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DISCREPANCIES IN MEASUREMENT OF ALANINE AMINOTRANSFERASE (GPT *) IN HUMAN PLASMA, USING A SHORT-INTERVAL ENZYME ANALYZER WITH THE GERMAN AND SCANDINAVIAN METHODS

H.J. HENDRIKS **, ANNIE M. VAN KOUWEN and H.W. ANTONISSE

Department of Clinical Chemistry of the Small Animal Clinic and the Department of Animal Husbandry, University of Utrecht (The Netherlands)

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Our interest in the problems of method- and instrument-dependency in clinical chemistry prompted us to study the measurement of GOT in human plasma by two different methods, using one of the recently introduced double-beam rapid analyzers (Beckman True Rate (TR) enzyme analyzer), which has minimal facilities for pre-incubation. The instrument calculates enzyme activity when the second derivative of the reaction rate, d^2A/dt^2, remains at zero for 17 sec. Measurements were made by the German [1] and Scandinavian [2] methods, in which a pre-incubation step is imperative. The results have been published in this journal [3]. It was concluded that when the Beckman TR was used in the single-beam mode with the Scandinavian method, enzyme activity was calculated before "other" NADH consumers (e.g., pyruvate) were removed, resulting in artificially high values. This was in contrast to the findings with the German method. Double-beam determinations gave essentially the same results as single-beam determinations.

We studied SGPT in a new series of experiments using the same instrument. The measurements were again made by the Scandinavian [1] and the German [2] methods. The composition of the reactants in the assay mixture was the same as has been specified for these methods. For the German method we followed a Boehringer method sheet [4].

In the double-beam (db) experiments the test cuvet contained all reagents and sample (heparinized plasma) and the reference cuvet the same minus 2-oxoglutarate. In the single-beam (sb) experiments the test cuvet contained all reagents and plasma, and the reference cuvet air only. The experimental design was that proposed by Rodgerson and Osberg [5].

Since the distinction between normal and slightly elevated values is impor-

* Non-standard abbreviation used: GPT = ALAT = L-alanine:2-oxoglutarate aminotransferase (EC 2.6.1.2).

** Reprint requests: Small Animal Clinic, Veterinary Faculty, University of Utrecht, 8 Yalelaan, Utrecht, The Netherlands.

tant, only values between 0 and 70 I.U./l were used for statistical treatment. All measurements were carried out at a reaction temperature of 37° C, as advocated for the Scandinavian method, which showed the discrepancies.

In the first experiment the German method was used and the following linear regression line was obtaines:

y (sb) = 3.340 + 0.957x (db)

degrees of freedom = 23

a_0 (intercept of the line) differs significantly from zero (P = 0.26 — 2), but the difference is very small.

a_1 (slope of the line) does not differ from 1 (P = 0.11)

r = 0.992; residual variance = 2.64

In the second experiment the same samples were analyzed by the Scandinavian method.

The best fitting linear equation was:

y (sb) = —12.18 + 0.8883x

degrees of freedom = 23

a_0 differs significantly from zero (P = 0.67 — 4)

a_1 does not differ from one (P = 0.61—1)

r = 0.958; residual variance = 5.82

These results indicate that the db and sb experiments produced the same results with the German method, but not with the Scandinavian method.

We also calculated the correlation between the results of the db experiments (Scandinavian method) and those of the sb experiments (German method). The best fitting linear equation was:

y = 2.154 + 1.039x

degrees of freedom = 23

a_0 does not differ from zero (P = 0.34)

a_1 does not differ from one (P = 0.49)

r = 0.968; residual variance = 5.49

Hence the German method in the sb mode gave the same results as the Scandinavian method in the db mode.

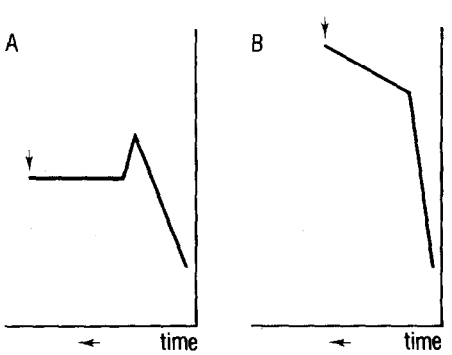

Fig. 1. Change in absorbance during (a) German method and (b) Scandinavian method. ↓ denotes the point in time at which the Beckman TR calculates the enzyme activity.

TABLE I

INSTRUMENT CALCULATED VALUES OF SGPT OF SIX KINDS OF DETERMINATION

In all cases mentioned in the seventh column the tracing rose during the determination.

Sample No.	German method			Scandinavian method		
	db-mode	sb-mode	sb-mode —oxoglutarate	db-mode	sb-mode	sb-mode —oxoglutarate
3	14	16	0	26	6	25
4	67	69	0	78	59	11
5	59	57	2	56	38	17
8	21	24	1	31	13	13
9	22	23	2	41	14	11
12	68	67	3	72	44	29
20	15	17	0	16	3	16
21	32	33	3	38	18	18
25	10	12	3	14	1	15
33	66	64	3	69	53	11

In the third experiment the test cuvet contained all reagents and plasma minus 2-oxoglutarate, while the reference cuvet contained air only. The reaction pattern was the same as that in the preincubation period, as required by the German and the Scandinavian methods. Using the capability of the TR to monitor the change in absorbance continuously, we found that the absorbance was stabilized before the instrument computed the pseudo enzyme level in the German method. This was in contrast to the findings with the Scandinavian method. During measuring time the absorbance rose (Fig. 1), sometimes to rather high pseudo-enzyme levels. Some examples are given in Table I. In the last series of experiments the first and second experiment were repeated using pure GPT (Sigma, No. G-9880), dissolved in 6% bovine albumin. The results obtained with both methods were essentially the same.

It is concluded that when the Beckman TR was used in the sb mode with the Scandinavian method, artificially low values were found. The use of the sb or db mode in the German method or the db mode in the Scandinavian method is imperative for obtaining accurate results.

The authors want to stress again their opinion [3] that it is necessary to study method-instrument dependency carefully and constantly, particularly before instruments designed along new conceptual lines are put into routine use.

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