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A novel and economic method to assess clinical transaminase assays

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ABSTRACT *Amino-transaminases are involved in the transfer of amino group from α amino acid to α oxo acid, an important step in the metabolism of amino acids. The two clinically significant enzymes of this category of aminotransaminases are alanine -aminotransferase, aspartine-aminotransferases which have diagnostic importance. The standard method of estimation has been developed by Reitman and Frankel(1957) method based on colorimetry. The modified versions have been used in the assessment of these two enzymes in the form of commercial kits developed by different companies for the assay and education purpose. An attempt has been made to develop a kit only for educational purpose where the serum need not be used. The kit is easy , cheap and simple to develop, with shelf life of six months and can be utilised at large scale.*

Introduction

Enzymes are the proteins capable of acting as biocatalysts of chemical reactions in the living systems. They are substrate specific, catalyse particular reaction, specific for a particular type of chemical bond or functional group. The serum contains two types of enzymes such as functional plasma enzymes which are in circulation and perform physiological function in the blood; and non functional plasma enzymes available in low concentrations and have no functional activity. Nonfunctional enzymes are intracellular in origin and their levels in the serum indicate the tissue damage or necrosis resulting from injury or disease and often accompanied by increase levels in the serum. Thus they have clinically diagnostic importance eg., Alanine transaminase, Aspartine transaminase. These are also called as transferases which are capable of transferring amino group from alpha to keto amino acids. The increase in the levels of Aspartate aminotransferase (AST, or SGOT) and Alanine aminotransferase (ALT or SGPT) used for the diagnosis of myocardial infarction and liver problems such as viral hepatitis.

Extensive research was carried out to understand the mechanism (Oshima T and Tamiya N,1961). The detection techniques of transaminases include colorimetric, spectrophotometric, chemiluminescence, chromatography, fluorescence and UV absorbance, radiochemical, and electrochemical techniques which can also help to assess the levels of these enzymes (Xuang et.al.,,2006).

Principle:

The action of serum transaminases is mainly associated with transfer of amino group, resulting the formation of direct pyruvate or an indirect action resulting in the formation of an unstable product which will be converted later into pyruvate AST and ALT are biological catalysts. The assays of AST and ALT activties are all based on their original enzyme reactions (1,2) and succeding reactions (Xing- Jiu Huang et.al.,2006). The development of identification and assessment of transaminases procedure is basically derived by using different concentration of pyruvate , buffered enzyme substrate, colouring reagent DNPH and the developed hydrazone in alkaline conditions as the standard graph.

The chemical reaction of AST and ALT as follows:

i) L Aspartate + α ketoglutarate $\xrightarrow{GOT}$ oxaloacetate + L glutamate

ii) L Alanine + α ketoglutarate $\xrightarrow{GPT}$ Pyruvate + L glutamate

iii) Oxaloacetate $\xrightarrow{\text{oxalacetate decarboxylase}}$ Pyruvate + CO_2

iv) Pyruvate + O_2 + Phosphate $\xrightarrow{\text{pyruvate oxidase}}$ Acetylphosphate + CO_2 + H_2O_2

v) L glutamate + O_2 $\xrightarrow{\text{glutamate oxidase}}$ α- oxaloglutrate + NH_3 + H_2O_2

vi) Oxaloacetate + NADH + H^+ $\xrightarrow{pH\ 7.8}$ Malate + NAD^+

vii) Oxaloacetate + NADH + H^+ $\xrightarrow{pH\ 7.4}$ L lactate + NAD^+

The colorimetric method of Reitman and Frankel (modified method) 1957 uses Di nitro Phyenyl hydrazine as colouring reagent which reacts with pyruvate standard (2mM) and forms brown coloured hydrazone complex in the alkaline conditions. The different pyruvate standards were considered as an equivalents to that different enzyme concentrations to plot standard graph from which the unknown serum SGPT/SGOT is assessed. The clinical importance of AST(GOT) and ALT(GPT) helps in monitoring patients with liver diseases, cardiac problems, viral infections as well as imbalance in metabolic activities. The normal range of concentration in the blood are from 5 to 40 U /l for AST and from 5 to 35 U/ l for ALT.

