

Original article

Study of the quality of leukodepleted pooled platelet concentrates (LDPPC) treated with pathogen inactivation: amotosalen - UVA light treatment through 7 days of storage

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Abstracts:

Introduction: The problem of microbial contamination of platelets is widespread. Currently, National Blood Centre, Thai Red Cross Society has prevented and detected microbial contamination by using bacterial cultures in all platelet units. However, this method has its limitations. The incubation period for the growth of the microorganisms should be long enough to obtain the optimal number of organisms detectable by the machine. To solve this problem, pathogen inactivation technology, a technology to inhibit the growth of microorganisms, was studied in LDPPC production. **Objective:** To investigate the quality of pathogen inactivation treated leukodepleted pooled platelet concentrates (LDPPC): amotosalen - UVA light treatment after 7 days of storage. **Materials and Methods:** A total of 120 units of whole blood were used to prepare 30 units LDPPC by the buffy coat method. The quality of LDPPC was assessed before pathogen inactivation by testing for volume, platelet count, leukocyte content, pH and swirling phenomenon. Pathogen inactivation was performed by the amotosalen - UVA light treatment method. LDPPC with PI were stored at 20-24°C in platelet incubator and examined for their quality on day 2, day 5 and day 7 for preparation volume, platelet count, pH, swirling phenomenon and bacterial cultures. **Results:** Testing before pathogen inactivation treatment, volume was 314.31±9.82 mL, platelet count was 327.21±26.19 ×10⁹ per bag, leukocyte content was 0.04±0.02 ×10⁶ per bag, pH was 7.15±0.03, swirling phenomenon was 5+ and red blood cell was 0.03±0.01 ×10⁶ per bag. After treatment with pathogen inactivation on day 2, volume was 289.02±9.73 mL, platelet count was 279.11±22.24 ×10⁹ per bag, pH was 7.04±0.03, and swirling phenomenon was 5+. On day 5, volume was 289.02±9.73 mL, platelet count was 266.12±22.32 ×10⁹ per bag, pH was 7.26±0.04, and swirling phenomenon was 5+. On day 7, volume was 289.02±9.73 mL, platelet count was 263.89±23.13 ×10⁹ per bag, pH was 7.13±0.07, and swirling phenomenon was 5+. The bacterial culture test was negative at day 2, 5 and 7. **Conclusion:** The results of LDPPC treated with pathogen inactivation were within the standard of National Blood Centre, Thai Red Cross Society during the 7 days of storage.

Keywords : ● Platelet ● Leukodepleted pooled platelet concentrates
● Leukodepleted pooled platelet concentrates with pathogen inactivation

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นิพนธ์ต้นฉบับ

การศึกษาคุณภาพของ leukodepleted pooled platelet concentrates (LDPPC) ที่ผ่านการทำ pathogen inactivation ด้วยวิธี amotosalen - UVA light treatment เมื่อเก็บรักษาเป็นระยะเวลา 7 วัน

