STABILITY OF TOTAL AND FREE PROSTATE SPECIFIC ANTIGEN IN SERUM SUBMITTED TO INTERMITTENT COLD STORAGE CONDITIONS

Asmahan A EL Ezzi and Mohammed A EL-Saidi*

Laboratory of Radioimmunoassay, Lebanese Atomic Energy Commission (LAEC), National Council for Scientific Research (NCSR), Airport Avenue - B.P. 11-8281, Beirut, Lebanon and Rafik Hariri Campus, Lebanese University, College of Science, Biochemistry department, Hadath, Lebanon.

*Utah Valley University, 800 W and University Parkway, Orem, UT 84058-5999, USA.

ABSTRACT

The goal of this study was to determine the stability of Total Prostrate Specific antigen (PSA-T) and Free Prostrate Specific Antigen (PSA-F) in archival serum stored at 4°C and -20°C and subjected to temperature shift due to interruption in power supply. Our study showed that PSA-T was stable up to 285 days and PSA-F was stable for 158 days under these conditions. Since power supply interruption is an unavoidable problem in developing nations, our study has implication on the validity of measurement of PSA-T and PSA-F in serum that was not properly stored due to emergency situations and for certain types of retrospective studies.

KEY WORDS

Prostate Specific Antigen, PSA Total, PSA Free, Immunoradiometric Assay, Interrupted power supply, Stability.

INTRODUCTION

The Prostate Specific Antigen (PSA) is one of the most useful tumor markers. It is proved valuable in determining the stage of prostate cancer and monitoring the response to therapy. PSA is one marker used for the diagnosis of prostate disorders as well as in the cancer screening trials such as the Prostate, Lung, Colorectal and Ovarian Cancer Screening trial (PLCO) and the European Randomized Study of Screening for Prostate Cancer (ERSPC) (1-5). Since the PSA test is not normally considered an urgent test, small and medium - size laboratories, as a cost-effective measure, commonly store their serum for some time until there is an adequate number to assay. Tests take place once or twice a week or even less frequently. Besides, serum samples, frozen or refrigerated, are sometimes transported for very long distances in an uncontrolled environment.

Due to the war in Lebanon that began in 1975 and the ensuing political instability, power outage was a common problem. Lebanese faced periods in which electric infrastructures were completely destroyed and so the nation relied completely on generators. These generators function intermittently, usually 6 hours on and 6 hours off. As a result, blood samples cannot be stored under optimum temperature conditions, particularly in small clinical and research laboratories. Furthermore, patients may refuse to return and give blood samples in case the first samples turn out to be defective. Therefore, PSA values should be interpreted with caution when the samples have been stored in such detrimental conditions.

Stability of PSA stored at various conditions and length of time has been investigated before but in cases the samples were stored at stable temperatures (6-20). To our knowledge, there is no previous work pertaining to interrupted power supply (and therefore unstable storage temperatures). In this study we used archival serum that was previously stored at -20°C for 5 months before being thawed and stability of PSA was monitored and studied over a time. The stability of PSA (PSA-T and PSA-F) was investigated at RT, 4°C and -20°C under continuous power supply. In the second study, stability of PSA was investigated only at -20°C but power supply was interrupted every six hours. Our study determined the extent to which PSA can be reliably assayed in frozen or refrigerated

Address for Correspondence :

Dr. Asmahan A. EL Ezzi

Laboratory of Radioimmunoassay,
Lebanese Atomic Energy Commission,
NCSR, Airport Avenue, B.P. 11- 8281, Beirut, Lebanon.
Phone: 961 3 630 759 (Cell) and 961 1 450 812 Ext. 204 (O)
E-mail: ichheisenana@gmail.com

serum subjected to unstable storage condition.

