

Chapter 7

Assessment of Equipment Programming Efficiency for Blood Component Preparation to Enhance Accuracy in Service

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1. Introduction and Background

Component preparation plays a crucial role in blood center services, requiring adherence to standardized procedures for optimal outcomes. The introduction of CPD solution for blood collection has notably enhanced the viability of whole blood, facilitating standard component preparation processes [1]. Utilizing specialized equipment like refrigerated centrifuge machines, which operate on centrifugal and centripetal forces, has been pivotal since the inception of the single-step heavy spin centrifugation method in the 1960s [2]. Over time, component preparation techniques have evolved from manual methods to fully automated centrifuge cryocentrifuge systems, categorized into first to third generations, each continually improving in performance. The fundamental principle underlying all generations is the utilization of centrifugal and centripetal forces to separate components, departing from conventional whole blood holding methods [3]. Foreign reaction in the recipient is a natural tendency of blood products to avoid this always rational use of blood product is always necessary. So component separation is a need of hours for the blood transfusion. The success of component preparation hinges on various factors, with proper instrument programming being paramount. Quality outcomes are achievable only through optimal programming, ensuring standardized products. The aim of the above study is to evaluate the impact of Differential centrifugation. Evaluating programming's impact on centripetal and centrifugal forces, acceleration, deceleration, and time intervals is crucial in selecting the most effective program to elevate standards.

2. Methodology

Advanced Whole Blood Handling and Component Processing

2.1 Whole Blood Collection and Transportation

Whole blood (WB) collected from either outdoor blood donation camps or indoor blood donation centers must be transported to the component preparation laboratory with minimal delay to ensure optimal component yield and quality. The integrity of labile components, such as platelets and plasma, depends on stringent adherence to temperature and time constraints during handling and transport.

- Platelet concentrates and fresh frozen plasma (FFP) must be separated within six hours of blood collection to maintain their functional efficacy.
- The Council of Europe guidelines permit platelet preparation from WB units stored at 20–24°C for up to 24 hours, provided they are rapidly cooled and stored under validated conditions.

Temperature-Controlled Transportation

The storage and transportation conditions of WB vary based on the intended component preparation:

1. For Platelet Preparation

- Blood units should be maintained at 20–24°C during storage and transport.
- Specialized 1,4-butanediol wax cooling plates can lower the WB temperature to 20°C within two hours, ensuring optimal conditions for platelet recovery.

2. For Plasma and Cryoprecipitate Preparation

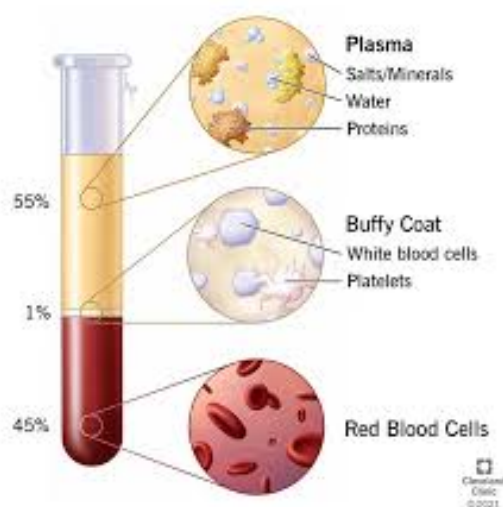
- WB should be transported in insulated blood transport containers with ice or gel packs to maintain temperatures below 10°C.
- Direct contact between ice and blood bags must be avoided to prevent localized hemolysis due to freezing.
- For long-distance transport or in high-temperature environments, the ice-to-blood volume ratio should be at least 1:1 to ensure sustained cooling.

To enhance process control, portable temperature monitoring devices can be employed for real-time logging of blood transport conditions, ensuring compliance with regulatory standards.

