

Short Communication

Transaminase Extraction and Stability from Dried Serum Samples

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Abstract: The stability of transaminase enzymes in the filter paper matrix, at different temperatures for different lengths of time is of considerable importance. A method for extraction and estimation of these enzymes from serum spotted on filter paper (whatman no.3) has been developed. The enzymes were efficiently extracted and measured from dried serum spots. For estimation, a commercially available enzymatic method was used. The enzyme levels in serum and corresponding dried serum showed good correlation with an intraclass correlation of 0.85 and 0.92 for aspartate and alanine aminotransferases respectively, on the day of collection. The enzymes were found to be stable for upto 15 days at room temperature (16-28 degrees Celsius) and upto one month at 4 degrees Celsius.

Keywords: Aminotransaminases, Dried serum, Filter paper, Stability, Alanine amino transferase (ALT), Aspartate amino transferase (AST)

INTRODUCTION

Measurement of serum alanine aminotransaminases (EC 2.6.1.2) and aspartate aminotransaminases (EC 2.6.1.1) is useful in the diagnosis and monitoring of hepatocellular diseases [1]. The stability of these enzymes in serum has been reported to be few hours at ambient temperature (38 degrees) [2, 3, 4]. Stability of many analytes has been reported to be extended in serum/ blood dried on a filter paper matrix [5, 6]. The present article describes a method for extraction and measurement of these enzymes from serum samples dried on filter paper. Stability of these enzymes in dried serum was studied at room temperature, 37 and 4 degree celsius for upto 30 days.

MATERIAL AND METHODS

For the study 55 patients coming to the department of laboratory medicine, All India Institute of Medical Sciences (AIIMS) for routine biochemistry investigations were selected randomly. Ethical clearance for the conduct of the study was obtained from institutional ethics committee. 2ml of blood was collected in tubes without anticoagulant and serum was separated within 1 hour of collection. An aliquot of the serum was kept for analysis of alanine and aspartate aminotransferases. The remaining serum was spotted on whatman 3MM filter paper. 30 spots of 10 ul each were prepared and allowed to dry at room temperature. After drying filter disc were preserved in sealed plastic zip lock bags to protect from dust and moisture. The filter

discs were stored at three different temperatures: room temperature (16-28°C), 37°C and 4°C.

For extraction of transaminases from dried sera, five (6mm diameter) discs were punched and taken into a 2 ml eppendoff tube and 250 µL of the substrate buffer containing L-alanine for alanine aminotransferase and L-aspartate for aspartate aminotransferase with alpha oxoglutarate in phosphate buffer was added (Randox) [7]. The extraction was carried out for a period of 1 hour with gentle shaking at 100 rpm in an environ shaker (Lab Line Inc. ILL, USA) at 37°C. The elute was transferred to fresh glass tube and 250 µL dinitrophenylhydrazine was added. The tubes were vortexed and kept at room temperature for 20 minutes. With the addition of 2.5 ml sodium hydroxide, red colour was produced and this was measured at 540 nm wavelength, against blank [7]. For comparison transaminase were determined in fresh serum using the same protocol. Multisera controls level II and III (Randox) were prepared and treated the same way as samples and run with each batch.

Inter assay precision of the dried sera method was obtained by estimating five samples in duplicate on five separate assays and intra assay precision was obtained by running triplicates of three samples assay.

RESULTS AND DISCUSSION

The relationship between dried sera and serum analysed on the day of collection was linear with intra class correlation (ICC) value of 0.85 and 0.92 for aspartate and alanine aminotransferases respectively (fig. 1). The mean (95% confidence interval) transaminase values in serum and their dried serum samples on the day of collection were 0.54 (0.51 to 0.58) and 0.52 (0.48 to 0.56) $\mu\text{kat/l}$ for aspartate and 0.69 (0.59 to 0.79) and 0.65 (0.56 to 0.73) $\mu\text{kat/l}$ for alanine aminotransferase respectively. Inter and intra assay CV for aspartate and alanine aminotransferase was found to be 6.0 %, 5.4% and 6.2 %, 5.8% respectively.

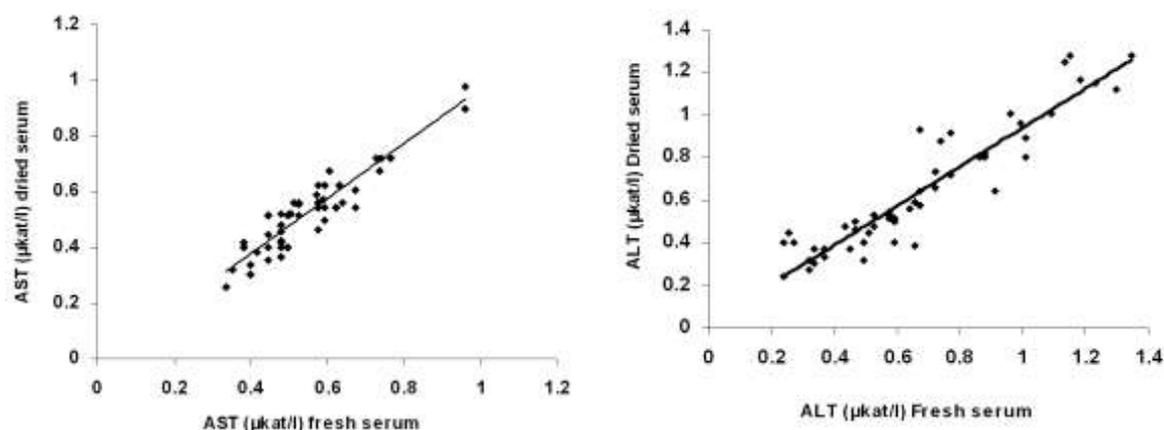


Fig. 1: Comparison of transaminases (AST and ALT) levels in paired serum and dried serum samples spots

The correlations of serum and corresponding dried serum AST ($y = 0.98x - 0.01$; $r = 0.93$; $p < 0.001$) and ALT ($y = 0.81x + 0.08$; $r = 0.93$; $p < 0.001$) of randomly selected samples are shown ($n = 55$). Values represent the mean of duplicate measurements.

We conclude that serum aminotransaminases are stable in dried serum and are readily transferable to a liquid phase. The good agreement between values in dried serum and fresh serum supports the validity of the measurement of these analytes in dried serum.

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To assess stability, the transaminase levels were analysed in dried sera stored at different temperature by the same method as described. Aspartate and alanine aminotransferases concentration remained stable for 15 days, while at the end of 30 days a mean decrease of 46% and 20% was observed at room temperature. A mean decrease of 62.3% and 45% was observed in disc stored at 37°C for 15 days. Transaminase levels remained stable for upto 30 days at 4°C.

Previous studies on the stability of transaminases in serum found the enzymes to be stable for 8 days at 38°C and upto 120 days at 4°C and at -20°C (2). With the use of matrix the stability was found to be increased upto 15 days at room temperatures.

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