International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN **Original Research Article**

Effects of conserving plasma pools on coagulation exploration tests

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Abstract

Introduction: Coagulation exploration tests are part of the most commonly performed hemobiologic practice in the hematology laboratory of the Joseph Ravoahangy Andrianavalona University Hospital Center (JRA UHC) Antananarivo. As an internal quality control, it uses commercial reference plasmas. These are expensive with minimal shelf life. This study aims to identify the preservation method that allows more extended stability of coagulation proteins.
Methods: This is a study spanning a period of 31 days, ranging from October 02nd to November 03rd, 2018. A series of two tests: prothrombin time (PT) and Partial thromboplastin time (APTT), was performed on normal and pathological plasmas stored in 3 different ways: refrigeration at 8-10 ° C, freezing at -20 °C and thawing in a water bath at 37 °C for 5 minutes and freezing at -20 °C and then thawing at room temperature 20-24 °C.
Results: Over the 31 days, PT and APTT median values from the normal plasma pool according to the conservation methods are: 29.4 s, 14.3 s, 15.3 s and 66.9 s, 35.8 s, and 36.8 s. And those from the pathological plasma pool according to the methods of preservation are 37.9 s, 17.2 s, 17.2 s and 88s, 40.1s, and 40s. Freezing methods stabilize coagulation proteins more than the refrigeration method. Thawing in a water bath is better than that at room temperature to stabilize the pool of plasmas.
Conclusion: Method number two, freezing at -20 °C and then thawing in a water bath at 37 °C for 5 minutes before testing, is the most effective for maintaining stable plasma.
Keywords: Prothrombin time, partial thromboplastin time, plasma pools, Madagascar.

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***Article History:**
Received: 16/06/2020
Revised: 23/07/2020
Accepted: 23/07/2020
DOI: https://doi.org/10.7439/ijbar.v11i7.5445

QR Code

How to cite: Manantsoa S. N, Rabearison J, Randriamahazo T. R, Raheritiana T. M, Rasamoelina A, Rakotovao L. A and Alson A. O. R. Effects of conserving plasma pools on coagulation exploration tests. *International Journal of Biomedical and Advance Research* 2020; 11(07): e5445. Doi: 10.7439/ijbar.v11i7.5445 Available from: https://ssjournals.com/index.php/ijbar/article/view/5445

1. Introduction

Hemostasis is the set of physiological cellular and biochemical phenomena that contribute to stopping bleeding by the formation of a fibrin clot [1]. The prothrombin time (PT) and the activated partial thromboplastin time (APTT) are part of the routine tests exploring coagulation [2]. According to quality standards, medical analysis laboratories must use reference plasmas marketed every day as internal quality control (IQC). However, these plasmas are expensive and have a very limited shelf life. Therefore, the use of plasma pools from normal or pathological patients under the same pre-analytical and analytical laboratory conditions can be recommended to reduce costs in developing countries like Madagascar. We have set the objective of determining the optimal storage factors (storage temperature, storage time and defrosting mode) for a pool of plasma reconstituted from patient samples received in the laboratory.

2. Materials and methods

This is a prospective analytical study from September 2017 to February 2019 carried out at the laboratory of hematology Joseph Ravoahangy Andrianavalona University Hospital Center (JRA UHC). The study population consists of two groups of plasma pools received at the laboratory for hemostasis tests during the study period.

Pool number 1 consisted of ten (10) normal plasma samples with a PT greater than 70% and Quick time (QT) between 11.5 s to 15.5 s and APTT ratio between 0.8 to 1.2. Pool number 2 consisted of ten (10) pathological plasma samples with a PT between 45% to 70% and QT greater than 15.5 s and APTT ratio greater than 1.2.

These plasmas were aliquoted to carry out the daily tests and the conservations. Three groups of aliquots were formed with, in each group, 30 aliquots of 200 µl of plasma each. Three conservation methods have been chosen:

- Storage in the refrigerator at + 8 °C to + 10 °C (Method 1)
- Freezing at -20 °C then thawing in a water bath at 37 °C (Method 2)
- Freezing at -20 °C then thawing at room temperature + 20 °C to + 24 °C (Method 3)

All the samples were analyzed on a Star-T4®Stago France semi-automatic device according to a chronometric measurement, the QT measurements by NEOPLASTINE® CI PLUS stago (France) and the APTT by APTT Cephalin Silica® CYPRESS DIAGNOSTICS (Belgium).

