

## Aggregates in platelet concentrates

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Collection and storage of platelets presents many challenges in avoiding platelet activation on one hand, while on the other hand, preserving their functional capacity to respond when transfused. Many of the platelet activation processes are calcium dependent, and reduction of the ionized calcium level with citrate-based anticoagulants, in addition to preventing coagulation factor activation, turned out to have a major role in inhibiting platelet activation during collection. From the early days of platelet transfusion therapy, aggregates were observed and preventive measures were developed, for example lowering of pH [1] and introducing a resting period before further processing [2]. However, other changes in the processing methods, such as the introduction of leucoreduction filters, have led to platelet activation resulting in irreversible aggregate formation [3]. Also, with the ongoing drive to collect more platelets per apheresis donation in a shorter donation time, the risk of donor citrate reactions has been mitigated by lowering the anticoagulant ratio from approximately 1:6 (ACDA:whole blood) to a 1:12 ratio; the trade-off was that this increased the starting pH of platelet preparations and decreased citrate ion concentration from approximately 30 to 13 mM. Similar modifications in the process may result in the occurrence of platelet aggregates in platelet concentrates.

Platelet concentrates may have aggregates immediately after collection by apheresis or after production from whole blood independent of technology or device. These 'intra production' aggregates normally disappear within hours with appropriate resting, agitation and storage temperature. However, in a small number of platelet products, macroaggregates do not resolve and remain visible to the unaided eye. There is little literature on the clinical relevance of platelet aggregates. For baboons, it has been shown that autologously radiolabeled (ADP-induced) aggregates initially sequester in the lung, but radioactivity was released in circulation, suggesting that these platelets may circulate and function [4]. In any case, the vital signs of the baboons remained within normal limits during 24 h after transfusion. Moreover, in the hospital setting, persistent aggregates will be caught by infusion filters during transfusion of the platelet product. It is,

nevertheless, sometimes a reason not to administer a platelet unit, resulting in discarding valuable donor material.

The aim of this forum was to gather current information on the experiences of blood collection establishments with aggregates in platelet concentrates. Requests were sent out to 26 blood establishments (both blood centres and hospitals) and 21 responses were received (Table 1). The replies are summarized here, and the detailed comments by each respondent are available in the online supplement.

### Question 1

How do you inspect the platelet concentrates for the presence of aggregates? Do you have a formal standard operating procedure to address the problem of platelet aggregates? Please describe.

All sites participating in this International Forum have visual inspection of the platelet concentrate at some point during the production process. Most perform the visual check immediately after production. If aggregates are visible, the units are rested for varying times until the aggregates have disappeared. One site that performs pathogen reduction does not treat units with aggregates, which are therefore effectively removed from inventory.

There is no consensus how units with aggregates are rested. Some leave the units unagitated for a couple of hours, followed by placing them on a platelet agitator. Others immediately place the units on an agitator for the aggregates to dissolve. Some sites hold the units in quarantine until the next day, and if aggregates persist, the units are discarded. At other places, the units might be held all the way to the day of expiry (or issue, if earlier) and inspected at the time the units are ordered by the ward. The presence of large persisting aggregates is for all centres a reason to discard units.

About half of the respondents report that they have SOPs that describe the visual inspection of platelet concentrates for the presence of aggregates. It can be assumed that the decision is taken according to a yes/

**Table 1** Respondents to the IF Query

Respondent	Location
Emma Castro, Irene Muñoz	Madrid, Spain
Hans Gulliksson, Per Sandgren	Stockholm, Sweden
Jed B. Gorlin	St. Paul, MN, USA
Teresa Jimenez-Marco	Balearic Islands, Spain
Paolo Perseghin, Patrizia Proserpio	Monza, Italy
Juergen Ringwald, Erwin F. Strasser, Reinhold Eckstein	Erlangen, Germany
Diana Teo, Pei Huey Shu, Sze Sze Chua	Singapore
Stephen Thomas	Brentwood, United Kingdom
Laurie J. Sutor, Doug Heath, Merlyn H. Sayers	Bedford, TX, USA
Juraj Petrik, Kevin McColl	Edinburgh, UK
Walter Nussbaumer	Innsbruck, Austria
Noemi Bondar, Dr Janet Wong, Sue Ismay, Joanne Pink	Alexandria and Kelvin Grove, Australia
Shinobu Wakamoto, Mitsuki Akino and Shigeru Takamoto, Kenji Tadokoro	Sapporo and Tokyo, Japan
Axel Seltam	Springe, Germany
Che Kit Lin	Hong Kong, China
Jenny Sun	Shanghai, China
Joseph D. Sweeney	Providence, RI, USA
José Coene, Hendrik B. Feys, Veerle Compennolle	Ghent, Belgium
Miguel Lozano, Joan Cid	Barcelona, Spain
Hany Kamel, James Dugger	Scottsdale, AZ, USA
Dana V. Devine David Howe	Ottawa, ON, Canada

no criterion. Only some specify in detail the size and number of aggregates that are allowed to be present.

Do you distribute products containing some macroaggregates, and what are your criteria? Are these units always accepted by the treating physician or receiving blood bank? If not, can you describe why not?

A minority of the sites distribute units with small visible aggregates. The blood centre in Singapore allows units to be issued if there are less than five aggregates with a size >3 mm are present. Carter BloodCare may issue units with 50 small aggregates or 10 somewhat larger aggregates. The Rhode Island Hospital reports that units with 'a few' aggregates may be shipped to the ward. The Belgian Red Cross-Flanders has developed an arbitrary scale where, based on size and number of aggregates, guidance is given on the acceptability of units with aggregates. The University Hospital of Erlangen leaves it up to the judgment of the responsible physician to issue a unit with some aggregates present.

The sites that issue units with a limited number of small aggregates report that these units are mostly accepted by the physicians.

## Question 2

With regard to apheresis platelet concentrates, do you rest the platelets after collection before further handling? If so, for how long? In what percentage of units do you observe persisting macroaggregates following the rest period? Have aggregates led to lower split rates?

Are there collection characteristics that you have observed that might impact the formation of platelet aggregates?

The resting period of apheresis units can be anywhere between no resting period to up to 12 h, resting before placing the units on an agitator. Most sites report a resting period between 15 min and 2 h. The percentage of units with aggregates varies widely; most respondents state that aggregates are found in a vanishingly small number of units, while others find percentages up to 20% post-collection. There probably is an association with the apheresis device, as one device collects hyperconcentrated platelets to which plasma is added in process; upon leucoreduction, platelet aggregates are caught in the filter. Other devices have different collection principles and have no need for filtration, which may ultimately result in aggregates in platelet units. Others find an association with donor characteristics such as gender and low haematocrit. (Note, for any particular ACD ratio, the final citrate concentration in plasma is proportional to the haematocrit.) Also, an association with high platelet yield was mentioned, as well as with transportation time. A number of respondents describe that the ACD ratio is critical in the prevention of aggregates. Nevertheless, aggregates usually disappear, and none report lower split rates caused by aggregates.

With regard to whole blood-derived platelet concentrates, do you observe macroaggregates during the first day after production, and if so, by estimation, in which percentage of the units? Do you have experience with certain collection characteristics that could influence the occurrence of platelet aggregates?

Many sites report that they rarely see, if at all, platelet aggregates in whole blood-derived platelet concentrates, while others report percentages of 10% or 15%. Aggregates are seen with PRP-derived platelets but also with BC-derived platelets. Canadian Blood Services reports unexplained sporadic-persistent aggregates in PRP-derived platelet units in the past, resulting in significant loss of products, which was a driver for switching to the BC method. The problem indeed disappeared. As indicated, other sites using PRP never see problems, and one can only speculate that a certain combination of blood collection specifics, holding and/or transportation time, temperature and (timing of) filtration might lead to different

frequencies of aggregates. Platelet concentrates from buffy coats; nowadays, all have a prestorage leucoreduction step in place, whereby aggregates are removed in the filter, and also some methods to prestorage pool PRP-derived platelet concentrates have a filtration step in place. Because in general pooled platelet products are made when derived from whole blood, the chance is smaller that certain donor-associated effects, unlike seen for apheresis units, are demonstrable. One site reports that adequate mixing with anticoagulant is a factor in preventing aggregate formation. Again, persistent aggregates in whole blood-derived platelets, resulting in discard of units, are rare.

### Question 3

Describe your environmental conditions during product handling once a unit is collected: is the temperature controlled, and if so, by what means? In which percentage of units do you observe macroaggregates beyond the first day after collection? Do they dissolve over time, and how long does it take them to dissolve? By estimation, in what percentage of the units do you have to discard because of non-dissolving aggregates? Do you see aggregates appear during storage that were not present at the time of collection/processing?

Sites often have temperature control in place using air conditioning both at the collection and processing site, blood can be cooled to room temperature with cooling plates, and transport takes mostly place in special shipping boxes to prevent temperature extremes. If no specific temperature control is present, staff is in general aware that units should not be placed in areas where the temperature is too low, for example on cold (metal) surfaces. Overall, the chance that blood products can reach a temperature outside 18–25°C is small. One site reports that appearance of aggregates was only found in units where the temperature had fallen outside the range of 20–24°C.

Depending on the site, the percentage of units with aggregates beyond the first day is small, in the order of 1–2% at most. Agitation, sedimentation of the aggregates, massaging of the aggregates or addition of 10% ACD-A in general result in dissolving of the aggregates. One site applying pathogen reduction said that small aggregates are trapped in the filter of the illumination set. While in general the frequency is low, both Belgium and Canada report occasional extremes of 33% and 40%, respectively, without clear cause, and these episodes would again disappear, without clear cause.

It is very rare that aggregates (newly) form after the initial production phase. If so, observers should be aware of a bacterial contamination.

### Question 4

Do you use plasma or PAS for storage of platelets? Do you believe that either one or the other makes the platelets more susceptible for the formation of macroaggregates?

Of the sites using PAS or having experience with PAS, none definitely attribute the occurrence of aggregates with the use of PAS. A number of replies indicate that factors in the process to facilitate the use of PAS, such as a concentration step during an apheresis procedure needed to remove plasma, might be underlying causes for aggregates and not the use of PAS itself. One site uses PAS for their washed platelets and found that aggregates would not disintegrate after centrifugation, but again it is likely that the concentration step and not the sole use of PAS is the cause of the aggregates observed. Only Devine and Howe found a suggestion that some PASs might induce aggregates during storage of platelet concentrates, but this was under R&D conditions.

### Question 5

Do you see donor dependence, for example a repeat donor whose platelets formed aggregates at multiple donations? Have you observed certain donor characteristics that could impact the formation of platelet aggregates?

A number of respondents state that they think there are 'clumpy' donors, but evidence is lacking. These donors would have a 'sensitive' phenotype, making them more susceptible to activation during for example apheresis, centrifugation or the use of PASs. A couple of respondents indicate that a small number of apheresis donors that repeatedly showed aggregates are asked to donate other components. Clumping in units from these donors may occur on a number of occasions, but is not after all procedures, showing that donor predisposition is not the only factor. Smoking is also mentioned as risk factor, as well as gender, haematocrit (see Question 2), high platelet count and the type of apheresis device. Others have looked and found no specific factor that might be responsible for repeated aggregate formation. Studies are being initiated to investigate in more depth why some donors repeatedly form platelet aggregates.