มัลลิกา เล็บครุฑ นฤมล วระขุน ภคมน จันทรัมย์ และ สิริพร เสนีโสด

ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

บทคัดย่อ

บทนำ การปนเปื้อนเชื้อจุลินทรีย์ในส่วนประกอบโลหิตประเภทเกล็ดเลือดเป็นปัญหาที่พบบ่อย ปัจจุบันศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย มีมาตรการในการป้องกันและตรวจสอบการปนเปื้อนของเชื้อจุลินทรีย์ โดยใช้วิธี bacterial culture ในส่วนประกอบโลหิตประเภทเกล็ดเลือดเข้มข้นทุกถุง ซึ่งวิธีนี้ยังมีข้อจำกัด ในด้านระยะเวลาที่ใช้เพื่อการเจริญเติบโตของเชื้อจุลินทรีย์ให้มีจำนวนมากพอที่เครื่องจะสามารถตรวจวัดได้ ดังนั้นเพื่อแก้ปัญหาเรื่องนี้จึงทำการศึกษาโดยใช้เทคโนโลยี pathogen inactivation ซึ่งเป็นเทคโนโลยีการยับยั้งการเจริญเติบโตของเชื้อจุลินทรีย์ในการผลิต LDPPC **วัตถุประสงค์** เพื่อศึกษาถึงคุณภาพของ LDPPC ที่ผ่านการทำ pathogen inactivation ด้วยวิธี amotosalen-UVA light treatment เมื่อเก็บรักษาเป็นระยะเวลา 7 วัน **วัสดุและวิธีการ** ใช้โลหิตรวมจำนวน 120 ยูนิต นำมาผลิตเป็น LDPPC จำนวน 30 ยูนิต โดยวิธี buffy coat method และนำ LDPPC ไปตรวจคุณภาพก่อนการทำ pathogen inactivation โดยวัด volume, platelet count, leukocyte content, pH และ swirling phenomenon จากนั้นนำ LDPPC ที่ผลิตได้ไปผ่านการทำ pathogen inactivation และนำไปตรวจคุณภาพในวันที่ 2, 5 และ 7 ของวันที่ผลิตโดยวัด volume, platelet count, pH, swirling phenomenon และทำการทดสอบ bacterial culture **ผลการศึกษา** การทดสอบก่อนทำ pathogen inactivation พบว่า ใน 1 ถุงของ LDPPC มีปริมาตร 314.31 ± 9.82 มิลลิลิตร ปริมาณเกล็ดเลือด $327.21 \pm 26.19 \times 10^9$ เซลล์ ปริมาณเม็ดเลือดขาว $0.04 \pm 0.02 \times 10^6$ เซลล์ ค่า pH 7.15 ± 0.03 ค่า swirling 5+ และปริมาณเม็ดเลือดแดง $0.03 \pm 0.01 \times 10^6$ เซลล์ การทดสอบหลังการทำ pathogen inactivation ในวันที่ 2 พบว่า LDPPC with PI มีปริมาตร 289.02 ± 9.73 มิลลิลิตร ปริมาณเกล็ดเลือด $279.11 \pm 22.24 \times 10^9$ เซลล์ ค่า pH 7.04 ± 0.03 และ swirling 5+ ในวันที่ 5 พบว่า LDPPC with PI มีปริมาตร 289.02 ± 9.73 มิลลิลิตร ปริมาณเกล็ดเลือด $266.12 \pm 22.32 \times 10^9$ เซลล์ ค่า pH 7.26 ± 0.04 และ swirling 5+ ในวันที่ 7 พบว่า LDPPC with PI มีปริมาตร 289.02 ± 9.73 มิลลิลิตร ปริมาณเกล็ดเลือด $263.89 \pm 23.13 \times 10^9$ เซลล์ ค่า pH 7.13 ± 0.07 และ swirling 5+ สำหรับผลการทดสอบ bacterial culture negative ในวันที่ 2, 5 และ 7 **สรุป** การศึกษาถึงคุณภาพของ leukodepleted pooled platelet concentrates (LDPPC) ที่ผ่านการทำ pathogen inactivation ด้วยวิธี amotosalen-UVA light treatment เมื่อเก็บรักษาเป็นระยะเวลา 7 วัน พบว่าปริมาณเกล็ดเลือด ปริมาณเม็ดเลือดขาว ค่า pH swirling และผลการทดสอบ bacterial culture อยู่ในเกณฑ์มาตรฐานของศูนย์บริการโลหิตแห่งชาติตลอดระยะเวลา 7 วัน ที่ทำการศึกษา ดังนั้น การทำ pathogen inactivation ด้วยวิธี amotosalen-UVA light treatment สามารถนำมาใช้ในการผลิต LDPPC ได้

คำสำคัญ : ● เกล็ดเลือด ● เกล็ดเลือดที่ลดเม็ดเลือดขาวด้วยวิธีการกรอง
● เกล็ดเลือดที่ผ่านการยับยั้งการเจริญเติบโตของเชื้อจุลินทรีย์

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2566;33:269-76.

Introduction

Microbial contamination of blood components is a problem that is dangerous to patients and can lead to death. Many countries need to prevent and detect microbial contamination in blood components. The National Blood Centre, Thai Red Cross Society (NBC, TRCS) is committed to providing patients with high quality and safe blood components.

Microbial contamination of blood components¹⁻³ may be caused by the blood donors having bacteremia or by the donor's and the blood collector's skin being contaminated during the blood donation process or by the equipment used during blood collection. Widely accepted and proven methods to reduce the risk of microbial contamination are applying antiseptic to the donor's skin and collecting approximately 30 mL of donor blood in the diversion pouch (which the blood is most likely to contain microorganisms) before entering into the bag³. In addition, the cause of microbial contamination may happen during the processing of blood components⁴⁻⁶, such as in the preparation of LDPPC. LDPPC is prepared by combining the buffy coats from four donors of the same ABO blood type using a sterile connecting device and adding platelet additive solution (PAS), a process that carries the risk of contamination with microorganisms. In addition, storage temperature of platelet concentrate is another factor that affects the growth of microbial normal skin flora⁷⁻¹⁰ coagulase-negative Staphylococci such as *S. epidermidis* which is the most common bacteria detected.

