

# Original Article

## Evaluation of Serum PSA in Different Storage Environments

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### ABSTRACT

**Background and Objective** Prostate cancer is a prevalent disease around the world. The prostatic specific Antigen (PSA) test has recently proved itself as a useful method for the diagnosis and examination of patients with prostatic cancer. The objective of this study was to compare the stability of free PSA and total PSA in different storage settings.

**Materials and Methods** Samples were obtained from 12 men, within the range of 50-70 yr old, who referred to Hazrat-e-Ghaem Hospital, Mashhad, Iran . The sera were separated via centrifuge and stored at room temperature (21-24°C) for 3, 6, and 9 hours; at 4°C for 24, 48, and 72 hours; and at -20°C for 1, 2, and 3 months. Finally, the stability of PSA was compared to that of the control group. The data were analyzed using the statistical software SPSS and paired t-test and repeated measure.

**Results:** In comparison with the average of the control samples, after 3 hours of storage at room temperature, the free PSA concentration had a 30% drop; and after 72 hours of storage in the refrigerator, the average of free PSA had a 34% fall. In addition, the average of the free PSA concentration kept in the freezer for 3 months exhibited an 11% drop. However, the average of total PSA kept in the refrigerator for 72 hours dropped by 6.9%. Finally, over 2 months of storage in the freezer, the average of the total PSA concentration exhibited a decrease of 10.6%.

**Conclusion:** Free PSA, when compared to total PSA in terms of time and storage temperature, shows less stability.

**Keywords:** Prostate-Specific Antigen, Temperatures, Chemical Analysis

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## Introduction

Prostate cancer is the second leading cause of cancer-related death amongst American men (1): more than 11,000 people are diagnosed with cancer and in excess of 2,300 people die annually in the U.S.A. In Iran, prostate cancer ranked fourth amongst the 10 most common cancers in 2003 (2). Early follow-up for the prostate cancer diagnosis of patients in the early stages of the disease is believed to confer a more effective treatment (3). The discovery of Prostate Specific Antigen (PSA), the most accurate and well-known tumor marker for prostate cancer to date, has been an important step toward the early diagnosis of this disease.

The PSA test is utilized as a serum marker in the diagnosis and differentiation of prostate cancer from benign prostate hyperplasia (4) and is more efficient in the disclosure of cancer than are the physical rectal examination and trans-rectal ultrasound test (5). It should be noted, however, that the U.S. Food and Drug Administration (FDA) stipulates that the PSA be conducted in tandem with the digital rectal examination for the final diagnosis.

PSA is a 34-kDa glycoprotein that circulates in both free and attached forms in the serum, with the bulk of the circulating PSA in the serum having a complex form (6). Prostate degradation triggers the release of PSA into the prostate tissue and circulation (7). Indeed, a rise in PSA levels can be due to cancer, prostatic adenoma, prostatitis, acute bacterial prostatitis, urinary retention, and prostatic needle biopsy (8-10). Furthermore, the stress of hospitalization for radiotherapy or hormone therapy can bring about a reduction in PSA levels (11-13).

Evaluations of different prostate cancer diagnostic methods show that the PSA measurement is the optimal test by virtue of its high positive predictive value (14), although it should be stressed that a simultaneous use of PSA and digital rectal examination for early follow-up is recommended (15). Screening for prostate cancer with PSA should be carried out every two years, starting at the age of 40-45 years; and if the test at ages 65-75 years shows PSA levels of 1-5/0 sa, the test should be discontinued (16). Normal PSA values vary according to age. For a more accurate diagnosis, any limitations of the PSA test must be identified to eliminate sources of error. There has been concern

over the stability of PSA and the factors that could compromise this stability, especially when in vitro. In this study, we sought to assess the relationship between the storage temperature and duration and PSA stability.

## Materials and Methods

Of all the men registered at Hazrat-e-Ghaem Hospital, Mashhad, Iran, 12 men, within the range of 50-70 years old, were selected based on clinical medical biography and medical history. The inclusion criteria were comprised of the absence of diabetes, hypertension, and hyperlipidemia, as well as a family history of cancer, prostate surgery, and vasectomy. In addition, the prostate size and consistency had to be normal in the examination.

