Video Article

Routine Screening Method for Microparticles in Platelet Transfusions

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Abstract

Platelet inventory management based on screening microparticle content in platelet concentrates is a new quality improvement initiative for hospital blood banks. Cells fragment off microparticles (MP) when they are stressed. Blood and blood components may contain cellular fragments from a variety of cells, most notably from activated platelets. When performing their roles as innate immune cells and major players in coagulation and hemostasis, platelets change shape and generate microparticles. With dynamic light scattering (DLS)-based microparticle detection, it is possible to differentiate activated (high microparticle) from non-activated (low microparticle) platelets in transfusions, and optimize the use of this scarce blood product. Previous research suggests that providing non-activated platelets for prophylactic use in hematology-oncology patients could reduce their risk of becoming refractory and improve patient care. The goal of this screening method is to routinely differentiate activated from non-activated platelets. The method described here outlines the steps to be performed for routine platelet inventory management in a hospital blood bank: obtaining a sample from a platelet transfusion, loading the sample into the capillary for DLS measurement, performing the DLS test to identify microparticles, and using the reported microparticle content to identify activated platelets.

Video Link

The video component of this article can be found at https://www.jove.com/video/56893/

Introduction

The interest in microparticles has revolved mostly around their involvement in cell to-cell communication and biological processes.[1,2,3] More recently, microparticles have also attracted interest as potential early diagnostic markers of autoimmune and cardiovascular diseases.[4,5] Microparticles, also known as extracellular vesicles or exosomes, have been widely investigated by flow cytometry. Unfortunately, despite efforts to standardize conventional flow cytometry protocols there is no consensus on the optimal protocol to use.[6,7,8,9,10]

Although conventional flow cytometry can characterize specific MP subpopulations,[11] it has several reported limitations.[11,12,13,14] Some of these limitations have been addressed by using higher power lasers and detectors in a so-called "small particles option",[15,16] as well as detection at 15°-25° forward scatter angle and modified sheath pressure.[17,18] Nevertheless, a quick and easy-to-use screening method that can be combined with these sophisticated methods is still needed to utilize microparticles as early diagnostic markers. Elevated levels of microparticles are detectable in about one third of normal blood donors[19] and might indicate subclinical conditions. Consequently, high microparticle levels in donated blood products might be incompatible with vulnerable recipients.[20]

When providing platelet transfusions, there is a risk of non-immune refractoriness - a condition wherein a patient's body rejects consecutive transfusions and does not allow a significant portion of platelets to circulate.[21,22] Non-immune refractoriness can result in wasted transfusions, health complications for patients, and extended hospital stays.[23] Platelet transfusions containing high numbers of microparticles can be a contributing factor for the development of non-immune refractoriness in vulnerable hematology-oncology patients.[20] It is possible to manage the platelet inventory according to the composition of platelet transfusions by screening for microparticles thereby reducing the risk for vulnerable patients. However, most microparticle tests require isolation of microparticles from platelets[5,12] or are otherwise very labor intensive[24,25] and can therefore not be implemented routinely in hospital blood banks.

The technique described here uses dynamic light scattering (DLS) - also known as photon correlation spectroscopy or quasi-elastic light scattering. For decades, dynamic light scattering has been extensively used in the pharmaceutical industry to characterize liposomal drug formulations or emulsions where particle sizes are in the sub-micron range.[26,27] However, tools developed for these applications are not optimized to screen blood products. A new DLS system was developed to overcome technical limitations and make dynamic light scattering useful for screening of platelet transfusions.[28]

Dynamic light scattering measurements are performed by illuminating the suspended particles with laser light and analyzing the time variation of the scattered light intensity which is a consequence of particles moving in suspension. Further, this method uses the inverse relationship between particle speed and size - small particles move fast and large particles move slowly - to provide information on the size distributions and relative concentrations of the sample components. Using DLS, the mean microparticle content and mean radius of the microparticle component can be quantified. The content of microparticles in the blood product is given as %MP based on the area in the measured histogram for particle radii from 50 - 550 nm. While particles with radii below 50 nm are detected by DLS and reported by the DLS system, they are not included in %MP. Rather than isolating microparticles from platelets, the heterogeneity of platelet transfusions is determined based on the relative content of microparticles and platelets in a sample. In fact, the ratio of the platelet and microparticle peaks can be used to calculate the absolute MP concentration when the platelet count is known.[19]