MATERIALS AND METHODS

The serum samples used in this study were collected from volunteers who participated in a prostate disease screening study conducted in 2005 in our laboratory. The volunteers (3,000 men) consented to have the left over serum to be used in research. Serum samples with known PSA-T values that were left over from these campaigns were stored in the laboratory for 5 months at -20°C . At the beginning of our study, the samples were thawed, and serum having similar values was grouped in different pools according to PSA-T values, regardless of the clinical diagnosis of the samples. New PSA-T and PSA-F assays were performed on each pool (but not on each thawed serum), and the values obtained were considered as its PSA-T and PSA-F initial value (at day 0).

Sample material : Plain Vacutainer tubes (BD, Franklyn Lake, NJ, USA) of 5 ml each were used for the collection of blood. The blood samples were withdrawn from men under non-fasting conditions and prior to digital rectal examination (DRE) or any other procedure related to the prostate. The men ranged in age from 45 to 78 years, with a mean age of 64 years and median age of 62 years. The blood was left to clot, and the serum was separated within 3 hours of the vein puncture. The serum samples were then directly assayed for PSA-T and PSA-F, or kept in the refrigerator at 4°C to be assayed within 24 hours or at -20°C if to be assayed at later time.

Immunoradiometric assay (IRMA) procedures: Total and free PSA were assayed using IRMA kits (Beckman Coulter, Paris, France). Briefly, PSA-T was assayed as follows: Duplicates (100 μl) of calibrators and serum samples (100 μl) were placed into anti-PSA coated tubes (triplicates of serum for the first study and quadruplets for the second study.) A 100 μl of ^{125}I labeled anti-PSA was added to each tube. The tubes were continuously shaken for 2 hours at room temperature (RT). After washing the tubes using a buffer supplied with the kit, the radioactivity was measured for 3 minutes by a gamma counter. The lower limit of detection (LLD) of the PSA-T assay was 0.1 $\mu\text{g/L}$. The coefficient of variation (C.V) calculated from the profiles based on the PSA-T assays of serum samples was $\leq 7\%$ in intra-assay and $\leq 6\%$ in inter-assay for the assay in the range of 1 - 100 $\mu\text{g/L}$.

PSA-F was assayed as follows: Duplicates (200 μl) of calibrators and serum samples (triplicates of serum were used for the first study and quadruplets for the second study) were placed into anti-PSA-F coated tubes. A 100 μl of ^{125}I labeled

anti- PSA-F was added to each tube. The tubes were continuously shaken for 2 hours at RT. After washing the tubes using a buffer supplied with the kit, the radioactivity was measured for 3 minutes by a gamma counter. The LLD of the PSA-F assay was 0.3 $\mu\text{g/L}$. The coefficient of variation (CV) calculated from the profiles based on the PSA-F assays of serum samples was $\leq 2.5\%$ in intra-assay and $\leq 4.3\%$ in inter-assay for the assay in the range of 0.3-30 $\mu\text{g/L}$.

Distribution of serum: We began the stability study by dividing the serum into three different volumes (determined based on the length of each study at each temperature). The first was kept at RT, the second was stored in the refrigerator at 4°C , and the third was kept frozen at -20°C (day 0).

Storage conditions: The first study was performed at RT, 4°C and -20°C and its RT varied between 18°C and 28°C , depending on the seasonal changes. The second study was performed only at -20°C . The samples were studied either under continuous power supply that maintained cold storage temperatures at 4°C or -20°C (the first study), or at -20°C under intermittent cold storage conditions, with the power supply 6 hours on and 6 hours off, day and night (the second study). Furthermore, the doors of the refrigerator and the freezer were opened once or twice during the day, even when the electrical current was off. The temperature in the freezer was measured every 6 hours (when off), and it was between 0 and -8°C .

In addition, the container containing the total amount of each pool of serum was taken out of the refrigerator (just for the time necessary to take the serum) and was put back every day an assay was performed (for the study at 4°C). Now concerning the samples stored in the freezer (for the study at -20°C), they were parceled out into a number of portions, each intended to be thawed and refrozen approximately for 6 times repeatedly. Samples were thawed by placing them first in the refrigerator for about an hour then at RT and were transferred to the freezer soon after pipeting the serum.

