

# Cold-Stored Platelets Hospital-Based Blood Bank Experience

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# Cold-Stored Platelets

- In 2013, because of existing evidence supporting the potential for providing added benefit in bleeding patients, a joint decision was made between the Trauma and Transfusion Services at Mayo Clinic in Rochester, Minnesota to pursue and obtain regulatory and accreditation approvals for the use of cold-stored platelets (CS-PLTS) for patients cared for by the Trauma Service.

This process of obtaining regulatory and accreditation approvals for cold-stored platelets was described in detail in the 2017 scientific paper entitled - Cold platelets for trauma-associated bleeding: regulatory approval, accreditation approval, and practice implementation – just the “tip of the iceberg”

This paper was published in the journal, Transfusion, in the year 2017, Volume 57, pages 2836 to 2844

# Cold-Stored Platelets - Approval

- On November 18, 2013, the Transfusion Service at Mayo Clinic in Rochester, Minnesota submitted a variance request to the AABB Standards and Global Development Department. The nature of the request was: although Blood Banking/Transfusion Medicine Standard 5.1.8A, pertaining to platelet storage states that “Platelet components intended for transfusion must be stored at 20°C to 24°C with agitation,” the Transfusion Service at Mayo Clinic in Rochester, Minnesota would like to be allowed to store apheresis platelet components at 1°C to 6°C without agitation for 5 days for specific use in actively bleeding trauma patients.

# Cold-Stored Platelets - Approval

- In a correspondence dated June 16, 2014, the AABB replied to the variance request in the following manner:
  - DENIED
    - “The Standards Program Unit (SPU) noted that because the bag manufacturer your facility uses does not have a claim to support cold storage we cannot grant your request.”
    - “The SPU feels that an appropriate course of action at this time would be for your facility to contact your Consumer Safety Officer at the Food and Drug Administration to determine if a variance would be needed to store platelets at the temperature described.”

# Cold-Stored Platelets - Approval

- In a letter to the Food and Drug Administration dated June 27, 2014, the Transfusion Service at Mayo Clinic in Rochester, Minnesota requested approval to store apheresis platelets at 1°C to 6°C in monitored, controlled blood storage refrigerators for a maximum of 5 days without agitation for specific use in actively bleeding trauma patients. This approval was sought because 21 CFR 640.25(a) allows the storage of platelet products intended for transfusion at room temperature (20°C to 24°C) with agitation and at refrigerated temperature (1°C to 6°C) without agitation.

# Cold-Stored Platelets - Approval

- The June 27, 2014 letter to FDA included validation data collected on platelets stored for up to 7 days at 1°C to 6°C without agitation. The validation parameters evaluated included bag pliability and translucency, adherence of the blood label to the platelet storage bag, the ability of bar codes on the product to be scanned, lack of smearing of ink on the product label, and absence of leaks in the platelet storage bag. All validation parameters satisfied acceptance criteria.

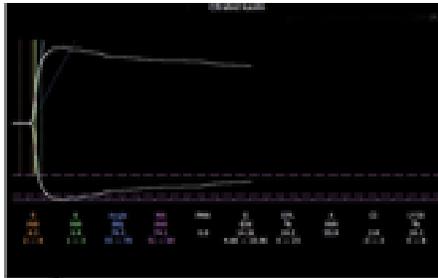
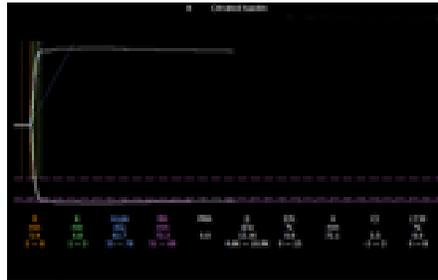
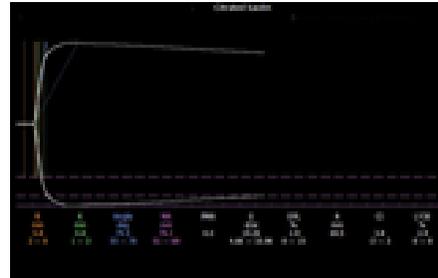
# Cold-Stored Platelets - Approval

- Also included in the June 27, 2014 letter to FDA were additional quality studies comparing cold-stored platelets (CS-PLTS) with room temperature platelets (RT-PLTS). Three blood group A double apheresis platelet components were collected with one platelet component stored as RT-PLTS and the other stored as CS-PLTS for 6 days. The following results were obtained:
  - Platelet swirling
    - Present in RT-PLTs
    - Not present in CS-PLTS days 3 through 6
  - Mean platelet count
    - RT-PLTS –  $1206 \times 10^3/\mu\text{L}$
    - CS-PLTS –  $1181 \times 10^3/\mu\text{L}$
  - Mean pH
    - RT-PLTS – 7.55
    - CS-PLTS – 7.51