2.2 Blood Component Separation and Processing

Centrifugation Principles and Optimization Blood components—red cells, platelets, plasma, and cryoprecipitate—are separated using differential centrifugation, exploiting variations in density, size, and sedimentation rate under centrifugal force.

1. Red blood cells (RBCs), being the most dense, settle at the bottom.
2. White blood cells (WBCs) and platelets form the intermediate “buffy coat” layer.
3. Plasma, the least dense component, remains at the top.



Centrifuge Calibration and Quality Control

Refrigerated centrifuges used for component preparation must undergo:

- Initial calibration before use,
- Recalibration after major repairs/adjustments, and
- Routine calibration (annually or every six months) as per institutional policies.

Key process variables influencing component separation include

- Rotor size (fixed for a given centrifuge),
- Centrifuge speed (RPM and G-force),
- Centrifugation duration,
- Acceleration/deceleration protocols, and
- Temperature control during centrifugation (ensuring optimal conditions for each component).

Optimized Centrifugation Protocols

Centrifugation generally follows a two-step spin cycle:

1. Heavy Spin (High G-force) – 5000 G for 10–15 min
 - Used for separating RBCs from plasma and buffy coat
2. Light Spin (Low G-force) – 1500 G for 5–7 min
 - Used for further separation of platelets and plasma

Centrifuge settings may vary based on manufacturer specifications, requiring standardization of protocols for reproducibility and efficiency.

2.3 Automated vs. Manual Component Separation

Manual vs. Automated Plasma Expression

After centrifugation, blood components must be expressed and separated using either:

- Manual plasma expressors, which rely on operator precision, or
- Automated/semi-automated extractors, which enhance standardization, consistency, and yield optimization.

Component Yield Maximization

Automated component separators allow for:

- Higher platelet recovery (~90% of whole blood-derived platelets)
- Lower residual RBC contamination, preserving component purity
- Reduced operator-dependent variability

Whole Blood Component Utilization

Whole blood, a heterogeneous mixture of cells, colloids, and crystalloids, is fractionated into:

1. Packed Red Blood Cell (PRBC) Concentrate – Used for anemia and blood loss management.
2. Platelet Concentrate – Essential for thrombocytopenic patients.
3. Fresh Frozen Plasma (FFP) – Rich in coagulation factors, used for clotting disorders.
4. Cryoprecipitate – High in fibrinogen, Factor VIII, and von Willebrand factor, used in coagulopathies.

Each component has unique storage, transport, and therapeutic requirements, necessitating.

2.4 Advanced Component Processing Techniques

Precise control over environmental conditions throughout the process.

Fully Automated Blood Component Processing.

Advanced automated component processing systems integrate high-precision centrifugation, optical detection, and robotic extraction to optimize blood component yield and quality. Key benefits include:

- Real-time component volume assessment
- Reduced processing time and contamination risk
- Enhanced traceability and standardization

Centripetal vs. Centrifugal Forces in Blood Separation

- Centripetal Force: Acts towards the center of rotation, maintaining component separation stability.
- Centrifugal Force: A pseudo-force that acts outward, facilitating the migration of denser components (RBCs) toward the periphery while lighter components (plasma) remain near the center.

Optimized centrifugal force application is critical for achieving high-purity blood components while minimizing hemolysis and platelet activation.

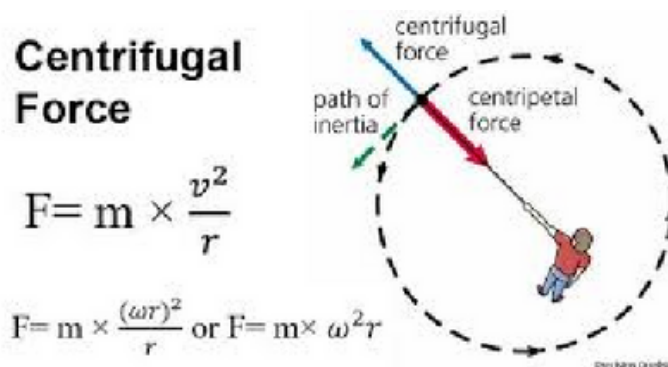
State-of-the-art whole blood processing integrates rigorous quality control, automated separation technologies, and real-time monitoring systems to ensure the highest standards in blood component preparation. The transition from manual handling to precision-driven automation enhances:

- Component yield and purity,
- Process standardization, and
- Overall transfusion efficacy.