Currently, bacterial cultures are used at the NBC, TRCS to detect microbial contamination of all platelet bags. This method still has a limitation in terms of the time needed for enough microbial growth to be detectable by the device and the short life span of platelets lasting only 7 days, so some microbially contaminated platelet concentrates may be used in patients, causing infection and can be fatal¹⁰. However, the severity depends on several factors, such as the number of microorganisms

in the blood components, the type of microorganism present, the ability to cause disease, and the clinical condition of the patients^{11,12}. This also affects the hospital's confidence in the products of NBC, TRCS.

In addition, some countries use pathogen inactivation (PI)¹³ which is a technology to inhibit the growth of microorganisms from 3 mechanisms^{13-15,16} 1) physical mechanisms such as the use of heat inactivation, 2) photochemical mechanism e.g. methylene blue (MB) - light treatment¹⁴, amotosalen - UV light treatment, riboflavin - UV light treatment, 3) chemical inactivation e.g. solvent detergent (SD). Pathogen inactivation damages the nucleic acid of bacteria and inhibits the mechanism of microbial division. This method inhibits the white blood cells and can be used as a substitute for gamma or X-ray irradiation to prevent transfusion-associated graft-versus-host disease (TA-GVHD)¹⁷⁻¹⁹ in patients who are immunocompromised, such as undergoing bone marrow transplantation. This is a rare disease but the mortality rate is 90-100%.

The NBC, TRCS used pathogen inactivation (PI) in single donor platelet collection (SDP with PI) by apheresis using amotosalen (INTERCEPT, Concord, CA, USA) since 2017. And recently, inactivation of pathogens in apheresis plasma was performed in the research project for plasma from people recovered from COVID-19 infection using the amotosalen (INTERCEPT, Concord, CA, USA)

Therefore, we aimed to study the quality of leukodepleted pooled platelet concentrates (LDPPC) treated with pathogen inactivation: amotosalen - UVA light treatment, through 7 days of storage. In this way, patients can use the product with greater safety¹³ because the lead time to delivery of the product is much shorter than the bacterial culture methods currently used to detect microbial contamination of platelets in each bag, but this production method is still limited by the price of the equipment used, so it is not available for every unit. This service can only be provided on request by the hospitals.

Materials and Methods

A total of 120 whole blood units were separated into 30 units of LDPPC and treated by the amotosalen UVA light treatment method in the Blood Component Preparation Department, NBC, TRCS from December 2022 to February 2023. This study was approved by the Human Research Committee of the National Blood Centre, Thai Red Cross Society under COA. number 5/2565.

Whole blood containing the anticoagulant CPD was used. The total blood volume of 468 to 558 mL was collected in 450 mL blood bags at a temperature between 20 and 24°C. A temperature controlled centrifuge (Heraeus Cryofuge 16, Thermo Scientific, Germany) was used to centrifuge at 3,400 rpm/minute ($3,838\times g$) at $22\pm 2^\circ\text{C}$ for 12 minutes, after which blood components were squeezed out using an automated separator (KAWASUMI KL 521) including leukocyte poor packed red cells (LPRC), buffy coat and fresh plasma. After that, stored the buffy coat in platelet incubator at $22\pm 2^\circ\text{C}$ for 24 hours pending testing for infectious markers and ABO blood groups. Four bags of buffy

coats with the same ABO blood group were combined with platelet additive solution (PAS) (Terumo, Belgium) in a 40:60 plasma: PAS ratio using a sterile connection device. PAS was the first bag followed by four buffy coat bags and the last bag was for platelet storage with leukocyte filter. The buffy coats were connected to the last buffy coat bag and suspended, then the buffy coat was rinsed with PAS so that each bag had no buffy coat residue. The pooled buffy coats with PAS was left at room temperature ($20\text{--}25^\circ\text{C}$) for at least 1 hour and mixed gently before centrifugation at 2,100 rpm/minute ($1,464\times g$) at $22\pm 2^\circ\text{C}$ for 3 minutes and then squeezed out by flowing the platelet rich plasma fraction through a leukocyte filter to obtain LDPPC and buffy coat remnant (Figure 1). From the segment of LDPPC by mixing every 5 sets of 10 times each to put samples in bags for quantification of cells using an Automated Hematology Analyzer (SYSMEX® XN-1000, Sysmex Corporation, Kobe, Japan). Add amotosalen and LDPPC in illumination container, mixed well. Exposed the illumination container to light using a UVA light