The stability of the serum samples was monitored by assaying the PSA-T and PSA-F values for each day studied. The percentage change of PSA for any given concentration was calculated for each day by making use of the initial value at day 0. The length and design of each study were dependent on the quantity of serum available.

Description of the first study: The serum samples previously frozen at -20°C for about 5 months were thawed on day 0 and those with close PSA-T values were grouped together

($n = 120$) to form five pools with PSA-T values: 1.53, 3.02, 6.08, 10.4 and 25.2 $\mu\text{g/L}$ (rounded to two decimals). Their PSA-F values were: 0.3, 0.7, 1.2, 2.0 and 2.1 $\mu\text{g/L}$ (rounded to one decimal). The concentration of PSA-T and PSA-F was measured simultaneously for all samples every working day over a period of 22 days for samples stored at RT and up to 32 days for samples stored at 4°C and -20°C. The daily results of PSA-T and PSA-F were compared to their initial values on day 0. It should be noted that this study was performed under continuous power supply.

Description of the second study: As for the first study, the serum samples previously frozen for 5 months were thawed on day 0 and those with close PSA-T values were grouped to form seven pools ($n = 80$). The PSA-T levels for these pools were measured and rounded: 0.5, 1.06, 2.0, 5.2, 7.8, 15.9 and 51.95 $\mu\text{g/L}$. Their PSA-F levels were: 0.0, 0.27, 0.37, 0.925, 1.49, 1.64 and 2.1 $\mu\text{g/L}$. The stability of the serum samples was then studied only at -20°C, and the freezer was subject to power outage, 6 hours on and 6 hours off. The stability of the serum was monitored once after a week, then after 32, 138, 158, 172, 188, 201, 231 and 285 days.

Statistical analysis: The total number of observations in the first study was 285 for PSA-T and 150 for PSA-F. Each observation represents the mean of a triplet, which makes the total number of sample values 1,305. In the second study, the total number of observations was 77 for PSA-T and 66 for PSA-F. Each observation represents the mean of a quadruplet, which makes the total number of sample values 572.

It should be noted that the mean value of serum concentration was used to represent each concentration. The average change in serum PSA-T or PSA-F concentration (y) as a function of storage time (t) in days, was determined by the simple linear regression model $y = \alpha + \beta t + \varepsilon$, where α (the y -intercept) and β (the slope) are the regression parameters to be estimated from the data, and ε is the error term. This model was found to be accurate in describing the data, and was subsequently used throughout our current study. The significance of the relationship between serum concentration (y) and storage time (t) was tested at the $\alpha=0.05$ level of significance using the p -value corresponding to the t -test (or equivalently the F -test) available in the computer output of the simple linear regression analysis. The p -value approach to hypothesis testing states that if $P\text{-value} \leq \alpha$, the relationship is considered significant, but if $P\text{-value} > \alpha$, the relationship is judged to be insignificant at the α level of significance. For illustrative purposes, we showed the regression analysis results only from the second study (Table 1).

The percentage change (P_c) of PSA-T or PSA-F for any given concentration (c) at a particular day was also calculated using the formula $P_c = [(c - c_0)/c_0] \cdot 100$, where c_0 is the concentration at day 0 (initial value) corresponding to the given concentration c . The percentage change was then displayed in a time-series plot where the horizontal axis represents the storage time in days, and the vertical axis represents the concentration. For illustrative purposes, we showed the time-series plots only for the first study (Figure 1).

Moreover, the changes in PSA-T, PSA-F, PSA-C and the ratio F/T PSA were analyzed by analysis of variance (ANOVA) for repeated measures available in SYSTAT-12 statistical program.

