www.jmscr.igmpublication.org
Impact Factor 5.244
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: http://dx.doi.org/10.18535/jmscr/v4i9.36

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Evaluation of the Influence of Storage time in the Determination of Serum Adenosine Deaminase Activity

Authors
***Dr Radhika Krishnaswamy[1], Sr Daisy Kunnathupurambil[2], Priyadharshini K[3]**
[1]Associate Professor, [2]Post Graduate [3]Undergraduate
Dept of Biochemistry, St. John's Medical College, Bangalore-560034 (Karnataka), India
*Corresponding Author
Dr Radhika Krishnaswamy
Address: B2, 1080/2, Vars Ville,13 A Main, HAL II Stage, Bangalore-560008
Telephone: 9448352907
E-mail: *lakdorai@gmail.com*

ABSTRACT
Measurement of adenosine deaminase (ADA) activity in various biological fluids is useful for the diagnosis of tuberculosis .It is a rapid and noninvasive test unlike the other routine tests used for the diagnosis of tuberculosis especially extra pulmonary tuberculosis. The time limit at which the analysis is done after the sample collection is crucial because the activity of the enzyme can decline with time at ambient temperature. The purpose of the present study is to identify, test, and determine the influence of storage time in the determination of serum adenosine deaminase activity and optimize the time at which the activity of ADA is best, consequently eliminating the error that can occur on delayed testing.. Serum samples collected from 20 healthy individuals both male and females in the age group of 20-60 years was used for the study Serum samples were analyzed on the same day of collection and then stored at -20^{0}C and the analysis was done on the following days 3,7,10,28. ADA analysis was done by the colorimetric procedure of Guisti and Galanti employing reagents optimized by Kaplan. All chemicals employed as stabilizing agents were of reagent grade. Results showed significant decline in the enzyme activity as the number of days on storage increases even though it is stored at -20^{0}C. The study shows that on storage without any additives there is a significant decline of the enzyme activity and it is advisable to do the analysis on the same day of sample collection.
Keywords: *ADA – Adenosine Deaminase, storage, day, enzyme, activity.*

INTRODUCTION

Measurements of enzymes are used in medicine in two major ways: enzymes are measured in serum and other bodily fluids to detect injury to a tissue that makes the enzyme. Enzymes are also measured, often within a tissue, to identify abnormalities or absence of the enzyme, which may cause disease. Many of the clinically useful markers of cellular damage are enzymes. For a substance to serve as a biochemical marker of damage to a specific or tissue, it must arise predominantly from the organ or tissue of interest. The timing of the enzymes diagnostic window is another important aspect to be considered when

these markers are used to evaluate acute injuries. [1] In vitro, during storage, the amount of a blood constituent in the specimen may change as a result of various processes. These changes occur, though to varying degree, at ambient temperature and during refrigeration or freezing.[2]

Adenosine Deaminase (ADA), an enzyme which is produced by T lymphocytes during cellular immune response and also plays a role in the maturation of T lymphocytes is found to be useful in diagnosis of tuberculosis. Several studies have demonstrated the use of ADA in the diagnosis of tuberculosis in fluids including serum, meningeal, pleural, peritoneal and pericardial. The immune cellular response which occurs in the body against mycobacterium tuberculosis, suggests the possible role of ADA in the diagnosis of mycobacterium tuberculosis. Confirmation of tuberculosis is difficult and slow due to the need for histological confirmation of caseous granulomas or bacteriological confirmation by acid-fast smears or mycobacterial cultures. So a quick, non-invasive test like ADA is very useful in the early diagnosis of tuberculosis especially. Extra pulmonary tuberculosis. [3,4,5,6]

Although measurement of ADA is simple, controversies exist regarding potential errors caused by time elapsed between sample collection and analysis as the levels of this enzyme decline with time. As it is necessary to get an accurate report eliminating all the possible pre-analytical errors, it is essential to determine the optimum time limit for ADA analysis after sample collection and also to understand the possible changes that can occur on storage at -20^0C at particular intervals.

The objective of this study is to evaluate the influence of time in the determination of ADA in serum samples stored at – 20^0C and to identify, and optimize the time at which the activity of ADA or the diagnosis of ADA is best, consequently eliminating the error that can occur on delayed testing. In this study, the enzyme activity of Adenosine Deaminase was measured immediately on the same day of sample collection and stored at -20^0C and estimated on the following days: Day – 3 (3rd day)Day – 7 (7th day) Day – 10 (10th day)Day – 28 (28th day)

MATERIAL & METHODS

The study was conducted in tertiary care center in Bangalore, Karnataka. Twenty apparently healthy males and females aged between 20 – 50years who don't have the history of alcoholism and cigarette smoking and a past history of acute or chronic illness were selected for this study. Blood samples were collected from the subjects in sitting posture from the median cubital vein in a plain glass tube. Blood samples were left undisturbed for 30 minutes. Following which were they centrifuged for 5 minutes and the serum was separated. The separated serum was made into 5 aliquots of each sample and was stored at -20^0C until the day of analysis. ADA analysis was done by the colorimetric procedure of Guisti andGalanti[7] employing reagents optimized by Kaplan.[8] All chemicals employed as stabilizing agents were of reagent grade. The analysis was done in all 5 aliquots of each sample on 5 different days.

STATISTICAL METHODS

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Student t test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale within each group. The significance was assessed as follows. Suggestive significance (P value: $0.05<P<0.10$); Moderately significant (P value: $0.01<P \leq 0.05$);S trongly significant (P value: $P \leq 0.01$).Statistical software: SPSS 16 used for this study .