Initially, 10 ml of venous blood were obtained in sterilized conditions and centrifuged at 2000 rpm for 3-4 minutes to separate the sera. Each of the separated sera was divided into 10 tubes. Three sample tubes from each patient, i.e. 36 samples from all the patients, were placed in the laboratory at room temperature, 3 sample tubes of each patient in the refrigerator at 4°C, and 3 tubes of each patient in the freezer at -20°C. Our measurement unit was ng/ml.

Immediately after sampling, the free PSA (fPSA) and total PSA (tPSA) levels of all the 12 subjects were measured and recorded as control samples. The test was conducted according to the kit (Imunotec, Germany) instructions and with the use of a Gamma counter device (Contron, Germany).

The fPSA and tPSA levels of the samples stored at room temperature were measured after 3, 6, and 9 hours and the average was calculated and recorded. The fPSA and tPSA levels of the samples stored in the refrigerator at 4°C were measured after 24, 48, and 72 hours of sampling and the average was calculated. Finally, the fPSA and tPSA levels of the samples stored in the freezer at -20°C were measured after 30, 60, and 90 days of sampling and the average was calculated.

The data were analyzed using the statistical software SPSS and paired t-test and repeated measure.

## Results

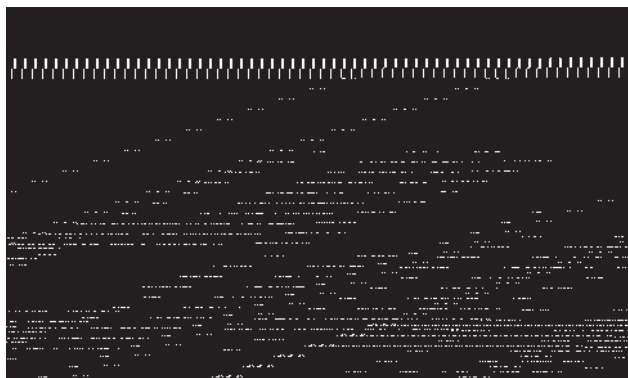
Immediately after sampling, the fPSA and tPSA levels were measured and the results were considered as control samples. Then, 3, 6, and 9 hours later, the

fPSA and tPSA levels were measured again and an average was calculated, which is shown in Fig. 1.

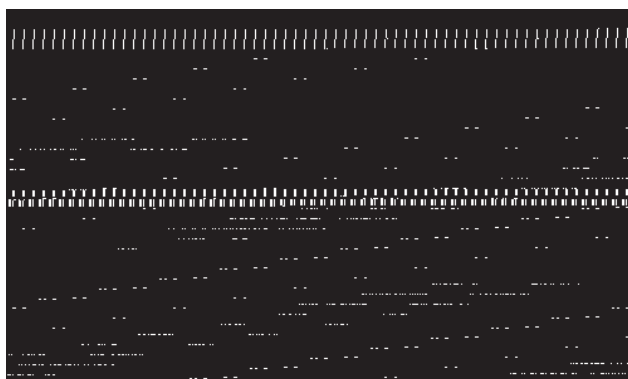
The PSA levels of the samples stored in the refrigerator at 4°C were measured after 24, 48, and 72 hours and the average was calculated, which is shown in Fig. 2. The PSA levels of the samples stored in the freezer were measured after 30, 60, and 90 days following sampling and the average was calculated, which is presented in Fig. 3.



**Fig.1:** Average of PSA levels of the samples after 3, 6 and 9 hours of sampling of storage in the laboratory at room temperature



**Fig. 2:** Average of serum PSA after 24, 48, and 72 hours of storage in the refrigerator at 4°C



**Fig. 3:** Average of serum PSA after 30,60 and 90 days of storage in the freezer at -20°C

## Discussion

We evaluated changes in the fPSA and tPSA levels with respect to different storage conditions. In the first three hours, the fPSA levels of the samples stored at room temperature underwent a significant change and we observed a 30% decrease in the control level.

There are a few studies regarding PSA stability in the existing medical literature. Piironen reported some degrees of statistical change in fPSA over 1-6 hours of storage (17). Same wise and Woodrum demonstrated changes in fPSA levels after the first 3 hours of storage (18). We witnessed significant changes in the first 6 hours of storage in the fPSA levels, which chimes in with the findings in the Piironen study. Nevertheless, the decrease in the fPSA levels in the first 9 hours of our study does not tally with the results of similar studies published previously. In addition, Woodrum showed changes in fPSA levels after 8 hours of storage at room temperature (18). Also, Jung and Cartledge reported a drop in fPSA (19, 20).