### Question 6

Have you observed a seasonal effect on the occurrence of platelet aggregates? If so, which season or time of year do you think is related to the formation of aggregates in platelet concentrates?

Two sites said they found an increased frequency of platelet aggregates during summer. One indicated that units were affected by excessive exposure to the air conditioning in summer; the other mentioned that maybe donor hydration was the cause of elevated platelet aggregate formation. In Belgium, two periods of elevated aggregation frequency were seen, both during the colder months. In Canada, there was a suggestion that aggregates were more common in winter, but upon a closer look, no seasonal effect could be proven. All other sites see no relation between season and aggregates.

### Question 7

Have you taken specific interventions to prevent or mitigate the formation of aggregates?

Most sites have taken interventions to prevent aggregate formation. During (apheresis) collection, a strict control of the ACD ratio seems important, and sometimes more ACD is added if there is a suspicion that aggregates may form. Stripping of lines has been mentioned as a way to prevent stasis of highly concentrated platelets. Others switched apheresis devices to prevent aggregates. Sufficient resting before placing on an agitator is mentioned by a couple of people. In the most extreme case, donors are moved from platelet apheresis to the donation of other components.

For whole blood, also adequate mixing with anticoagulant is important, and one respondent moved to automated mixers. Canada switched from PRP-derived platelets to buffy coat.

Careful handling without vigorous shaking has been introduced, as well as a guideline not to stack units unless needed during transportation. Temperature control, including not placing units in the airstream of air conditioning units, and not placing platelet concentrates on cold surfaces, is mentioned. Leucoreduction as a means to remove aggregates was indicated, as well as making sure that the platelet concentration is not too high. Finally, the composition of the additive solution is mentioned, though the respondents disagree whether potassium and magnesium should be present, or not.

### Question 8

What is your level of concern regarding macroaggregates in platelet concentrates when transfused (1 = low, 5 = high) and why?

Varying levels of concern are expressed, but for very different reasons. Some are really not worried, because they feel that removal of the aggregates by the transfusion filter is sufficient to provide a safe and effective platelet transfusion. Others show more concern.

Aggregates may be a sign of bacterial contamination. Some respondents indicate that with removal of aggregates, the platelet dose becomes lower, resulting in a lower CCI than if no aggregates were present. Others state that the larger aggregates may be captured in the transfusion set, but that smaller aggregates still can cause problems in the patient. Also, there is worry that aggregates are indicative of general platelet activation, and otherwise poor quality of transfused platelets, leading to lower increments.

The potential of transfusing platelet aggregates to a patient is a major concern of respondents, but the prevalence of aggregates in current practice is generally very low. All respondents inspect 100% of platelet products for aggregates, usually at several points in the platelet history from collection (if by apheresis) through the point of issue. Inspection methods vary from a non-descript 'visual inspection' to detailed requirements for backlighting and determination of size and quantity of the aggregates. Likewise, acceptance criteria vary widely from 'no visible aggregates' allowed to 'no macroaggregates issued' to a formal algorithm based on the size and quantity. The subjectivity of these assessments by blood bank staff as well as by clinical staff is recognized. One respondent noted that clinical staff sensitivity may have been affected by a recent bacterial contamination case. One group may release platelets with some aggregates noting that the transfusion filter (nominally 170–220  $\mu\text{m}$ ) would prevent the macroaggregates from reaching that patient. No one used microscopic inspection.

Platelet aggregation seems to be an episodic problem, coming and going at various times with no consistent cause and effect association identified. Some have noted that this is associated with specific donors. Practices to mitigate and allow aggregates to dissociate include resting the platelet undisturbed for 1–3 h and re-examination at varying periods after the platelets have been on the platelet agitator. Prevention strategies range from precise mixing with the anticoagulant to the blood to changing processing and collection techniques or equipment. The use of PAS per se does not appear a generalizable cause of aggregation although there may be specific formulation exceptions. Identification of fundamental cause and effect with corrective, generalizable strategies has been elusive, no doubt because this appears to be a multifactorial phenomenon [5]. The investigations of several groups suggest that the presence of large, persistent platelet aggregates may be indicative of an elevated activation state in the remaining unaggregated platelets [3, 6], thus supporting the long-standing practice reported by many international forum respondents to reject concentrates with large aggregates, though one group has the practice

to sediment and remove the macroaggregates. For units with transient aggregates, the studies showed that aggregates fully disintegrate without remaining microaggregates [7] and that the platelets have *in vitro* quality similar [6–8] or almost similar [9] to platelets from concentrates without aggregates. These *in vitro* data substantiate the common practice to hold platelet concentrates with aggregates until these dissolve and then allow the units to be released for transfusion. Finally, some respondents allow units with smaller aggregates to be sent to the hospital ward. These aggregates are effectively removed by the transfusion set [8], and this results in minor platelet loss [5].

There are differences in prevention, mitigation, detection and distribution strategies between the various practitioners responding in this forum. The fundamental principles of reducing the storage medium ionized calcium with adequate citrate administration, reducing the pH and resting of the platelet are varyingly applied by these international forum participants. However, identification of specific causes for this phenomenon has been elusive. Thus, data-free practices have evolved by practitioners, using informed judgment based on fundamental principles, but lacking systematic development of evidence to support these practices. Additional research into specific causes including donor-related causes is needed. Resolution of this unpredictable problem with the collection and preparation of platelets for transfusion may avoid the loss of platelets and time expenditures of blood centre staff, device manufacturers' personnel and blood donors.

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### Question 1

- (a) Apheresis platelets are visually inspected for aggregates immediately after collection. Following collection, apheresis platelets are rested for 1 h, after which they are again inspected for aggregates prior to sampling for routine process control testing and consignment to the processing centre. Apheresis platelets are also inspected for aggregates immediately prior to supply.

Whole blood-derived pooled platelets are inspected for aggregates immediately after completion of the manufacturing process and immediately prior to supply.

Platelet units (apheresis and whole blood-derived pool) that are randomly selected for testing at expiry are inspected for aggregates as part of routine process control testing.

Our standard operating procedures require all platelet concentrates to be visually inspected at each stage of the collection and manufacturing process as noted above. While platelet aggregates are not specifically

mentioned in the instructions, they are addressed as part of routine staff training.

- (b) The blood service does not issue components with any visible aggregates.

### Question 2

- (a) Apheresis platelets are rested for 1 h after collection. Apheresis platelet donations collected at the end of a collection session are rested for a minimum of 15 min in the donor centre. If the platelets are not able to be rested for the full hour at the donor centre, the platelets are then rested for 1 h upon receipt at the processing centre.

With our current apheresis platelet collection process, aggregates have very rarely been observed immediately following collection (<1% of collections). If any aggregates are present at the end of the resting period, these appear to be completely dispersed by the time the platelets are received at the processing centre.

As we have not observed persistent platelet aggregates with our current processes, our split rates have not been affected.

- (b) We have not observed aggregates in our whole blood-derived pooled platelets. There is a leucodepletion filtration step involved in process. Any macroaggregates, if present, would be captured by the filter.

### Question 3

Once the apheresis platelets are collected, the packs are placed in a temperature-monitored environment (20–24°C), away from any direct source of cold or hot air, draft or vent. Following the resting period, the platelets are packed in a temperature-controlled transport shipper, which maintains a temperature between 20–24°C, for transporting to the processing centre. On arrival at the processing centre, the platelets are handled in a temperature-controlled and monitored environment and placed on platelet agitators (platelet incubators at 20–24°C) as soon as possible. Any aggregates that may be present at collection and persist after the resting period are always dispersed by the time the platelets arrive at the processing centre.

Pooled platelets are manufactured in a temperature-controlled and monitored environment (20–24°C) and are placed on an agitator (platelet incubators at 20–24°C) after manufacture.

We have not observed any aggregate formation during the storage of either apheresis platelets or whole blood-derived pooled platelets.

### Question 4

Currently, apheresis platelets are resuspended in 100% plasma. Whole blood-derived pooled platelets are resuspended in approximately 30% plasma and 70% PAS (SSP+). As noted above, we have not observed aggregate formation during the storage of either apheresis or whole blood-derived pooled platelets produced using our current processes.

Collection of apheresis platelets in PAS has been evaluated; however, our initial validation was unsuccessful due to approximately 10–15% of collected units showing irreversible aggregation (macroaggregates). These aggregates formed during the collection process and persisted for more than 24 h after collection. Our evaluation studies are currently ongoing.

### Question 5

We have not observed any correlation between donor characteristics and platelet aggregation.

### Question 6

We have not observed any seasonal effect on the occurrence of platelet aggregates; however, there have been some anecdotal reports that aggregate formation during the apheresis platelet collection process seems to occur with greater frequency the day after a very hot day, suggesting that donor hydration could possibly be a contributing factor.

### Question 7

Apheresis platelets are rested for 1 h after collection and whole blood-derived pooled platelets are rested for 1–2 h after manufacture.

In developing our platelet collection and manufacturing processes, factors that may predispose platelets to aggregate formation have been taken into consideration, such as the final platelet concentration, the anticoagulant to whole blood ratio, the type of additive solution and the residual plasma volume.

### Question 8

Very high, 5. In Australia, all platelet components are transfused through blood administration sets that incorporate a standard 170- to 200- $\mu$ m filter. While this filter will remove visible macroaggregates, it is possible that smaller aggregates may be able to pass through. As the clinical relevance of the presence of any aggregates is currently unclear in terms of their effects on component quality, safety and efficacy, the Blood Service does not issue any components that contain visible aggregates.

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#### *Question 1*

- (a) We control our concentrates 2 h after the collection for visible aggregates. We check the units in back-light. The check is also done immediately before we choose a unit for transfusion and accept it only if there are no visible aggregates. The process is described in our SOP.
- (b) No, we do not distribute units with visible aggregates. Our concerns are that in units with macroaggregates, we will also have lots of microaggregates, not hold back by a standard transfusion filter.

#### *Question 2*

- (a) Resting period is 2 h. Persisting visible aggregates occur at a vanishingly low rate. If the aggregates are also visible at the time point of distribution, the unit is not used.

- (b) Maybe up to 10%, what we observed is that filtration of apheresis products on the day of production will lead to a high rate of filter blockages; therefore, we perform necessary filtration of apheresis-based units on the following days with excellent results and nearly no blockages of the filters. The problem is more accurate for Amicus units than for Trima units.

#### *Question 3*

- (a) Our production unit is air-conditioned at room temperature, after the 2-h rest, units are stored between 20 and 24°C on a horizontal agitator inside an incubator.
- (b) As long if we do not manipulate the units (e.g. washing), we have only episodic reports of new visible aggregates beyond the first day of collection. As an estimate, percentage of units discarded due to not resolved aggregates is clearly below 1%. Aggregates, appearing during storage and not visible after production, are not an issue in our centre.

#### *Question 4*

We use PAS 3M in a 40:60 ratio for all our platelets. We are using PAS now for more than 4 years for all our platelet concentrates (pool and apheresis) and did not see a problem of aggregate formation in a higher percentage compared with the production of units in 100% plasma. Again, especially for Amicus-derived platelets, we would not recommend a filtration on the day of production.

