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THE pH ESTIMATION IN STORED PLATELETS: AN INSTITUTIONAL STUDY

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ABSTRACT

Background : As per the current quality control standards the platelets can be assessed by using several parameters such as swirling, volume, platelet count and WBC count per bag and pH changes which in-turn have also been endorsed by The American Association of Blood Banks (AABB). In recognizing the limited capacity of transfusion services to perform a wide array of checks, AABB has restricted the basic minimum requirements to at least include the platelet count, pH, volume, red cell content, and residual WBC count respectively.

Materials and Methods: 625 random donors platelets were studied after obtaining the written consent. The results were analyzed by the analysis of the variance (ANOVA) and a two-tailed Student's test.

Results: The pH values showed a significant fall over 7 days of storage but were within the acceptable limits. Over the these seven days of storage period, significant changes in Po2 values Pco2 was observed along with the LDH levels.

Conclusion: Therapeutic storage of platelet concentrate remains a challenge and hence this study was carried out with the aim of improving the duration of storage of platelet concentrates.

Key words: platelet concentrate, biochemical parameters, pH , storage lesions.

INTRODUCTION

pH is the simplest parameter indicator of the platelet storage lesion and probably the most important quality parameter that gives an indication of viability and potential recovery of platelet concentrates at the end of the storage period. pH has been identified as the parameter having the highest correlation with recovery and survival platelets. In absence of oxygen stored platelets revert to glycolytic metabolism with increased generation of lactic acid and consequent fall in pH within 3 days of preparation.[1]

Platelet viability is markedly affected by pH. The final pH of platelet concentrate and hence in-vivo recovery and survival will depend on the type f storage container, storage conditions and the volume of residual plasma.[1] Therefore platelet storage bags must allow for free gaseous exchange. Platelets must be stored in sufficient plasma, whose bicarbonate content acts as a buffer, to maintain pH at greater than 6.2. Depletion of bicarbonate by high content acts as a buffer, to maintain pH at greater than 6.2. Depletion of bicarbonate by high lactic acid level, typically at 20-25 mmol/l, lowest pH and results in loss of platelet viability.[1]

Platelets utilize energy for a variety of intracellular biochemical reactions associated with their role in hemostatsis.[2] Unlike many other cells, platelet do not contain creatine or creatine kinase and rely on large amounts of intracellular, stored glycogen to fuel glycolysis. The principal source of energy for platelets is the hydrolysis of ATP. ATP is essential for signal transduction and, once released, creates calcium cross-linkages. In order to maintain adequate platelet function, platelets must generate ATP continuously to meet their energy needs.[2]

For stored platelets, in vitro tests of platelets have generally not correlated well with platelet performance in vivo. However, platelet cellular levels of ATP , glucose , and lactate can offer some

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indication of platelet performance. A drop in platelet count with an increase in the level of lactate dehydrogenase in medium can be used as a measure of cellular lysis. The Ph of platelet suspension above 7.6 and below 6.2 at the end of the storage period has been shown to correlate with decreased in vivo performance.[3]

METHODOLOGY

Blood was collected from eligible donors as per Drugs and Cosmetic Act, 1945, by Ministry of Health, Govt. of India along with written consent. Donors who had taken analgesics and antibiotics for last 7-10 days have excluded from the study. 625 Random Donors Platelets (RDP) were studied.

Phlebotomy site was prepared with a povidine-iodine scrub followed by povidine-iodine application (2-3times). For donors who were allergic to iodine a chlorhexidine scrub was substituted and kept for 30 seconds. The first portion (20 ml) of Whole Blood (WB) collection was diverted in diversion pouch.

