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Reference Ranges for α -Amylase in Serum and Urine with 4,6-Ethylidene-(G7)-1-4-nitrophenyl-(G1)- α ,D-maltoheptaoside as Substrate

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Summary: Reference ranges for α -amylase in serum, spontaneously voided urine, and 24 h urine were determined, using 4,6-ethylidene-(G7)-1-4-nitrophenyl-(G1)- α ,D-maltoheptaoside as the substrate (EPS method), at 25, 30, and 37 °C. The measured values were evaluated with and without the use of a factor which converts the results of the α -amylase EPS method into values comparable to those obtained with the α -amylase PNP method (substrate: 4-nitrophenyl- α ,D-maltoheptaoside); comparison with the established reference ranges of the PNP method was therefore possible. The values for urine sometimes deviated markedly from the PNP reference ranges, but the values for serum showed close agreement. With the use of the conversion factor, the following reference ranges are proposed for the new α -amylase method:

Serum (186 males and 131 females): up to 120 U/l (25 °C), up to 160 U/l (30 °C), and up to 220 U/l (37 °C).

Spontaneously voided urine: up to 600 U/l (n = 323, 25 °C), up to 800 U/l (n = 373, 30 °C), and up to 1000 U/l (n = 373, 37 °C).

24 h urine: up to 450 U/24 h (n = 90, 25 °C), up to 650 U/24 h (n = 129, 30 °C), and up to 900 U/24 h (n = 129, 37 °C).

Introduction

Determination of the catalytic concentration of α -amylase (EC 3.2.1.1) in serum and urine in cases where acute pancreatitis is suspected is currently the most frequently requested test, despite its limited specificity

(1). Analytical procedures that use oligosaccharides with chromophore groups as substrates have proved to be effective. As a rule, substrates of this kind have 4–7 glucose residues joined by an α -glycosidic linkage (2–4) and are converted under the test conditions

into structurally and stoichiometrically defined products. However, different substrate conversion rates lead to substrate-specific reference ranges.

We determined the reference ranges of α -amylase in serum, spontaneously voided urine, and 24 h urine for a new method that uses the protected substrate 4,6-ethylidene-(G7)-1-4-nitrophenyl-(G1)- α ,*D*-maltoheptaoside (5). A comparison with earlier reference ranges determined with the unprotected substrate 4-nitrophenyl- α ,*D*-maltoheptaoside was of particular interest (6), since, with the use of a method conversion factor, the same reference ranges would be expected in both cases (7).

Materials and Methods

Reagents, methods, equipment

Since the reference ranges for the α -amylase EPS method were determined in the course of the evaluation of this method in 5 laboratories, details of the assay conditions and methodology as well as instrumentation and temperatures used in the individual laboratories are described in that evaluation report (7).

The reagents used in the α -amylase determination were from Boehringer Mannheim GmbH, Mannheim, FRG:

- α -Amylase EPS,
Cat. No. 882 682 (buffer/enzyme) and
Cat. No. 882 747 (substrate).
- α -Amylase PNP,
Cat. No. 568 686 (buffer/enzyme) and
Cat. No. 568 651 (substrate).

The determinations were performed using the following analytical systems, in accordance with the reagent manufacturers' instructions (7): Eppendorf ACP 5040, Boehringer Mannheim/Hitachi 737, IL-Multistat[®] III, Boehringer Mannheim/Hitachi 705. Precinorm[®] U and Precipath[®] U control sera were used for quality control.

The α -amylase activities and reference ranges were calculated both on the basis of the original factor for the amylase EPS method (2652 for sample/reagent ratio 1:21 and $\lambda = 405$ nm) and using the same factor multiplied by the conversion factor 2.5, which was derived from the median of slopes of the regression lines evaluated for 14 method comparison studies (7).

Reference groups

The following reference groups were enrolled in Linz and Tübingen to determine the reference ranges in serum:

Linz: 100 men and 100 women aged between 18 and 60, selected from ambulatory patients of the hospital. Selection criteria were normal values for: aspartate aminotransferase, alanine aminotransferase, total bilirubin, γ -glutamyltransferase, alkaline phosphatase, total protein, glucose, lipase and α -amylase with the maltotetraose method.

Tübingen: 31 women and 86 men aged between 18 and 66. This group were blood donors to whom the following selection criteria were applied: apparent good health, exclusion of drug taking, normal values for alanine aminotransferase.

The α -amylase determinations in spontaneously voided urine were carried out in two groups in Linz (160 women, 163 men) and Nijmegen (29 women, 21 men).

The group used for the determination of α -amylase reference ranges in 24 h urine consisted of 129 volunteers; the samples were collected in the 4 laboratories participating in the evaluation of the assay: Linz, Tübingen, Nijmegen and Stuttgart.