The DLS system provides health care professionals with qualitative and quantitative information about microparticles found in specimens derived from human blood or blood products. The main advantage of the described DLS technique over alternative techniques such as flow cytometry, electron microscopy,[29] nanoparticle tracking analysis[30] or tunable resistive pulse sensing[31] is the sample preparation: aliquots of platelet concentrates can be directly measured without the need to isolate MP from platelets, sample dilution or other modifications.[9,17] In addition, DLS is an absolute sizing method and does not suffer from the lack of calibration beads with appropriate refractive index.[14,32]

A correlation between platelet activation and MP content has previously been shown by microscopy[33,20] and can be inferred from the increase in MP content in pathological conditions[15,34,35,36] and under *in vitro* conditions known to activate platelets.[19,37,38] However, further studies are needed to fully understand the relationship of DLS-measured microparticle content and platelet activation. Based on our current knowledge that activated platelets contain high numbers of microparticles, they are best used for therapeutic treatment of actively bleeding patients[39], while hematology-oncology patients benefit from non-activated platelets with no or low levels of microparticles[20]. It has recently been reported that *in vitro* responsiveness of donor platelets did not significantly impact the recovery and survival of these platelets in single transfusions to stable, mostly non-bleeding hematology-oncology patients[40]. From this finding, it might be concluded that platelet activation identified by high MP content also plays no significant role in the prophylactic treatment of patients. However, due to the narrow selection of patients, this study did not address the impact of donor factors for complex patients who are febrile (excluded from the study), not stable and receive many more than just one platelet transfusion. The question how the complexity of these patient cases can be reduced - which holds the promise to reduce refractoriness - remains unanswered.

Microparticles are early markers of inflammation[41,42,43,44] and platelet activation[45] and are therefore detected in many normal donors.[19] Consequently, activated platelets and microparticles are present in the platelet donations. It is reasonable to hypothesize that patients with fever, i.e., a fully activated innate immune system, cannot tolerate the additional challenge of a transfusion of activated platelets. However, studies are needed to prove this hypothesis. Microparticle screening can alleviate the current uncertainty about the content of platelet transfusions and reduce the complexity of patient treatment.

The ratio of activated to non-activated platelets in a hospital blood bank depends primarily on the donor population and to a much lesser degree on transport, irradiation, pathogen inactivation and other processes that might increase platelet activation in concentrates.[19] Data from major hospital blood banks in the United States showed that the average composition of the platelet inventory is 49% activated and 51% non-activated (range for activated platelets: 38 - 62%, personal communications). If blood product providers or hospital blood banks want to know how many activated and non-activated platelet concentrates they produce or receive, and want to manage their inventory based on platelet activation as indicated by microparticle content, this protocol might be appropriate for them.

Based on platelet composition a hospital blood bank will be able to direct non-activated, homogeneous platelets for prophylactic use and activated, heterogeneous platelets for therapeutic use. Platelet screening allows hospitals to maximize usage of available inventory which improves patient care and decreases cost. This protocol is intended for laboratory personnel who are familiar with basic handling and manipulation of blood products.

Presented here is a screening method for microparticles in platelet transfusions that can be routinely applied to manage hospital blood bank inventory where selecting product based on microparticle content is desirable. The objective of this protocol is to outline the implementation and evaluation of DLS for screening of donated platelets. The described protocol addresses the common questions of non-invasive access to samples, integration of the testing into the blood bank work flow, and performance characteristics.

Protocol

The following protocol has been performed in compliance with all Canadian Blood Services (CBS) guidelines. Volunteers gave consent that their donations could be used to carry out these studies. Platelet concentrates were prepared according to CBS standard operating procedures. All performance testing described here was carried out at the Centre for Blood Research at the University of British Columbia, Vancouver, Canada with institutional Research Ethics Board approval.