By continuously refining transport logistics, centrifugation protocols, and separation techniques, blood banks can significantly improve blood product safety, availability, and therapeutic outcomes. This advanced version incorporates greater technical depth, process optimization insights, and emerging automation trends for modern blood component processing.

The blood components are prepared by centrifuging at different relative centrifugal force in g at different time. Conversion of relative centrifugal force (RCF) to rpm depends upon the radius of centrifuge rotor. It can be calculated by:

1. Nomogram illustrated in figure .
2. By any one of the formulae .



- i) Relative centrifugal force in g = $28.38 R \{1000 \mid 2\}^2 \text{rpm}$
R = radius of centrifuge rotor in inches
- ii) Relative centrifugal force in g = $118 \times 10^{-7} X_r \times N^2$
r =: radius of centrifuge rotor in cm N = speed of rotation (rpm)

Although component preparation is an evolving science. The present study was observational study carried out in a tertiary care institute in eastern India over three months. Evaluation of 20 each as pilot samples for both equipment has been selected. performance run carried out according to standard operative procedure of the equipment.

There were present (working programmes) of both equipment e.g 1. Thermofisher cryocentrifuge 2. Rota Silenta Agile were modified with five closely matched programs and their performance was observed.

1. Present program for Thermofisher cryocentrifuge.

RPM (2350/3450), RCF (1048x2/2054x2), Acceleration (9), Deceleration (10) and Mid interval (8).

2. Present program for Agile centrifuge

RPM (2190/3250), RCF (671x2/1509x2), Acceleration (7), Deceleration (9) and Mid interval (9).

3. Parameters such as acceleration, deceleration, mid-interval time, RPM, relative centrifugal force (RCF), and gravitational force (G) were studied.

4. The quality and frequency of prepared blood products of modified programs (PRBC, FFP, PC, and WBC) were assessed using a Fully Automated Hematology Analyzer.

Modified/Applied Cryocentrifuge (programs)

| PROGRAM ME | RPM- Hard spin/S oft spin | RCF | DIAMETE R | TIME | MID- TIME | ACCELER ATION | DEACCEL ARATION |
|---------------|---------------------------------|--------------------|--------------|------|--------------|------------------|--------------------|
| Thermofisher | 3450/2350 | 2054X2/1048 0X2 | 30/29 | | | | |
| P1 | do | do | do | 21 | 9 | 8 | 4 |
| P2 | do | do | do | 19 | 8 | 8 | 3 |
| P3 | do | do | do | 20 | 9 | 8 | 3 |
| P4 | do | do | do | 24 | 10 | 10 | 4 |
| P5 | | | | 25 | 10 | 10 | 5 |
| Rota silenta | 3250/2190 | 1509X2/671 X2 | 29.5/28.5 | | | | |
| PI | do | do | do | 23 | 12 | 8 | 3 |
| P2 | do | do | do | 20 | 11 | 7 | 2 |
| P3 | do | do | do | 21 | 12 | 7 | 2 |
| P4 | do | do | do | 25 | 13 | 8 | 4 |
| P5 | do | do | do | 27 | 13 | 9 | 5 |

The module of the different programs estimated over RCF, RPM, MID-INTERVAL, ACCELERATION and ACCELERATION.