#### *Question 5*

Not in a remarkable number. But what we found are two or three donors with high number of residual leucocytes, despite the use of Amicus and/or Trima devices. Until now, we could not find an explanation for that phenomenon.

#### *Question 6*

No, or at least not to my knowledge.

#### *Question 7*

In general, a 2-h rest without agitation after the production, in particular we follow the recommendations of shaking, given by the company, for Amicus platelets, and introduced a similar process for Trima concentrates before we resuspend the highly concentrated units (>3000/ $\mu$ l) with PAS 3M.

**Question 8**

I would rate it with 4, comparing problems caused by microaggregates before BC removal of whole blood and the resulting ARDS.

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**Question 1**

All platelet concentrates are visually inspected prior to distribution. Platelet concentrates that are clearly visually abnormal or have a swirling score below 2 are discarded. Clear instructions have been given on how to judge aggregates, including a scoring system for size and number. The scoring system, detailed in Table 1, serves to aid decisions on acceptability of platelet concentrates in case aggregates are present. Platelets are only released and distributed if the global aggregate score is below 5. By applying this cut-off, platelet concentrates with, for example, more than 10 aggregates of 1–2 mm, with more than 5 aggregates of 2–3 mm, or with a single aggregate larger than 3 mm are considered not acceptable.

Obviously, this scoring method and the acceptance criterion are, at least partially, arbitrary. In determining this scoring system, the safety and quality of the product as well as the acceptability to the clinician were

**Table 1** Score for aggregates

Score A	Size of the largest aggregates
0	Not larger than a pinpoint
1	Up to 1 mm
2	Up to 2 mm
3	Up to 3 mm
4	Larger than 3 mm
Score B	Number of aggregates
0	None
1	1–5
2	6–10
3	11–20
4	More than 20
Score for aggregates = Score A + Score B	

crucial elements. Initial findings on quality of the product (see question 8 for more details) and efficacy in removing aggregates by transfusion set filters, indicated that a zero tolerance is unnecessary and would cause unacceptable loss of platelets. The upper limit was set to avoid that hospitals would either be uncomfortable with the quality of the product or start neglecting the result of visual inspection of products. This scoring system has been applied for 2 years within our blood establishment. So far, we did not receive complaints from treating physicians on acceptability of platelet products.

**Question 2**

We leave apheresis platelets to rest for 1 h after collection, after which products are placed on a flatbed agitator. Platelet additive solution is only added some hours later. We do not inspect the platelet concentrates for the presence of aggregates immediately after the resting period, but at the time of release of the product, usually on day 1.

Our split rates have not been influenced by the presence of aggregates. When comparing procedural and product characteristics of apheresis platelets with aggregates-versus-apheresis products without aggregates, we noted that the donated volume, product volume, predicted yield and effective yield were significantly higher in the group of products with aggregates compared to the control group. We did not detect differences in procedure time nor in the amount of ACD-A that went to the donor. We have seldom observed macroaggregates in platelet concentrates prepared from whole blood.

**Question 3**

The platelet collections and the early storage take place at 'normal' ambient temperatures, without climate control. We do take care not to put the platelet concentrates on 'cold' metal surfaces, and they are not stacked until transportation from the collection site to the component laboratory. They are transported in insulated boxes to protect them against extreme outside temperatures. The component laboratory and the dispatching room are kept at 20°C. The PAS added is kept at room temperature.

The percentage of platelet concentrates with aggregates is very variable. In the recent past, we experienced three episodes with increased percentages of products with aggregates: one short period at the end of 2011 and two prolonged episodes mid–end of 2012 and mid–end of 2013. During the most affected months of this period, on average 4.5% of the products displayed



aggregates at the time for release. In nearly half of the aggregates, 2% persisted in unacceptable amount till the end of storage. However, during 1 week, in one production site, 33% of products were affected. The aggregates—if present—were always visible from the beginning. Outside these episodes, the occurrence of aggregates is rare.

#### Question 4

All our platelets are stored in PAS. Both high and low prevalence of clumps occurred while using the same solution (SSP+; MacoPharma, Mouvaux, France), so other factors seem to be involved in provoking the occurrence of aggregates in apheresis platelets.

#### Question 5

Data collected during the 2012 episode do not suggest a correlation between donor characteristics such as height, weight, gender, blood volume, donor platelet concentration or haematocrit from donations and the occurrence of aggregates in apheresis products.

On the other hand, during the same episode, several donors gave multiple affected products. This finding needs further investigation.

#### Question 6

As described under question 3, we have in the recent past observed three episodes with increased prevalence of aggregates: one short episode at the end of 2011 and two prolonged episodes (mid–end of 2012 and mid–end of 2013).

#### Question 7

A number of measures were taken to reduce the occurrence of aggregates: no storage on 'cold' stainless steel surfaces, earlier transfer of the freshly collected platelet concentrates to the flatbed agitator after only 1 h of rest, no stacking of the platelet concentrates (unless for transportation), storage of the PAS at a slightly higher temperature. Temporarily, we introduced stripping of the line between the Trima cassette (Trima, Terumo BCT, Colorado) and the platelet bag so as to avoid stasis of concentrated platelets in the tubing. Such stasis can also be avoided by the early addition of the PAS, at the end of collection instead of later on in the component laboratory.

#### Question 8

We are not too concerned to transfuse platelet products with presence of the macroaggregates in the platelet

concentrates (level of concern = 2). Validation tests conducted demonstrated that the aggregates are efficiently removed by in-line filtration on transfusion sets with no significant effect on platelet counts or MPV. Moreover, an initial *in vitro* study on a limited number of platelet concentrates indicated that flow cytometric and aggregation parameters were not significantly different in apheresis products with aggregates-versus-apheresis products without aggregates [1]. Larger groups are needed to reveal less pronounced differences. The pH of all products was within limits of acceptance on day 6 of post-donation. Of note however, the pH of products with aggregates was significantly lower than the pH of products without aggregates, a finding that requires further attention.

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D. V. Devine & D. Howe

#### Question 1

- (a) All platelet concentrates are subjected to visual inspection prior to issue. We have no formal SOP for investigation of aggregated platelet units. CBS employees and hospital personnel do have access to a Visual Assessment Guide, which assists with the determination of the suitability of our products. This guide covers issues such as platelet aggregates. When unanticipated product issues arise, employees follow the corporate deviation management process to determine the product disposition. For issues that are not readily resolved, the research and development scientists work with production staff to investigate such problems; historically, this approach was taken with unexplained aggregation in multiple platelet concentrates [1].
- (b) We do not distribute products that contain visible aggregates.

### Question 2

- (a) Apheresis platelet concentrates are left to rest for 2 h after collection. We very rarely see platelet concentrates containing macroaggregates that are visible to the naked eye. This is so rare that we do not keep statistics on the event. Overall unsuitability of apheresis platelets for all visual issues that cause discard occurred at a rate of below 1:2000.
- (b) Until about 5 years ago, CBS prepared its whole blood-derived platelets using the platelet-rich plasma method in a system that contained an in-line leucoreduction filter. Irreversibly aggregated platelet concentrates were a common problem, which occurred sporadically in most of our production sites, and would impact a significant proportion of inventory and resulted in the discard of many units of platelets. Despite significant effort to resolve this problem, with and without the assistance of the blood bag vendor, we were never able to clearly identify a single root cause. Owing to the manual nature of the processing, the increased care and attention of the staff to processing conditions was emphasized, and this sometimes but not always reduced the level of aggregated platelet concentrates. Frustration with this situation was one of the drivers for Canadian Blood Services to implement buffy-coat-derived whole blood processing. Since conversion to buffy-coat production, we no longer see platelet aggregates in whole blood-derived platelet concentrates. One factor that was identified as contributing to the formation of aggregates in platelet concentrates is the presence of air in the platelet concentrate bag. Our current SOPs recommend removal of the air prior to incubation.

### Question 3

Whole blood collections that are destined for platelet concentrate production are collected into a top-and-bottom bag system, in a temperature-controlled clinic, and placed on butane diol cooling trays, once the collection is completed. We do not see macroaggregates in these platelet products. Historically, the aggregated platelet concentrates produced using the PRP method never returned to a disaggregated state within their allowable storage period. The percentage of discards under the former production protocol varied widely, but did at times reach over 40% of a production run.

The appearance of aggregates during storage would raise a high degree of suspicion of bacterial contamination. This is very rare phenomenon in our production laboratories.

### Question 4

There is no platelet additive solution licensed in Canada, so our platelets are suspended in plasma from one of the male donors in each buffy-coat pool of four donors. We do not have real production laboratory experience with PAS, but have worked with PAS formulations in our research laboratory. Some PAS formulations appear to have slightly increased chance of aggregate formation, but only after day 5 of storage. We have not examined this systematically.

### Question 5

Because we no longer see aggregated platelets in either our apheresis or whole blood-derived platelet concentrates, we have not investigated a donor dependence in this phenomenon. That said that we believe that it is highly likely that there are donor-specific factors involved for the 'repeat offenders'.

### Question 6

In our PRP platelet production days, there was a suggestion made that aggregate formation was more common in the winter, but when analysed across 14 production sites, no seasonality could be reliably predicted.

### Question 7

We changed our whole blood production method to buffy coat with overnight hold of the whole blood using butane diol cooling platelets. Upon doing so, the problem of irreversible aggregates in platelet concentrates disappeared.

### Question 8

My level of concern is a '2'. Even in platelet concentrates with numerous visible macroaggregates, there are still adequate numbers of singlet platelets. Aggregates of any significant size will be trapped in the filter of the infusion set. There is of course the possibility that the filter could clog and the transfusion discontinued. There is also a school of thought that would suggest that if aggregated platelet units are a reflection of platelet activation state, such platelet concentrates might actually be more efficacious in a bleeding patient if the platelets are already somewhat activated.

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### Question 1

Yes, before blood components (including platelet concentrates) are distributed to hospital, they are examined for abnormalities (including aggregates) by means of visual inspection according to our protocol similar to the Canadian Blood Services' Visual Assessment Guide [1]. Platelet concentrates with macroaggregates, that is white and opaque masses that do not dissipate with gentle manipulation, are not distributed for clinical transfusion.

### Question 2

- (a) There is no resting period for apheresis platelets. The collected apheresis platelet units, after registration in our blood bank computer system, will be transferred to and stored in platelet agitators with continuous agitation. Macroaggregates are seldom noticed in apheresis platelets.
- (b) Whole blood-derived platelet concentrates, prepared by PRP method, will not be examined for macroaggregates during the first day after production. We have no data regarding the appearance of platelet aggregates on the first day and the collection characteristics.

### Question 3

Any whole blood collection exceeding 15 min from needle insertion will not be used for platelet production. Annually, around 0.1% of collected whole blood cannot meet this criterion. Immediately after collection, whole blood units are rapidly cooled down to 18–24°C by sandwiching with ThermaSure™ coolants and stored

in an insulated container. They are then transported back to our processing laboratory, stored in a temperature-controlled cool room of 20–24°C overnight and processed into blood components, including platelet concentrates, on the day after collection. The separation process must be completed within 24 h from the time of collection and the environmental temperature of processing area is kept at 18–24°C and logged regularly.