WB derived platelets can be prepared using platelet rich plasma (PRP) and buffy coat (BC) techniques. For our study PRP method was followed where the WB is collected into an anticoagulant solution and then subjected to a soft spin centrifuged with a soft spin to separate the PRP. The PRP is centrifuged again to prepare the platelet concentrate (PC) which contains 60-75% of the platelets present in the whole blood unit from which it is derived. The second generation tri-(ethylhexyl)-trimellitate (TEHTM plasticizer) bags (Terumo-Penpol, Baxter) were used and the PCs bags were left stationary, with the label side down, at room temperature for one 1 hour to rest. PCs were stored with continuous agitation in a quarantine platelet incubator (Termopenpol) at 20-24 ^{o} C all the PCs were agitated by a rotatory motion. The speed was fixed at four rotations per second. A total 625 Platelet Concentrate (PC) were analyzed.

Two units of ABO group specific platelets were pooled using sterile connecting device (TSCD209A, Terumo, Japan), which prevents contamination, to ensure that a minimum of 50ml of plasma is left on day 5 after sampling.

Sampling was done on day 0,3,5 7 and 8 aseptically, through sample site coupler with bacterial filter 4C2405 (Baxter, USA), and large bore needle to avoid artificial activation of platelets. The sealed 5ml syringe sample was used for measurement of pH, pO_2 and Pco_2 using blood gas analyzer (Novaultra C, Nova Biomedical Corporation, USA)at 37^{o}C following the manufacture's instructions. The result of ABG analyser were temperature corrected to 22 ^{o} C.

The volumes of PCs were determined by subtracting the weight of empty bag from that of full bag. To convert weight to volume, resultant weight was divided by 1.03 specific gravity of PRP-PC.

$$\text{Volume} = \{\text{weightofconcentrate(g)}\} / 1.03 \{\text{-weighttofemptybag(g)}\}$$

The electrode of pH meter was placed in PCs and swirled in the solution. The pH reading had to be stabilized before the pH result of PCs taken. When the reading was freezing, the pH of PCs was recorded.

Sterility was performed on Day 5 of the PCs shelf-life. Bacterial growth examination was performed by using automated blood culture system (BacT/ALERT, BioMerieus Inc.,). The plastic flip-top from the culture bottle was removed and disinfected with an alcohol pad. 10 ml of PCs was obtained by using syringe with aseptic technique and it was transferred into the culture bottle. The PCs sample had to transfer into the anaerobic culture bottle first before putting in aerobic culture bottle so that any oxygen trapped in the syringe was not be transferred to the anaerobic bottle. The bottle was loaded into the automated blood culture system to be scanned by the bottle barcode label. The culture bottle has to be incubated in the automated blood culture system for seven days. If there was positive culture, light was seen flashing from the automated blood culture system.

RESULTS

The results of the 625 PCs were analyzed by analysis of the variance (ANOVA) and a two-tailed Student's test.

Among metabolic parameters pH showed a significant fall over seven days of storage. Fall in pH may affect the quality of the final platelet product. Though, a significant drop in pH was observed in the present study, mean pH was 7.278 even on day 7 of storage (Table 1: Fig 1)

Over the these seven days of storage period, an increase in mean Po_2 values with concurrent decreased in Pco_2 was observed (Table 2: Fig 2) & (Table 3: Fig 3) respectively. This is a documented effect of platelet storage lesions with second generation platelet storage containers.[4] During storage, metabolic activity of platelets continues

leading to O_2 consumption and CO_2 production that leads to trend of decreasing O_2 and increasing CO_2 in container. However, second generation platelet storage containers are more permeable to gases, gaseous exchange take place readily across the container walls which resists accumulation of CO_2 and depletion of O_2. If the storage container walls were not gas permeable, as in case of first generation plasticizer, oxygen levels would diminish and carbon dioxide levels would increase within the storage containers. Therefore, it can be surmised that second generation plasticizer used by us an had a good gas exchange capability.[5]

It was observed that there was no significant change in pH on day 3, 5 & 7 with respect to Day 0 values. There was statistically significant difference in pH between day 0 and day 8.But on all days pH was in normal range (Table1 and Fig1)

