Original Research Article

Preanalytical Variables Influencing Total Prostate-Specific Antigen: Sample Storage Temperature and Duration

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ABSTRACT

Background: Prostate cancer is one of the leading cancers in men, globally. Total Prostate-specific antigen (total PSA) has been widely used for diagnosis of Prostate cancer. For accurate testing and interpretation of total PSA values, sample storage conditions must be taken into account, as these might affect the stability of total PSA levels in the serum. In this study, we evaluated the impact of Preanalytical variables -including sample storage temperature and duration- on stability of serum total PSA.
Materials and Methods: Study group consisted of 11 subjects who were chosen from those attending Urology, Surgery and other Departments of St. John's Medical College Hospital. Blood samples were collected, processed and sera analyzed for total PSA levels on day 0 (baseline values), and after storage at room temperature (23-28^0 C) and 4-8^0 C on days 1 and 3. Stability of serum total PSA levels over 3 days was evaluated using Friedman test.
Results: Serum total PSA on day 1 and 3 did not change significantly as compared to day 0 (baseline values), either at room temperature (23 - 28^0C) or at 4 - 8^0C.
Conclusion: Total PSA levels are stable when the serum samples are stored at room temperature (23 - 28^0C) or at 4 - 8^0C over a period of 3 days. These results suggest that after routine processing, serum samples may be stored for 3 days at above mentioned temperatures without adversely affecting the total PSA levels.
Key Words: Prostate- specific antigen; tumor marker; stability; temperature.

INTRODUCTION

Globally 899,000 new cases of Prostate cancer occur every year. Annually, it accounts for 258,000 deaths globally. [1] Total Prostate Specific antigen (total PSA) has been widely used in diagnosis and monitoring of Prostate Cancer. Total PSA has also been used in screening for Prostate Cancer. [2]

Preanalytical phase which includes variables like sample collection, storage and processing is an important part of Laboratory testing cycle. In fact, 60-70% of errors occur in the Preanalytical phase. Hence, determination of optimum sample processing and storage conditions are important for accurate test results. [3]

Studies to evaluate the influence of Preanalytical variables like sample storage temperature and duration on total PSA levels have yielded conflicting results. In a study by Sokoll et al. which included 8 subjects, it was observed that total PSA was not stable beyond 2 days at room temperature and 4^0C. [4] In contrast, other studies have found that total PSA was stable for 1 week when stored at room temperature (23^0C) and 4^0 C. [5,6] None of the above studies have studied the impact of storage on total PSA levels at temperature ranges {example, room temperature (23 - 28^0C) and 4-8^0 C} , seen routinely in small and medium sized laboratories.

In addition, total PSA being a non-urgent analyte, samples collected over consecutive days are stored at various temperatures (example at 4-8^0 C) over short durations, to be analysed in batches in order to economise on the efforts and reagents. [7] Delay in analysis may also occur in cases where samples are transported over long distances from Peripheral to Referral laboratories for testing and in situations of instrument breakdown. The impact of storage temperature and duration on total PSA levels in the above mentioned situations is unknown. Hence, standardisation of sample storage conditions is imperative for accurate interpretation of total PSA results. In this study, we evaluated the impact of sample storage temperature and duration on serum total PSA levels.

MATERIALS AND METHODS

Eleven male (age 69.3±9.8 years) subjects for the study were chosen from among the individuals attending the Surgery, Urology and other Departments of St. John's Medical College and Hospital and who were referred to Clinical Biochemistry laboratory for total PSA analysis. The study was approved by Institutional ethical review board at St John's Medical College, Bangalore. This study was in conformance with standards of *Declaration of Helsinki* and it followed ethical standards as laid down by the ICMR-Ethical Guidelines for Biomedical Research on Human Participants

2 ml of blood was collected from each subject by venepuncture avoiding hemolysis into plain vacutainer tubes, under aseptic precautions. Blood was allowed to clot and serum separated by centrifugation within 4 hours of collection. Serum was aliquoted and stored at -20 ^{0}C for 1 month and if the storage extended beyond 1 month, serum samples were stored at -70 ^{0}C to maintain the stability of total PSA, till further analysis. [6,8]

Serum samples were thawed before analyzing them for total PSA in batches. Each serum sample was subjected to one freeze-thaw cycle throughout study. Upto three freeze-thaw cycles do not alter total PSA levels. [7] The levels of total PSA in serum after thawing were considered as baseline values or day 0 values. After thawing, four aliquots (from each subject's sample) were prepared to be stored-two each at 4 - 8^0C and room temperature (23 - 28^0C) for 3 days. Further analysis was done on each sample at day 1 (24 hours after baseline analysis on day 0) and day 3 (72 hours after baseline analysis on day 0). So, repeated measurements of total PSA were performed on day 1 and day 3 with day 0 being baseline values.