RESULTS

Stability of PSA stored under continuous power supply:

In the first study (Figure 1 and Table 1), the PSA-T at RT showed low and insignificant change ($P = 0.52$) up to day 7 with mean decrease value of 2.27%. This decrease becomes significant ($P = 0.0003$) and increases gradually over time from 9.1% on day 8 to 94.7 % on day 22. The change was not dependent on concentration. For example, on day 15, the mean change was 84% for the concentration 3.02 $\mu\text{g/L}$ and 52.6 % for the concentration 25.2 $\mu\text{g/L}$. At 4°C, the change in PSA-T was low up to 11 days with mean decrease value of 4.3%. At day 22, the decrease was statistically significant ($P < 0.001$) but low, with mean value of 9.5%. At -20°C and after 11 days of storage, the change in PSA-T was low and insignificant ($P = 0.11$), with mean value of 1.02%. At day 22, the change in PSA-T remains insignificant ($P = 0.306$) with mean value of 1.19%.

The PSA-F (Figure 1 and Table 1) showed significant change ($P = 0.0001$) after a day of storage at RT, with a mean decrease value of 6.3%. The decrease rate gradually increases over time to reach 54% on day 11 and 84.3% on day 22 ($P < 0.0001$). At 4°C, the change in PSA-F after a day was low with a mean decrease value of 2.4%. At day 2, the decrease becomes significant and noticeable ($P = 0.0051$), with a mean value of 6.3%, reaching 27.6% on day 11 and 42.5% on day 22 ($P < 0.0001$). At -20°C, PSA-F remained stable for the entire 22 - day period of study, showing insignificant change ($P = 0.28$), with mean value of 3.1% on day 22.

The complexed PSA (PSA-C), which is defined as the difference between PSA-T and PSA-F; namely PSA-T - PSA-F, showed insignificant changes (Table 1) for the entire period of study of 22 days at 4°C, with mean decrease value of 4.3%

Table 1: Archival serum stored for 5 months at -20°C before being thawed and divided into 3 parts: the first was placed at RT, the second at 4°C, and the third at -20°C under continuous power supply. The stability of PSA-T, PSA-F, PSA-C and the ratio F/T PSA of the serum was monitored at these three temperatures for a period of 22 days (The first study)