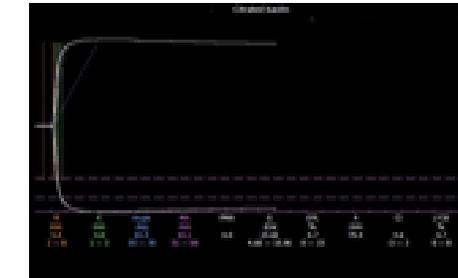
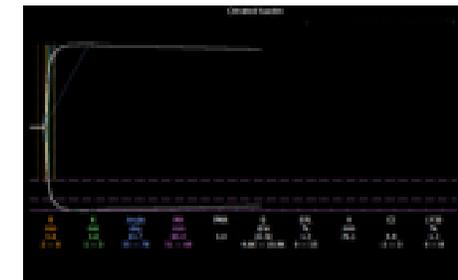
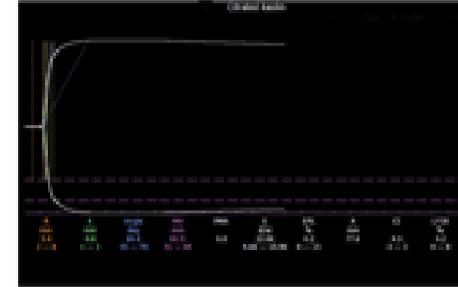
# Thromboelastography

Thromboelastography (TEG) results were also included in the June 27, 2014 letter to FDA. TEG performed on room temperature platelets (RT-PLTS) and cold platelets (CS-PLTS) on Days 2, 3, and 6 of storage showed, at the very least, equivalence between the two products, and there is some evidence to support superior function of CS-PLTS as reflected by TEG. For example, the day 6 TEG results show evidence of superior function of CS-PLTS compared to RT-PLTS

RT Platelets Days 2, 3, and 6



Cold Platelets Days 2, 3, and 6



# Cold-Stored Platelets - Approval

- In a correspondence dated March 27, 2015 from the FDA we received the following response:
- “We have approved your request to include an alternative procedure from 21 CFR 610.53(c) and 606.65(e) under the provisions of 21 CFR 640.120 to store apheresis platelets at refrigerator temperature without agitation for up to 3 days. You will restrict the use of these cold-stored platelets to use in the resuscitation of actively bleeding patients.”

# Cold-Stored Platelets - Approval

- Upon receiving approval to store apheresis platelets at refrigerator temperature without agitation for up to 3 days from the FDA on March 27, 2015, the Transfusion Service at Mayo Clinic in Rochester, Minnesota asked the FDA if bacterial testing of CS-PLTS would be required. The FDA responded with the following:
- “Regarding your question about bacterial testing for cold platelets, we are working on a guidance relating to this issue and we will take your question into consideration. Testing isn’t required right now.”

# Cold-Stored Platelets - Approval

- After obtaining FDA approval, the Transfusion Service at Mayo Clinic in Rochester, Minnesota again approached the AABB about obtaining a variance to use CS-PLTS stored for 3 days without agitation and without bacterial testing for use in actively bleeding trauma patients. In a letter dated October 8, 2015, the AABB's Blood Banking and Transfusion Standards Program Unit (BBTS SPU) replied in the following manner:
- “The BBTS SPU reviewed your request for variance and has elected to grant your request.”
- “The variance applies only to apheresis platelet components collected using your automated blood collection systems and intended for use in the resuscitation of actively bleeding trauma patients as defined by your facility.”
- “These components may be stored for a maximum of 3 days at 1-6°C without agitation.”
- “Use is restricted only to the resuscitation of actively-bleeding trauma patients.”
- “These components shall not be released to the general transfusable inventory.”

# Cold-Stored Platelets - Implementation

- Regarding implementation of CS-PLTS for actively bleeding trauma patients, the goal of the Transfusion Service at Mayo Clinic in Rochester was to collect 3 group A CS-PLTS per week. The group A CS-PLTS were stored in a blood bank refrigerator located in the Emergency Department at Saint Marys Hospital starting in October 2015. A major challenge for the CS-PLT program that became readily apparent early was product wastage. The 3-day storage period created a tight window for utilization prior to product expiration. Due to the requirement to perform infectious disease testing on blood donors, the CS-PLTS had to be held in quarantine until the results of such testing were complete. This resulted in a true availability for transfusion of 2 days for CS-PLTS. Another problem encountered during the early days of the CS-PLT program was clot formation. The CS-PLTS collected by the Transfusion Service at Mayo Clinic in Rochester were stored in 100% plasma, and clots were commonly found in these components after placement in the refrigerator.

# Cold-Stored Platelets - Implementation

- As previously stated, a major challenge faced with the CS-PLT program for the Transfusion Service at Mayo Clinic in Rochester was product wastage. Between October 2015 through August 2016, 119 CS-PLTS were produced. Nine CS-PLTS were discarded prior to distribution to the Transfusion Laboratory due to clots. 110 CS-PLTS were delivered to the Transfusion Laboratory with the following final dispositions:
  - 21 (19.1%) CS-PLTS transfused
  - 89 (80.9%) CS-PLTS discarded
    - 20 developed clots
    - 65 outdated prior to use
    - 1 was returned after issue and then outdated
    - One transfusion was stopped due to a suspected transfusion reaction and the component was ultimately discarded
    - Two components were discarded following a transport failure within the pneumatic tube delivery system

# Cold-Stored Platelets - Implementation

- From October 2015 until July 2017, 31 CS-PLTS were transfused in the Emergency Department at Saint Marys Hospital, with the yearly totals as follows:
  - 4 units – 2015
  - 22 units – 2016
  - 5 units - 2017
- During the same time period, 152 RT-PLTS were transfused in the Emergency Department. The wastage rate (product outdating or other reasons for product discard) for CS-PLTS remained extremely high.
- Therefore a decision was made to move CS-PLTS to the Air Ambulance Service's refrigerator for prehospital treatment of trauma patients on July 24, 2017. The rationale was that the use of CS-PLTS in the prehospital setting would enhance the quality of remote damage control resuscitation and making platelets available for the Air Ambulances might increase utilization and decrease component outdating.