AST(GOT) and ALT(GPT) detection have been researched by a number of scientists all over the world as well as International Federation of Clinical Chemistry (IFCC) and The Scandinavian Committee on Enzymes (SCE) (Xing-Jiu Huang,et. al.,2006). The quantitative estimation of these diagnostic enzymes has an important aspect of biochemical and clinical biochemistry experiments in the academic circles. The present method of assessing diagnostic serum enzymes is a similar method but with a different set of substrate composition. The intention of developing this method was to reduce the possible health hazards in the usage of different sera in the academic circles and to reduce the costs incurred.

Materials and methods:

Standard pyruvate: 44 mg Sodium Pyruvate dissolved in 20ml of Phosphate buffer and diluted to 200ml using the phosphate buffer. Store it in amber coloured bottle under refrigerated conditions

Buffered Substrate : 15ml of 2mM Sodium pyruvate made upto 100ml with phosphate buffer. Store it in amber coloured bottle under refrigerated conditions

1mM 2,5,DNPH: Dissolve 39.6 mg of 2,5,DNPH in 200 ml of warm 1N HCl. Store it in amber coloured bottle under refrigerated conditions

/ 0.4 N NaOH: Dissolve 24 gm of NaOH in 150 ml of distilled water and make upto 1500 ml.

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The preparation of buffer varies with respect to Aspartine and Alanine transaminases estimations.

Colorimeter with 505 wavelength filter.
GPT buffer : 3.39 gm of Di Sodium hydrogen Phosphate anhydrous and 0.81gm Potassium di hydrogen Phosphate dissolved in 300ml of distilled water.(pH 7.4)

GOT buffer : Prepare (A) 3.561 gm of DiSodium hydrogen Phosphate anhydrous in 300ml distilled water and (B) 0.4539 gm Potassium dihydrogen Phosphate in 50 ml distilled water . Mix 21ml Of A and 4 ml 0f B solutions to prepare the buffer. (pH 7.8) Stored at room conditions.

All the chemicals used are reagent grades (Loba) only
The protocols (Table 1, and 1a) followed are based on the modified Reitman and Frankel method with different enzyme activities and colorimetrically the method is referred as fixed-time method. Altogether minimum six samples each time were assessed for four times. The statistical analysis of t test, was performed to assess the significance of the method.

Table 1 : Protocol for SGPT assay

Addition sequence	1	2	3	4	5	6
Enzyme activity (U/l)	0	28	57	97	150	Test
Substrate(ml)	0.5	0.45	0.40	0.35	0.3	0.5ml (incubate 5min/37°C)
Pyruvate standard(ml)	-	0.05	0.10	0.15	0.20	Add 0.5 ml serum=ukn
Distilled water(ml)	0.1	0.1	0.1	0.1	0.1	(incubate for 30 min /37°C)
2,5,DNPH(ml)	0.5	0.5	0.5	0.5	0.5	0.5
Mix well. Allow to stand for 20 min.						Mix well and allow to stand for 20 min/RT
0.4 N NaOH(ml)	5	5	5	5	5	5
Mix well and allow it to stand for 10 min at RT. Check the absorbance at 505nm						

Table 1A: protocol for SGOT assay

Addition sequence	1	2	3	4	5	6
Enzyme activity (U/l)	0	24	61	114	190	Test
Substrate(ml)	0.5	0.45	0.40	0.35	0.3	0.5ml (incubate 5min/37°C)
Pyruvate standard(ml)	-	0.05	0.10	0.15	0.20	Add 0.5 ml serum=ukn
Distilled water(ml)	0.1	0.1	0.1	0.1	0.1	(incubate for 20 min /37°C)
2,5,DNPH(ml)	0.5	0.5	0.5	0.5	0.5	0.5
Mix well. Allow to stand for 20 m						Mix well and allow to stand for 20 min/ RT
0.4 N NaOH(ml)	5	5	5	5	5	5
Mix well and allow it to stand for 10 min at RT. Check the absorbance at 505nm						

Results :
The SGPT/SGOT experiments were performed as per the protocol given by modified method of Reitman and Frankles' method suggested (Table -1, 1a). The readings were read by using Digital Colorimeter EQ-650-A (Equiptronics Company) at 490-505 nm wave length. Minimum six readings were read at each time and the mean values were compared with standard values expected (Table -2).