PSA												
Days		0	1	2	3	4	7	8	9	11	22	
PSA-T, µg/l												p value
RT	Mean ± SD	9.3 ± 9.6	8.9 ± 9.1	8.7 ± 8.9	9.1 ± 9.5	9.4 ± 9.9	9.4 ± 9.6	8.4 ± 9.1	7.7 ± 8.4	6.7 ± 7.6	0.5 ± 0.6	0.0001
	Median	6.08	5.96	5.93	5.84	5.65	5.01	3.91	3.08	2.28	0.18	
4°C	Mean ± SD	9.3 ± 9.6	9.2 ± 9.7	9.1 ± 9.5	9.1 ± 9.3	9.3 ± 9.6	9.4 ± 9.8	9.4 ± 9.7	9.1 ± 9.4	8.9 ± 9.2	8.4 ± 8.7	< 0.0001
	Median	6.08	5.88	5.77	5.88	6.06	5.96	5.81	5.80	5.89	5.40	
-20°C	Mean ± SD	9.3 ± 9.6	9.3 ± 9.7	9.1 ± 9.5	9.2 ± 9.3	9.4 ± 9.6	9.4 ± 9.8	9.4 ± 9.7	9.3 ± 9.4	9.5 ± 9.2	9.4 ± 8.7	0.3064
	Median	6.08	5.82	5.50	5.60	5.98	6.03	5.96	6.09	6.30	5.78	
PSA-F, µg/l												p value
RT	Mean ± SD	1.3 ± 0.8	1.2 ± 0.7	1.1 ± 0.7	1.1 ± 0.7	1.0 ± 0.7	0.9 ± 0.7	0.8 ± 0.7	0.7 ± 0.7	0.6 ± 0.5	0.2 ± 0.3	< 0.0001
	Median	1.22	1.17	1.12	1.10	1.01	0.71	0.47	0.36	0.26	0.07	
4°C	Mean ± SD	1.3 ± 0.8	1.2 ± 0.8	1.2 ± 0.8	1.2 ± 0.7	1.1 ± 0.7	1.1 ± 0.7	1.0 ± 0.7	1.0 ± 0.7	0.9 ± 0.6	0.7 ± 0.5	< 0.0001
	Median	1.22	1.21	1.18	1.15	1.05	1.04	1.02	0.98	0.96	0.83	
-20°C	Mean ± SD	1.3 ± 0.8	1.3 ± 0.8	1.3 ± 0.8	1.3 ± 0.8	1.3 ± 0.8	1.3 ± 0.8	1.2 ± 0.8	1.3 ± 0.7	1.3 ± 0.9	1.2 ± 0.8	0.2810
	Median	1.22	1.24	1.24	1.24	1.22	1.27	1.24	1.23	1.22	1.19	
PSA-C, µg/l												p value
RT	Mean ± SD	8.0 ± 8.9	7.7 ± 8.4	7.6 ± 8.3	8.0 ± 8.9	8.4 ± 9.3	8.1 ± 9.0	7.6 ± 8.5	6.9 ± 7.8	6.1 ± 7.1	0.3 ± 0.3	0.0004
	Median	4.87	4.79	4.81	4.73	4.64	4.29	3.44	2.73	2.06	0.16	
4°C	Mean ± SD	8.0 ± 8.9	8.0 ± 9.0	7.9 ± 8.8	8.0 ± 8.7	8.2 ± 9.1	8.3 ± 9.2	8.3 ± 9.1	8.1 ± 8.8	7.9 ± 8.7	7.6 ± 8.4	0.2609
	Median	4.87	4.67	4.59	4.73	5.01	4.92	4.80	4.82	4.93	4.57	
-20°C	Mean ± SD	8.0 ± 8.9	8.0 ± 9.1	7.8 ± 9.2	7.9 ± 9.2	8.1 ± 9.3	8.1 ± 9.1	8.1 ± 9.0	8.1 ± 8.9	8.1 ± 8.7	8.1 ± 9.0	0.4072
	Median	4.87	4.59	4.26	4.36	4.76	4.77	4.73	4.86	5.08	4.59	
F/T PSA µg/l												p value
RT	Mean ± SD	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	0.3609
	Median	0.20	0.20	0.19	0.18	0.15	0.14	0.12	0.12	0.11	0.31	
4°C	Mean ± SD	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0007
	Median	0.20	0.21	0.20	0.18	0.17	0.16	0.16	0.16	0.15	0.12	
-20°C	Mean ± SD	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.0065
	Median	0.20	0.21	0.22	0.22	0.20	0.21	0.20	0.20	0.19	0.20	

($P = 0.261$), and also at -20°C, with mean decrease value of 1.88% ($P = 0.407$). However, the decrease of PSA-C was significant at RT after 7 days ($P = 0.0004$), with mean value of 5%, reaching 23.5% on day 11, and 96.2% on day 22 ($P < 0.0004$). The change in the ratio F/T PSA showed significant change after a day of storage at RT and also at 4°C, with mean value of 5.3%, reaching 31.5% on day 11 and 36.8% on day 22 at 4°C ($P = 0.0007$). The change was also significant but low at -20°C, with mean change of 5.2% on day 11 and

10.5% on day 22 ($P = 0.006$).

Stability of PSA stored under disrupted power supply: In the second study (Table 2), the regression analysis results show there is a significant relationship between the mean change in serum PSA-T or PSA-F and the storage time, in days. The P -values for PSA-T ranged from 0.0001 to 0.0137 for all mean concentrations, except for the highest, 51.94, while the p -values for PSA-F ranged from 0.0000 to 0.0115 for all

Figure 1: Effect of time and temperature on PSA-T and PSA-F in archival samples stored under continuous power supply and investigated in the first study

Table 2: Archival serum stored for 5 months at -20°C before being thawed and the stability of PSA-T, PSA-F, PSA-C, and the ratio F/T PSA of the serum was monitored at -20°C when the freezer was subject to power outage of 6 hours on and 6 hours off around the clock for 285 days (The second study)