The measurements were carried out in duplicate for each sample and each test. The coagulation times were measured daily from day 0 to day 30 between 6 AM and 8 AM in the same conditions, by the same operator and using the same reagents.

The data was analyzed using Epi INFO® version 7.0 software. The results of the coagulation times obtained were compared with the comparison of the means with a *p*-value <0.05 as a significant difference.

3. Results

During our study, the PT and APTT values of the normal and pathological pool for Method 1 decrease over time. Those of Method 2 and 3 are more stable.

Method 1 (storage in the refrigerator at + 8 ° C to + 10 ° C)

The PT for the normal pool showed a drift (100% to 67%) from the third day of storage in the refrigerator at + 8 ° C to + 10 ° C (Figure 1). For the pathological pool, the PT began to drift on the 6th day of storage (60.1% to 31%). (Figure 2)

The APTT for the normal pool showed a drift (33 s to 43.9s) from the second day of storage in the refrigerator at + 8 ° C to + 10 ° C (Figure 3). For the pathological pool, the APTT began to drift on the third day of storage (41.5s to 48.6s). (Figure 4)

For the normal pool, we found a median of 29.6s for QT and 29% for PT and APTT at 66.9s and the pathological pool QT at 17.2s, i.e. PT at 64% and APTT 88 s.

Method 2 (freezing at -20 ° C then thawing in a water bath at 37 ° C)

The PT for the normal pool showed a drift (100% to 54%) from the 8th day of conservation (Figure 1). For the pathological pool, the PT also began to drift on the 22nd day of storage (from 60.1% to 39%) (Figure 2).

The APTT for the normal pool showed a drift (33 s to 51.1 s) from the seventh day of storage (Figure 3). For the pathological pool, the APTT began to drift on the 28th day of conservation (i.e. from 41.5 s to 51.1 s). (Figure 4)

For the normal pool, we found a median of 14.3 s for QT, 90.5% for PT and APTT at 35.8 s and the pathological pool QT at 17.2 s, i.e. PT at 64% and APTT 40.1 s. (Figure 2)

Method 3 (freezing at -20 ° C then thawing at room temperature + 20 ° C to + 24 ° C)

The PT for the normal pool showed a drift (from 100% to 66%) from the 2nd day of storage (Figure 1). For the pathological pool, the PT began to drift around the 13th day (60.1% to 43%) of conservation. (Figure 2)

The APTT for the normal pool showed a drift (from 33 s to 41.6 s) from the 17th day of storage (Figure 3). For the pathological pool, the APTT began to drift around the 29th day (i.e. 60.1% to 30.8%) of conservation. (Figure 4)

For the normal pool, we found a median of 15.3 s for QT and 79% for PT and APTT at 35.8 s and the pathological pool QT at 17.2 s, PT at 63.5% and APTT 40 s.

For PT and APTT, the comparison of the medians by the Wilcoxon Mann-Witney tests are significantly different between the values of PT and APTT of the normal (table 1) and pathological (table 2) pool according to the method of conservation of method 1, method two and method 3 with p <0.05.

Statistically, method 2 is the best method for maintaining the stability of coagulation proteins, then method three and finally method 1.