3. Results

The HERAEUS Thermofisher cryocentrifuge 16 Centrifuge outperformed the ROTA Silenta 630 RS. The optimal programming for the HERAEUS CRYOFUGE involved acceleration (8 minutes), deceleration (8 minutes), mid-interval (3 minutes), and RPM (2350 and 3450), with calculated RCF values of 2096 and 4018. This programming resulted in the highest platelet yield (287ug/dl), minimal FFP contamination by RBC and platelets (0.01 and 24ug/dl, respectively), PRBC with a hematocrit of 69%, RBC concentration of 9.6ug/dl, and platelet contamination of 186ug/dl.

In comparison to applied programming (P2) to present programs, the present program observed inferior platelets yield (189ug/dl), FFP-contamination by RBC and platelets observed (.02 and 26Ug/dl) respectively).

For the ROTA Silenta, the best programming included acceleration (8 minutes), mid-interval (3 minutes), deceleration (4 minutes), RPM (2190 and 3250), with a radius of 29.5cm and computed RCF values of (1342 and 3018), respectively, resulting in the highest quality products.

Comparison to applied programming (P4) of ROTA present programs also observed platelets yield (198ug/dl) FFP Contamination by RBC and platelets (.02 and 24Ug/dl) respectively.

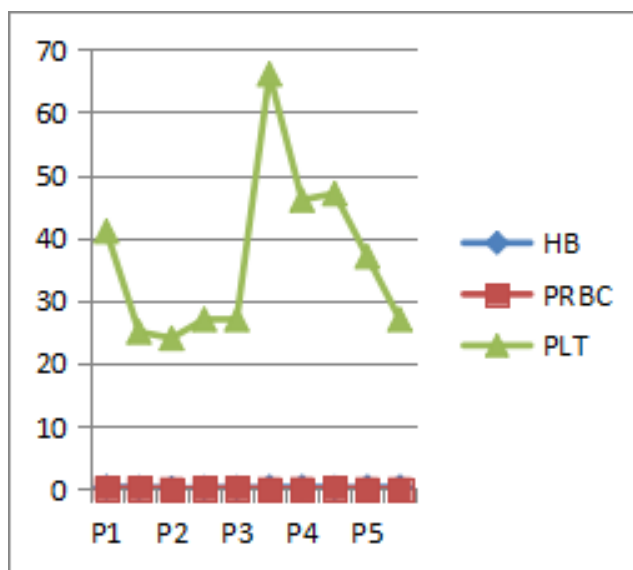


Figure :1 FFP (Thermofisher)

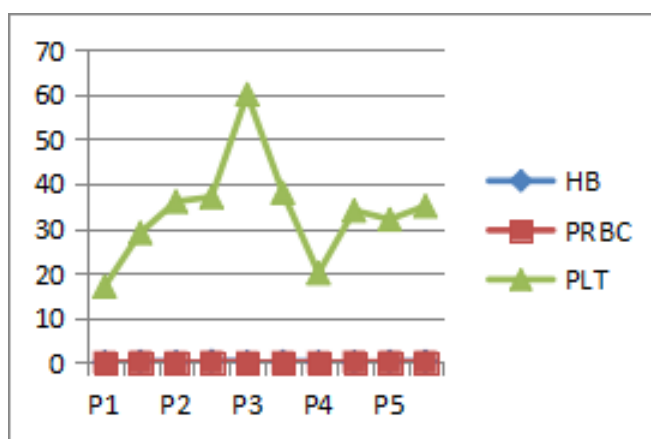


Figure :2 FFP(Rota)

Figure1, (Thermofisher)

Above graphical presentation of the outcome of programs of cryocentrifuge which represent HB and PRBC lies over the lower side while contamination of prepared FFP products is highest noted with platelets. P2 was associated with the lowest contamination of HB and PRBC (.1) and (.01) respectively. Platelets was 24 u/dl in P2. The highest contamination of HB, PRBC and platelets was in P3 (.2), (.01) and (27) respectively.