	Days											p value
	0	7	22	32	138	158	172	188	201	231	285	
PSA-T, µg/l at -20°C												
Mean ± SD	12.1 ± 2.2	12.2 ± 2.3	12.5 ± 2.5	11.4 ± 1.9	11.8 ± 2.1	11.2 ± 1.9	11.2 ± 1.9	11.5 ± 2.0	11.2 ± 1.9	11.3 ± 1.9	11.6 ± 2.1	0.0195
Median	5.21	5.49	5.39	5.40	5.35	4.72	4.58	4.64	4.47	4.45	4.44	
PSA-F, µg/l at -20°C												
Mean ± SD	1.6 ± 0.5	1.5 ± 0.5	1.6 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.4 ± 0.6	1.4 ± 0.5	1.3 ± 0.5	1.3 ± 0.5	0.0005
Median	1.56	1.53	1.560	1.50	1.51	1.51	1.51	1.41	1.34	1.29	1.28	
PSA-C, µg/l t -20°C												
Mean ± SD	18.7 ± 4.0	18.9 ± 4.1	19.4 ± 4.4	17.5 ± 3.3	18.2 ± 3.7	17.1 ± 3.2	17.3 ± 3.4	17.8 ± 3.6	17.5 ± 3.4	17.6 ± 3.5	18.1 ± 3.8	0.0568
Median	10.27	10.220	10.200	10.11	10.09	10.08	10.05	9.99	10.14	10.06	10.01	
F/T PSA, µg/l at -20°C												
Mean ± SD	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0005
Median	0.14	0.13	0.150	0.14	0.14	0.15	0.14	0.14	0.13	0.13	0.13	

concentrations, except for the lowest, 0.0038. Moreover, based on the ANOVA for repeated measures results (Table 3), we found that the mean change in PSA-T at -20°C was insignificant over the entire 285 days of the study ($P = 0.1905$). However, we noticed that the change in PSA-T was insignificant ($P = 0.525$) and low up to day 138, with mean decrease value of 2.24% and from day 158 till day 285, while the change continued to remain low with mean decrease value of 4.15% on day 285, it was almost significant ($P = 0.053$) at the 0.05 level of significance. The change in PSA-F was insignificant and low ($P = 0.244$) up to day 158, with a mean decrease value of 1.29 %. Then, the change increased gradually over time, became significant and reached a mean decrease value of 12.9% on day 201, and its highest change was on day 285, with a mean decrease value of 14.9% ($P = 0.0005$). The PSA-C did not change significantly over the period of 285 days, and this is consistent with our earlier results regarding the insignificant changes in PSA-T over the same time period. The change in the ratio of F/T PSA was significant ($P = 0.0005$) for 285 days but still low, with a mean decrease value of 7.69%.

Discussion

Most laboratories store the remaining of blood samples in freezers at -20°C or at lower temperatures, depending on the space availability, to use them in future retrospective studies

or for other applications. If for any reason a power outage occurs while these archival samples are stored, and no space is available in other freezers in the laboratory, what will happen to the values of PSA in these samples? We made a bibliographic search to find an answer to this question in previous studies of PSA stability, but failed to find any. The purpose of this paper is to investigate the stability of PSA-T, PSA-F, PSA-C and the ratio F/T PSA in archival serum samples that are stored in a refrigerator or a freezer which failed to function properly for a certain period of time due to power outage. Under such conditions, refrigerators and freezers are operated using generators. We studied one case when these generators are turned on and off for 6 hours, alternately around the clock. We also studied the extreme case where a generator is not available and the serum samples are stored at RT.

To the best of our knowledge, such conditions of intermittent cold storage were not investigated before. Consequently, there was no point of reference to compare the results of our study with, or to predict how long the serum may remain stable under these cooling conditions. For this reason, we started with the first study in which we tested the stability of PSA in the archival serum, under normal conditions of continuous power supply. The archival serum samples had been left over from prostate cancer screening campaigns done earlier in our laboratory and were stored at -20°C for about 5 months.