# Helicopter Blood Transport

In order to successfully transition CS-PLTS to the Air Ambulances, the packing of the in-flight cooler needed to be configured and validated. The configuration of the cooler was as follows:

- 2 RBCs, WB, and 1 FFP in a row
- 1 FFP on the side
- Flattened Cold Platelet on top (with part tucked on side with FFP)



# CS-PLTS – Functional Validation

- An assessment of whether CS-PLTS retained similar functional capabilities in a cooler for up to six hours compared to storage in a refrigerator also was performed. Three CS-PLTS stored in a refrigerator were compared to 5 CS-PLTS stored in the Air Ambulance cooler for up to 6 hours. Parameters evaluated included platelet count, platelet aggregation, expression of platelet surface phosphatidylserine, expression of platelet surface P-selectin, expression of platelet surface fibrinogen receptor, and platelet-derived microvesicles. The study demonstrated that CS-PLTS stored in the Air Ambulance cooler showed no evidence, by in vitro parameters, of inferior function compared to CS-PLTS stored in a refrigerator.

# Cold-Stored Platelets – Air Ambulances

- Even after transferring CS-PLTs to the Air Ambulance Service, the number of units actually transfused remained low as reflected here:
  - Mayo Clinic Medical Transport - 2 units - 2017
  - Mayo Clinic Medical Transport - 12 units - 2018
  - Mayo Clinic Medical Transport – 1 unit - 2019
- The CS-PLT program was put on hiatus on February 25, 2019. The Mayo Clinic Medical Transport Service decided that it would prefer to carry two group O negative low-titer whole blood units on the air ambulances rather than one group O negative low-titer whole blood unit and one group A CS-PLT.

# Comparison of RT and CSP Platelets: Retrospective Clinical Study

As part of being granted the variance by the AABB to use CS-PLTS in actively bleeding trauma patients, the Transfusion Service at Mayo Clinic in Rochester, Minnesota was asked to submit clinical outcome data. A retrospective study of trauma patients who received CS-PLTS compared to trauma patients who received RT-PLTS was conducted. There were 20 patients in each arm of the study. There were no statistically significant between-group differences for Age, Male Sex, Blunt Trauma, Emergency Department Glasgow Coma Scale (GCS), or Emergency Department Trauma Score. The Injury Severity Score was significantly higher in the patients who received CS-PLTS.

# Comparison of RT and CSP Platelets: Retrospective Clinical Study

- There were no between-group differences in CS-PLT versus RT-PLT recipients for the number of RBC, FFP, or Cryoprecipitate units transfused. The pre-transfusion and post-transfusion hemoglobin, platelet count, INR, and thromboelastography results were not significantly different. The percentage of patients who experienced re-bleeding after initial hemostasis did not differ between groups, and there were no transfusion reactions identified in either group.

# Comparison of RT and CSP Platelets: Retrospective Clinical Study

There were no between-group differences for CS-PLT versus RT-PLT recipients with regard to mortality within 24 hours of admission, mortality within 30 days of admission, or hospital length of stay.

In conclusion, this retrospective comparison study is too small to draw any definitive conclusions, but there were no adverse events and CS-PLTS did not show evidence of being worse than RT-PLTS even when used on a more severely injured group of patients.

# Cold-Stored Platelets – Final Numbers

At the time that the CS-PLT program was put on hiatus by the Transfusion Service at Mayo Clinic in Rochester, Minnesota, 169 conventional and 212 pathogen-reduced CS-PLTS had been put into inventory. A total of 46 CS-PLTS were transfused (28 conventional and 18 pathogen-reduced CS-PLTS). A total of 223 CS-PLTS were discarded (141 conventional and 82 pathogen-reduced CS-PLTS). Only 16.6% of conventional CS-PLTS and 8.5% of pathogen-reduced CS-PLTS entered into inventory were ultimately transfused. The short 3-day shelf life and the restriction on use for trauma patients only contributed to low utilization. If a cost of \$600 dollars is used for apheresis platelets, then \$133,800 of CS-PLTS were discarded. A THREE-DAY COLD-STORED PLATELET PROGRAM FOR TRAUMA IS NOT SUSTAINABLE!

# Cold-Stored Platelets – How Can This Work?

- Can a CS-PLT program work? A follow-up conversation with the Mayo Clinic Medical Transport team revealed that it would be their preference to be able to carry CS-PLTS as an option for their Air Ambulance Service. However, the Mayo Clinic Medical Transport team and the Transfusion Service at Mayo Clinic in Rochester, Minnesota both agreed that this option is not feasible until the shelf-life of CS-PLTS can be extended beyond 3 days. It was agreed that the goal should be to pursue approval of a pathogen-reduced CS-PLT product that can be stored for 14-days. The thought process is that the combination of cold storage and pathogen reduction will result in a product with minimal risk for infectious disease transmission (including bacteria) and, at 14-days, the number and function of the platelets in the CS-PLT product will be sufficient to be effective in actively bleeding patients.