Figure 1 Represents the production process of LDPPC

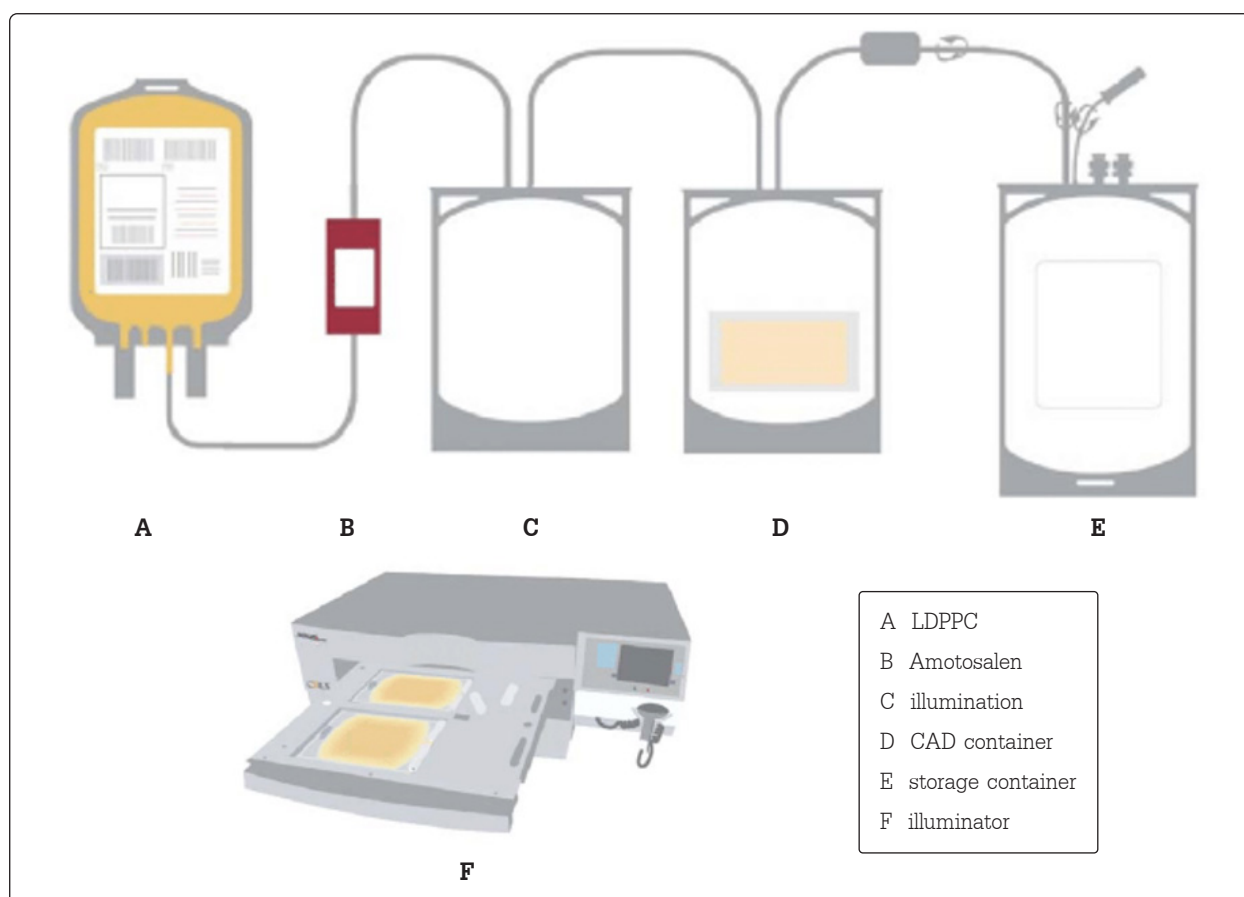


Figure 2 Represents the production process of LDPPC with PI

illuminator, for 5 minutes. Then removed the illumination container from the illuminator, hung the illumination container and kinked the cannula to allow the LDPPC with PI to flow into the CAD container. Place the storage container in an incubator and shook the platelets for 4.5 hours, then transferred to the storage container for LDPPC with PI (Figure 2). Samples were prepared from segments of LDPPC with PI by stripping wire to mix 5 sets of 10 times each to represent samples in bags for cell quantification using LDPPC with PI samples were visually examined for swirling and scored (5+ = strongest swirling, 3+ = moderate swirling, 1+ = slight swirling, and 0 = no swirling) together with pH measure on days 2, 5, and 7. In addition, bacterial screening was also performed.