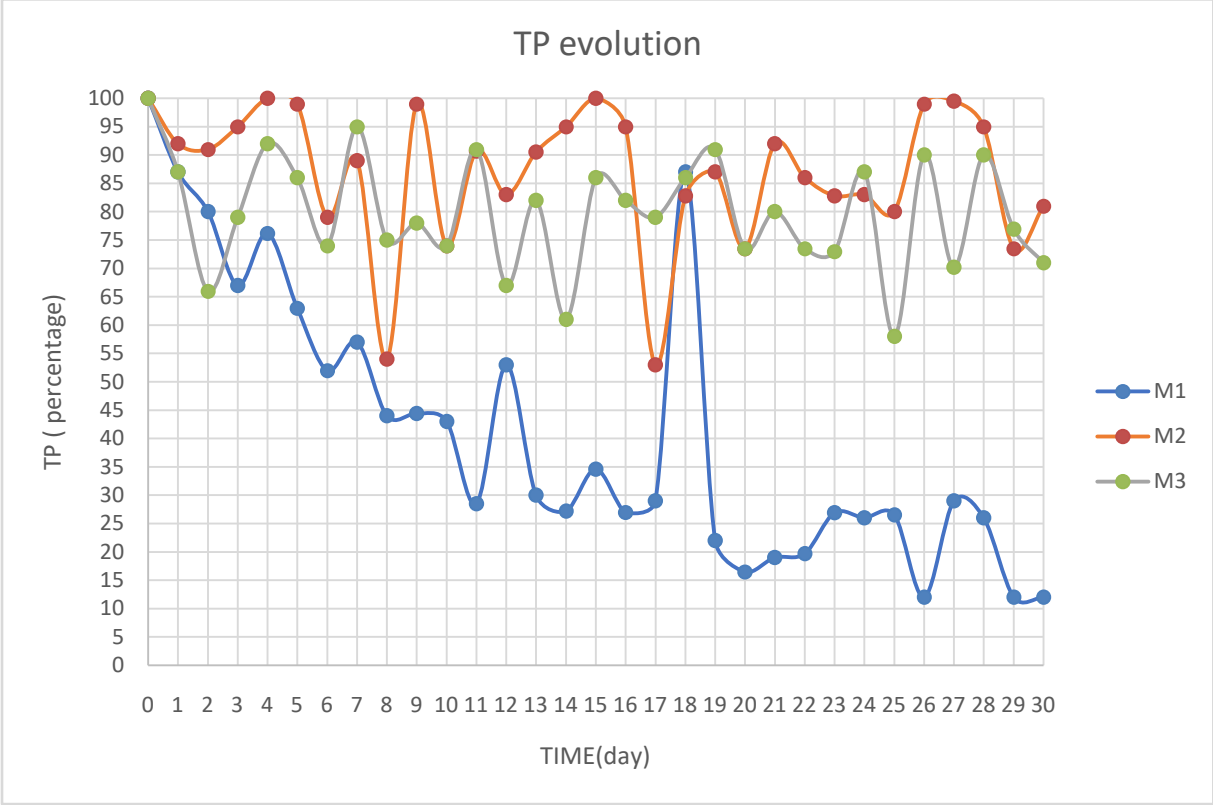

Figure 1: Evolution of the PT of normal pools according to conservation methods

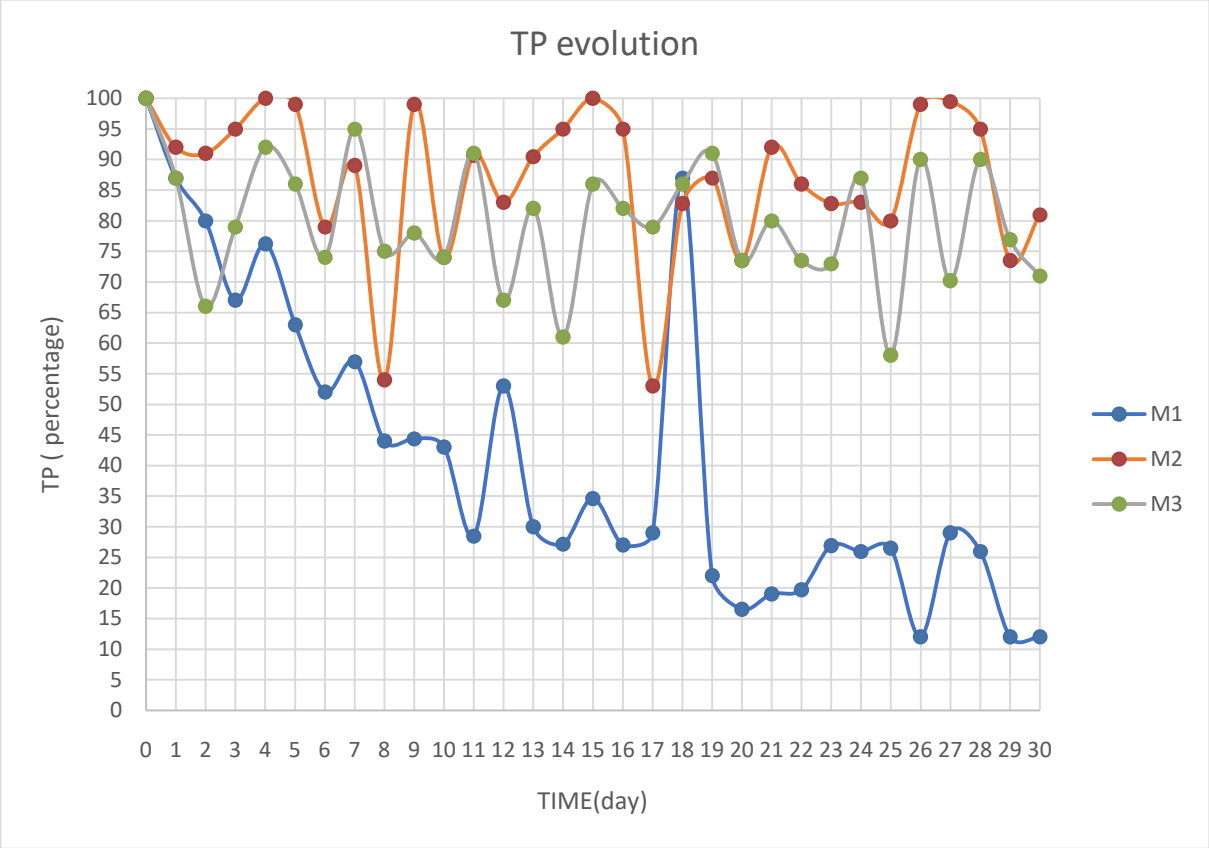

Figure 2: Evolution of the PT of pathological pools according to conservation methods