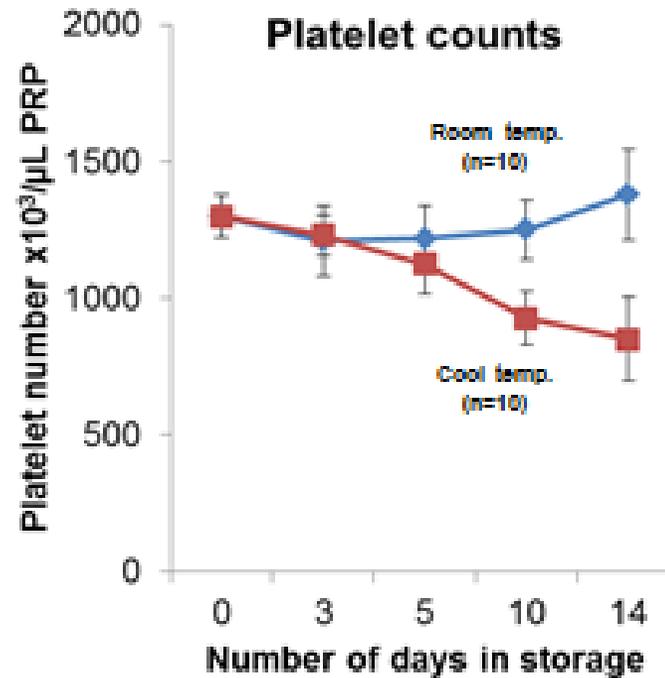
# Cold-Stored Pathogen-Reduced Platelets – 14 Days

Laboratory Studies



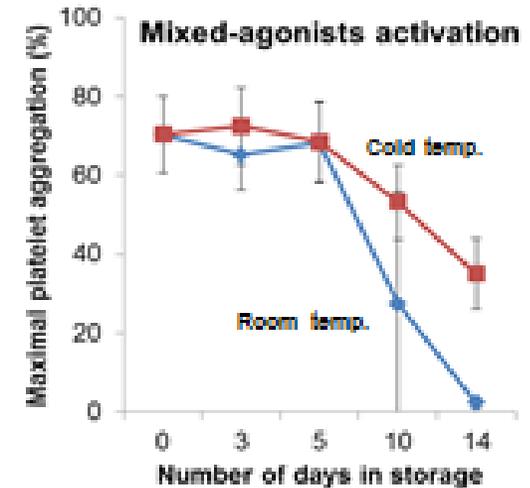
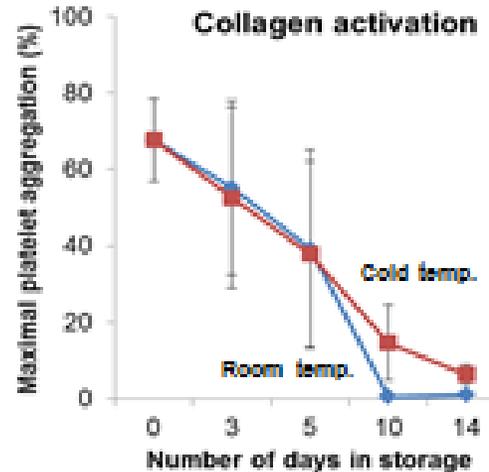
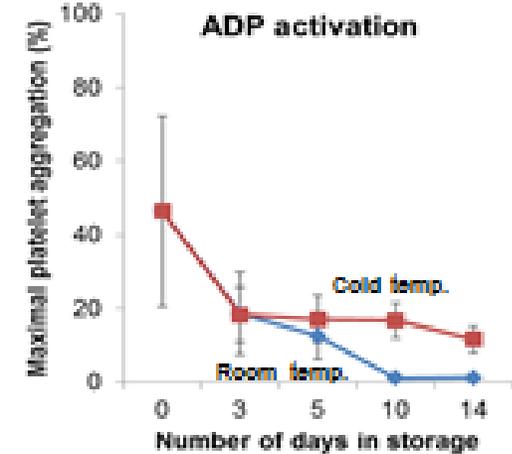
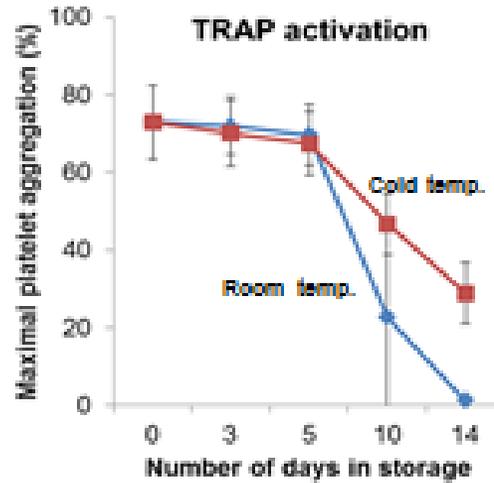
## Total platelet counts from platelet rich plasma stored in room and cold temperature

Platelet counts were monitored over the 14-day storage period, and the data showed that RT-PLTS maintain higher platelet counts over the storage period compared to the CS-PLTS. The platelet counts showed evidence of divergence (i.e., the lower platelet count for CS-PLTS) after 3 days of storage.