In our practice, we do not inspect platelet concentrates until issue. Our issue staff are responsible for the final inspection for non-conformity (including macroaggregates) before distribution. All products with visual gross non-conformities, such as clots, red cell contamination, lipaemia, abnormal coloration, etc., will be taken out. We observed that some 1.5–2% of the whole blood-derived platelet concentrates were discarded due to the presence of platelet aggregates. We also noted that the number of whole blood-derived platelets returned by hospitals after issue due to macroaggregates ranged from 0 to 2 units per year during the past decade.

### Question 4

Platelets are suspended in 48–50 ml donor plasma depending on the donation volume.

Our limited experience with PAS (MacoPharma SSP+) was from a brief project studying the preparation of Intercept™-treated platelets concentrates (pathogen-reduced platelets derived from pools of five buffy coats suspended in PAS and treated with INTERCEPT; no macroaggregates were observed).

### Question 5

We have not studied the relationship between platelet aggregates and donor dependence.

### Question 6

According to our data, relationship between seasonal effect and occurrence of platelet aggregates is not apparent.

### Question 7

Yes, the environmental temperature for processing and storage of platelet concentrates are well controlled to prevent formation of platelet aggregates.

### Question 8

Level 5; platelet units with the presence of aggregates may indicate improper handling (with possible impaired platelet

functions) or the first sign of bacterial contamination which mean that they may not be able to achieve the expected therapeutic efficacy or may cause adverse transfusion reactions.

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J. Sun

### Question 1

- No. So far we haven't formal standard operating procedure to address the problem of platelet aggregates.
- We haven't found that products contain some macro aggregates. It should be considered that the criteria how to distribute these products as we don't have such criteria by now.

### Question 2

- Yes, we will rest the platelets for more than 30 min after collection before further handling, with regard to apheresis platelet concentrates. There will be no persisting macro aggregates following the rest period as we observed.

The ratio of anticoagulant and platelet and the device maybe impact the formation of platelet aggregates.

- We observe macro aggregates during the first day after production, with regard to whole blood-derived platelet concentrates. But no macro aggregates have been found during the first day after production as the storage time of whole blood-derived platelet concentrates is <24 h in China.

So far we have no experience with certain collection characteristics that could influence the occurrence of platelet aggregates.

### Question 3

The temperature is controlled at 20–25°C by air-conditioning during product handling once a unit is collected. No macro aggregates have been found beyond the first day after collection according to our observation.

### Question 4

We use plasma for storage of platelets. We have no idea which makes the platelets more susceptible for the formation of macro aggregates as we haven't ever used the other.

### Question 5

We don't know whether a repeat donor whose platelets formed aggregates at multiple donations. As we observed, the high platelet count (>300 × 10<sup>9</sup>/l) maybe impact the formation of platelet aggregates, but the ratio is <1% or so.

### Question 6

We don't believe that a seasonal effect on the occurrence of platelet aggregates as we haven't met with such examples.

### Question 7

We take some interventions to prevent or mitigate the formation of aggregates for example we control the temperature when collection, introduce a rest period before further processing and agitation when storage etc.

### Question 8

The level of our concern regarding macro aggregates in platelet concentrates maybe two because the occurrence of platelet aggregates in platelet concentrates is low and there is little literature on the clinical relevance of platelet aggregates in China.

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J. Ringwald, E. F. Strasser, R. Eckstein

### Question 1

- First of all, we would like to give some short information about the production of platelet (PLT) concentrates in our department. Only single-donor platelet concentrates (SDPs) using the two devices Trima Accel (TerumoBCT, Zaventem, Belgium) or Amicus (Fresenius-Kabi, Bad Homburg, Germany) are produced in our

university hospital-based blood donation service. All SDPs (approximately 5000 per year) are exclusively transfused to in- or outpatients of our university hospital (approximately 1400 beds). So, we do not serve other hospitals or blood banks regularly. Until October 2012, we collected SDPs by single (target  $3 \times 10^{11}$  PLTs in 220 ml) or double ( $6 \times 10^{11}$  PLTs in 220 ml) collection depended on the PLT concentrations and blood volume of the donor. In November 2012, we changed to collecting exclusively double-dosed SDPs. We do still collect and store our SDPs in 100% plasma but intend to change to PAS 70% in the future.

Our policy in quality control of produced SDPs is a 100% control of all PLT products for PLT content and concentration, residual white blood cell count, swirling and aggregation. Therefore, every single SDP produced in our department is checked for these parameters. In routine, all SDPs are checked for the appearance of macroscopically visible PLT aggregates by an operator within 1 h after production together with the check for the swirling. If macroscopically PLT aggregates are visible, this has to be documented on the production protocol. Six to 24 h later, dependent on the availability of the final results of donor testing, the responsible physician for the release of the products has to check every single protocol and, if macroaggregates had been seen by the operator, SDPs have to be checked again by this physician. If the aggregates are no more present, SDPs can be released. If the aggregates are still present, it is up to the decision of the responsible physician to give the SDPs another 24-h rest to be checked again or to discard the SDP. In summary, SDPs with persisting macroscopically visible PLT aggregates should not be released in our department.

- (b) The aim of the above-described procedure is to prevent the release of SDPs with persisting easily macroscopically visible PLT aggregates for transfusion. Yet, we do not have defined exactly the upper limits with regard to number or size of aggregates that definitely should lead to discarding an SDP as there is, to our knowledge, no evidence about the clinical relevance of this phenomenon. Therefore, the final decision in terms of discarding a unit due to the presence of aggregates is up to the individual decision of the responsible physician for the final release who is always a specialist for transfusion medicine in our department.

To our knowledge, there has been just one SDP within the last few years that was released by us but had not been accepted by the treating physician for transfusion due to the presence of a low number of macroaggregates. So overall, this happens very rarely (<1:5000 released SDPs).

### Question 2

- (a) We give the SDPs a rest of 30 min after collection without agitation. Then, the products are put on a flatbed agitator where they are kept under continuous agitation throughout the whole storage period.

So far, we have not been documenting routinely the number of SDPs with macroaggregates in our department. However, we performed a retrospective analysis of randomly chosen SDPs of several periods in 2012 [1]. In brief, in 100 of 803 SDPs (12.5%), the presence of visible macroaggregates was documented by the operator initially. In 81 SDPs, the aggregates had been disappeared completely within the next 24 h. Overall, 10 SDPs (1.2%) had not been released due to persisting aggregates at least for 24 h or even beyond.

In this retrospective analysis, we also have compared different variables such as PLT concentration, PLT content, separation volume, collection time, donor's pre/post-haematocrit (hct)/WBC or PLT count, donor's total blood volume, volume of ACD-A in PC, and total ACD-A used between SDPs with and without PA for single and double collections for each device separately. In brief, SDPs with or without aggregates did not differ in variables such as separation volume, collection time, anticoagulant volume in SDPs, donor's total blood volume, WBC or PLT count. However, SDPs with aggregates were donated more often by females and donors with lower haematocrit. For double collection with the Trima Accel, SDPs with aggregates also showed a higher PLT concentration and content. In summary, our retrospectively analysed data might indicate that aggregates might be found more often in SDPs derived from female donors or donors with a lower haematocrit in general. A higher PLT concentration or content in the SDPs could be also associated with this phenomenon. However, prospective studies are needed to get more information.

- (b) We do not produce whole blood-derived platelet concentrates in our department. Therefore, we cannot give information on this.

### Question 3

Of course the temperature is controlled throughout the whole process. Immediately after the apheresis procedure that is performed in air-conditioned room (temperature 20–25°C), the SDPs are transferred to a temperature-controlled storage container with a temperature ranging between 20 and 24°C. In such a storage container, the SDPs are kept throughout the whole storage period. Temperature is controlled electronically and also using a disc recorder. So far, we are not definitely aware of aggregates appearing during storage that were not present at the time of collection/processing. However, retrospectively we

cannot exclude that the one SDP the treating physician was refusing to transfuse (see above) might have been such a case. With regard to further answers to questions in this paragraph, we refer to our detailed answer to question 2a.

#### Question 4

As mentioned above, we still are using plasma for storage of platelets but intend to change to PAS in the future (see answer to question 1a). However, we performed a number of studies within the last decade with different PAS solutions, and therefore, we have quite some experience using PAS for PLT storage. Specially with regard to the most recently performed studies, we do not believe that either one or the other makes the platelets more susceptible for the formation of macroaggregates as we had not been observing aggregate formation [2].

#### Question 5

We would like to refer to our detailed answer to question 2a first. Furthermore, indeed within the retrospective study described briefly above, we became aware of one or two donors (females) whose PLTs formed aggregates at multiple but, however, not all donations. However, most of the aggregates of these donors disappeared within 6–24 h after collection.

#### Question 6

We have not observed a seasonal effect on the occurrence of PLT aggregates.

#### Question 7

As the number of persisting aggregates is low and, therefore, also the number of discarded SDPs due to this phenomenon is low, we have not taken specific interventions. However, due to the slightly higher discard rate of SDPs due to persistent aggregates collected with the Amicus device (1.6% vs. 1.0%), we advised our operators to focus even more intensively as they did this so far on the resuspension of the PLT pellet after the collection.

#### Question 8

This question is difficult to answer. No clear conclusions can be drawn with regard to the clinical relevance of aggregates in PLT products as reliable data are very rare. Overall, one can consider mainly two possible threats. First, SDPs with macroaggregates may have a lower clinical efficiency as the PLTs lost in the aggregates may not be functional anymore. To our knowledge, such a correlation has not been investigated so far. However, Jimenez

*et al.* [3] showed that transfusion of PLT concentrates derived from donors with an increased likelihood to form microaggregates in an *in vitro* functional test (ACD microaggregation assay) resulted in a lower PLT recovery *in vivo*. The real clinical relevance of this observation remains unsure. Second, the present aggregates might harm the patient. However, as any visible aggregates should be captured by the filter in the transfusion set, this should reduce the potential risk to the patient. To our knowledge, report of adverse events due to persisting aggregates in transfused PLT products is lacking which is also in accordance with our experience, our level of concern is rather low (2–3).

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A. Seltsam

#### Question 1

- (a) The platelet concentrates are visually inspected for the presence of aggregates during the production process and before release of the products. It is a macroscopic inspection defined by a standard operating procedure.
- (b) Products containing macroaggregates are generally not distributed.

#### Question 2

- (a) Apheresis platelet concentrates have a resting period of 1 h after collection. Then, they are agitated for at least another hour before further handling. In 1–3% of apheresis platelet concentrates, macroaggregates are observed following the resting period. In our insti-

tution, we see an association between the collection machine and the presence of macroaggregates. We perform platelet collection with the Amicus (Fresenius) and Trima (Terumo) cell separators. Aggregates are more often observed in platelet concentrates collected with the Amicus machine. This phenomenon may be due to platelet activation by the collection process of the Amicus machine (highly concentrated PLTs are stored in small bags within the centrifuge, exposed to centrifugal forces and shear stress during the entire separation procedure) [1, 2].

- (b) We do not observe macroaggregates in whole blood-derived platelet concentrates, most probably due to the fact that the platelet units are filtered at the end of production.