Figure-2FFP (Rota)

The above graphical presentation of the outcome of programs of cryocentrifuge which represent HB and PRBC lies over the lower side while contamination of prepared FFP products is highest noted with platelets. P2 was associated with the lowest contamination of HB and PRBC (.04) and (.01) respectively. Platelets were 36 u/dl P2 at the lowest in P4 (20) ul/dl. Highest contamination of HB, PRBC and platelets was in P3 (.3) gm/dl, (.1) ul/dl and (60) ul/dl respectively.

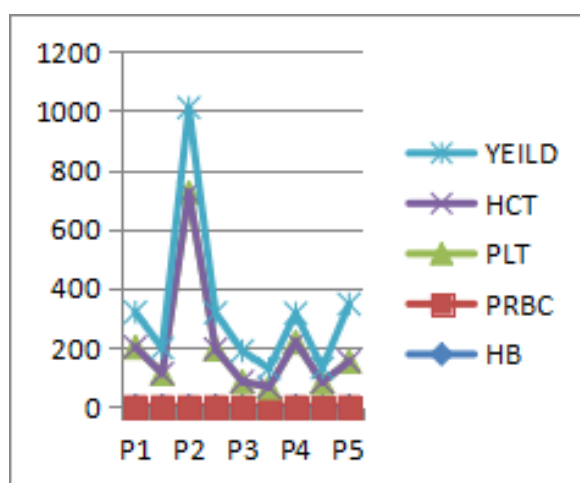


Figure:3 PLT (Thermocentrifuge)

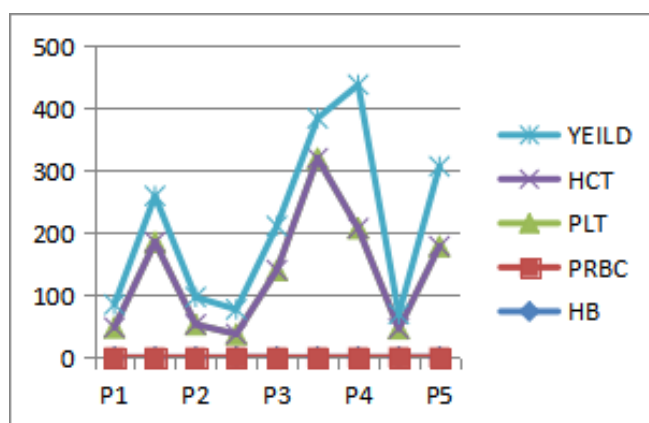


Figure:4 PLT (Rota)

Figure 3 (Thermocentrifuge)

Above graphical presentation of the outcome of programs of cryocentrifuge which represent HB, PLT, HCT, YEILD and PRBC. Here PRBC lies over the lower side while contamination of prepared PLT products is highest noted with PRBC and negligible with wbc (.1) ul/dl. P2 was associated with the lowest contamination of HB and PRBC (.2) and (.02) respectively. Platelets were 722 u/dl in P2. The highest contamination of HB, PRBC and HCT was in P1 (.4), (.04) and (.3) respectively. The highest yield of PLT was achieved in P2 (288) and the lowest was P4 (48).

Figure 4PLT (Rota)

Above graphical presentation of the outcome of programs of cryocentrifuge which represent HB, PLT, HCT, YEILD and PRBC. Here PRBC lies over the lower side while contamination of prepared PLT products is highest noted with PRBC and negligible with WBC (.1) ul/dl. P1 was associated with the lowest contamination of HB and PRBC (.2) ul/dl and (.02) ul/dl respectively. Platelets were 319u/dl in P3. The highest contamination of HB, PRBC and HCT was in P4 (.4), (.04) and (.3) respectively. The highest yield of PLT was achieved in P4 (230) and the lowest was P3 (74).

