10µl citrate provides sufficient anticoagulant action for a 20µl aliquot of finger-prick blood. Both whole blood samples and finger-prick samples can be kept in the fridge at 6°C for 24 hours before analysis since no platelet activation occurs. This will aid studies where analysis can’t immediately be performed due to travelling distance from flow cytometer or time constraints.

**ACKNOWLEDGEMENTS**

We gratefully acknowledge Peter Smith for his assistance with the statistical analysis.

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