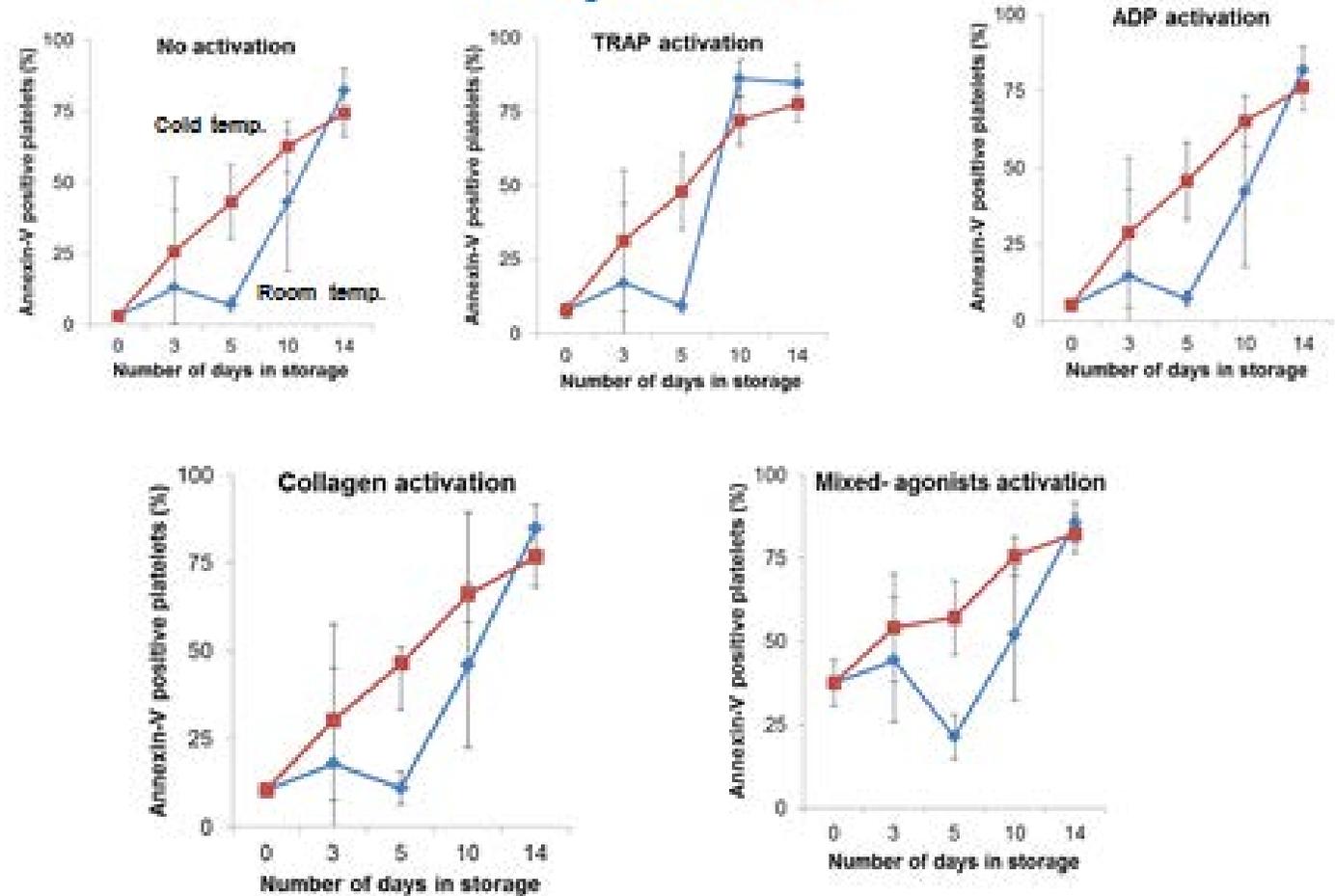
Platelet aggregation studies were performed on CS-PLTS and RT-PLTs with TRAP, ADP, Collagen, and Mixed-Agonist activation. In all cases, over the 14-day storage period, platelet aggregation was better preserved in the CS-PLTS. Evidence of divergence (i.e. better maintenance of platelet aggregation for CS-PLTS) occurred after 3 to 5 days of storage. RT-PLTS showed a total lack of aggregation in their responses to ADP and collagen activation at 10 days of storage.