Table 2 : GOT and GPT values estimated using the new method

Enzyme activity	0	24	61	114	190	Enzyme activity	0	28	57	97	150
std.	0.303	0.403	0.5	0.592	0.675	std.	0.345	0.459	0.53	0.63	0.7
test 1	0.33	0.47	0.61	0.69	0.76	test 1	0.27	0.425	0.53	0.64	0.73
test 2	0.33	0.42	0.6	0.69	0.75	test 2	0.38	0.56	0.63	0.72	0.85
test 3	0.32	0.49	0.56	0.67	0.79	test 3	0.34	0.48	0.62	0.74	0.8
test 4	0.32	0.46	0.54	0.66	0.73	test 4	0.34	0.49	0.61	0.73	0.8
mean	0.31	0.46	0.58	0.68	0.76	mean	0.33	0.48	0.59	0.70	0.79
std dev	0.04	0.029	0.033	0.015	0.025	stdev	0.04	0.055	0.045	0.045	0.049
error	0.0025	0.014	0.016	0.007	0.012	error	0.022	0.027	0.023	0.02	0.023

(TEST 1,2,3,4, ARE THE AVERAGES OF 6 OBSERVATIONS)

The overall readings indicated with values (mean±SE) of 0.31±0.002 , 0.46±0.014, 0.58±0.016, 0.68±0.007 and 0.76±0.012 for enzyme activity of 0,24,61,114 and 190U/l of SGOT while the standard readings as per the kit remained 0.303,0.403,0.5,0.592 and 0.675 respectively.

GPT values (mean±SE) were 0.33±0.022, 0.48±0.027, 0.59±0.023, 0.70±0.02 and 0.79±0.024 for enzyme activity of 0,28,57,97 and 150U/l of SGOT while the standard readings as per the span kit remained 0.345,0.459,0.53,0.63 and 0.70 respectively. The present kit SGOT and SGPT values are significantly similar to that of commercially available kit (Fig.1 and Fig.2). The statistical analysis of the significance was tested for the new version of GPT/GOT assessment. The t test results (calculated t value=0.27 < table value 1.72at α=0.1) indicated no significance difference between the standard kit values and the new method. Each time a set of chemicals prepared can give 100 test results instead of 25

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tests of the kit preparations at the cost of one kit.

Fig. 1. GPT assay efficacy of newly developed to that of commercial kit (Std)

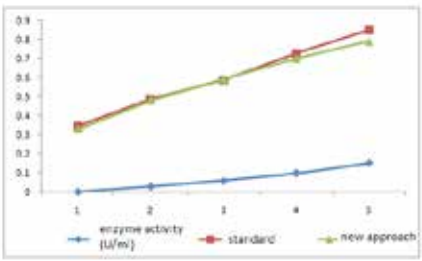
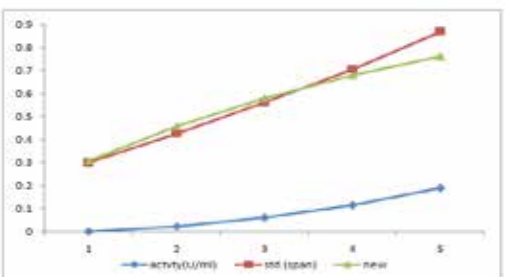


Fig. 2 GOT assay efficacy of newly developed to that of commercial kit (Std)



Conclusion:

Comparative studies of different test methods to that of reference method of Reitman and Frankel method were conducted earlier (In Seinong et.al.,1975). Studies also revealed certain aspects such as accuracy and precision of same serum sample value may change over the period of time with respect to level of enzyme. The assays based on commercially prepared reagents and standard solutions are accurate and comparable hence in widespread use (Witter and Linda,1966). In the current scenario of academics, the importance of practical dissections was reduced to minimise the cruelty against animals. A similar condition should be applied as the blood samples used for the biochemical studies can be of hazardous, infectious which can cause negative impact on the student's health. The calculation of (GPT/GOT) enzyme activity is based on the calorimetrically determined pyruvate which is produced after incubation of the enzyme and substrate at fixed conditions and reacts with DNPH reaction. The levels are in comparison with enzyme activity and expressed as Reitman-Frankel units. The representative kit has the artificial substrate, pyruvate standard , DNPH to produce coloured hydrazone complex. as reagents which produce hydrazone in alkaline conditions. The kit with an serum sample (dilution of standard 1:1,1:3 with the buffer) of serum is more useful healthwise, economical and cheap for the laboratory purposes.

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