1. Quality Control Check

1. Perform a quality control check at least once per testing day to verify the proper operation of the DLS system. Measure the provided control beads (50 nm radius) following the manufacturer's instructions for use.
2. Retrieve the control vial from cold storage and allow 15 min to warm to room temperature. The beads must equilibrate to room temperature prior to use.
3. Turn the DLS system on prior to preparing the sample so the 15 min laser warm up period is completed by the time the first sample is ready for testing.
4. Once the console has started up, follow the instructions on the touch screen to log in and select the System Test option to measure the Control sample.
5. Vortex mix the vial for 10 s to ensure a representative sample.

6. Fill a capillary with the control beads using a 100 µL fixed volume pipette, pipette tip and capillary from the test kit.
7. When the test is completed follow the instructions on the screen of the DLS system.
    NOTE: The control bead test results are automatically compared to the information stored in the control bead barcode label and a PASS or FAIL notification as well as sample information are shown on the DLS system screen at the end of the test.

2. Obtaining a sample from a platelet concentrate

NOTE: These steps describe the process for non-invasive sampling from the platelet transfusion into the DLS testing capillary for routine platelet inventory management. An overview is shown in **Figure 1**. Required accessories: tube sealer, manual tube stripper, scissors, and splash shield.

1. Remove the platelet concentrate from the untested inventory.
2. Obtain the sample of the platelet product either from an unused sampling pouch (proceed to step 2.3) or a tubing segment (proceed to step 2.4 if empty or step 2.5 if not empty).
    To disconnect the pouch tubing or tubing segment use a tube sealer. To operate the tube sealer, follow the manufacturer's instructions. Briefly, clamp the tubing to be sealed in a precise position between two heated jaws. In a handheld device, press the molten tubing together and cool under high pressure while pressing the lever (the duration is indicated by the green light) resulting in a permanent, leak-proof seal.
3. **Sampling from a pouch**
    1. Mix the content of the platelet bag well by gentle horizontal movement for 5 s (tipping from end to end five times).
    2. Open the clamp to the pouch. The pouch is evacuated and will fill by itself. It is not necessary to fill the pouch completely as only 100 µL sample will be required for DLS testing.
    3. Disconnect the pouch from the bag by heat sealing the connecting tubing with a tube sealer. Continue with step 3.
4. **Sampling from empty tubing**
    1. Verify that the platelet bag tubing has been stored empty and the tubing block is still in place. Visually inspect the tubing to verify that there is not a significant amount of platelet concentrate in the tubing. If the tubing is not empty continue with step 2.5.
    2. Close the tube stripper on the tubing as close to the tubing block as possible by compressing the handles to squeeze the tubing between the rollers.
    3. Hang the bag vertically and keep compressing the stripper handles.
    4. While keeping the stripper closed, release the tubing block.
    5. Slowly pull the stripper down the empty tubing allowing for the tubing to slowly fill behind the stripper. Continue until the section below the stripper has fully inflated or until the stripper is within 1 inch of the end of the tubing.
    6. Once the stopping point is reached with the stripper, use the tube sealer to heat seal the tubing 1 inch above the stripper.
    7. Release the handles of the manual tube stripper.
    8. Heat seal again 1 to 2 inches above the previous seal to create the testing segment.
    9. Cut off the testing segment from the platelet unit. This segment will be used for DLS testing.
5. **Sampling from tubing that is not empty**
    1. Hang the bag vertically and release the tubing block if in place.
    2. Visually inspect the tubing to determine if there are any significant solid clumps. If solid clumps do exist, seal above them such that they cannot be stripped into the bag.
    3. Close the tube stripper on the tubing as close to the sealed end of the tubing as possible.
    4. Strip the contents of the tubing into the bag by compressing the handles to squeeze the tubing between the rollers and, while maintaining the clamping force, move the tube stripper along the tubing towards the bag.
    5. Remove the platelet bag from the hanging hook and gently mix the bag for 5 s by tipping it from end to end five times while keeping the stripper closed.
    6. Hang the bag vertically while keeping the stripper closed.
    7. Slowly pull the stripper down the empty tubing, allowing for the tubing to slowly fill behind the stripper. Continue until the section below the stripper has fully inflated or until the stripper is within 1 inch of the end of the tubing.
    8. Once the stopping point is reached with the stripper, use the tube sealer to heat seal the tubing 1 inch above the stripper.
    9. Release the stripper.
    10. Heat seal again 1 to 2 inches above the previous seal to create the testing segment.
    11. Cut off and discard the last portion of the tubing.
    12. Cut off the testing segment from the platelet unit. This segment will be used for DLS testing.