Criteria for group selection for the determination of all urine values were: exclusion of participants showing pathological results either with the test strip Combur[®]-Test[®] Nephur-Test[®] (Cat. No. 203245, Boehringer Mannheim GmbH), insignificant urinary sedimentation, normal creatinine clearance and no indication of pancreatic disease.

Sample collection

The serum samples were taken from fasting people in a resting position and analysed within a period of 24 hours. All urine samples were analysed directly after collection.

Spontaneously voided urine samples were predominantly those first voided in the morning.

Evaluation of the data

The statistical evaluation was done with a non parametric procedure. The 97.5th percentiles were taken as a basis for the upper decision limits in the serum reference groups, and the 95th percentiles were taken as cut-off points in the urine reference groups. The reasons for selecting the 95th percentiles in the urine reference groups are:

- The method has limited precision when using urine as the sample material, so that a precise borderline cannot be expected.
- In addition, the 24 h urine group is too small for taking the 97.5th percentile as the decision limit, and comparability between spontaneously voided and 24 h urine is required.

Since the activities of α -amylase in serum and urine are independent of sex and age (8, 9), no evaluation specific for these conditions was undertaken.

Results

Quality control

The interlaboratory comparability of the methods using different instruments was shown by the results obtained in the round-robin and recovery studies of the assay evaluation (7).

Reference ranges in serum

Figure 1 shows the serum α -amylase activities in the Linz and Tübingen reference groups at 25 °C.

Slight differences in distribution can be seen between the blood donor group and the "hospital group". In the blood donor group the median of α -amylase values at 25 °C is 10 U/l lower; a remarkably good agreement is obtained at the upper limit of the reference range: the 95th percentiles are 116 U/l in both groups and the 97.5th percentiles are 128 and 126 U/l.

Similarly good agreement between the groups was obtained at 30 °C and 37 °C.

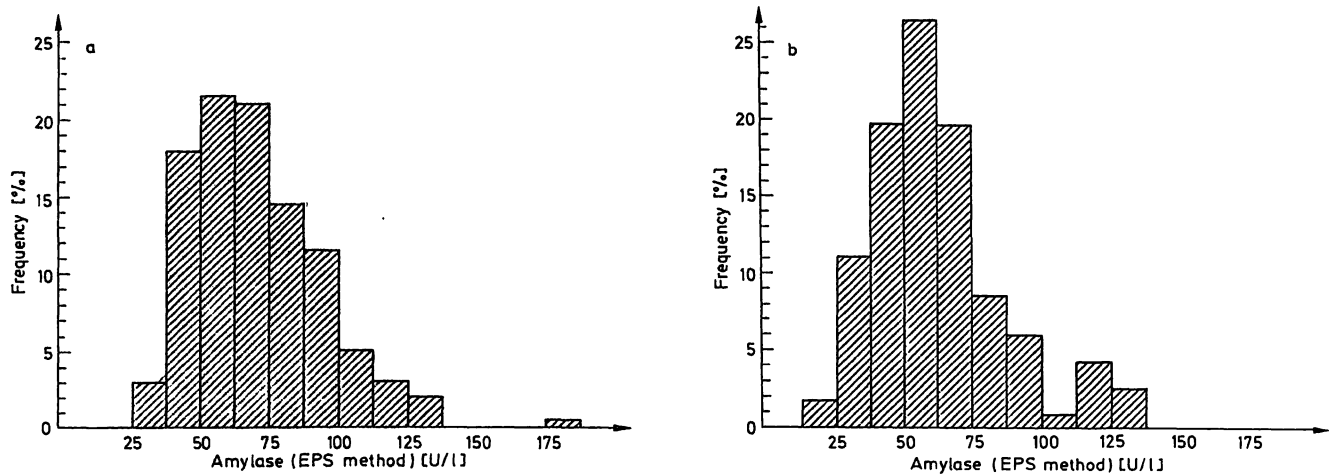


Fig. 1. Distribution of serum α -amylase activities in the Linz and Tübingen reference groups; measuring temperature 25 °C. The values are calculated using the method conversion factor.

a) Linz, n = 200: median = 68 U/l;
95th percentile = 115 U/l;
97.5th percentile = 128 U/l

b) Tübingen, n = 117: median = 58 U/l;
95th percentile = 116 U/l;
97.5th percentile = 126 U/l

Both the Linz and the Tübingen groups represent an unbalanced selection of volunteers each comprising only a part of non-pathological people. That is why, for medical reasons, the data from the two groups were pooled for evaluation. By this combination the reference basis is extended, and the group enlargement moreover increases the statistical significance of the upper decision limit. The most important percentiles

of serum α -amylase activities for the individual and the combined groups (with and without the use of the method conversion factor) at 25, 30 and 37 °C are presented in table 1. Table 1 also contains the results for the PNP method from the Tübingen subgroup, in which the EPS and the PNP methods were simultaneously used.