Criteria for the evaluation of the test^{20,21}

Standardized LDPPC approval criteria before treatment with pathogen inactivation were:

- Volume between 230-430 mL and 100% of bags tested passed.

- Platelet count must be greater than 240×10^9 per bag and 75% of bags tested passed.
- Leukocyte content must be less than 1×10^6 per bag and 90% of bags tested passed.
- Swirling and scored greater than 3+ and 100% of bags tested passed.
- pH must be greater than or equal to 6.4 per bag and 100% of bags tested passed.

Standardized LDPPC approval criteria after treatment with pathogen inactivation were:

- Volume between 230-430 mL and 100% of bags tested passed.
- Platelet count must be greater than 240×10^9 per bag and 75% of bags tested passed.
- Swirling and scored greater than 3+ and 100% of bags tested passed.
- pH must be greater than or equal to 6.4 per bag and 100% of bags tested passed.
- Bacterial screening test results must be negative for each bag and 100% of bags tested passed.

Table 1 Quality parameters of LDPPC before pathogen inactivation treatment: amotosalen-UVA light method

Parameters	Min	Max	$\bar{X} \pm SD$
Volume (mL)	293.24	327.80	314.31±9.82
Platelet count (10^9 /bag)	264.65	326.74	327.21±26.19
Red blood cell (10^6 /bag)	0.02	0.06	0.03±0.01
Leukocyte count (10^6 /bag)	0.03	0.11	0.04±0.02
pH	7.09	7.20	7.15±0.03
Swirling		5+	

Table 2 Quality parameters of LDPPC after treatment with pathogen inactivation: amotosalen-UVA light method on day 2, day 5 and day 7.

Parameters	LDPPC with PI								
	Day 2			Day 5			Day 7		
	Min	Max	$\bar{X} \pm SD$	Min	Max	$\bar{X} \pm SD$	Min	Max	$\bar{X} \pm SD$
Volume (mL)	266.38	307.83	289.02±9.73	266.38	307.83	289.02±9.73	266.38	307.83	289.02±9.73
Platelet count (10^9 /bag)	222.45	309.94	279.11±22.24	207.39	300.75	266.12±22.32	202.85	300.16	263.89±23.13
pH	6.97	7.10	7.04±0.03	7.16	7.35	7.26±0.04	6.95	7.24	7.13±0.07
Swirling						5+			
Bacterial screening						Negative			

Statistical Analysis

The statistical data analysis was performed using IBM SPSS Statistics version 28.0.1.0. Data were averaged ($\bar{x}$) to compare volume, platelet count, leukocyte counts, pH, and swirling, and standard deviation (SD) was calculated for each parameter of LDPPC before pathogen inactivation and of LDPPC after pathogen inactivation were recorded at different time points (days 2, 5, and 7) to assess whether the platelets were in good quality after stored for 7 days.

Results

Table 1 shows LDPPC parameters before pathogen inactivation including volume, platelet count, leukocyte count, pH and swirling. The maximum and minimum volumes were 327.80 and 293.24 mL. The mean and standard deviation volumes were 314.31 mL and 9.82 mL. The maximum and minimum platelet counts were

326.74 $\times 10^9$ per bag and 264.65 $\times 10^9$ per bag. The mean and standard deviation of platelet counts were 327.21 $\times 10^9$ per bag and 26.19 $\times 10^9$ per bag. The maximum and minimum leukocyte counts were 0.11 $\times 10^6$ per bag and 0.03 $\times 10^6$ per bag. The mean and standard deviation of leukocyte counts were 0.04 $\times 10^6$ per bag and 0.02 $\times 10^6$ per bag. The maximum and minimum pH were 7.20 and 7.09. The mean and standard deviation of pH were 7.15 and 0.03 and swirling scores were 5+.