It has been suggested that fPSA analysis be carried out instantly after sampling bearing in mind that storage at room temperature has a reducing effect on fPSA levels.

In the first 3 hours in our study, the tPSA levels did not exhibit a significant change. Similarly, Woodrum reported no change in the tPSA levels after 3 hours of storage at room temperature. We witnessed no significant change in the tPSA levels after 6 hours of storage, which is not concordant with the results of the previous studies, although it is worthy of note that Woodrum reported no change in tPSA after 5 and 8 hours at room temperature. There was a significant change in the tPSA levels in our samples after 9 hours of storage; to the best of our knowledge, no other studies have hitherto referred to this point. Cartledge witnessed no change in the tPSA levels after 24 hours of storage, followed up by a decline (20). Our results show that tPSA can be stored for less than 9 hours at room temperature.

The fPSA levels in our study after 48 hours showed a significant difference, there being a decrease of 38% in the fPSA levels compared to those of the controls. Similarly, Woodrum reported a decrease in fPSA in his study (18).

We observed a reduction in fPSA after 72 hours of storage; no other studies have to date referred to this matter. Sokoll showed a decrease in fPSA stored at 4°C for 1 week (21). Cartledge reported a 25% fall in the fPSA levels compared to those of the controls (20).

Piironen reported a 28.8% reduction in fPSA stored at 4°C after 2 weeks of storage (17). Also, Arcangeli showed a 28% reduction in the fPSA levels compared to those of the controls after 1 and 2 weeks of storage (22). Our study as well as those previously published shows that fPSA cannot be stored in the refrigerator, for it has a reducing effect.

There was a significant change in the tPSA levels at 24 hours, with the average showing a reduction of 4.8% when compared to that of the controls. Arcangeli reported a decrease in the tPSA levels stored at 4°C for 24 hours (22). We found the average of the tPSA levels in the first 48 hours compared to that of the controls significant. Along the same lines, Woodrum reported changes in tPSA after 2 days (18).

At 72 hours, the tPSA levels in our study were reduced by 6.9%; our literature review showed no other similar studies. The previous investigations stipulate that tPSA cannot be stored in the refrigerator. We stored the serum samples for 1, 2, and 3 months at -20°C.

We observed a 17% drop in the fPSA levels at the first month. Cartledge also had a 14% decrease in their fPSA levels in the control samples (20). At 3 months, the fPSA levels of our samples increased by 11.3%; no other similar studies have been carried out thus far. However, Leinonen reported an increase in the fPSA levels after storage in the freezer for 2 weeks and 2 years (23).

Woodrum observed no change in 60% of the samples stored at -20°C and 100% of the samples stored at -70°C, and nor did he witness any change in the samples stored at -20°C and -60°C. Ulmert showed no significant change in the sample levels stored at -20°C for 20 years (24).

Previous studies stress that fPSA stored in the freezer is liable to change; it, therefore, must be analyzed immediately and if analysis cannot be made, samples should be stored in the freezer at -70°C.

As regards the tPSA levels in our study, a 5.8% drop in the samples was observed at the first month. The averages of the tPSA levels compared to those of the control samples at the first month had a significant change. In similar studies, in 1999, Cartledge reported a change in tPSA after the first month (20). In the second month of storage, tPSA was down by 10.6%. Our literature review yielded no other similar studies in this aspect. At three months of storage, the tPSA levels of our samples had a rise of 16.4%.

Sokoll reported no difference in tPSA stored in the freezer for 2 weeks (21). Arcangeli observed no

difference in the samples stored for 6 months at -20°C (22). In a similar study, Leinonen showed a decrease in the tPSA levels when compared to the controls (23). Woodrum reported tPSA change at -20°C but no difference at -70°C (18).

The results of the present study and those of the previous ones show that tPSA cannot be stored in the freezer at -20°C and must be stored at -70°C.

## Conclusion

fPSA maintains less stability in different storage conditions compared to tPSA; accordingly, it must be analyzed immediately. Nevertheless, tPSA analysis can be delayed for up to 8 hours as long as it is stored in a refrigerator, although the storage of samples in a refrigerator or freezer reduces the speed of change considerably.

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