Tab. 1. Distribution of α -amylase activities in serum from the reference groups for the amylase EPS method at 25 °C, 30 °C and 37 °C (N = 317).

The data indicate the 2.5th, 5th, 50th (median), 95th and 97.5th percentiles as well as the lowest and highest values for N people. In Tübingen, the PNP method for amylase was performed in parallel (N = 117). Data in parenthesis: Values for α -amylase EPS without method conversion factor.

T	Method	N	Group	α -Amylase (U/l)						
				Min.	2,5%	5%	50%	95%	97,5%	Max.
25 °C	α -Amylase EPS	317	Linz + Tübingen	20	33 (13)	38 (15)	65 (26)	116 (47)	126 (51)	183
	α -Amylase EPS	200	Linz	30	38	40	68	116	128	183
	α -Amylase EPS	117	Tübingen	20	28	33	58	116	126	135
	α -Amylase PNP	117	Tübingen	18	26	32	55	104	113	132
30 °C	α -Amylase EPS	317	Linz + Tübingen	28	41 (17)	48 (19)	88 (35)	146 (59)	156 (63)	193
	α -Amylase EPS	200	Linz	35	46	55	94	146	159	193
	α -Amylase EPS	117	Tübingen	28	34	41	78	145	156	185
	α -Amylase PNP	117	Tübingen	27	34	42	77	143	154	178
37 °C	α -Amylase EPS	317	Linz + Tübingen	33	51 (21)	64 (26)	120 (48)	193 (77)	208 (83)	241
	α -Amylase EPS	200	Linz	45	60	68	125	195	205	235
	α -Amylase EPS	117	Tübingen	33	48	56	103	189	209	245
	α -Amylase PNP	117	Tübingen	38	49	59	103	188	204	241

Tab. 2. Percentiles of α -amylase activities in urine from the reference groups for the amylase EPS method at 25 °C, 30 °C and 37 °C.

Data in parenthesis: Values for α -amylase EPS without method conversion factor.

T	N	α -Amylase (U/l)						
		Min.	2,5%	5%	50%	95%	97,5%	Max.
Spontaneously voided urine								
25 °C	323	35	71 (29)	89 (36)	243 (97)	574 (230)	674 (270)	1698
30 °C	373	38	70 (28)	85 (34)	290 (116)	806 (323)	850 (340)	1565
37 °C	373	35	96 (39)	123 (49)	420 (168)	996 (399)	1125 (450)	1545
24 h urine								
25 °C	90	0	12 (5)	18 (7)	137 (55)	456 (182)	526 (210)	864
30 °C	129	1	22 (9)	30 (12)	232 (93)	671 (268)	786 (314)	1144
37 °C	129	0	25 (10)	42 (17)	310 (124)	936 (374)	1081 (432)	1505

Amylase activities in spontaneously voided and 24 h urine

Table 2 presents the percentiles of α -amylase activities (with and without the use of the method conversion factor) in the spontaneously voided and the 24 h urine at 25, 30, and 37 °C.

Figure 2 shows the distribution of α -amylase activities in spontaneously voided urine samples at 37 °C according to the two methods, α -amylase EPS and α -amylase PNP. It can be seen that the two methods yield a near-identical distribution of urine α -amylase values.

Discussion

The results of the determination of reference ranges in serum provide further confirmation that the use of the conversion factor 2.5 (7), derived from method comparison studies, leads to near-identical values with the two methods, α -amylase PNP and α -amylase EPS. This is obvious in a comparison of the medians obtained in the parallel performance of the two methods in Tübingen (see tab. 1); slightly larger deviations were observed at the upper limit of the reference range, but were always within a $\pm 10\%$ range.

There were slight differences in the 95th and the 97.5th percentiles between the newly determined EPS values and the previously published PNP values (6). The upper limit of 120 U/l established for 25 °C corresponds approximately to the 95th to 96th percentile in the new reference group, while at 30 °C virtually no deviation is observed in the 97.5th percentile. At 37 °C only, the value corresponding to the established reference limit (220 U/l) in the new reference group is somewhat above the usual cut off point (97.5th percentile). The α -amylase PNP values determined in

