

STANDARDIZATION OF TEMPERATURE IN HANDLING AND STORAGE OF PLASMA FOR COAGULATION TESTING

by
David L. McGlasson, M.S., CLS
Wilford Hall Medical Center
Hematology Department
Lackland AFB, TX. 78236

INTRODUCTION

One of the most frequently asked questions in hemostasis and coagulation laboratory testing deals with specimen handling and processing of samples for coagulation factor assays, particularly Factor VIII procoagulant activity (VIII:C), a thermolabile factor. How long can we keep it in the refrigerator? How do we freeze it? How can we store the sample for a VIII:C if it comes in at 3 a.m.? These questions are rarely, if at all, referred to in textbooks or other literature. We found little consistency in the information supplied as to specimen handling. Recommendations range from immediate testing to storage at -20°C and -70°C for indefinite periods with only one exception that deals with stored plasma for use in activity curves.¹⁰ At times one even finds controversial information concerning the handling of fresh frozen plasma for transfusion purposes where preservation of VIII:C is crucial.^{1,4,6,11,12,13,15}

This study was conducted in an effort to establish proper handling and storage of plasma for laboratory testing of VIII:C activity.

Materials and Methods

The test sample population consisted of eleven male volunteers between the ages of 20 and 35 years of age. Prothrombin times, APTT's, Factor VIII:C, Factor VIII Associated Antigen (Factor VIII:Ag) and Factor VII Levels were assayed. Males were used so that estrogen induced variations would not cause fluctuation of Factor VIII:C and Factor VIII:Ag.⁹ The test subjects had no bleeding histories nor were they taking any medications. Factor VII activity was assayed so that a stable factor activity could be used for comparison.^{16,17}

The samples were collected in the blue topped vacutainer tubes containing 3.8% sodium citrate with a 9:1 ratio of blood to anticoagulant. These samples were checked for clots and then centrifuged at 3200 rpm for 12 minutes at room temperature. The supernatant plasma was separated within 20 minutes of collection and transferred to 12 x 75 mm plastic tubes with caps. The samples were visually checked for hemolysis. Each specimen was divided into three containers. One set was snap frozen in liquid nitrogen and stored at -70°C for 5 days. A second set of samples was frozen at -20°C for 5 days. The third set was kept on ice and the assays were performed within 90 minutes from obtaining the sample. All reagents used to perform the coagulation tests were Dade products. One stage prothrombin times¹⁴, and APTT's^{3,8} were performed using Thromboplastin and Actin reagents. Controls used were Citrol I and III. Factor VII and Factor VIII deficient plasmas used for corresponding factor

assays were Dade products. The Factor VIII:C and Factor VII assays were performed by one stage methods using APTT^{2,3,5,7} and PT^{2,3,5,10} methods correspondingly. The Factor VIII:Ag was assayed by the Laurell-Rocket immunoelectrophoresis technique⁸, using Factor VIII from Cal-Biochem-Behring Co.

The instrument used to perform all the PT, APTT's was the MLA-700. The coagulation factor assays were performed using the BBL fibrometer. The VIII:Ag determinations required a Buchler power supply and a Cal-Biochem-Behring water cooled electrophoresis chamber.

Each group of samples had normal and abnormal controls tested with them. All assays except the VIII:Ag were run in duplicate. The statistical analysis was done using mean, coefficient of variation, and standard deviation of each group. The groups test precision were compared by using the Spearman-Rank correlation coefficient. Differences are indicated by $P < .01$ significance level.

Results

The results of this study are shown in Table 1. The statistics reflect that the group means are significantly different ($P < 0.1$) in the Factor VIII:C. This reflects a drastic reduction of the thermolabile VIII:C due to improper storage. A loss of 50.4% was evident in the -20°C samples when compared to the baseline activity level of the unrefrigerated samples. All of the other parameters of Table 1 show no statistically significant differences.

Discussion

This study does not directly reflect the variability between laboratory test method differences and reagent differences. The true importance of this study can be shown in a possible misdiagnosis of patients that have borderline Von Willebrand disease and its variants or those individuals who may be Hemophiliacs. This is particularly reflected by the mean results for the Factor VIII:C in plasma of 139.97% in the unrefrigerated samples assayed within 90 minutes, 147.4% in the -70°C specimens and the low activity at -20°C at 89.6%. The study emphasizes the importance of standardization in specimen processing and handling of sample in both clinical and reference lab settings. Shipping of specimens must include strict instructions to insure that thermolabile blood coagulation factors are protected from deterioration. Samples for blood coagulation factor testing should be run as quickly as possible or stored at -70°C after snap freezing to ensure the complete integrity of the sample. Storage of samples for long periods at -20°C or in regular refrigerator freezers is not sufficient for specimen storage.

TABLE 1

	PT	APTT	F.VIII:C	F.VIII:Ag	F.VII
Unrefrigerated					
MEAN	11.21	24.63	139.97	92.77	123.48
STANDARD DEVIATION (SD)	0.55	1.88	44.40	11.14	32.44
COEFFICIENT OF VARIATION (CV)	4.86	7.63	31.7	12.0	26.27
RANGE (2SD)	10.13-12.31 sec.	20.57-28.39 sec.	51.18-228.76%	70.49-115.05%	58.6-188.4%
STANDARD ERROR	.190	.745	8.17 ^{13.4}	4.47	8.26
-70°C					
MEAN	11.37	22.5	157.4	95.9	118.1
STANDARD DEVIATION (SD)	0.48	2.53	52.9	11.7	29.3
COEFFICIENT OF VARIATION (CV)	4.18	11.2	33.6	12.2	24.8
RANGE (2SD)	10.42-12.32 sec.	17.47-27.6 sec.	51.5-263.3%	72.6-119.3%	59.4-176.8%
STANDARD ERROR	.190	.745	7.75 ^{15.7}	4.47	8.26
-20°C					
MEAN	11.16	24.06	89.6	100.1	114.88
STANDARD DEVIATION (SD)	0.68	2.22	33.1	16.2	20.84
COEFFICIENT OF VARIATION (CV)	6.09	9.21	36.9	16.15	18.1
RANGE (2SD)	9.8-12.5 sec.	19.6-28.5 sec.	23.4-155.8%	67.8-132.5%	73.2-156.6%
STANDARD ERROR	.190	.745	7.75 ^{7.7}	4.47	0.26

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Research Definitions

The following phrases, frequently found in technical writings, are defined here for your amusement and enlightenment. This list was plagiarized from some unknown worker who had evidently read too many scientific papers.

"It has long been known that...." — I haven't bothered to look up the original reference.

"...of great theoretical and practical importance." — It is interesting to me.

"Three of the samples were chosen for detailed study." — The results on the others didn't make sense and were ignored.

"Typical results are shown." — The best results I ever had are shown.

"It is believed that..." — I think.

"It is generally believed that..." — A couple of my friends think so too.

"It is clear that much additional work will be required before a complete understanding..." — I don't understand it.

"Thanks are due to Joe Glotz for assistance with the experiment and to Joe Schwartz for valuable discussions." Glotz did the work and Schwartz explained it to me.

Linda A. Smith