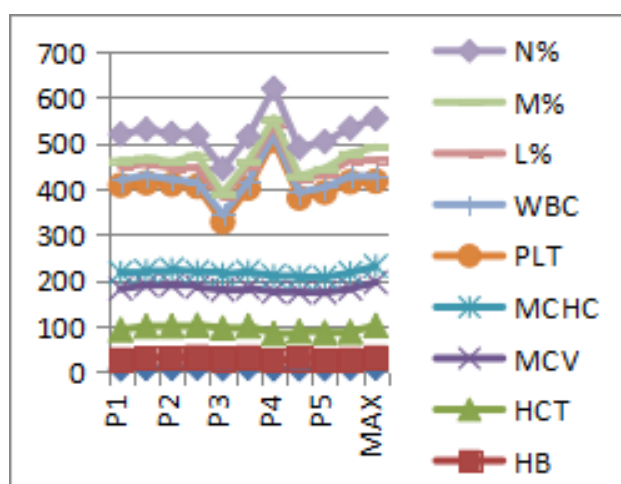


Figure :5 PRBC (Thermocentrifuge)

Figure 5 Thermo

Above graphical presentation of the outcome of programs of cryocentrifuge which represent HB, PLT, HCT, MCV, MCHC, WBC, L%, M%, N% and RBC. Here concentrated PRBC lies over the lower side (8.4) ul/dl while contamination of prepared PRBC products Lowest noted with PLT, HCT and WBC (186) ul/dl, (68.8%) and (8.25) ul/dl in P2. Maximum MCV and MCH were in P5 and P3 respectively.

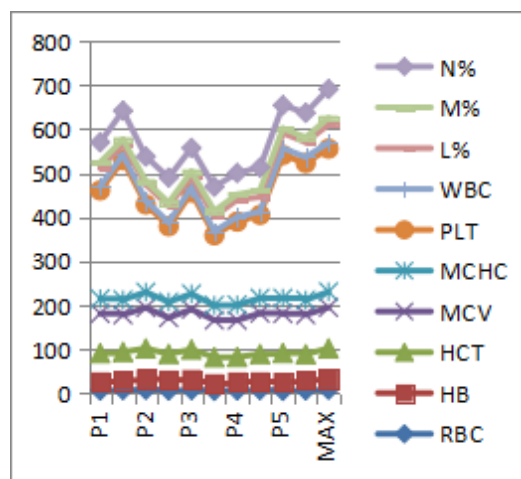


Figure :6 PRBC Rota

Figure 6. PRBC Rota

Above graphical presentation of the outcome of programs of cryocentrifuge which represent HB, PLT, HCT, MCV, MCHC, WBC, L%, M%, N% and RBC. Here concentrated PRBC lies over the lower side (8.4) ul/dl while contamination of prepared PRBC products Lowest noted with PLT, HCT and WBC (153) ul/dl, (68.8%) and (69.7) ul/dl in P4. Maximum MCV and MCH were in P4 and P5 respectively.

4. Statistical analysis

Conducting a chi-square test, multivariate programming for component preparation was assessed. P2 yielded the highest platelets (PLT) at 288, while P4 showed the lowest at 48 for the cryocentrifuge. In the case of Rota Silent, P4 had the highest PLT yield at 230, with P3 having the lowest at 74. Overall, P2 demonstrated the most significant product effect across all variables, with statistical significance at an estimated p-value of <0.05. In terms of PRBC (Rota Silent), P4 emerged as the best-programmed choice.

| programs | PLT count($10^3/\text{ul}$) | FFP contamination with plt($10^3/\text{ul}$) | Chi-square | P value at <.05 |
|----------------------------------|-------------------------------|--|------------|-----------------|
| P2 Thermofisher | 287 | 24 | .32 | |
| Present program for Thermofisher | 183 | 26 | .53 | |
| P4 Rota | 240 | 24 | .05 | |
| Present programs for rota | 198 | 26 | .81 | |
| P2 Thermofisher | 287 | 24 | .37 | |
| P4 Rota | 240 | 24 | .53 | |
| | | | | |

T-test statics at the confidence of variance (.01) computed as (.04) with degree of freedom (18) estimated p-value obtained (.4842) which was not significant at <.05.