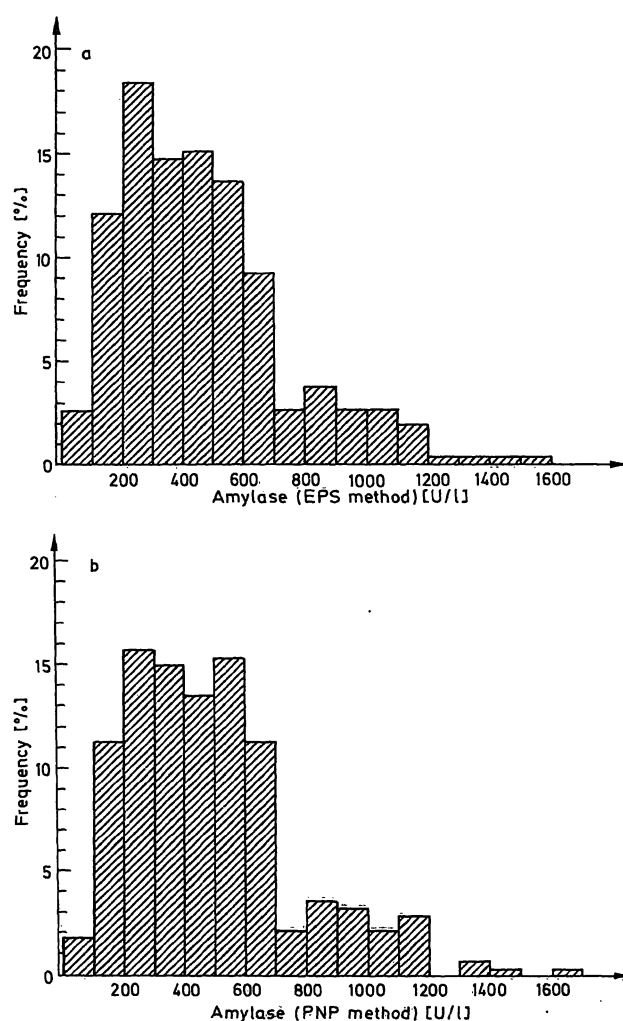


Fig. 2. Distribution of α -amylase activities in spontaneously voided urine at 37 °C. Comparison between the methods, α -amylase PNP and α -amylase EPS at 273 volunteers (subgroup Linz/Nijmegen, where both methods were performed in parallel).

- a) EPS, n = 273: median = 420 U/l;
 95th percentile = 1019 U/l;
 97.5th percentile = 1166 U/l
- b) PNP, n = 273: median = 454 U/l;
 95th percentile = 1079 U/l;
 97.5th percentile = 1133 U/l

parallel in Tübingen provide excellent confirmation of the new EPS values (tab. 1), so that the divergence between the new and the old values can be attributed to a group difference rather than to a methodological difference. Other studies confirm the somewhat higher reference ranges for 37 °C (10, 11).

The divergence between new and old reference ranges for serum cannot be ascribed to any substrate-related characteristics of the PNP and the EPS assays. It may therefore be recommended without any loss of diagnostic reliability that the reference ranges determined with the α -amylase PNP method (6) should be retained for the α -amylase EPS method (Tab. 3).

Tab. 3. Recommended upper reference limits for the EPS method for α -amylase in serum and urine.

	α -Amylase EPS			
	Dimension	25 °C	30 °C	37 °C
Serum	U/l	120	160	220
Spontaneously voided urine	U/l	600	800	1000
24 h urine	U/24 h	450	650	900

In contrast, larger divergences from the published ranges for the α -amylase PNP method (6) were found for spontaneously voided urine. The magnitude of the differences varied with the measuring temperature. The relative differences between our results (95th percentile) and the published results are as follows: + 3% at 25 °C, + 15% at 30 °C, and -20% at 37 °C (referred to the established values). Thus, the largest

deviations are seen at 37 °C. Conversely, in the present study a nearly congruent distribution of the α -amylase activities for identical groups was obtained with the two methods, α -amylase EPS and α -amylase PNP (see fig. 2). Therefore, it is improbable that this deviation is due to the substrate. Any explanations of the differences between new and old reference ranges for spontaneously voided urine can, of course, be only hypothetical. A possible reason for the differences may be the fact that no correction for the urine density was made; it is true that the diagnostic value of urinary amylase activity can be enhanced if related to another urine constituent such as creatinine (12), but this needs an additional determination and that is why this relationship has so far not been widely used in the routine diagnosis. A considerable feature of the cited study (6) is the different size of the groups at different temperatures and the non-uniform temperature coefficients in the comparisons of serum and urine reference ranges. The groups enrolled in our study were almost identical at the three temperatures, and the ratios of the enzyme activities at the various temperatures are very similar in serum and spontaneously voided urine. For these reasons, and on the basis of the large size of our groups, we propose the values listed for spontaneously voided urine in table 3 as reference ranges.

Table 3 also contains our proposals for reference ranges in 24 h urine. It should be noted that α -amylase values in 24 h urine must be interpreted with reservation, owing to the instability of α -amylase in this sample material (13).

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