Table1 : pH changes from Day 0 to Day 8

Day	Mean	N	Std. Deviation	t value	P Value
Day 0	7.276	625	0.4147		
Day 3	7.274	625	0.4208	-0.312	0.755
Day 5	7.389	625	2.8086	-0.984	0.326
Day 7	7.278	625	0.4128	-0.420	0.674
Day 8	6.967	625	0.5425	11.185	<0.0001

Mean Percentage change in pH from Day 0 to Day 8 was – 3.89%

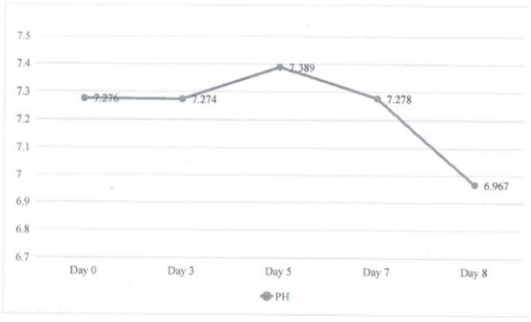

Figure 1: Line diagram showing PH changes

Several factors including dissolved gases also affect platelet metabolism and survival in storage. The partial pressures of dissolved gases such as Po_2 and Pco_2 are a function of equilibration with their respective solubility at the beginning of storage (po_2 approx, 160mm Hg in plasma pCo_2 62 mmHg in plasma, assuming Ph 7.2, T=295k, [HCO_3] = 24.0 mmol/Lin plasma, the nature of the breathable container, gas consumption and /or production via oxidation metabolism, and bicarbonate decomposition via pH changes. [6]

Table 2 : PCo2 changes from Day 0 to Day 8

Day	Mean	N	Std Deviation	T value	P value
Day 0	119.482	625	6.8044		
Day 3	119.556	625	6.7827	-6.181	<0.0001
Day 5	119.634	625	6.8417	-10.661	<0.0001
Day 7	119.721	625	6.8334	-14.524	<0.0001
Day 8	119.771	625	6.8296	-13.855	<0.0001

Mean percentage change in Pco2 from Day 0 to Day 8 was 0.24%

Mean PCo2 levels when compared to Day 0, it was observed that Pco2 values increased on all the days and this difference was also statistically significant on all the days (Table 2 and Fig 2)

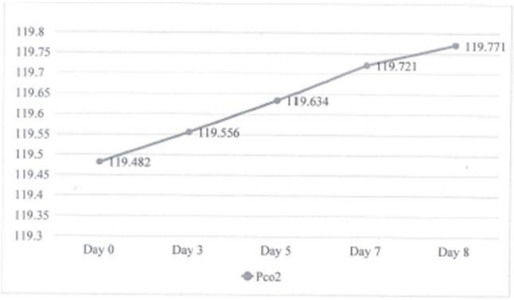

Figure 2: Line diagram showing Pco2 Changes

The primary contributor to the decrease of pH in PCs is glucose metabolism via glycolysis and the production of lactate. Platelet metabolism required some direct correlations such as those between glucose and lactate, where glucose consumption directly results in lactate production, as well as between pH and pCo_2 [7] where the pH correlates negatively with pCo_2 (as H+ ions drive the reaction of carbonates toward production of $Co_{2\,(aq)}$) (Table 4: Fig 4)

The change of blood gas levels reflects the gas exchanging ability of the storage container. Maintaining high po_2 and low pCo_2 is crucial during Platelet storage because hypoxia may lead to increased glycolysis, lactate production, and subsequent Ph decrease. O_2 equilibration is faster

than O_2 consumption because Po_2 increases steadily during storage. [8] On day 1 and all the PCs contain a pO_2 of 75 to 80 m HG; therefore they all experience the same driving force toward equilibration with atmosphere pO_2 at 180 to 200 mmHg.