## Cumulative data of maximal platelet aggregation in platelet rich plasma



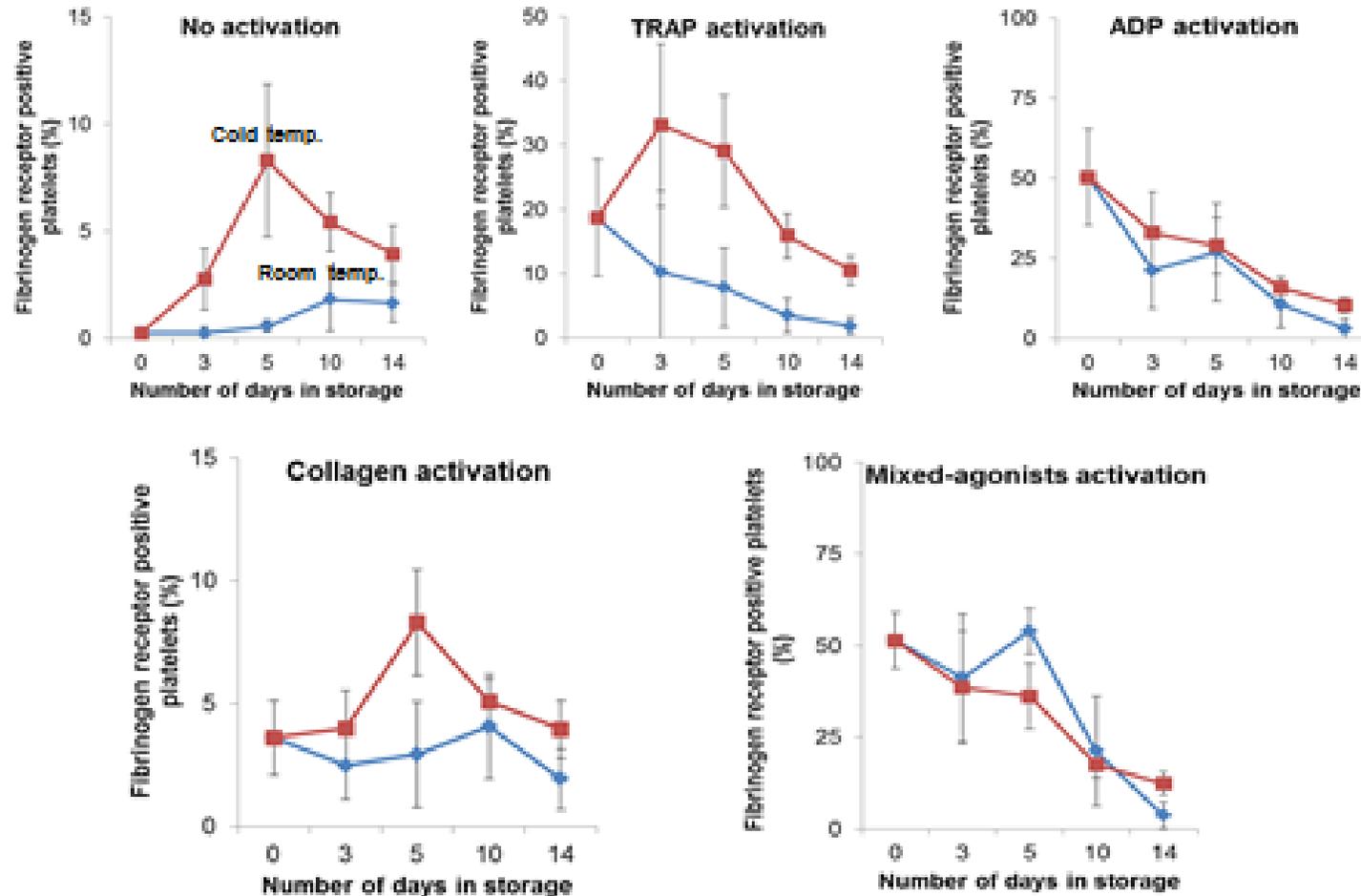
## Percentage of phosphatidylserine (annexin-V) positive platelets stored in room and cold temperature

The expression of phosphatidylserine (PS) on the platelet surface represents a procoagulant surface. PS expression throughout the 14-day storage of CS-PLTS and RT-PLTS was compared in the presence of no activation and with TRAP, ADP, Collagen, and Mixed-Agonist activation. CS-PLTS showed higher PS levels earlier in storage in all test conditions with RT-PLTs catching up in PS expression at 10 to 14-days of storage.



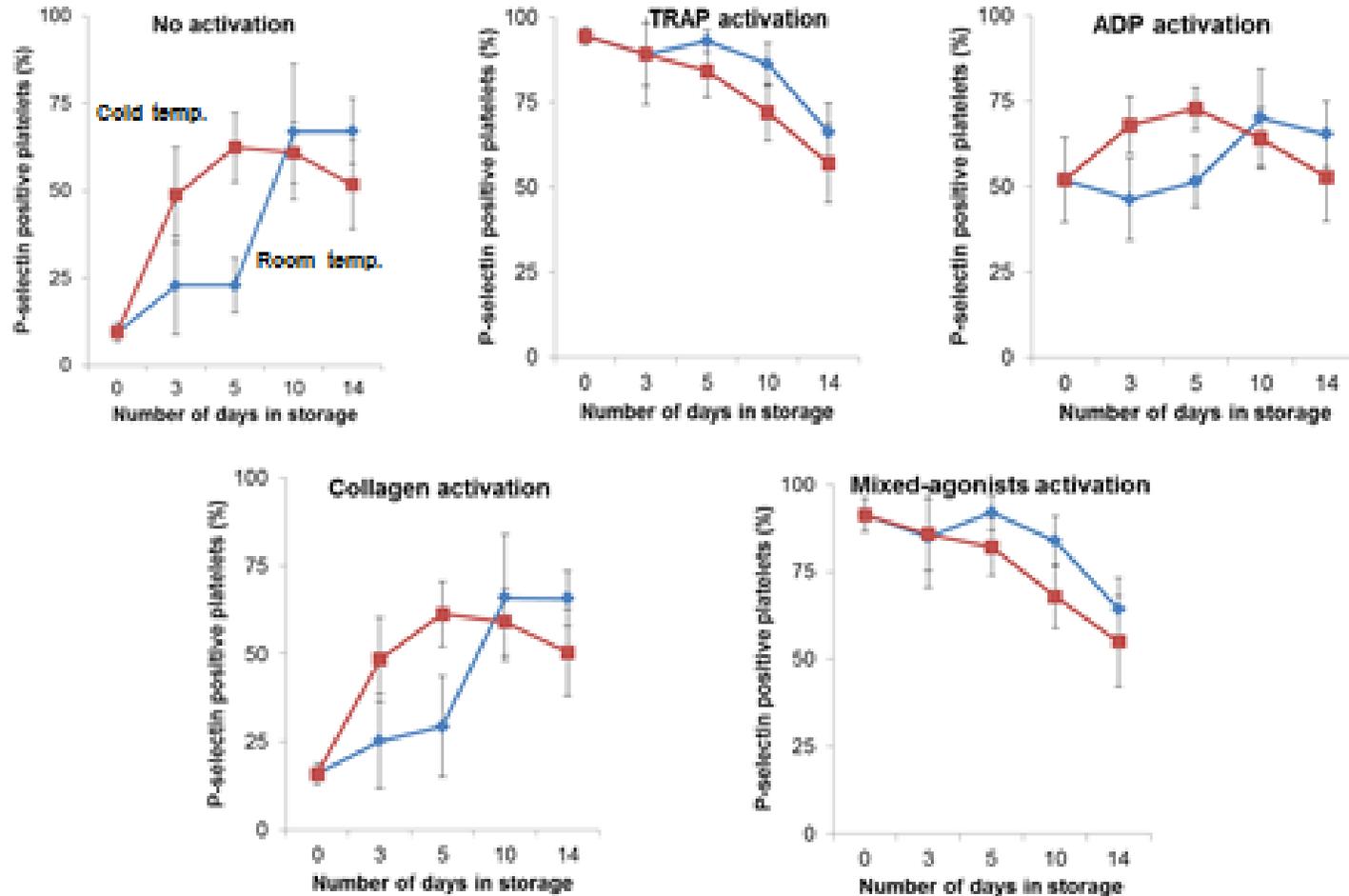
The expression of the fibrinogen receptor, PAC-1 on the platelet surface pertains to the ability of platelets to aggregate. PAC-1 is responsible for the binding of fibrinogen to platelet glycoprotein IIb/IIIa, which mediates platelet aggregation. PAC-1 expression throughout the 14-day storage of CS-PLTS and RT-PLTS was compared in the presence of no activation and with TRAP, ADP, Collagen, and Mixed-Agonist activation.