3. Filling the sample into the DLS testing capillary

1. Using a splash shield and clean, dry scissors, cut one end of the pouch tubing or testing segment.
    NOTE: The capillary used for DLS testing should be filled immediately.
2. Fill the capillary by using the sampling tool (assembled 100 µL fixed volume pipette, pipette tip and capillary) to draw the sample directly from the opened pouch or tubing segment into the capillary.
3. Seal the bottom of the filled capillary by gently pushing the filled capillary into the capillary tube sealant while applying a gentle twist and some pressure against the tray.
4. Disconnect the 100 µL fixed volume pipette and tip from the capillary, ensuring the capillary remains firmly embedded in the capillary tube sealant.
5. Remove the capillary from the capillary tube sealant tray, wipe with an isopropyl alcohol pad and ensure that no air bubbles are trapped by gently flicking the bottom of the capillary.
6. Place the capillary into the DLS system.

4. Performing the DLS test

1. Select the MP test option to initiate a new DLS test for measuring the microparticle content of a platelet sample.
2. Enter the sample information (Donation Identification Number (ISBT), Collection/Production Expiration Date, and Product Code) using the barcode scanner or manually.
3. If no product code is available from which the DLS system can extract the fluid medium information, select the appropriate fluid medium manually (for most platelet concentrates select plasma but for samples containing Platelet Additive Solution (PAS), enter the percentage of residual plasma).
NOTE: A small deviation in the PAS percentage from the nominal 65% will cause a small error in the viscosity (automatically calculated by the DLS system) which minimally affects the calculated MP radius but not %MP.
4. Place the capillary into the DLS system when instructed and start the test.
5. When the test is completed, remove the sample from the capillary holder and dispose of the consumables appropriately according to facility guidelines. Remove the sample from the capillary holder and dispose of the consumables appropriately according to facility guidelines.
6. Tag the platelet bag with the color corresponding to the result, for example orange for non-activated (homogeneous) with %MP equal or below 15% and pink for activated (heterogeneous) with %MP above 15%.
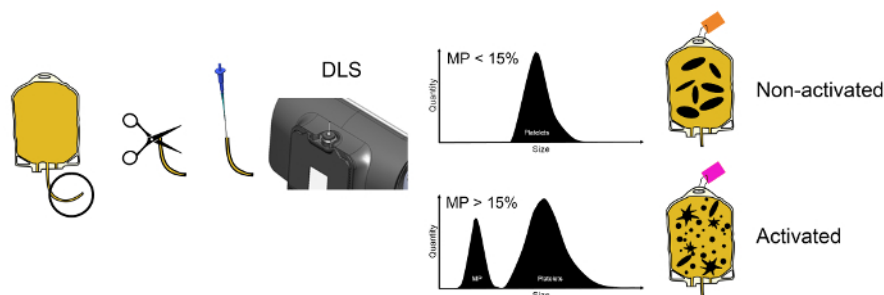

Figure 1: Method overview. Overview of the steps to be performed for routine platelet inventory management in a hospital blood bank: obtaining a sample from a platelet concentrate, loading the sample into the capillary for DLS measurement, performing the DLS test to identify microparticles and using the reported microparticle content to identify activated platelets. Please click here to view a larger version of this figure.