It was observed that there was statistically significant difference in Po2 on day 3, 5, 7 and 8 days when compared to day 0 values. There was increase in Po2 levels with increase in storage duration. Though there was statistical significance the values of Po2 were in normal range of 80 — 120 mmHg. (Table 3 and Fig 3)

Table 3: pH2 Changes from Day 0 to Day 8

Day	Mean	N	Std. Deviation	t value	p value
Day 0	108.848	625	4.14		
Day 3	109.635	625	3.94	-47.332	<0.0001
Day 5	110.520	625	3.81	-33.229	<0.0001
Day 7	111.092	625	3.55	-37.511	<0.0001
Day 8	111.884	625	3.38	-42.045	<0.0001

Mean Percentage change in Po2 from Day 0 to Day 8 was 2.82%.

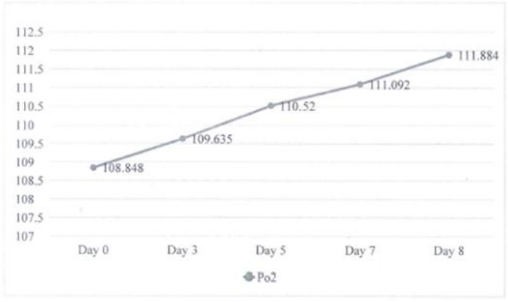

Figure 3: Line diagram showing Po2 changes

DISCUSSION

Since platelets were discovered in the year 1881, there has been considerable efforts to know the exact structure and function of the platelets along with their clinical use.Normal discoid platelets, when exposed to a light source and gently rotated or squeezed, refract light and produce a "swirling" phenomenon that can be identified by trained personnel in Blood Banks for transfusion. Studies done during the 1970s show that maintenance of normal discoid morphology during storage may be essential for maintenance of platelet viability and also as an effective quality control measure with levels of 1-4 depending upon their grading which can range from poor – excellent respectively.[3] Swirling – negative PCs should be investigated further and discarded if they fail to meet other standards of quality.[3]

Table 4: Lactate Changes from Day 0 to Day 8

Day	Mean	N	Standard deviation	T value	P value
Day 0	23.958	625	4.77		
Day3	24.080	625	4.76	-43.352	<0.0001
Day5	24.121	625	4.72	-16.694	<0.0001
Day 7	24.193	625	4.72	-22.490	<0.0001
Day 8	24.275	625	4.71	-27.962	<0.0001

Mean Percentage change in Lactate from Day 0 to Day 8 was 1.43%.

Mean LDH levels when compared to Day 0, it was observed that LDH values increased on all the days and this difference was also statistically significant on all the days. (Table 4 and Fig 4)

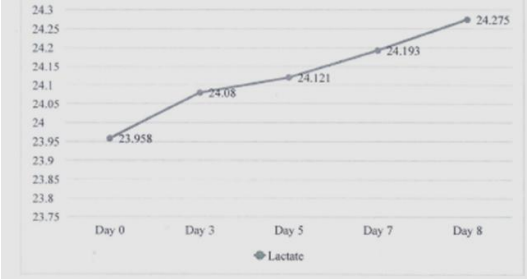

Figure 4: Line diagram showing Lactate changes

Platelets carry active mitochondria which generate 85% of the adenosine triphosphate supply through oxidative phosphorylation. The remaining energy demand is largely fulfilled by anaerobic respiration) leading to an accumulation of lactic acid in suspensions like on concentrates for transfusion. Acidosis adversely effects platelet behavior (in) directly causing the cell to swell and adopt a spherical instead of discoid shape. Therefore, pH-determination is a straightforward method commonly used to report product quality.[3] Sometimes, pH and other indicators of quality correlate, but this is not always the case with precedents on both sides, that is, issued concentrates with acceptable pH but poor quality or vice versa. This especially holds for platelet products prepared in additive solutions, which often contain buffering salts that this may hold pH, but not necessarily biological integrity.[3]