Table 2 shows LDPPC parameters after pathogen inactivation including volume, platelet counts, pH, swirling and bacterial screening. The mean volumes on days 2, 5 and 7 were the same. The mean platelet counts on days 2, 5 and 7 decreased. The mean pH on days 2, 5 and 7 decreased. Swirling scores were 5+ and bacterial screening test results were negative for all 7 days of the study.

Discussion

The platelets storage temperature was between 20-24°C¹ which is the optimal temperature for microbial growth, that's making platelets more at risk of microbial contamination than other blood components. In addition, data showed that in some countries the technology of pathogen inactivation are used in the manufacture of blood components. This technology inhibits the growth of microbial organisms by damaging the nucleic acid of bacteria, inhibiting the division of microbial organisms. The advantage of using the pathogen inactivation system is that it can also inactivate viruses and parasites, especially emerging pathogens that cannot be detected by current immunological or molecular screening assays. Another advantage of pathogen inactivation is that it can inactivate viable lymphocytes and prevent transfusion-associated graft-versus-host diseases (TA-GVHD) so it can substitute blood irradiation. In this way, patients can use the product with greater safety¹³ because the lead time to delivery of the product is much shorter than the bacterial culture methods currently used to detect microbial contamination of platelets in each bag, The National Blood Centre, Thai Red Cross Society has been performing pathogen inactivation in single donor platelets (SDP with PI) collected using an apheresis machine since 2017.

When compared to other studies, Dr Helen New²² studies validation of plasma and platelet pathogen inactivation. The study found that after 7 days of storage the platelet counts, volume, pH and swirling were still within standard. The researchers also studied additional parameter such as activation markers CD26P, LDH, glucose/lactate, ATP, function platelet, cytokines, chemokines, coagulation activation markers (FXIIa) and microvesicles to ensure that the quality remained good throughout the 7 days of storage.

Canadian Blood Services²³ studies pooled pathogen-reduced platelets, manufactured from seven donor buffy coats, which are pooled together to create an individual double-dose unit that is then divided into two separate units. In contrast, current untreated pooled

platelets in plasma units. While platelets are derived from seven donors (PPPT) than untreated pooled platelets from four donors, the total volume of plasma in a unit of PPPT is less than the total volume of plasma in a unit of untreated pooled platelets. This is because the PPPT product is pooled in PAS-E rather than male plasma (PAS-E: plasma ratio is approximately 60:40). the lower platelet yield, and differences in volume and mean platelet count, of PPPT compared to the untreated pooled platelet component. The residual white blood cell count post-filtration in a double-dose unit of PPPT is $0.04 \pm 0.06 \times 10^6$ cells/unit, which is comparable with the residual white blood cell count post-filtration in untreated pooled platelets in plasma ($0.04 \pm 0.09 \times 10^6$ cells/unit). Additionally, the few residual white blood cells in PPPT are inactivated by amotosalen - UVA light treatment.

From this study, all units before treated with pathogen inactivation were checked for leukocyte content and passed the standard criteria, with a small amount of red blood cells found. The results on days 2, 5 and 7 showed the average volume of LDPPC with PI on days 2, 5 and 7 was 289.02 mL, which had a standard value of 230-430 mL which was considered to pass the criteria. The average platelet counts on days 2, 5 and 7 was 240×10^9 per bag. All criteria were met. LDPPC with PI showed normalized platelet counts throughout storage. The swirling phenomenon was scored as 5+ throughout the storage period. The pH on days 2, 5, and 7 was ≥ 6.4 which was the standard value. All bacterial screening tests were negative throughout the storage period.

Statistical analysis compared LDPPC before pathogen inactivation with LDPPC after pathogen inactivation at day 7 to determine if LDPPC was of lower quality after pathogen inactivation. It was found that platelet counts and pH had significantly decreased. This is due to the fact that platelet death increases with shelf life. However, the study showed that pH and platelet counts at day 7 are still able to pass the criteria, therefore, good quality LDPPC with PI can be delivered to patients at 7 days of age.

Conclusion

The quantitative study of LDPPC with PI showed that platelet count, pH, swirling and bacterial screening were all within standard values during the 7 days study period. Pathogen inactivation may replace the current method of detecting microbial contamination in LDPPC by bacterial cultures in all bags. Inactivation of pathogens achieves good standard LDPPC quality and shortens the waiting time before dispensing and increases patient safety.

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