### Question 3

Product handling is performed in rooms with a permanent temperature control. We sometimes observe macroaggregates in apheresis platelet concentrates that dissolve after a few hours. However, we also observe products with more stable aggregates. These platelet concentrates are usually not immediately discarded but followed-up by regular visual inspection. If the aggregates disappear during storage, the platelet concentrates can be used for transfusion. We do not see aggregates appear during storage that were not present at the time of collection and processing.

### Question 4

Whole blood-derived platelet concentrates are stored in 30% plasma and 70% PASIII-M, while apheresis platelets are stored in 100% plasma. Platelet activation during collection and processing may be the main cause for aggregate formation in platelet concentrates.

### Question 5

We observe a donor dependence of aggregate formation in apheresis platelet concentrates, which seems to be related to the type of cell separator used for collection. We no longer observe aggregates with these donors when another machine is used for collection (see also Q2). We could not identify any donor characteristics (age, gender, pre-collection cell count, etc.) that make the platelets more susceptible for macroaggregate formation.

### Question 6

A seasonal effect on the occurrence of aggregates in platelet concentrates is not observed.

### Question 7

Leucocyte filtration of whole blood-derived platelet concentrates is considered as an effective measure to also remove macro- and microaggregates. If macroaggregates repeatedly occur in the platelet concentrates of an apheresis donor with one type of cell separator, another type of separator is used for this donor. There are no other systematic interventions to prevent aggregate formation in platelet concentrates in our institute.

### Question 8

As long as infusion filters are used, macroaggregates should be caught during the transfusion of platelet products. Therefore, the risk of side effects by macroaggregates in transfusion recipients may be near to zero. If macroaggregates are accidentally transfused, they may cause side effects. However, the database is too small to make any reasonable conclusion on the clinical relevance of macroaggregates in platelet concentrates.

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P. Perseghin & P. Proserpio

### Question 1

- (a) In our institution are available both single-donor platelet concentrates from apheresis donors (PLT-A, approximately 1200/year) and platelet concentrates from pooled buffy coats obtained from whole blood donation (PLT-BC, approximately 3000/year). PLT-A dry platelets are obtained by single-vein cell separators and subsequently stored in an artificial medium.

All platelet concentrates are visually inspected before being available for clinical use. Moreover, since our

specific SOP includes a platelet count performed on each PLT unit (either PLT-A or PLT-BC) with an automated device, thus, both macroaggregates from visual inspection and smaller aggregates can be detected. In the case that either macro- or microaggregates are detected (these last impairing a correct platelet count), platelet concentrates are allowed to rest in permanent flat agitation up to 24 h, and then a further visual inspection and/or repeated platelet count are performed [1–4].

(b) We do not deliver for clinical use any platelet concentrate with macroscopically evident platelet aggregates.

#### Question 2

(a) Yes, platelet concentrates are allowed to rest on the bench for 1 h before further handling. In these last years, we had a very low percentage (<0.5%) of persisting macroaggregates (that means macroaggregates persisting after 24-h flat agitation). These units are discarded.

(b) We did not observe macroaggregates at visual inspection, but sometimes microaggregates are detected when assessing platelet content by means of an automatic analyser. In this case, as mentioned above, PLT-BC undergo a 24-h flat agitation before further controls. In the majority of cases, further aggregates are not detected and PLT-BC are made available for clinical use.

#### Question 3

Environmental temperature is automatically controlled in the processing laboratory. PLT concentrates are stored under flat agitation in a temperature-controlled device. In most instances, macroaggregates dissolve after a 24-h flat agitation period, only episodically (<0.5% of total PLT units, the persistence of macroaggregates result in product discard).

No, we do not observe platelet aggregation during storage if not pre-existing.

#### Question 4

All our PLT concentrates are stored in an artificial medium (SSP; MacoPharma, Mouvoux, France).

#### Question 5

We sporadically observed a high incidence of spontaneous platelet aggregation in few apheresis donors (almost exclusively males), most of them being heavy smokers. These donors have been discouraged to undergo plateletpheresis (until they modify their habit).

#### Question 6

No.

#### Question 7

In the case of male donors undergoing plateletpheresis at first, we increased the ACD-A ratio, but this resulted in a higher percentage of apheresis-related side effects (i.e. paresthesias, in spite of pre-donation oral calcium) without a decrease of the formation of macroaggregates.

To date, our policy is that if a plateletpheresis donor shows at least two donations with visually evident macroaggregates that do not disappear after 24 h flat agitation, we discourage him/her to further PLT-A donations.

#### Question 8

Our concerns (level = 4) are due to a possible lack of clinical efficacy of those PLT units with visually persistent macroaggregates, since the presence of these last, as a consequence of *ex-vivo* platelet activation/aggregation, might result in a further platelet loss firstly due to entrapment within the transfusion filters and in a facilitated removal by the recipient, with subsequently reduced post-transfusion platelet increment [5, 6].

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S. Wakamoto, M. Akino, S. Takamoto & K. Tadokoro

### Question 1

(a) We have a standard operating procedure (SOP) to examine any abnormal appearance, including aggregates, of the PCs. The SOP of the Japanese Red Cross Blood Center requires inspection of the appearances of PCs, including colour, swirling, contamination of foreign substances, presence of aggregates and clotting, in the processing section as well as the supply section. The first inspection is performed under a bright light source such as fluorescent light in the processing section. The PCs with aggregates are re-examined after storage with agitation at 20–24°C. Most aggregates dissolve within a day of agitation after collection. However, some PCs with persistent aggregates are processed to static sedimentation for sterile removal of the aggregates.

The details of the inspection method to determine the presence of platelet aggregates in PCs are not provided in the SOP. In our blood centre, we hang the PC bags statically for 5 min, and then, we observe whether there is sedimentation of the aggregates at the bottom of the bags under fluorescent light.

(b) We do not distribute any PCs in which aggregates are observed visually.

### Question 2

(a) We do not have a mandatory period to rest PCs after collection. However, PCs wait for the transportation to manufacturing centre at 15–25°C without agitation. We believe that the waiting time corresponds to resting period for PCs. The waiting time varies from 20 min to 6 h.

During the year 2012, PCs with aggregates were observed in 7957 of 36 603 bags, namely, 21.7%. However, most aggregates dissolve within a day of storage with agitation. Since PCs are not generally split into parts in Japan, we have not experienced that the aggregates cause lower split rates.

The cell separator might impact the formation of aggregates. We use three different apheresis cell separators (Trima Accel, Terumo BCT Inc., Lakewood, CO, USA, Haemonetics Corp, Braintree, MA, USA; TERUSYS-S, Terumo Corporation, Tokyo, Japan). In the case of Trima Accel, aggregates are formed during collection. In contrast, in the cases of CCS and TERUSYS-S, aggregates are not observed during collection, but at the manufacturing centre after a certain time for transportation. In addition, the percentage of PCs with aggregates tends to increase depending on the transportation distance. However, we have to investigate further in detail to conclude.

(b) Since all PCs are collected by apheresis in Japan, the question about whole blood-derived PCs is not applicable.

### Question 3

After the collection of apheresis PCs, the environmental temperature is controlled in the range of 15–25°C before and during transportation, with the use of a special carrier box, which keeps the inside temperature at a certain level. At the manufacturing centre, the temperature of storage of PCs is controlled in a similar range. The percentage of PCs in which aggregates were detected the day after collection was 0.07% in the year 2012. Since the shelf life of PCs is rather short (4 days including the day of collection) in Japan, we cannot wait longer than overnight for the aggregates to dissolve. Therefore, in the case of PCs with aggregates, we remove them by static sedimentation. We have not observed any PCs in which aggregates appear during storage that were not present at the time of the first inspection.

### Question 4

We use plasma for the storage of PCs, so the question about a difference in susceptibility to the formation of aggregates between plasma and solution for PC storage is not applicable.

### Question 5

We experienced some donor dependence. We have observed that there are some donors whose platelets form aggregates repeatedly. In 628 repeat donors who donated apheresis platelets from 10 to 12 times in the year 2012, the percentage of donors whose platelets never formed aggregates was 31.2%, those that formed aggregates 1 time was 21.2%, 2–5 times was 36.0% and 6–11 times was 10.7%. Therefore, we believe that a donor-specific factor is involved in the formation of aggregates. The characteristics of the donors associated with the formation of platelet aggregates are not clear at present. We are undertaking studies to determine the factors specific to the donors.

### Question 6

We have not found a seasonal effect on the occurrence of platelet aggregates.

### Question 7

Although we have been examining the relevant conditions and factors that could contribute to the formation of aggregates, we have not found any possible answers to

this. Therefore, we have not taken any interventions to prevent the formation of aggregates.

### Question 8

Level = 5.

We consider that the issue of aggregates in PCs is very important because we found that the platelets in PCs with persisting macroaggregates are activated compared with those in PCs without them [1]. Therefore, the quality of the former platelets may be poor, although the aggregates are caught by infusion filters during transfusion. In our previous study, we compared the *in vitro* platelet characteristics in PCs containing no aggregates (group 1), PCs whose aggregates disappeared during the first 48 h of storage (group 2) and PCs that had aggregates consistently through 72 h of storage (group 3). We found that, in comparison with group 1, group 3 showed a relatively high percentage of CD62P-positive platelets, and high levels of  $\beta$ -thromboglobulin and platelet factor 4, and lower percentages of hypotonic shock response and discoid platelets. In contrast, group 2 showed no difference compared to group [1].

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### Question 1

(a) We perform a visual inspection on all our platelet products. There is an SOP in place to address

the problem of platelet aggregates, which includes visual guides. The blood component will not be issued for clinical transfusion if more than five platelet aggregates with size  $>3$  mm are visualized.

(b) Platelet products with platelet aggregates are issued for clinical use if less than five aggregates are observed and are  $<3$  mm in size. We have not observed rejection of the platelet products by our clinical end users with these criteria in use.

### Question 2

(a) Yes. All apheresis platelets are 'rested' for 1 h before further processing. We have not observed any macroaggregates following the rest period.

(b) We have recently converted our platelet preparation from the PRP method to the buffy-coat method and have not observed any platelet aggregates with the buffy-coat method.

### Question 3

Once the whole blood is collected, it is placed in a validated blood storage container or at room environment, which is able to hold temperatures between 20 and 24°C. We do not observe platelet aggregates in platelet concentrates prepared using the buffy-coat preparation method. Previously, we observed platelet aggregates in platelets prepared using the PRP method and the aggregates usually do not dissolve overnight. From past experience, platelet aggregates usually do not appear during storage if they were initially not present at the time of processing.

### Question 4

We use plasma as the suspending media for storage of platelets.

### Question 5

We have not studied the issue of platelet aggregates' dependency on repeat donors whose platelets formed aggregates at multiple donations.

### Question 6

No. Singapore does not have four seasons.

### Question 7

No. We have conducted a study to identify possible root causes in the work processes that led to platelet aggregates formation but did not find any significant root causes.

**Question 8**

Our concern over macroaggregates in platelet concentrates is low as platelet infusion sets are used for platelet transfusion.