Table 3: Summary of PSA-T and PSA-F regression analysis in frozen serum samples stored underinterrupted power supply at -20°C from 0 to 285 days (The second study)

PSA-T						
PSA µg/l	Intercept	Slope	St. Error	R-Square	Test Stat. <i>t</i>	<i>P</i> -Value
0.5635	0.5581	-0.0002	0.0000	0.7751	-5.5688	0.0003
1.0593	1.0608	-0.0003	0.0001	0.5089	-3.0537	0.0137
1.9558	2.0244	-0.0013	0.0003	0.7334	-4.9757	0.0008
5.2123	5.4403	-0.0040	0.0006	0.8083	-6.1592	0.0002
7.7550	7.6627	-0.0022	0.0003	0.8157	-6.3104	0.0001
15.9125	15.8924	-0.0011	0.0002	0.8478	-7.0793	0.0001
51.9425	51.6311	-0.0133	0.0076	0.2534	-1.7479	0.1144
PSA-F						
PSA µg/l	Intercept	Slope	St. Error	R-Square	Test Stat. <i>t</i>	<i>P</i> -Value
0.0038	0.0044	0.0000	0.0000	0.0011	0.1006	0.9221
0.2642	0.2755	-0.0006	0.0001	0.8743	-7.9123	0.0000
0.3696	0.3646	-0.0007	0.0001	0.8997	-8.9861	0.0000
0.9248	0.9611	-0.0007	0.0001	0.7102	-4.6965	0.0011
1.4844	1.4607	-0.0009	0.0002	0.7478	-5.1652	0.0006
1.6388	1.6833	-0.0009	0.0002	0.5971	-3.6520	0.0053
2.1681	2.2023	-0.0009	0.0003	0.5264	-3.1627	0.0115

After the archival serum was thawed, the first study was carried out for 32 days, in which the serum was stored at RT, 4°C and -20°C (under continuous power supply). The results showed that when archival serum was further stored at -20°C, PSA-T was stable for the entire period of study (32 days), while PSA-F was stable for only 22 days. These results assured us that the archival serum used in our investigation wouldn't have lost its immunoreactivity when we started our stability study. In fact, PSA may remain stable for over a year if stored at -20°C as shown by previous studies performed on archival samples (11, 16).

When this serum was stored at 4°C, PSA-T was stable for up to 21 days and the PSA-F was stable for only one day. At RT, PSA-T showed low and insignificant change ($P = 0.52$) up to one week. The PSA-F showed significant change ($P = 0.0000$) after one day of storage at RT, with a mean decline of 5.9%. Although testing the stability at RT was not one of the main objectives of our study, and knowing that serum should never be stored at RT, we did it as a way to test if the immunoreactivity of the archival serum used in our study was lost during the five month storage period. Earlier studies of PSA stability were carried out at room temperatures of approximately 22°C (14, 16-17). The RT in our study, however, varied between 18°C and 28°C due to the fact that

the air conditioners were turned off on weekends to save electricity in a country dealing with power crisis. Piironen et al. (17) showed that room temperatures higher than 22°C might accelerate the decay of PSA-F. Our results are consistent with this finding since the PSA-F showed significant change ($P = 0.0000$) after one day of storage at RT. Previous studies on archival samples were performed by measuring the PSA just once after thawing the serum that has been stored frozen for a certain period of time. The only study we are aware of that monitored stability of PSA on archival samples after they were thawed was conducted by Leinonen and Stenmen (14) who stored serum at -20°C for 2 years before thawing them and then monitoring their stability for 11 days at 22°C and 4°C. According to their findings, PSA-T decreased by 10% after 11 days of storage at 4°C, whereas this decrease was attained in our study after 21 days. Concerning PSA-F, their results showed initial decrease followed by return to initial level after 11 days. In our study, the PSA-F was stable for only one day. In Leinonen and Stenmen study, the PSA-T was less stable than PSA-F after 11 days of storage at 4°C which is an unusual result since most previous studies showed that PSA-F is less stable than PSA-T under every condition of serum storage. However we noticed there was large inter individual differences in PSA-F and this was consistent with the results reported in

many previous studies about PSA-F, especially after prolonged storage (7, 13-14, 16-17).