Serum total PSA were estimated by Microplate Immunoenzymometric assay provided by Monobind, inc., CA (USA). In this method, either PSA calibrator, patient specimen or control (all of which contain PSA antigen) was added to Streptavidin coated well. Biotinylated monoclonal and enzyme labelled antibodies (directed against distinct and different epitopes of PSA) were added and the reactants mixed. Reaction between various PSA antibodies and native PSA forms a sandwich complex that binds

to Streptavidin coated onto the well. After the completion of required incubation period, enzyme - PSA antibody bound conjugate was separated from the unbound enzyme - PSA conjugate by decantation. The activity of the enzyme present on surface of the well was quantitated by the reaction with a suitable substrate to produce colour. The intensity of colour was directly proportional to the concentration of the antigen PSA. By utilizing several serum references of known antigen values, a dose response curve was generated from which an antigen concentration of an unknown sample was ascertained.

Statistical Methods

Total PSA values were non-normally distributed. Friedman's test was used to determine the difference in values on days 1 and 3 as compared to day 0. Friedman's test statistic (χ^2_R) and the 'p' value were calculated. $p<0.05$ was considered to suggest statistically significant difference in the values on days 1 and 3 as compared to day 0 (baseline values). Median values were used because of non-normal distribution of total PSA values and Trimean was used as the standard deviation was high due to scatter in day 0 values.

RESULTS

There was no significant difference between the values of total PSA on days 1 and 3 as compared to baseline (day 0), at room temperature and $4 – 8^0$ C ("p' >0.05 in both the cases) (Tables 1, 2).

Table 1. Friedman test and total PSA levels at different time intervals at room temperature (23-28 ^{0} C)

Days	PSA levels at Room temperature			
	Range (ng/ml)	Median (ng/ml)	Tri-mean (ng/ml)	Mean±SD (ng/ml)
Day 0	2.0-41.0	5.5	8.88	11.0±12.02
Day 1	2.5-43.0	5.5	8.75	11.0±12.52
Day 3	1.5-35.5	5.5	8.38	11.0±11.78
Significance (Friedman test)	χ_R^2=0.750, p=0.687			

Table 2. Friedman test and total PSA levels at different time intervals at 4-8^{0}C

Days	PSA levels at 4-8^{0}C			
	Range (ng/ml)	Median (ng/ml)	Tri-mean (ng/ml)	Mean ±SD (ng/ml)
Day 0	2.0-41.0	5.5	8.88	11.0±12.02
Day 1	1.0-34.0	5.5	8.50	10.1±10.46
Day 3	0.0-31.0	5.0	6.13	8.7±9.51
Significance (Friedman test)	χ_R^2=1.077, p=0.584			

DISCUSSION

In our study, total PSA levels were found to be stable when the serum was stored over a period of 3 days at room temperature (23 - 28^{0}C) and 4-8^0 C. Woodrum et al. have observed similar results in their study, the difference being temperature points (that were close to our temperature ranges) were used in their experiments as opposed to ranges in our study. [5] In contrast, our results differ from that of Sokoll et al who found that total PSA

values declined beyond two days of storage at room temperature and 4^0 C. [4] Use of commonly observed temperature ranges like 23 - 28^0C and 4-8^0 C is a salient feature of our study, as this is more likely a scenario in practical laboratory conditions. Total PSA has also been found to be stable for longer duration of 1 year at -20^0 C and -70^0 C. [5] To summarise, total PSA is stable for short durations like 3 days at (23 - 28^0C) and 4-8^0 C and for longer duration of 1 year at -20^0 C and -70^0 C. A limitation in our study was that stability was evaluated over short duration of 3 days. It would have been desirable to use longer storage interval at the same temperatures (example, 1 week of storage), which in turn would have provided data about feasibility of storing samples for longer terms for routine diagnostic purposes.

Based on above findings, after routine processing, we recommend that serum samples may be stored for 3 days at room temperature (23 - 28^0C) and (4-8^0 C) without adversely affecting total PSA levels. This has following applications: 1. Samples may be stored in small and medium sized laboratories at recommended temperatures over a course of 3 days before being analysed in batches to economise on reagents and efforts. 2. Samples may be transported from Peripheral to referral laboratories over long distances provided the samples are maintained at recommended temperature throughout. 3. In cases of instrument breakdown, short term delays in analysis are acceptable provided, samples are stored at temperature (mentioned above) and delay is not beyond 3 days.

CONCLUSIONS
Serum total PSA levels were stable when samples were stored at room temperature (23 - 28^0C) and 4-8^0 C for 3 days. After routine sample processing, serum samples may be stored at above mentioned temperatures without adversely affecting total PSA levels.

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