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T. Jimenez-Marco

**Question 1**

- (a) Platelet (PLT) aggregates are assessed visually in PLT concentrates obtained by apheresis and from whole blood buffy coats. There is a standard operating procedure (SOP) for detecting and reporting platelet aggregates in whole blood-derived buffy-coat platelets after processing on day 1, and in platelets obtained by apheresis at different times. Apheresis nurses check for aggregates in the plateletapheresis units at different stages of the process, that is during the platelet collection procedure, immediately after collection of the PLT unit and on day 1 when the PLT count of the unit, serology and NAT testing are performed prior to pathogen reduction. The apheresis nurses send the quality control laboratory reports about whether or not small aggregates (microaggregates) or large stable platelet aggregates (macroaggregates) are observed at different stages, and whether or not they persist on day 1. If microaggregates are still detected on day 1, the unit is withheld from issue and re-inspected on day 2. If the microaggregates persist on day 2 and the platelet content is below the transfusion dose, the unit is discarded. There is also an apheresis registry in which apheresis procedure and donor data are recorded. If the plateletapheresis units collected from a specific donor, using different apheresis cell separations and on different occasions, repeatedly present aggregates, this donor is invited to donate other blood components as well as to undergo a study.
- (b) PLT units containing macroaggregates on day 1 are discarded and, therefore, not distributed. In contrast to microaggregates, we do not wait until day 2 to recheck since macroaggregates tend to persist throughout the PLT storage period. Their platelet count is usually lower than the yield required ensuring an appropriate adult transfusion dose after patho-

gen inactivation. It is worth noting that pathogen inactivation technology implies a platelet content loss of between 8% and 2%, depending on the method used, during the inactivation process.

**Question 2**

- (a) The rest period after apheresis collection is 2 h. During the rest period, PLT bags are held on a laboratory bench, without any wrapping and label side down, at 20–24°C.

The percentage of post-collection PLT microaggregates in plateletapheresis is around 5.6%, only 1.2% persist on day 1, and <0.5% on day 2. If the platelet content is below the transfusion dose, the unit is discarded. The percentage of post-collection PLT macroaggregates in plateletapheresis is below 1%, most persist after the rest period and beyond the first day of collection. These units are also discarded.

Indeed, platelet aggregates lower than the platelet count, with the missing platelets appearing as a leftward skew, or flagging, in the lymphocyte histogram. This could keep a double-dose unit from being split, or even lead to a single unit being discarded. To avoid this, PLT units with microaggregates are re-inspected on day 2.

In relation to aggregates in plateletapheresis units: in Trima Accel plateletapheresis, PLT aggregates can be observed during and after finishing the collection because the gradual collection of platelets in an external bag can be seen throughout the entire process. However, in Amicus procedures, aggregates can only be seen immediately after collection has finished. This is because, during the procedure, highly concentrated platelets are collected in a small interior chamber; therefore, the operator cannot check for aggregates. This can only be performed after, when the PLTs are manually diluted in plasma and additive solution in an external bag. PLT aggregates have not been detected in Haemonetics MCS plus plateletapheresis procedures, although unexplained low PLT yields are sometimes obtained, perhaps resulting from the presence of aggregates. One possible reason for low PLT yields could be the presence of aggregates which, if they occur, are trapped in the leucoreduction filter and are not seen in the final leucoreduced product.

- (b) In our Blood Bank, pooled buffy-coat platelet components are prepared using the COMPOMAT G5 automatic blood component processing system (Fresenius SE & Co. KGaA; Bad Homburg, Germany) and the automatic TACSI system (Terumo BCT Europe N.V., Zaventem Belgium). PLT aggregates are neither detected in the intermediate product during the process, nor in final whole blood-derived PLT units, however, if they do occur, may they again have been

trapped by the leucoreduction filter included in the TACSI kit.

### Question 3

Immediately after collection, whole blood donations are placed on butane diol plates for transportation from the mobile drive to the blood bank for processing. Whole blood units are gradually fractioned as they arrive at the blood bank, provided at least 2 h have passed since their collection. Buffy-coat units obtained from fractioning whole blood donations are maintained in the separation area at 20–24°C for 12 h overnight before the whole blood-derived PLT concentrates are produced the following morning. Apheresis collections are performed in the collection area at 20–24°C. Both, whole blood-derived PLTs and plateletapheresis units are pathogen inactivated in the separation area on day 1 and then stored under constant agitation at 20–24°C for 7 days. For further details related to temperature impact on aggregates, please refer to the answer to question 6.

We have never seen aggregates appear during storage unless the temperature has varied outside the range of 20–24°C.

### Question 4

To perform pathogen inactivation in our institution, all platelet components, whole blood-derived buffy-coat platelets, as well as plateletapheresis units, are prepared with PLT additive solution (AS) SSP+ (MacoPharma, Mouv-aux, France), in a ratio of approximately 35% plasma and 65% AS. Mg and/or K have been reported to have an inhibitory effect on aggregation, and both are present in SSP+ in contrast to other additive solutions [1]. However, the cause of the increased incidence in PLT aggregates could also be related to the method used to obtain highly concentrated PLTs, in which AS is a component.

### Question 5

We think donor-specific factors could contribute to aggregate formation in PLT concentrates. Donor-specific factors could be associated with a higher PLT sensitivity to functional damage resulting from preparation methods, additive solutions, storage conditions and, among other variables, the unavoidable isolation of PLTs from their normal contact with endothelium [2].

PLT units from a small pool of our regular apheresis donors contain aggregates when collected with different cell separations and on different occasions, as stated above. These donors are invited to donate other blood components and to undergo a study.

### Question 6

In the past, we certainly observed a higher incidence of PLT aggregates in summer time, probably related to the PLT units being excessively exposed to the air-conditioning fans and other devices in the collection area. As some authors have previously reported [3, 4], low temperatures can stimulate spontaneous aggregation in PLT concentrates.

### Question 7

We implemented an SOP to maintain a constant temperature of 20–24°C for PLT units and the AS to avoid exposure to extreme temperatures, either too cold or hot, throughout the whole PLT process, including collection, processing, storage, transportation and delivery to the hospitals.

### Question 8

PLT macroaggregates represent large stable platelet aggregates that, in contrast to microaggregates, and due to their stability, tend to persist throughout the PLT storage time. So, according to our policy, PLT macroaggregates are not transfused since these units are discarded.

In fact, these particles are large enough to be removed by the transfusion filter set. However, if the proportion of aggregated PLTs is high enough to leave a considerably reduced residual-free platelet concentration, the PLT yield would not reach the recommended transfusion dose. In addition, if aggregated PLTs reflect activated PLTs, increased platelet activation could therefore cause a population of platelets to undergo full granule release and irreversible aggregation in the PLT bag before being transfused into the patient's blood stream. Nevertheless, the impact that transfusing activated PLTs has on clinical outcome is a controversial topic in PLT transfusion medicine [5, 6]. Since further information is required, and as it would be wise to be cautious with respect to macroaggregates in PLT concentrates when transfused, our level of concern is 3.

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M. Lozano & J. Cid

#### Question 1

- (a) We inspect all platelet concentrates before issuing per visual inspection. We do not have a formal standard operating procedures dealing with the problem of platelet aggregates.
- (b) We do not issue to the wards platelet concentrates with visible platelet aggregates.

#### Question 2

- (a) Until April 2013, we collected platelet concentrates by apheresis using Amicus and Trima apheresis separators. Before further handling, we rested platelet concentrates for at least 12 h. We used to observe persisting macroaggregates in a very few numbers of collected products. Approximately between 1% and 2%, aggregates have not led to lower split rates. But it should be emphasized that in Spain, only about 4% of the platelet apheresis collections are double [1]. This is probably due to lower platelet counts in our population in comparison with populations of North European origin. The inverse relationship between platelet count and mean platelet volume also contributes to a poorer platelet collection efficiency since bigger platelets tend to sediment more easily [2].
- (b) We do not prepare whole blood-derived platelet concentrates.

#### Question 3

After collecting a platelet concentrates by apheresis, we keep them under continuous agitation at  $22 \pm 2^\circ\text{C}$ . As stated earlier, we see macroplatelet aggregates in around

1–2% of the concentrates after at least 12 h of continuous agitation. In cases when the platelet concentrate is of high value such as an HLA-matched platelet donation for an HLA alloimmunized patient, if macroaggregates are seen in the product we add an additional 10% volume of ACD-A to the unit and keep it between 30 and 60 min under agitation. In such cases, the platelet aggregates used to disappear and the unit can be transfused to the recipient with good tolerance.

We do not see aggregates appearing during storage.

#### Question 4

We use PAS, specifically PAS-E [3] for storing platelets collected by apheresis. Although we have not noticed a higher tendency to develop aggregates since the conversion from 100% plasma to PAS, we have received comments of colleagues stating that the implementation of PAS-E was associated to an increase in the development of aggregates.

#### Question 5

Yes, we have seen some donors to have tendency to form aggregates in the platelet concentrates although we have not been able to identify any donor characteristics that might be associated with the aggregate formation.

#### Question 6

No, we have not identified a seasonal effect in the occurrence of platelet aggregates in the apheresis products.

#### Question 7

In those platelet donors with tendency to form clumps, we tried to keep a high ratio ACD-A blood (around 1:8) during donation to prevent the development of aggregates.

#### Question 8

Our level of concern regarding macroaggregates in platelet concentrates is high (4) mainly due to the fact that they might impact negatively the transfused dose of platelets, since, the aggregates bigger than 170–200  $\mu\text{m}$  will be caught by the infusion filters.

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E. Castro & I. Muñoz

### Question 1

In our blood centre, all platelet concentrates are visually inspected before being submitted to the pathogen inactivation procedure. Platelets that show macroaggregates are neither treated with the inactivation system nor distributed to hospitals.

To avoid the generation of platelet aggregates, our standard operating procedure states that all apheresis concentrates have to rest without agitation for 1 h before being placed in the platelet incubator under continuous agitation, and similarly for buffy-coat-derived platelet concentrates that have to rest for 2 h before being placed in the incubator.

Platelets that show macroaggregates are considered unsuitable for transfusion and are therefore removed from the inventory.

### Question 2

All the apheresis platelets are placed on a flat surface to rest for 1 h immediately after collection and before further manipulation. In our experience, we hardly ever observe any kind of aggregates, either micro or macro, after apheresis procedures. The most critical collection issue is the ACD/blood ratio, which is maintained at 1:11 in all apheresis machines, and the use of Intersol as the platelet additive solution.

As in the case of apheresis platelets, whole blood-derived buffy coats and platelet concentrates always rest for 2 h after every centrifugation step. This strategy, in conjunction with using Intersol as the additive solution, seems to be very effective in preventing the formation of major aggregates. We rarely observe macroaggregates during the day following production of buffy-coat-derived platelets.

### Question 3

Cooling plates are used to maintain blood components between 20 and 22°C both after collection and during

transportation to the blood centre. Temperature is controlled after arrival in the blood centre in addition to whole blood being maintained in canisters, together with the cooling plates, until blood component separation starts. The blood component production laboratory temperature is maintained at 22°C and is monitored by an environmental bore connected to the general continuous temperature control system. As mentioned above, we hardly ever detect macroaggregates during production or storage procedures. The percentage detection of macroaggregates is around 0.1%. The smallest aggregates, when present, are trapped by the pathogen inactivation kit filter, and a small amount of product is discarded due to the presence of macroaggregates. We have never observed aggregates appearing during the storage process.