During the last 3 decades, platelet were stored in first generation containerers-composed of polyvinyl chloride plastic containing diethylhphthalate under continuous agitation.[1] However, even with continuous agitation and suspension of the platelets in plasma volume of 40 to 50 ml many PC exhibited a fall in pH from a initial value of 7.0 - 7.2 to less than 6.0 within 72 hours of storage at 20 to 24 ^{o} C in first generation containers, as stipulated by Food and Drug Administration Regulations published in 1974. [3]

The reduction is pH was problematic because of the association between reduced pH levels and substantially decreased posttransfusion viability. The reduction is pH was caused by formation of lactic acid which depleted the bicarbonate buffer present in the plasma. Enhanced lactic acid production was associated with increased glucose consumption.[1]

For energy production, platelets use glucose and free fatty acids from plasma and e.g., acetate added to the synthetic medium. Almost all glucose used is converted through pyruvate to lactate and a hydrogen ion. In storage medium, the hydrogen ion is buffered by bicarbonate, which is thereby converted to carbon dioxide that can leave the medium through the walls of the container. Very little, if any, pyruvate is decarboxylated to acetyl-CoA and carbon dioxide. Thus, storage solutions need to reduce the rate of glycolysis to a minimum and provide a mechanism to buffer the hydrogen ion that is produced by glycolysis. Also the amount of plasma should be optimal.[3]

The morphological transition from disc to sphere mentioned above is often exploited for platelet concentrate quality determination[3]. The method results in a 'swirl (ing)' score, referring to the level of anisotropic and nematic light scattering behavior of discoid particles in suspension. The elegance is mainly of practical nature as it is easy and quick allowing transfusion services to discard products below threshold scores if required. However, the assay hardly has dynamic range, is prone to subjectivity and does not entirely or necessarily correlate with other platelet (quality) markers.[3]

The biochemical, structural and functional changes that occur during platelet storage under blood bank conditions are collectively known as platelet storage lesions (PSL). These lesions may have a impact on platelet viability and haemostatic function. PSL is associated with morphological changes and platelet activation followed by microvesciculation and loss of function, leading to platelet transfusion failure. Various laboratory tests have been recommended to study PSL ranging from most simple test such as pH to more complex tests of platelet function. [9]

Although pH measurement using simple colorimetric dip sticks are widely available, economical and easy to use but these methods are highly susceptible to error, require a large sample volume for accurate measurement, and carry high risk of contamination. Similarly, blood gas analyzers (BGAs) also have the same deficiencies and in addition, the BGAs are expensive, labor-intensive and requires mandatory temperature corrections because BGAs are designed to measure pH of whole blood at 37°C at a pH of around 7.4 and pH values must be corrected to report pH at 22^{o}C. [9]

Platelet viability is markedly affected by pH. The final pH of platelet concentrate and hence in-vivo recovery and survival will depend on the type of storage container, storage conditions and the volume of residual plasma. [8] Therefore platelet storage bags must allow for free gaseous exchange. Platelets must be stored in sufficient plasma, whose bicarbonate content acts as a buffer, to maintain pH at greater than 6.2. Depletion of bicarbonate by high content acts as a buffer, to maintain pH at greater than 6.2. Depletion of bicarbonate by high lactic acid level, typically at 20-25 mmol/l, lowest pH and results in loss of platelet viability. [8]

The pH is an important and valuable parameter for the assessment of the in vitro quality of PCs.[1]

The American Association of Blood Banks (AABB) recommended that platelets with Ph < 6.2 should not be used for transfusion,[10] and in Europe the same recommendation applies to platelets with Ph > 7.4.[11] As per the Drugs and Cosmetics Act of India , minimum pH should not be < 6 at any given day of storage.[12]