Representative Results

Average preparation time

The platelet screening process using a DLS system is summarized in **Figure 1**. Platelets are tested with the DLS system at the time of receipt from the blood supplier. As detailed in **Table 1** , the average preparation time for trained users is 2 min 23 s while the clean-up and post-test work times are 14 s and 46 s, respectively. In total, the average user takes 3 min and 23 s of hands-on time per test.

Activity	Active time	Walk-away testing time	TOTAL
Prepare DLS System	14 s		
Assemble sampling tool	28 s		
Obtain segment	52 s		
Fill capillary and start test	49 s		
		5 min	
Clean-up	14 s		
Tag and inventory platelet bag	46 s		
Total time needed per sample	3 min 23 s	5 min	**8 min 23 s**

Table 1: Active testing time breakdown. The test itself is a walk away test with an average duration of 5 min. The average user takes 3 min 23 s to prepare the DLS system for a test, obtain and test a sample following this protocol, and tag the platelet bag.

Precision

The precision of the DLS system was assessed at three microparticle levels, 0-7%, 12-25% and 28-75%. The clinically relevant range of %MP is 3 - 75%. Two operators tested low, medium, and high control samples for 16 operating days on two DLS systems in parallel. Samples were tested in duplicate, but in random order on each testing day.

Table 2 summarizes the within-device precision of the DLS measurements for Percent Microparticles (%MP) relative to platelets.

	Microparticle Content		
	Low	Medium	High
Mean %MP (%)	4.4	19.5	53.8
Standard deviation (%)	1.8	2.6	5.8
CV (%)	40.4	13.2	10.6

Table 2: Within-device precision of Percent Microparticles (%MP). At very low microparticle content small platelets may contribute to %MP resulting in increased variability for low microparticle content samples.

Table 3 shows the within-device precision of the DLS measurements for average microparticle radius between 50-550 nm.

	Microparticle Content		
	Low	Medium	High
Mean Radius (nm)	331	161	188
Standard deviation (mm)	133	41	25
CV (%)	40.1	25.2	13.5

Table 3: Within-device precision of microparticle radius. At very low microparticle content small platelets may contribute to Percent Microparticles (%MP) resulting in increased variability for low microparticle content samples.

Table 4 shows the reproducibility of DLS measurements for Percent Microparticles (%MP).

	Microparticle Content		
	Low	Medium	High
Mean %MP (%)	4.4	29.2	53.6
Reproducibility (%)	1.5	2.3	5
CV (%)	35	11.8	9.4

Table 4: Reproducibility of Dynamic Light Scattering (DLS) measurements for Percent Microparticles (%MP).

Linearity
Figure 2 shows that DLS results are linear, *i.e.*, fit a straight line with respect to the assigned values of the samples. Seven samples were prepared with differing microparticle content. Samples with high (MP$_7$) and low (MP$_1$) microparticle content and matching platelet concentration were prepared and mixed at different ratios to create intermediate samples (MP$_x$). Samples were tested with flow cytometry as described previously[19] and DLS and %MP results at each concentration were plotted as input and output. The coefficient of determination was found to be 0.985. Examples of DLS histograms for low and high microparticle content are shown in **Figure 3A**. The DLS results were confirmed by flow cytometry (**Figure 3B**).
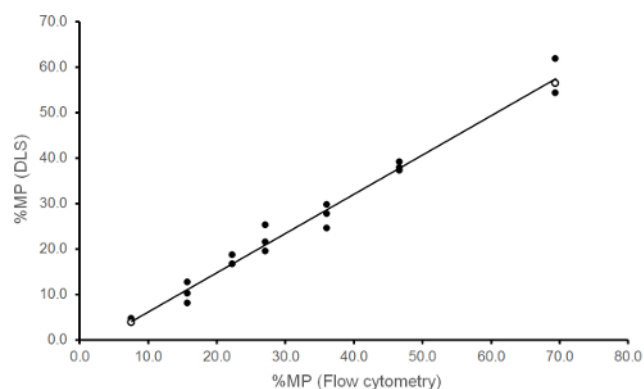