In the second study, we investigated the stability of the archival serum under intermittent cold storage conditions, with power supply 6 hours on and 6 hours off alternately. In this study, the stability of archival samples was investigated for over a period of 285 days (9.5 months) at only -20°C, because this is the temperature at which archival serum is stored in most laboratories.

The results of the second study demonstrated that PSA-T showed little change throughout the entire period of 285 days. On the other hand, the PSA-F showed little and insignificant change up to 158 days after which the change increased with time.

According to previous studies performed under continuous power supply, Hamm et al. (10) found that PSA-T was stable for up to 42 months (three and a half years) in the serum stored at -20°C. Now concerning the stability of PSA-F in serum stored at -20°C, there was no concordance between the results of previous studies. According to Woodrum and York (12), PSA-F was stable for 2 years, other authors (6, 14) found that the concentration of PSA-F increased in serum after 2 years of storage, in contrary to others (16) who showed that, after one year of storage, PSA-F was stable in some samples and declined in others, but not at the same rate in all specimens.

The reason that the PSA-F was not stable in our study for the entire period of 285 days could be interpreted as a result of the unstable temperature change between 0 and -8°C in the freezer when the power supply was off. Besides, these samples were subject to 6 cycles of thawing and freezing. According to previous studies (16, 17, 19), five cycles of freezing and thawing didn't introduce any significant changes in PSA-T and PSA-F. However, one study (8) reported that after a second freeze-thaw cycle, only 71% of samples were unchanged whereas an increase between 11 – 1740% in PSA levels was observed in 16% of samples and a decrease of 20 – 95% was noticed in 14% of samples.

The results obtained in the two studies showed that the stability of PSA-C was similar to that of PSA-T, in contrary to the PSA-F which was much less stable. This result is in accordance with the suggestion of Piironen et al. (17) that the loss of PSA occurs mostly in the smaller entity of free PSA.

We believe our study is unique in light of the novel conditions

of interrupted temperature control, in addition to the substantial numbers of serum samples (1305 sample points in the first study and 572 in the second study) and the frequent measurement of PSA-T at different temperatures over long periods of time. In this study, the pre-analytic management didn't have a substantial impact on the levels of PSA obtained. The serum was separated within 3 hours of vein puncture as recommended by previous authors (16-17) to prevent PSA degradation, especially the PSA-F.

We believe our findings have several important implications. First, they offer reassurance that PSA-T measurements (but not those of PSA-F) are valid when serum samples are sent by mail, even when they have not been kept in adequate cold conditions during the transportation for few days, when room temperatures are similar to those used in this study; that is, in the range 18°C to 28°C. Second, when there is a power outage or an interrupted power supply, as it is the case in an emergency situation, serum samples stored frozen in conditions similar to this study can be used for PSA-T measurements. The values of PSA-T can be applied for screening or retrospective studies but not for follow-up purposes.

However, there are several important limitations that need to be taken into consideration. First, the specimens used in this study had been previously frozen at -20°C for 5 months under continuous power supply. We don't know what would have happened if these samples were frozen for longer time period or if they were fresh. Leinonen and Stenmen (14) concluded that long term storage at -20°C (for 2 years) reduces the stability of PSA immunoreactivity. Second, the results of stability obtained are valid only under the conditions specified in this study, where serum samples were subjected to intermittent cold storage conditions of 6 hours on and 6 hours off around the clock. We didn't examine other possibilities, so we could not speculate what would have happened if the period was longer or shorter than 6 hours. Third, while serum samples were used in this study; it might be preferable in circumstances applied to our current study if plasma samples were used instead of serum. Previous authors recommended the use of plasma for retrospective studies because PSA-F is more stable in it than in serum (7, 17).

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