RESULTS

Table 1: Mean Levels of Adenosine Deaminase Activity
at Day 1, Day 3, day 7, Day 10 and Day 28

Study period	ADA	
Day	Range	Mean ± SD
Day 1	7.0-14.50	10.53±1.96
Day 3	6.90-14.10	9.91±2.04
Day 7	6.40-14.00	9.50±2.11
Day 10	6.30-13.90	9.06±1.97
Day 28	5.60-12.80	8.40±1.95

Figure 1: Mean Levels of Adenosine Deaminase Activity
at Day 1, Day 3, day 7 , Day 10 and Day 28

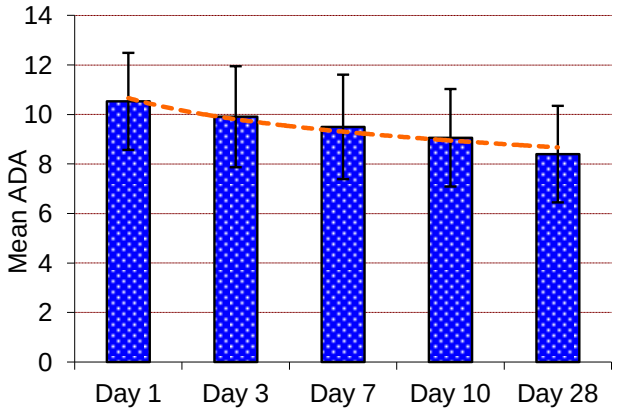

Table 2: Mean difference of ADA and significance

Pair	Mean difference	t value	P value
Day 1-Day 3	0.63±0.34	8.145	<0.001**
Day 1-day 7	1.03±0.55	8.408	<0.001**
Day 1-Day 10	1.47±0.59	11.036	<0.001**
Day 1-Day 28	2.13±0.78	12.264	<0.001**
Day 3-Day 7	0.41±0.33	5.360	<0.001**
Day 3- Day10	0.85±0.49	7.791	<0.001**
Day 3-Day 28	1.51±0.66	10.202	<0.001**
Day 7- Day10	0.44±0.35	5.550	<0.001**
Day 7-day 28	1.10±0.53	9.279	<0.001**
Day 10-day28	0.66±0.40	7.369	<0.001**

DISCUSSION

The values obtained in the study, such as the mean difference of ADA activity on various days and its significance, clearly indicates that there is a decline of the enzyme activity on storage at -20^{0}C. This is against some studies which showed that there is no decline in enzyme activity with storage at -20^{0}C [9,10]. In the present study serum samples were used without any addition of preservatives or enzyme stabilizing agents. It is clearly evident that on storage without any additives there is a decline of the enzyme activity. The results of the present study shows that it is always better to be analyze the sample for ADA activity on the same day of sample collection for an accurate report. There can be various factors that influence the decline of the ADA activity on storage at -20^{0}C. One may be the effect of freezing on storage and thawing for the analysis. During storage, the amount of a blood constituent in the specimen may change as a result of various processes, including adsorption to glass or plastic tubes, protein denaturation, evaporation of volatile compounds. These changes occur, though to varying degree, at ambient temperature and during refrigeration or freezing [2] Freezing often causes denaturation due to the stress and pH variation caused by ice-crystal formation.

In conclusion, any delay in the sample processing can alter the enzyme activity in the serum samples which is being stored without any addition of preservatives and additives. So the samples that are collected for the analysis of ADA activity needs to estimated on the same day without delay.

REFERENCES

1. Burtis C A, E R, Ashwood, Bruns.E, tietz text book of clinical chemistry and molecular diagnostics 4th ed. *Clinical enzymology*; 597.
2. Mcpherson. R. A, Pincus W.B, Henry's clinical diagnosis and management by laboratory medicine.21sted: 24.
3. Segura R M, Pascal C, ocana l, et al. Adenosine deaminase in body fluids: a

useful diagnostic tool in tuberculosis. *ClinBiochem*. 1989; 22: 141- 148.

4. Thamania. J P, R K. Jenaw, S. K. Luhada, D. K. Mather, H. C Parithan and S.K Shama. Serum adenosine deaminase in differential diagnosis of pulmonary tuberculosis and common nontubercular respiratory diseases. *Ind. J. tub* 1988, 35,25
5. Segura R M, Pascal C, ocana l, et al. Adenosine deaminase in body fluids: a useful diagnostic tool in tuberculosis. *ClinBiochem*. 1989; 22: 141- 148.
6. Aston N O. Abdominal tuberculosis. *World J Surg*. 1997; 21: 492- 499
7. Guisti, G, Galanti, B Adenosine deaminase. Bergmeyer, HU eds. *Methods of enzymatic analysis* 1974,1092-1096 Academic Press. New York, NY:
8. Kaplan, A The determination of urea, ammonia, and urease. *Methods Biochem Anal*1969;17,311-324
9. Antonangelo L[1], Vargas FS, Almeida LP, Acencio MM, Gomes FD, Sales RK, Seicento M, Teixeira LR. Influence of storage time and temperature on pleural fluid adenosine deaminase determination Respirology July 2006;11(4):488-92 ·
10. Bielsa S, Esquerda A, Palma RM, Criado A, Porcel JM. Influence of storage time on pleural fluid adenosine deaminase activity. Clin Lab. 2014; 60(3):501-4.