## Percentage of fibrinogen receptor (PAC-1) positive platelets from PRP stored in room and cold temperature



CS-PLTS showed clearly higher PAC-1 expression than RT-PLTS throughout the 14-day period when no activation was used and when TRAP and Collagen were used as activators. CS-PLTS showed higher PAC-1 expression when ADP was used as the activator throughout the 14-day storage period with the exception of Day 5. PAC-1 expression in the setting of Mixed-Agonist activation was higher in RT-PLTS on Day 5, but higher in CS-PLTS on Day 14.

## Percentage of P-selectin (CD62P) positive platelets from PRP stored in room and cold temperature

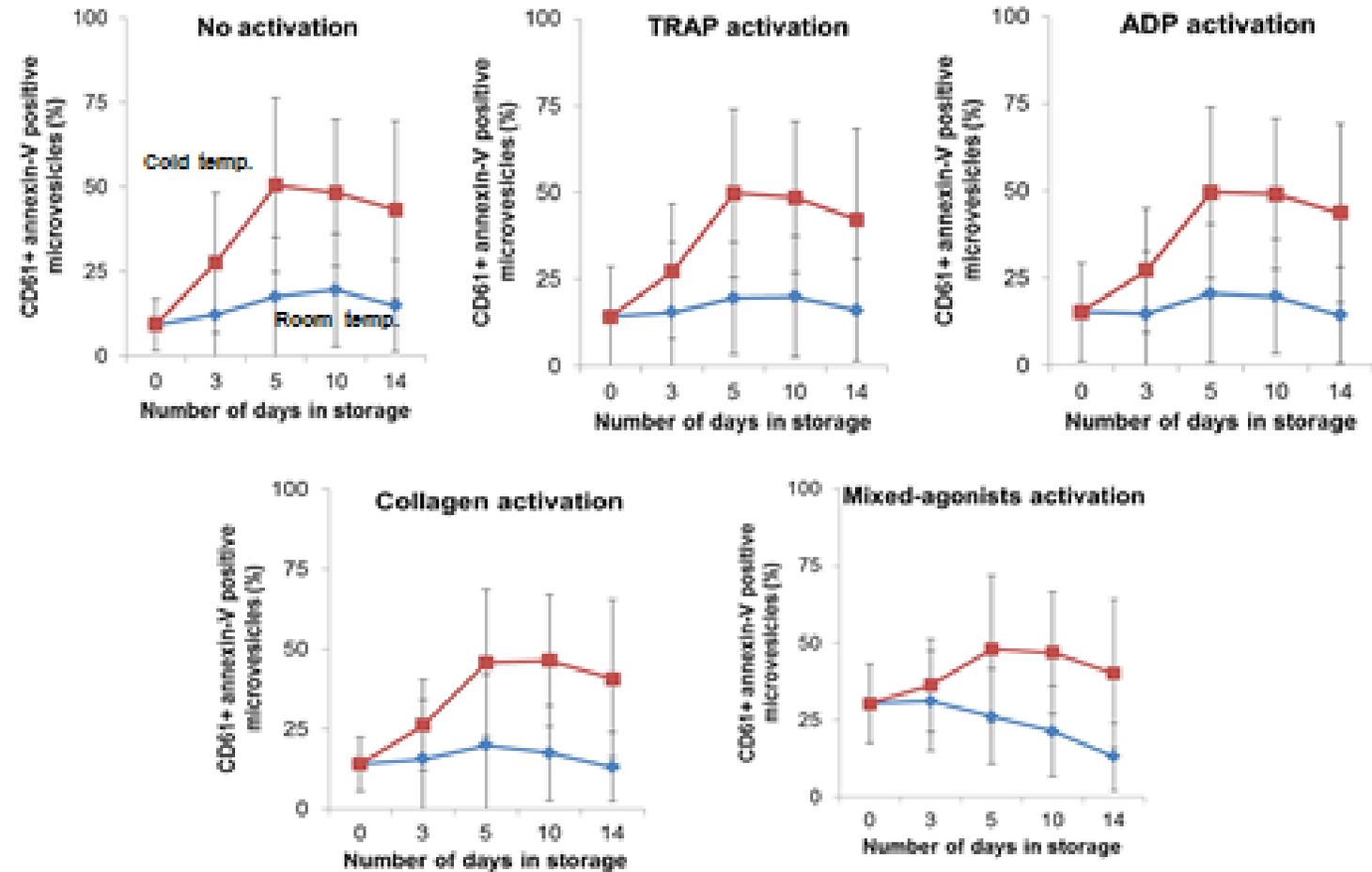


P-selectin resides on internal aspect of the platelet membrane, and when platelets become activated by fibrinogen, P-selectin migrates to the external platelet membrane and is involved in platelet aggregation. P-selectin expression throughout the 14-day storage of CS-PLTS and RT-PLTS was compared in the presence of no activation and with TRAP, ADP, Collagen, and Mixed-Agonist activation.

P-selectin expression on CS-PLTS and RT-PLTS throughout the 14-day storage period showed variable results based upon test conditions. With no activation, ADP activation, and Collagen activation, P-selectin was higher in CS-PLTS than RT-PLTS until day 10 and then P-selection expression was higher in RT-PLTS through Day 14. With TRAP and Mixed-Agonist activation, P-selectin expression was higher in RT-PLTs from day 5 through day 14 of storage.

## Percentage of both CD61+annexin-V positive microvesicles from platelets stored in room and cold temperature

The percentage of CD61+annexin V positive microvesicles throughout the 14-day storage of CS-PLTS and RT-PLTS was compared in the presence of no activation and with TRAP, ADP, Collagen, and Mixed-Agonist activation. In all test conditions, the percentage of thrombogenic (annexin-V positive), platelet-derived (CD61 positive) microvesicles was greater in CS-PLTS than RT-PLTS throughout the 14-day storage period.



# CS-PLTS vs. RT-PLTS 14 Day In Vitro Findings

- At 14-days of storage, both pathogen-reduced products, CS-PLTS and RT-PLTS, had bacterial culture results showing no growth.
- At 14-days of storage, CS-PLTS had uniformly acceptable pH results while RT-PLTS failed pH acceptability.