Figure 2: Comparison of flow cytometry and DLS results. Linear relationship between %MP determined by Flow Cytometry (input) and DLS (output), the original DLS data from the two samples marked by the open symbol ○ are shown in **Figure 3**. Please click here to view a larger version of this figure.
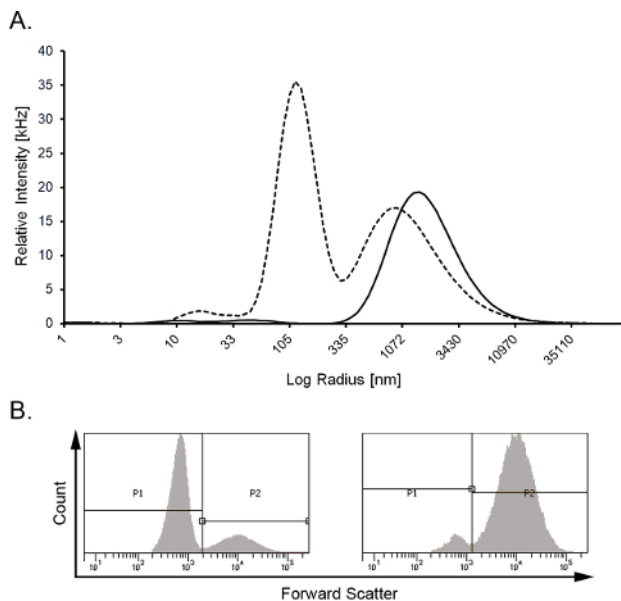
A.

B.

Figure 3: Microparticle content to differentiate between activated and non-activated platelets. (A) DLS results of MP content in activated platelets (dashed line) was 57% compared to 4% in non-activated platelets (solid line). Tests were performed at a measurement temperature of 37 °C, plasma viscosity setting of 1.06×10^{-3} Pa·s, and total intensity settings between 200-600 kHz. **(B)** Flow cytometry results (obtained as described previously[19]) of the same samples as shown in (A); in the forward scatter histograms P1 and P2 represent the MP and platelet gates, respectively; for activated platelets (left) 76% of events fell into the MP gate compared to 6% for non-activated platelets (right). The linear regression line suggests a constant relative contribution of background noise to %MP by flow cytometry leading to the consistently higher results. Please click here to view a larger version of this figure.

Specificity/Interference

The International Organization for Standardization (ISO) defines analytical specificity as the ability of a measurement procedure to detect or measure only the measurand while there are other quantities present in the sample. The analytical specificity of microparticle screening might be affected by red blood cells (RBC). DLS measures MP content relative to platelet content. RBC might interfere because the scattering contribution of RBC is included in the scattering contribution of platelets, reducing the relative contribution of MP. Regulatory limits for the allowable RBC content in blood products exist; a conservative conversion of the threshold recommended by AABB for allowable RBC concentration in platelet concentrates-2 mL of packed RBC in one unit of platelets-resulted in 8.0×10^{10} cells/L (assumptions: RBC volume is 8.5×10^{-14} L, hematocrit of packed RBC is 68%, volume of platelet unit is 200 mL). The reported residual RBC concentrations in different products are well below this threshold[46].

Three different donors donated platelets and red blood cells (RBC) on two different days, as eligible, for three independent experiments. The initial RBC content in the platelet concentrates (reference sample) was $0.05 - 0.15 \times 10^9$ cells/L as determined with a hemocytometer. Five additional samples were created by spiking-in known quantities of RBC into aliquots of the platelet concentrate; target RBC levels in these samples were 1.0, 5.0, 10, 40 and 80×10^9 cells/L.