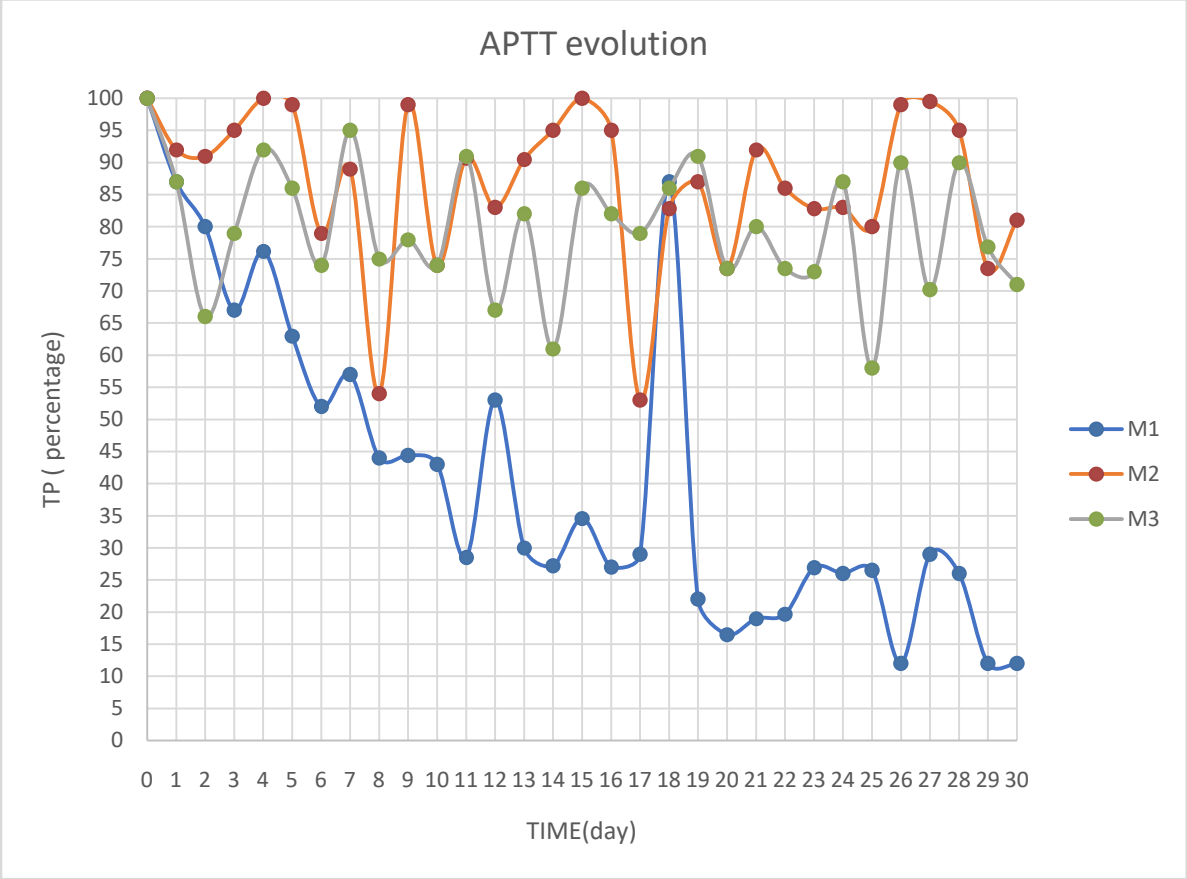

Figure 3: Evolution of the APTT of normal pools according to conservation methods