For instance, German, European and US guidelines require the measurement of pH as essential quality control parameter and define the reference ranges, e.g.6:4:7:8,>6:4 and $\geq$6:2,respectively.[13] Some of these guidelines recommend that the pH has to be reported at 22^{o} C. The US guidelines do not directly recommend a definite temperature but refer to FDA criteria recommending that the pH has to be measured at the storage temperature of the unit.[13] The Dutch guidelines, however, require a pH between 6:3 and 7:3 measured at 37^{o} C. [13] According to the European guidelines, that pH may be measured at

another temperature and converted by the calculation for reporting at 22 °C.[13]

The correct measurement of the 'true' pH in a PC, however, remains a challenge as pH may depend on the kind of sampling method of the product or the machine used for the determination Review literature regarding pH and PCs showed that 27.6%, 38.2% and 31.6% of the investigators reported pH at 22 ° C, 37 ° C or gave no reference to temperature, respectively [(10).] The fact that pH reading of PC may vary by as much as 0.22 if measured at 22 ° C vs. 37 ° C illustrates the possible bias when comparing published pH values.

Of late, usage of Platelet additive solutions (PAS) for the storage of Platelets has become popular. Hence, the accurate estimation of pH is important. Modern blood gas analyser (BGA), which are primarily manufactured for the measurement of the pH of the patient's whole blood, are widely used for the determination of pH of PCs 37° C. The same analyzer can calculate the pH also for other temperature such as 22° C, but this conversion is based on a formula or equation factor for whole blood . [14]

Many investigators had measured conversion factors defined as the change of the pH per 1° C difference (dpH/ dT) for plasma and whole blood with values between 0.0118 and 0.0170 OR 0.0147 and 0.0276, respectively.[14] Analysis of these data revealed that the temperature-dependent pH changes are higher in whole blood than in plasma. Therefore, the usage of the correction formula for whole blood will result in inaccurate estimation of pH values at 22 °C when applied for PCs stored in 100% plasma.[14] As the pH difference between plasma and whole blood seems to be negligible significance this may not be of great clinical relevance which may not necessarily be applicable for different types of PAS currently used.[14]

The dpH/ dT for mixtures of PAS and plasma had only been published by Gullikson et al. [(15)] who reported dpH/ dT for mixtures of 30-40% plasma and PAS-I or PAS-II with 0.00025 ± 0.0003 pH unit per 1 ° C, respectively. Studies conducted by Moroff et al . who concluded that one question factor is not suitable for all pH levels.[16] This association, however, is strong and higher significant for PCs containing a high plasma carryout but much weaker or not existing anymore for PCs containing 30-40% or 20% of plasma, respectively.[16]

Furthermore, it should be mentioned that the usage of conversion factors per 1° C (dpH/ dT) may not be correct as the change of pH does not happen in accordance with a linear function [(14)]. Therefore, studies conducted by Ringwald. J et al suggest that only specific dpH indicating that total temperature-dependent pH change between 22 and 37 °C should be used with regard to pH when using blood gas analyzers.[9]

Because of the underlying complexities and relevant guidelines it is recommend that when using blood gas analyzers calibrated for pH determination at 37 ° C aiming a high level of preciseness, the pH value may be reported as pH37 as long as no precise conversion factor is available for the actual mixture of the distinct PAS and plasma.[14] With regard to daily routine practice and the need to report pH22 at a level of one decimal in accordance with several guidelines , Recent studies suggest that at least for PC containing 20% or 30% plasma no correction factor and for PC containing more than 40% plasma a correction factor of 0.05 before rounding the value to one decimal may be appropriate to end up with a sufficient and satisfactory preciseness. [14] This approach would be definitely more correct than reporting pH22 after conversion using the formula for whole blood. [14]

The pH value is established as quality parameter for stored platelet concentrates (PCs). pH values below 6.8 are associated with significant reduction of the in vivo survival of the stored platelets. During storage, platelets generated metabolites, especially lactate which acidify the platelet concentrate. Correlation of pH decrease and morphological/physiological changes has been consistently demonstrated in several studies. [17]