The interference threshold of red blood cells was approximately 1.0×10^{10} cells/L (**Figure 4**), which also correlated with the level at which the presence of red blood cells was visually evident (**Figure 5**). Above this level, %MP was underestimated which means that in visually red samples-which contain RBC rather than hemoglobin-the reported microparticle content will be too low.
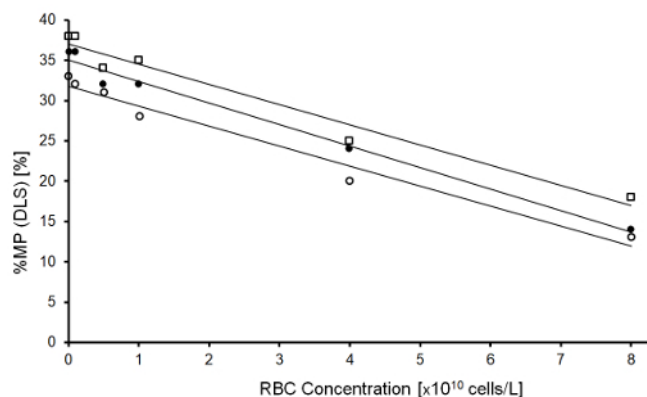
Figure 4: Linear relationship between %MP (DLS) and RBC Concentration (cells/L). Increasing RBC concentrations of 0.1 x10^9, 1.0 x10^9, 5.0 x10^9, 1.0 x10^{10}, 4.0 x10^{10}, and 8.0 x10^{10} cells/L (from left to right) from 3 independent experiments (○ experiment 1, ● experiment 2, □ experiment 3, linear regression lines are shown for each experiment). Above 1.0 x10^{10} RBC/L leads to underestimation of %MP. Visual appearance of RBC containing samples is shown in **Figure 5**. Please click here to view a larger version of this figure.
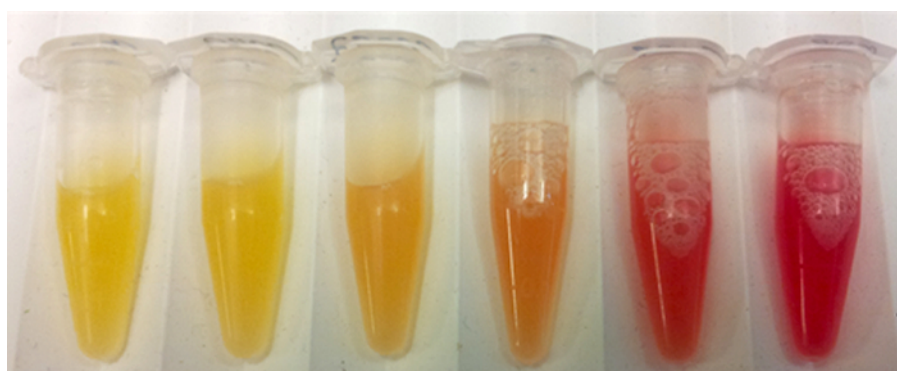

Figure 5: Visual appearance of RBC containing samples. Redness of platelet samples containing RBC concentrations of 0.1 x10^9, 1.0 x10^9, 5.0 x10^9, 1.0 x10^{10}, 4.0 x10^{10}, and 8.0 x10^{10} cells/L from left to right. Please click here to view a larger version of this figure.

Accuracy

Accuracy is defined as the difference between a single measurement result and a true quantity value assigned to the sample. This measurement error includes a systematic component estimated by measurement bias and a random component estimated by a standard deviation. Thus, the accuracy of a measurement result is a combination of trueness and precision.

A bead standard was used to determine the accuracy of the DLS test. Accuracy was evaluated at two concentrations within the clinically relevant range of the assay of 3 - 75% MP. Reference bead mixtures were used to prepare samples of known concentrations because reference beads have a known size and concentration. Consequently, reference beads can be mixed to obtain samples with desired particle sizes and concentrations.

Standard polystyrene beads with a 125 nm radius were used to represent microparticles and beads with 1.5 μm radius were used to represent platelets. The accuracy of the microparticle assay was assessed with bead mixtures of approximately 20% and 50% MP content. The accuracy of the measured particle radii was compared to the particle radii documented on the Certificates of Analysis for the reference beads as shown in **Table 5**.