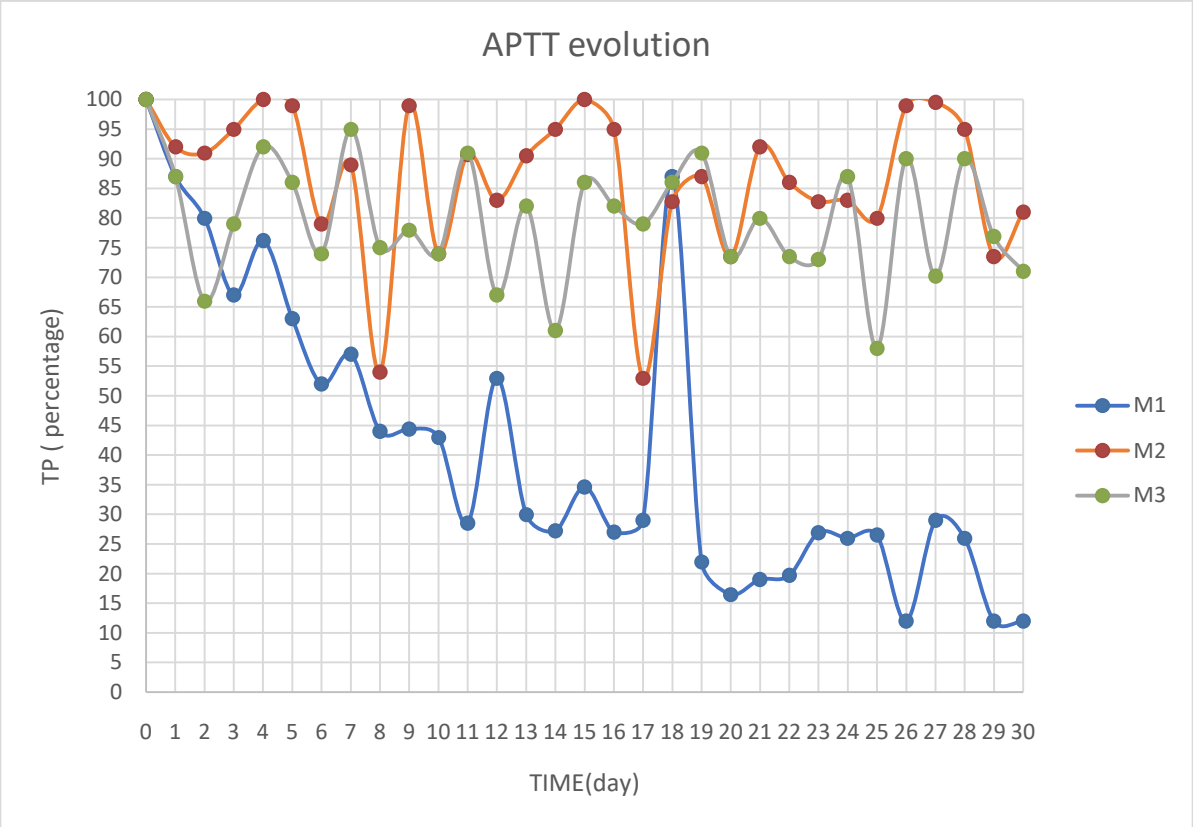

Figure 4: Evolution of the APTT of pathological pools according to conservation methods