# Cold-Stored Platelets - Summary

- In 2013, a joint decision was made by the Trauma and Transfusion Services at Mayo Clinic in Rochester, Minnesota to pursue approval to use CS-PLTS for actively bleeding trauma patients. On March 27, 2015, FDA approved the Transfusion Services at Mayo Clinic in Rochester, Minnesota's request to store apheresis platelets at refrigerator temperature for up to 3 days without agitation for use in actively bleeding patients. The Transfusion Services at Mayo Clinic in Rochester, Minnesota was subsequently informed by the FDA that bacterial testing of the CS-PLTS would not be required. On October 8, 2015, The AABB's Blood Banking and Transfusion Standards Program Unit approved the Transfusion Services at Mayo Clinic in Rochester, Minnesota's request to use platelets stored at 1°C to 6°C for 3 days without agitation for actively bleeding trauma patients only.

# Cold-Stored Platelets - Summary

- The CS-PLT program at Mayo Clinic in Rochester, Minnesota was active from October 2015 to February 2019. During that time, 46 CS-PLTS were transfused and 223 CS-PLTS were discarded. The use of pathogen-reduced CS-PLTS started at Mayo Clinic in Rochester, Minnesota on February 27, 2017. A retrospective clinical outcomes study comparing 20 CS-PLT trauma patient recipients with 20 RT-PLT trauma patient recipients, although underpowered to yield definitive conclusions, suggested that CS-PLTS stored for 3 days were at least equivalent to conventional RT-PLTS stored for 5 days. There were no adverse events (e.g., transfusion reactions or thromboembolism) documented in the 20 CS-PLT recipients.

# Cold-Stored Platelets - Summary

- Due to a high discard rate, the CS-PLT program was placed on hiatus on February 25, 2019. The goal is to bring back pathogen-reduced CS-PLTS for the treatment of actively bleeding patients, if and when, the allowable storage period for CS-PLTS can be extended to at least 14-days. With that goal in mind, the Transfusion Services at Mayo Clinic in Rochester, Minnesota conducted several in vitro analyses comparing pathogen-reduced CS-PLTS with pathogen-reduced RT-PLTS throughout 14-days of storage. The in vitro studies showed that, relative to RT-PLTS, CS-PLTS had lower platelet counts, better preserved platelet aggregation, and, in general, evidence of an increased level of platelet activation, including higher levels of procoagulant microvesicles. The pathogen-reduced CS-PLTS showed better and acceptable pH values throughout the 14-day storage period and bacterial cultures showed had no evidence of bacterial growth.

# Cold-Stored Platelets - Summary

- The Transfusion Services at Mayo Clinic in Rochester, Minnesota recommends that 14-day pathogen-reduced CS-PLTS be strongly considered as an acceptable blood component for the treatment of actively bleeding patients.

Thank You