	125 nm beads			1.5 μm beads		
	Certificate of Analysis			Certificate of Analysis		
		20% MP	50% MP		20% MP	50% MP
Mean Radius	122.0	113.8	124.4	1.5	1.6	1.60
Std Dev	4.5	2.83	3.6	0.035	0.06	0.07
CV	3.60%	2.48%	2.89%	2.20%	3.74%	4.05%
Accuracy		6.70%	2.00%		6.50%	6.30%

Table 5: Accuracy of Dynamic Light Scattering (DLS) measurements. Size comparison for DLS results against certificate of analysis for beads with 125 nm radius and 1.5 μm radius in mixtures.

Discussion

This protocol describes a dynamic light scattering method for microparticle screening optimized for the high particle concentrations found in biological samples such as platelet concentrates. The method of DLS is inherently standardized to accurately measure size. The relative concentration of microparticles can be converted into an absolute concentration if the platelet concentration is known and the platelet peak area is used as the reference peak[19]. As platelet concentrations are usually obtained with hematology analyzers or flow cytometers these methods can be considered companion technologies to DLS.

The functionality of the DLS system is insured by running control beads regularly. Distilled water can be measured to verify that background noise is minimal. Commercially available platelet standards can be analyzed as MP-negative controls and, after addition of 125 nm radius beads, as MP-positive controls. Within the biological range of MP concentrations of interest, the procedures are practical and quick to perform as part of the blood bank routine.

As opposed to flow cytometry, this method is not based on comparing the scattering intensities of particles but rather the speed of their Brownian motion. Thus, exosomes can also be detected despite their small size and are reported separately from MP.

Designed as a screening tool, the limitations of this method are related to its inability to differentiate between different types of microparticles. There is potential room for improvement if additional isolation steps are used; samples could be tested before and after the specific removal of microparticles through antibody coupled magnetic bead capture. In addition, it cannot be assumed that all detected microparticles are cell derived because chylomicrons formed in hyperlipidemia[47,48] and small bacteria or viruses[6] will also be reported in the microparticle range. However, other safeguards exist within the blood supply to avoid highly lipemic or contaminated platelets to enter the hospital blood bank inventory.

The choice of anticoagulant in the sample affects the extent of platelet activation and therefore the MP content[49]. For comparisons of different products this factor needs to be considered. Furthermore, exchange of plasma with MP free suspension media such as PAS will affect the MP content and the threshold for determining heterogeneity-if only about one third of the original MP content is left within the residual plasma in the concentrate an accordingly lower MP content threshold will indicate the same level of platelet activation as in 100% plasma. Percent MP is the MP content relative to platelets. It was previously reported that the average platelet count of PAS product was lower so that the average %MP was still 9.5%[19]. The %MP threshold for PAS platelets licensed in the US is currently set to 10%.

While the primary source of MP in platelet concentrates is the donor, processes that cause stress to platelets will increase the MP level depending on the susceptibility of the platelets to stress-if platelets are already highly activated, minor stressors such as extended shelf life, pathogen inactivation, washing, irradiation or long-distance transport could lead to significant increase in MP content. None of these stressors have been shown to significantly affect homogeneous, non-activated platelets[19]. In addition, attention should be paid to the potential for changes in sample composition within the capillary if not tested immediately after preparation (completion of Step 3 of this protocol).

The focus of this protocol is on determining the composition of particles present in platelet transfusions and to use microparticles as biomarkers of platelet activation. The platelet transfusions are tagged as either non-activated (orange) or activated (pink) based on a microparticle percentage threshold of 15%. The threshold of 15% MP for platelets in 100% plasma was empirically determined as the average 66th percentile from multiple sites.

The objective of platelet inventory management based on routine microparticle screening with DLS is to improve patient care and drive cost efficiencies by preventing non-immune platelet refractoriness. The implementation of the DLS system for screening of platelet bags will enable the user to direct non-activated platelets to patient populations most at risk for developing platelet refractoriness.

Disclosures

Acknowledgements

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