Table 1: Wilcoxon Mann Whitney test on the result of PT and APTT of normal pools

PT/APTT (%/sec)	Variance	Minimum	**Médiane**	Maximum	p
Method 1	615.03/638.54	12,00/31.50	**29,00/66.90**	100,00/122.10	0, 001
Method 2	148.09/23,97	53,00/26.10	**90,50/35.80**	100,00/51.10	0, 001
Method 3	101.90/40.13	58,00/27.60	**79,00/36.80**	100,00/63.10	0, 001

Table 2: Wilcoxon Mann Withney test on the result of PT and APTT of pathological pools

PT/APTT (%/sec)	Variance	Minimum	**Médiane**	Maximum	p
Method 1	283,95/1875,23	12,00/41,50	**21,00/88,00**	67,00/180,00	0, 001
Method 2	65,18/15,91	38,50/31,60	**64,00/40,10**	78,00/51,10	0, 001
Method 3	57,98/11,74	43,00/29,30	**63,50/40,00**	80,00/46,00	0, 001

4. Discussion

Knowledge of the effective method of preserving pools of normal and pathological plasmas allows not only reliability in the rendering of hemostasis assessments resulting inadequate patient care, but also the reduction of the hospital budget allocated to the purchase of reference plasmas in a developing country.

The study by Raoau on the effect of different storage conditions on the PT and APTT values showed that a significant difference was observed between the PT and APTT values for normal plasmas after 12 hours of storage at + 4 ° C [3]. Another study conducted by Sultan A.M. Saghir1 in Malaysia showed that the value of PT could not be accepted after 4 hours and APTT near 2 hours if it is stored at room temperature or in the refrigerator [36]. Other studies have claimed that coagulation test times are increased or decreased and that the activity of coagulation factor was significantly reduced at 4 ° C. [4] The hypothesis would be the possible activation of factor VII by the cold, loss of von Willebrand factor and platelet lysis [5]. This confirms our hypothesis on the large discrepancies in results on refrigerated plasmas at +8 to + 10 °C (Method 1).

In contrast, fresh frozen plasma (FFP) at temperatures below -25 °C helps maintain the integrity of labile coagulation factors and can be stored for up to 36 months [6]. A freeze-thaw study in a water bath at 37 ° C for 6 minutes concluded that the results of freeze-thaw stability were affected by time and temperature [7]. Another study, almost similar (method 2) to ours, showed that the value of PT is valid for one month and, 15 days that of APTT [48]. According to the literature, previously frozen samples should be quickly thawed at 37 ° C in a water bath for 5 to 10 minutes or until completely thawed [5]. Close monitoring during this period is necessary to avoid inadequate or excessive incubation at 37 ° C. The integrity of the samples may be compromised if the samples are not completely thawed or if they are kept for too long at 37 ° C. Thawed plasmas should be stored at 18-25 ° C (i.e. room temperature) and analyzed as soon as possible [5]. For optimal use, normal or pathological control plasma pools must then be renewed every seven days for this preservation method. Thus, the similarity of results could be observed with the study conducted by Ying Zhao [8].

A study carried out by Lamboo in Leiden in 2006 evaluated the coagulation parameters of thawed FFP during storage at room temperature (method 3) up to 6 h and storage at 4 ° C for two weeks. It was found that there were no significant differences between the PT values while the APTT values prolonged by 6% at the end of the study duration [9].

5. Conclusion

This study shows that the best preservation method is freezing at -20 ° C and then thawing in a water bath at 37 ° C, but it should be limited to 7 days for optimal use. However, each method has its advantages and disadvantages depending on the level of laboratory equipment.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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