5. Discussion

To meet quality standards, any prepared component must adhere to 60 to 75% of the D&GHS advisory guideline [4]. Currently, our prepared components meet 60% of the standard, requiring the reprogramming or discarding of the remaining components to meet the standard. Reprogramming can be time-consuming, especially when needed for contaminated products like FFP contaminated with RBC, necessitating their disposal. Modifications in platelet preparation programming are essential for achieving higher platelet yields. For RBC preparation, selecting more concentrated PCV-RBC is necessary, achievable through program modifications. Contamination of WBC in RBC products leading to non-hemolytic febrile transfusion reactions can be reduced by selecting appropriate programs. Effective centrifugation to separate RBC from the buffy coat simplifies the process. Contamination of RBC in FFP and platelets is detrimental to RH-negative patients, mitigated by optimal program selection. Therefore, modifying programs to search for optimal programming is imperative for ideal component preparation. The conventional method of refrigerated cryo-centrifuge operation relies on centripetal and centrifugal forces, facilitating component separation based on specific gravity [5]. Plasticizers used significantly affect blood product quality, impacting storage lesion and product quality. Studies compared SAGM and non-SAGM blood bags, assessing RPM and RCF according to guidelines same manners as Bostan et al [6,7]. Dzik et al had been described a formula over 5000/2000xg heavy and soft spin

over g(gravitational force) and radius of equipment. Calculated RPM/RCF was static for the present study while changes were made to the programming of the equipment. Component preparation algorithms align with AABB and DCGH guidelines, focusing on product-specific processing and storage [8,9]. Heather et al and Basu et al, had described the component preparation overview similarly for product-specific processing and storage of component products [9,10].

From gravitational methods to semi-automated and fully automated component separation techniques, advancements have led to improved efficiency and outcomes. While hollow fibre (Ere Sep) methods offer an alternative to centrifuge-based separation, they are more time-consuming (2hrs) and less effective in leukoreduction while above conventional separation technique took only 50 mins [11]. Johnson et al, has proven highest platelet yield (297.43ul/l) of their own methods while present studies highest noted with 283 ug/l of P2 programs of thermofisher. The MCV of RBC product of Ere sep methods was 92.6 femtoliter while P3 and P5 of thermofisher has shown highest MCV. The automation technique of Atreus 2C can only hold one component while the above programming can support 10 to 12 buckets of component programming [12]. Thomas et al, has shown in his study meanPCV of RBC (58.5%) while PCV of the applied programs{(Thermofisher(p2))} was 68% which was quite higher [11]. Automation technologies like Atreus 2C and Revous offer improved processing capabilities, with Revous providing high platelet yields and component pooling [12,13].

The FFP contamination with platelets prepared from Revous product was quite low in comparison with present study (24ul/l) [12]. Inappropriate programming compromises product quality and may damage blood bags, necessitating adherence to biomedical waste handling guidelines [14]. While present study has not experienced any tear or leakage of blood bags. Overall, the study's outcomes are satisfactory, with optimal programs identified, best platelet products selected, FFP contamination minimized, and discard rates reduced. This enhances inventory management and facilitates meeting different group requirement.

The limitation of present study conducted in very small sample size although, this has been carried out in pilot study.

6. Conclusion

Selecting the optimal programming parameters enhances product quality and meets established standards for platelet preparation, hematocrit, and PRBC preparation. FFP contamination with RBC aligns with the defined log reduction for WBC. The choice of programming can significantly improve product quality and adherence to standards. Every component laboratory should maintained to find out optimal programming, that could strengthen the blood transfusion services. unnecessary discard of time lay out can be avoided.

Ethical Approval : Ethical approval has been taken-Ref.no-RD/AIIMS/Pat/RAC/29

Competing Interests: There are no conflicts and competing interests here.

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