### Question 4

Platelets are always collected in additive solution and we are currently using Intersol (Fenwal). We have not observed any increase in aggregates since starting to use platelet additive solution compared to when platelets were collected in plasma.

### Question 5

We have not observed any special donor characteristics related to the development of macroaggregates.

### Question 6

We have not observed any relationship between the formation of platelet aggregates and any specific season of the year.

### Question 7

In our experience, the adoption of some measures during the production of platelets, such as selecting the right ACD/blood ratio for apheresis procedures, resting period of almost 1 h after collection/centrifugation, the use of a platelet additive solution without magnesium and potassium, as is the case of Intersol, and the proper control of all processing steps, all lead to the elimination of major platelet aggregates.

### Question 8

Our level of concern is intermediate (3). The problems deriving from the presence of aggregates in platelet concentrates are mostly related to the actual dose of platelets that patients receive because all aggregates are expected to be trapped by the transfusion filters.

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H. Gulliksson & P. Sandgren

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### Question 1

The assessment of the platelet units is based on visual inspection to confirm swirling and to detect visible aggregates without using any rating scale. The visual inspection is performed immediately after production. When aggregates are detected, the platelet units in a first step are rested at room temperature for 1 h without agitation. In a second step, such units are stored on a flatbed agitator in a temperature-controlled cabinet at  $22 \pm 2^\circ\text{C}$  to dissolve remaining aggregates. This is a standard operating procedure.

Platelet units are not distributed until all aggregates are dissolved or no longer are visually detectable. If a unit containing irreversibly aggregated platelets would be found, this unit will be discarded. We would most likely get comments from the clinical departments if such units would be distributed.

### Question 2

- (a) All apheresis platelets (Trima, TerumoBCT) are rested for 30–60 min according to our present standard operating procedures. If there would still be remaining aggregates, the platelet units are rested for an additional period of time (up to a few hours) until all aggregates are dissolved. It is very unusual to find platelet units containing irreversibly aggregated platelets. The split rate is not affected. We are not aware of any specific factors that might impact the formation of platelet aggregates, possibly except for the individual donor (cf. question 5).
- (b) The appearance of macroaggregates in whole blood-derived platelet units has been observed immediately after production. Over a period of 5 months, approximately 15% of all units prepared from pooled buffy coat showed some degree of transient macroscopically visible aggregates that subsequently dissolved at room temperature. Aggregates seem to occur randomly. We have not been able to associate the occurrence with certain collection characteristics, specific donors or blood groups.

### Question 3

Whole blood-derived platelet units are prepared in a temperature-controlled laboratory intended for blood component preparation. However, the automatic equipment for pooling of buffy coat and preparation of final platelet unit is not temperature controlled (OrbiSac; TerumoBCT). When no aggregates are detected post-production, no cases of detectable aggregates after the first day of storage have been reported. Non-dissolving aggregates are very rare, and the rate is negligible. Apheresis units are collected in a specific department of the blood donor premises without the same specific temperature control as for whole blood-derived platelets.

### Question 4

We use SSP+ (MacoPharma, Mouvaux, France) for whole blood-derived platelets (30% plasma/70% SSP+) and 100% plasma for apheresis platelets as storage environment. The presence of macroaggregates in either whole blood-derived platelet units or apheresis platelets is not experienced as a major problem. We are aware of two contributing factors. Lowering of pH will facilitate dissolving of aggregates and 'close cell contact' will increase formation of aggregates [1, 2]. On one hand, the use of SSP+ containing phosphate will probably increase the rate of aggregate formation compared with previous PAS used (T-Sol, Fenwal, Lake Zurich, IL, USA). On the other hand, apheresis technique may increase concentration of platelets during preparation, favouring aggregation by 'close cell contact'.

### Question 5

We have a feeling that some apheresis platelet donors are associated with more aggregates, but unfortunately we have no data.

### Question 6

We have not observed any recurrent seasonal effects on the occurrence of platelet aggregates, either in whole blood-derived platelet units or in apheresis platelets.

### Question 7

Since we do not experience major problems associated with the presence of aggregates, no specific measures have been taken in routine production other than those described in questions 1 and 2. On the other hand, we are well aware of the problem and in a recent *in vitro* study using whole blood-derived platelets stored in SSP+, units

containing aggregates were compared to units without aggregates stored in either SSP+ or plasma. Differences were observed for a number of factors [3].

### Question 8

We do not normally distribute platelet units containing aggregates, which would imply a high level of concern [3]. We do see negative effects in *in vitro* parameters in aggregate-containing units and such units would not be readily accepted by treating physicians.

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S. Thomas

### Question 1

(a) During collection of apheresis platelets, staff monitor the donor, machine and product in accordance with an SOP. Staff are trained to monitor the tubing and collection bag for evidence of aggregates although this is not specifically mentioned in the SOP. A review of incident reports logged in our quality system since October 2008 found four reports of clumped apheresis platelets being detected in donor centres, of approximately 500 000 donation procedures. On receipt at manufacturing, apheresis donations are handled according to an SOP that states 'Any platelet product showing clots, aggregation or clumping during processing that does not disappear after a short period of agitation should be discarded'. Three incident reports were retrieved relating to detection of clumps in apheresis platelets during manufacturing. Following

manufacturing (splitting and validation), on receipt into stock and selection for dispatch, staff check all components for lipaemia, icterus and haemolysis according to an SOP. Staff also check for platelet aggregates at this time but this is not documented in the SOP. Twenty-four incident reports were retrieved relating to detection of clumps at the point of issue.

(b) If aggregates are seen, then the platelet units are not issued. However, some packs do get returned by hospitals, suggesting that aggregates were present but undetected or have developed during transport and storage. In the 5 years of review, approximately 1 million apheresis platelet units were issued and 66 reports were logged relating to hospitals returning clumped platelets. The majority of these were from the same institution that had a high index of suspicion of aggregates following a bacterial contamination incident.

### Question 2

(a) Our post-collection procedures are under review. Currently, immediately after collection by apheresis, the platelet units are placed into a plastic bag and stored in an insulated transport container for up to 4 h. The platelets are then transported either (a) directly to a manufacturing site, which can take up to 23 h; or (b) to a stock holding unit for unpacking and agitation for up to 16 h before repacking and onwards transport to manufacturing.

Aggregates are observed in approximately 3% of apheresis platelets during and immediately after collection. There is no protocol for resting or rechecking these units in the donor centre so it is not known if these aggregates persist after resting. Although only anecdotal, platelets from donors with very high platelet counts are reported to aggregate more often than from those with lower counts.

(b) There are very few reports of pooled platelets developing clumps – only two recorded in 5 years (approximately 250 000 units). Whole blood collections that are to be used for buffy-coat production may be processed on day 0 (day of collection) or day 1, but the buffy coats are always rested for at least 4 h – if not overnight – before pooling.

### Question 3

Donor centres are temperature controlled between 20 and 24°C and we have shown that the apheresis platelet units equilibrate with the environmental temperature during collection. After collection, the units are placed in an insulated bag to protect against extreme environmental temperatures during transport. Manufacturing facilities are temperature controlled between 18 and 26°C.



A review of 5 years of incident reports revealed fewer than 70 reports of clumped platelets, against a denominator of over 1 million components or 0.5 million apheresis procedures (0.007%). As described earlier, if clumps are noted, then the platelets are allowed to rest for a short period, but it is not known what proportion this applies to nor how many are discarded at this point. However, if this was a significant issue affecting productivity, then it would have been raised through management lines.

#### Question 4

We currently collect only platelets in plasma so have no operational experience of collecting into PAS. However, we do supply platelets in 100% additive on request, and following a switch in the PAS used, we saw increased clumping (or failed resuspension) and therefore reverted back to the previous PAS.

#### Question 5

Some donors do appear to have platelets that regularly aggregate, and these donors are removed from the apheresis panel and returned to whole blood donation. A high platelet count is the only characteristic that is currently assessed that appears to be associated with aggregation.

#### Question 6

No seasonal effect on the proportion of aggregated platelets has been noted by the donor centres, nor is this detectable from the incident reports retrieved.

#### Question 7

The presence of aggregates has not presented a major operational issue and as such no active steps have been taken to address this. However, in a 2009 pre-contract validation study, we found aggregates were more likely to form in platelets collected using one particular device and this contributed to the decision to select a different device. We are currently in another contract round and steps have been taken to reduce the likelihood of persistent aggregates forming in components collected by the first device, including a 1-h rest period followed by temporary storage on an agitator prior to packing and transport of the units to a manufacturing site.

#### Question 8

We would not wish for aggregates to be transfused, but our level of concern is low given the checking procedures in place in the blood service and at hospitals. Units are returned by hospitals if large aggregates are seen because of concerns about bacterial contamination, and those with

large aggregates are sent to be cultured. Smaller aggregates would be removed by the in-line filter in the platelet-giving set.

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J. Petrik & K. McColl

#### Question 1

- Visually against the light. We have no specific SOP, but the SOP for dispatch contains an advice to inspect PC visually.
- If there are visible aggregates present, we do not distribute. The primary concern is about platelet aggregates formation linked to certain bacterial contamination. Visible aggregates are evident in <1% of products.

#### Question 2

- Yes, resting for 1 h after collection and prior to handling.
- Again <1% of platelets produced. In our experience, if aggregates are present, they will be evident immediately after production.

#### Question 3

Temperature-controlled laboratory ( $22 \pm 2^\circ\text{C}$ ).

We do not usually observe the macroaggregates after first day after collection, nor do we see new aggregates appearing (true for both, apheresis and WB).

#### Question 4

We routinely use plasma, except for a subset of patients (~1% or less) where we use SSP+. More aggregates are in SSP+, but this is most probably due to the centrifugation step, not necessarily due to PAS as such.

#### Question 5

Apheresis donors occasionally produce clumping during collection (<1%). The procedure will be stopped and the

donors taken off apheresis programme and referred to whole blood donation.

#### Question 6

We have not observed a seasonal effect.

#### Question 7

Many of the instances of aggregation that we have observed have, we feel, been due to the transportation of freshly drawn donations being transported too soon after donation. This is predominantly from more remote collection centres. As a mitigating action we have stressed the importance of the resting phase prior to handling and packaging for transportation.

#### Question 8

Staff are encouraged not to issue platelets containing aggregates, so the concern is high. However, the primary driver for this practice is link between bacterial contamination and platelet aggregate formation.

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H. Kamel & J. Dugger

#### Question 1

(a) The organization's standard operating procedures (SOP) for distribution of apheresis platelets instruct staff to inspect for the absence of irreversible aggregates.

The training materials (trainers to have products with examples of reversible and irreversible aggregates) include:

- Demonstrate how to inspect for aggregates.
- Must not contain grossly visible aggregates.

Inspecting with a light source may help detect aggregates.

Massage platelet to ensure aggregates are not irreversible.

The training materials for collection staff address platelet clumping or pasting; platelets are involved in the clotting process and therefore like to 'stick together'. This clumping can cause problems during the collection and can form aggregates if the clumping is not addressed.