The pH decreases during storage depending on the stabilizer in plastic platelet storage bags and storage conditions used. Increased platelet glycolysis resulting in a fall in pH to levels approaching 6.0 in PC stored in plasma is associated with substantial loss of viability.[18] The majority of fresh, un-stimulated platelets are discoid with few projections. In the early observations of PCs stored at 20-24 ° C, a gradual disc-to-sphere transformation was seen during storage. Some of these changes are reversible with incubation at 37 ° C in fresh plasma. Qualitatively similar changes occur during PC storage, but in first generation containers major additional variable is pH fall. If pH does not fall to less than 6.8, platelet volume decreased by approximately 10% during three days. However, if pH falls below this level, these is progressive rise in platelet volume and decrease in

density suggesting swelling due to influx of extracellular fluid.[18] The swelling begins at pH of 6.8 and reaches it maximum at a pH of 6.0, at which point platelet volume is increased almost two-fold. At the same time, there is an accelerated rate of disc-to-spheres transformation so that only swollen spheres are seen if pH reaches 5.7 to 5.9. These changes are almost entirely reversible if pH stays above 6.1, but they are not reversible if pH falls below 6.1. These morphological observations correlate well with the result of viability in vivo. [18]

Keeping in view of the numerous drawbacks regarding pH estimation of platelets by using the conventional methods Reed and Coworkers described a method for noninvasive pH measurement with a fiber optic fluorescence detector. Based on the ability of certain organic compounds to change color with pH, conjugated fluorescent dyes were immobilized on a nitrocellulose membrane. Light emitted through the polycarbonate window generates a pH –dependent signal at 600nm and a pH-independent signal at 568nm. The calculated ration of both signals represents the Ph value.[19]

This recent, non invasive, fluorescent method of pH monitoring in PCs require small 15 ml storage bags with integrated pH sensor offers the opportunity for pH screening in a small volume during storage of PCs. By sterile docking the 15-ml bag to the sampling bag of the cell separator the PC remain unattached and available for therapeutic treatment with excellent reproducibility.[20] Preparation of platelet units according standards operation procedures and sterile docking of the satellite bag for noninvasive pH measurement should excluded the possibility of a bacterial contamination.

This technique of noninvasive pH measurement is easy to perform and do not require any expertise in laboratory methods, offering the opportunity to include this method in the quality management of all blood banks particularly in a rural and resource constrain setup.[19] This could be done either in the blood bank with the small bag as backup sample or in the hospital with the PC in a storage bag with integrated pH device

CONCLUSIONS

The pH estimation of platelets remains a challenge for safe blood transfusion practices particularly in a rural and resource constrained setup like us because the current methods for pH measurement are not practical for 100% Screening of PCs. Bacterial detection by monitoring pH during PC storage is complementary to the BacT/ALERT culture method. The culture method measures CO2 evolution as a surrogate marker for bacterial growth and is faster since growth in enriched medium is at 37°C, but can fail due to sampling error at low CFUs/mL. pH measurement (atleast daily) detects growing bacteria in PC units without sampling, but is slower which needs further validation and standardization particularly in a rural and resource constrain setup.

AABB has recommended that each transfusion center formulate a policy to be followed if they are to transfuse ABO group incompatible PC.[21] Our institute has adopted the policy of issuing only ABO compatible PC. However, there are instances where ABO incompatible platelets have been used in emergency settings or when there were no compatible platelets available. In such cases stringent quality control measures such as swirling, volume, platelet count and WBC count per bag and pH changes [22] were strictly followed as per the AABB guidelines.

Our study has demonstrated that the Ph and platelet counts can form an integral part of a rapid, simple and practical method for validation of collection, processing and storage procedures that is useful for routine quality monitoring and pre-release testing of PC. In addition, this study shows that it is possible to sample all units and subject them to pre-release quality checks. Implementation of these will help in continuous quality improvement as well as entrench a standardization and harmonization program on platelet quality monitoring.

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