Monitoring of the collection process, products and donor will minimize the risk of product loss.

Each operator's manual has a section on remedies for platelet clumping.

(b) Yes, only if 'no irreversible aggregates'. We do not monitor apheresis platelets returns from hospitals by reason of the presence of macroaggregates. Subjectively, we believe this is rare and infrequent.

#### Question 2

(a) Yes, Collection and component production SOPs state: If aggregates are present from collections performed on:

Trima

Rest component label down for at least 1 h.

Place on rotator for at least 1 h prior to storage and sampling. Amicus

Rest component at room temperature for 2–4 h prior to placing in storage and sampling.

Inspect prior to sample collection for platelet yield testing, at component production and at labelling and distribution.

If aggregates in product disperse after gentle massage or agitation, then it is acceptable,

If persistent, requires further observation:

Re-inspect within 24 h of collection. . .

If grossly visible aggregates, discard product;

If no gross aggregates, continue to observe for dispersion until product expires. If irreversible, discard.

In our system, there are seven regions on one computer system (Progesa). In 2012, we performed 61 361 successful apheresis platelets procedures. Three hundred and twenty were discarded due to the presence of irreversible aggregates (0.52%). The majority (95%) was prior to separation and labelling of the components.

We believe that aggregates have minimal (if any) effect on split rates.

In 2012, the rate of discard due to irreversible macroaggregates varied from one region to another, from none in one region to as high as 1.8% at another. We have not identified specific collection characteristics that might impact the formation of platelets aggregates.

(b) We do not produce whole blood-derived platelets.

### Question 3

The temperature of collection areas is kept within donors' comfort range. To meet supply storage requirements, temperature must remain between 15 and 30°C. Our component production laboratories are kept between 20 and 24°C when platelets are being produced. The area is monitored by automated temperature-monitoring systems.

The per cent of units with macroaggregates beyond the first day after collection is not known. When observed, most dissolve by massaging and mixing. The data on discard due to irreversible aggregates are discussed under question 2a. The appearance of macroaggregates during storage that were not present at the time of collection/processing is extremely rare.

### Question 4

We do use plasma for storage of platelets. We have no opinion on whether plasma or PAS makes platelets more susceptible for the formation of macroaggregates.

### Question 5

We are not sure that we observed donor dependence. Some anecdotal incidents of macroaggregate formation are observed in more than one donation from a donor. We have not made any analyses to identify donor characteristics that are likely associated with the formation of platelet aggregates.

### Question 6

We have not observed a seasonal effect on the occurrence of platelet aggregates.

### Question 7

Due to lack of understanding of factors promoting the formation of macroaggregates, we are unable to implement interventions to prevent their formation. Massage or gentle mixing is a measure that helps dispersing reversible macroaggregates.

### Question 8

On scale of 1–5, I would say 3. This is largely due to uncertainties. Transfusion facility may refuse the infusion of a component with visible macroaggregates, leading to product loss. If they are infused, macroaggregates will be caught by the infusion filter reducing the therapeutic dose to the patient.

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J. D. Sweeney

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### Question 1

- (a) Apheresis-derived platelets are routinely inspected, and the written protocol is to rest the apheresis product for 1–2 h after collection to allow any macroaggregates to dissipate. Whole blood-derived pooled platelets are manufactured by in-line filtration of platelet-rich plasma on day 0 [1] with pooling on day 1 [2]. There is no protocol to inspect these pools but they are routinely held for 1–2 days because bacterial testing and macroaggregates have not been a significant observed problem [3].
- (b) Any product containing multiple macroaggregates are not shipped; products with minimal (a few macroaggregates) may be shipped but there has not been a problem with customer acceptance.

### Question 2

- (a) As stated, apheresis platelets are routinely rested for 1–2 h. Persistent macroaggregates are rarely if ever seen (<0.1%). There has been effect of this factor on split rates: no specific collection characteristics have been associated with the phenomenon of macroaggregate formation but this would be unlikely because of the rarity.
- (b) Macroaggregates are rare (<1%) in whole blood-PRP-derived platelet concentrates and rapidly dissipate. In an experimental system mixing A and O platelets during storage, microaggregates were observed as expected in the mixed platelets but solely Group O platelets had less aggregates than solely Group A platelets perhaps attributable to the higher levels of von Willebrand factor in A plasma [4].

**Question 3**

Platelets are kept unagitated at room ambient temperature or stored with agitation in a temperature-controlled chamber. Macroaggregates are observed in <1%. Macroaggregates have not been observed to form during storage.

**Question 4**

Only plasma is in use at the present time.

**Question 5**

A pattern of donor-related macroaggregates has not been observed—but the rarity of this problem would make such an observation unlikely.

**Question 6**

No seasonal effect is observed.

**Question 7**

No specific interventions have been taken since the phenomenon is so rare.

**Question 8**

There is minimal concern regarding transfusion: these macroaggregates may disperse *in vivo* post-transfusion or, if larger, are likely trapped in the macroaggregate nylon filter (180–270 µm).

**References**

- 1 Sweeney JD, Holme S, Heaton WAL, *et al.*: White cell reduced platelet concentrates prepared by in-line filtration of platelet rich plasma. *Transfusion* 1995; 35: 131–136
- 2 Sweeney JD, Koultab NM, Holme SH, *et al.*: Prestorage pooled whole blood derived leukoreduced platelets stored for seven days preserve acceptable quality and do not show evidence of a mixed lymphocyte reaction. *Transfusion* 2004; 44:1212–1219
- 3 Sweeney JD, Dupuis MP, Testa G: Lack of macroscopic aggregates in pools of prestorage leukoreduced whole blood derived platelets. *Transfusion* 2000; 40:615–616
- 4 Sweeney JD, Koultab NM, Holme SH, *et al.*: In vitro evaluation of prestorage pools consisting of mixed A and O platelets. *Transfusion* 2007; 47:1154–1161

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**Question 1**

(Note: At this juncture, our blood centre is only manufacturing apheresis platelets as we have no demand for whole blood-derived platelets).

(a) The component laboratory SOP has the following requirements:

- SOP-CL-03-004, visual inspection
- Visually inspect platelet unit(s) for:
- Presence of platelets
- Swirls
- Abnormal plasma colour and/or haemolysis—red, brown, purple or abnormal bright yellow
- Clots
- Grossly lipemic plasma (milky appearance)
- Platelet aggregates that do not separate
- Inadequate sealing or holes in the plastic
- Before it is released from hospital services to a customer, it is subjected to re-inspection at time of packaging for shipment:
- SOP-HS-03-001 visually inspect platelet unit(s) for:
- Abnormal plasma colour and/or haemolysis—red, brown or purple
- Inadequate sealing or holes in the plastic
- Hold unit up to the light and inspect for:
- Clots
- Platelet aggregates that do not separate.

Other findings that seem unusual, such as, but not limited to, debris or particulate matter.

(b) We do not intentionally release to customers products that do not meet acceptable release criteria as demonstrated above. Admittedly, some of the above criteria are intrinsically subjective. The ‘white particulate matter’ [1], so to speak, may be in the eye of the beholder...

**Question 2**

(a) Platelets collected on the Trima platform are rested for 15 min, and for those collected on the amicus, there is no set time. (The Amicus involves resuspension of a platelet concentrate with concurrent plasma as part of taking the set off the device.) It is rare that the laboratory observes persisting microaggregates after the rest period.

Aggregates could theoretically lead to lower split rates, but since they are rare, we have not measured a direct effect.

- (b) Memorial blood centres, now known as innovative blood resources no longer produces WB platelets.

### Question 3

Facilities ensure maintenance of 'room temperature' in all platelet collections areas within a specified range. (68–73°F = 20–23°C).

If units arrive in the laboratory with aggregates, it is rare; however, aggregates typically disappear after overnight agitation. <1% of units are discarded for visible aggregates.

If aggregates form during storages, these are typically the units where aggregation does not resolve and such units would not be released.

### Question 4

IBR does not use an additive solution.

### Question 5

No 'clumpy' donors have been identified to date.

### Question 6

No donor characteristics have been identified, since no such donors have been identified. No seasonal effect noted.

### Question 7

All laboratory personnel are instructed to handle units gently and avoid vigorous shaking.

### Question 8

Level (3). The 170- $\mu$ m filter required for all transfusions should catch any large clots. Primary concerns for units with macroaggregates are: (a) Is there potential for decreased platelet count increment if platelets have significantly aggregated? and (b) Does aggregation suggest other aetiology for predicted platelet dysfunction (e.g. low pH, platelet pre-activation)

### Reference

- 1 Rentas FJ, Macdonald VW, Rothwell SW, *et al.*: White particulate matter found in blood collection bags consist of platelets and leukocytes. *Transfusion* 2004; 44:959–966

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L. J. Sutor, D. Heath & M. H. Sayers

### Question 1

- (a) Standard operating procedures address inspection of platelets by staff. Any particulate matter is classified either as clots, that is clumps that cannot be broken up, or aggregates, which can be dispersed by gentle agitation. Staff training and level of experience are important in achieving proper recognition of suitable components. Components that do not meet the standard are returned to the platelet rotator for re-inspection the following day.
- (b) Our procedures allow us to distribute platelet components with up to approximately 50 small aggregates or approximately 10 somewhat larger aggregates, but nothing we would describe as clots or macroaggregates. One local hospital, of around 200 facilities, insists on platelets entirely clean of all aggregates. One other hospital will accept platelets with a few aggregates provided they are shipped no later than the day after collection.

### Question 2

Apheresis platelets are rested for 1 h after collection. Aggregates have been observed very rarely, so an impact on split rates has not been important.

- (b) We see aggregates in approximately 4% of whole blood-derived platelets. Our platelets are made from platelet-rich plasma, at least 2 h but <8 h after collection. The platelet is allowed to rest for 90 min post-processing. We have observed issues with improper mixing of the whole blood with the anticoagulant during the blood collection process as a periodic possible cause of aggregates.

### Question 3

Whole blood is held in an environment with cooling towards room temperature (20–24°C) from the time of collection until the platelets are separated. Apheresis platelet units are stored similarly after collection. They are held at a collection site with monitored room temperature and then placed in an insulated cooler, validated for room temperature, before transport to the component laboratory. Once in the laboratory, they are held in a monitored room temperature environment or platelet incubator. Temperature is controlled at all locations by heating and air conditioning.

Apheresis platelets with aggregates are very rare in our experience. If whole blood-derived platelets are going to

form aggregates, they seem to do so early in their shelf life. If they have not formed them by 24 h, we rarely see them form aggregates later. We discard about 4% of whole blood-derived platelets due to aggregates that persist past 48 h for observation.

*Question 4*

We have always used plasma for storage of platelets. We have no experience with PAS.

*Question 5*

We have not looked whether a repeated tendency to aggregates is related to specific donors.

*Question 6*

We have not observed a seasonal variation of aggregates.

*Question 7*

We have implemented automated mixing devices on blood drives and at donor centres to help improve the consistency of whole blood mixing with anticoagulant.

*Question 8*

Since the standard of practice in our area is for all transfused platelets to be administered with a bedside clot fil-

ter, our level of concern is low that any aggregates will be transfused to patients.

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