Evaluation of a New Inhibitor Test for Isoamylase on Hitachi 705 Analyser

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Summary: We describe a simple and rapid method for measuring pancreatic and salivary type amylases using the Hitachi 705 analyser. The determination is based on the inhibition of salivary amylase using an inhibitor isolated from wheat germ.

The precision of the proposed method was very good: within-day precision varied from 0.4 to 2.5% (CV) and day-to-day precision from 2.2 to 3.7% (CV). The new application correlated well with another commercially available inhibition method.

As the standardization is very stable and the assay procedure exactly the same as for the total amylase assay, the proposed method is suitable for routine isoamylase determination.

Reference values for pancreatic and salivary amylase activities are presented.

Materials and Methods

α-Amylase PNP reagents, pancreatic amylase and salivary amylase Standards and the inhibitor (from Boehringer Mannheim, Mannheim, Germany) were reconstituted and used according to the manufacturer's recommendations. Assay parameters are presented in figure 1. Except for the presence of inhibitor, the assay procedure for the measurement of the uninhibited amylase activity (residual activity in fig. 2) was the same as for the total amylase activity. The isoamylase activities were calculated using the following formulae:

\[
P = \frac{R - b \cdot T}{a - b} \quad \text{and} \quad S = T - P
\]

where

- \( P \) = total amylase activity
- \( P \) = activity of pancreatic isoamylase
- \( S \) = activity of salivary isoamylase
- \( R \) = uninhibited amylase activity (residual activity)
- \( a \) = \( R/T \) of P-standard
- \( b \) = \( R/T \) of S-standard

Alternatively, a standard curve can be used (fig. 2). Phadebas® Kinetic Amylase and KAI (Kinetic Amylase Inhibitor, (13)) reagents (Pharmacia Diagnostica, Uppsala, Sweden) were used according to the manufacturer's recommendations and enzyme activities were measured with Olli C (Kone Oy, Instrument Division, Espoo, Finland).
Chemistry parameters

Test code: AMY
Assay code: Rate-25-31
Sample volume: 10
R I vol.: 350 H (S)
R 2: 70—8 (S)
R 3:
Wavelength 1: 660 NM
Wavelength 2: 415 NM
Rgt. blk. abs:
Rgt. blk. conc:
St. conc: 0-0-0
Factor:
Std. abs. allowance:
Normal r nge L:
Normalized r nge H:
Abs. limit (rate):
Control id. no.:

In order to establish reference ranges, the sera of 173 healthy individuals (80 males and 93 females) were examined for total, pancreatic and salivary amylase at 30 °C and 37 °C. The group consisted of healthy employees checked routinely on a five yearly basis. Individuals with elevated γ-glutamyltransferase activities (37 °C: males 50 U/l, females 32 U/l) were excluded. The remaining reference group consisted of 70 male and 93 female individuals from 20 to 50 years.

Results

The inhibition was examined by mixing pancreatic and salivary amylase standards in different ratios and measuring total and uninhibited amylase activities. The inhibition curve was linear (fig. 2). The inhibition ratio was independent of the salivary amylase activity at least up to 2000 U/l. In practice, we always diluted the serum sample with saline if its total amylase activity was higher than 2000 U/l.

The precision of the proposed method was checked by measuring pancreatic and salivary α-amylase standards and three pooled sera teil times in one run (within-day) and once a day during two weeks (between-day). The precision data are presented in table 1.

We also measured 74 patient samples with Phadebas® Kinetic Amylase and KAI reagents, and by the present method. The correlation between these two methods was good (fig. 3). The slope of this method comparison for pancreatic amylase is 1.75, which is in good agreement with the conversion factor (1.77) for α-amylase PNP to Phadebas® units (10, 11).

Table 2 shows the distribution of total, pancreatic and salivary amylase activities in the sera of 163 apparently healthy individuals at 30 °C and 37 °C. The 97.5 percentile of total amylase activities demonstrates good agreement with established reference values (12). The reference values, assuming a log normal distribution established for another inhibitor method (16) that requires a preincubation step, are significantly higher for total, pancreatic and salivary amylase. In our evaluation, non-parametric statistics have been chosen and there is consistency with reference limits established for total amylase (12). Therefore, the ranges for pancreatic and salivary amylase activities given in table 2 can be recommended as reference limits.

Tab. 1. Precision of the proposed method.

<table>
<thead>
<tr>
<th>Within-day</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>n</th>
<th>Between-day</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
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<td></td>
<td>U/l</td>
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<td></td>
<td></td>
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<td>U/l</td>
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<tr>
<td>P-ST</td>
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<td></td>
<td></td>
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<td></td>
<td>T</td>
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<td></td>
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<tr>
<td>T</td>
<td>2324</td>
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<td>R</td>
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<td>0.2</td>
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<td></td>
<td>2102</td>
<td>57</td>
<td>2.7</td>
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<tr>
<td>S-ST</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>R/T (%)</td>
<td></td>
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<tr>
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<td>0.4</td>
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<td>14.9</td>
<td>1.0</td>
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<tr>
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<td></td>
<td>96</td>
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Tab. 2. Reference limits for pancreatic and salivary type amylase.

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<tr>
<td></td>
<td>Percentile</td>
<td>Percentile</td>
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<tr>
<td>Total amylase (U/l)</td>
<td>2.5 — 97.5</td>
<td>2.5 — 97.5</td>
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<tr>
<td>(♀)</td>
<td>(41 — 170)</td>
<td>(50 — 220)</td>
</tr>
<tr>
<td>(♂)</td>
<td>(46 — 147)</td>
<td>(63 — 198)</td>
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<tr>
<td>Pancreatic amylase (U/l)</td>
<td>18 — 94</td>
<td>28 — 126</td>
</tr>
<tr>
<td>(♀)</td>
<td>(19 — 70)</td>
<td>(26 — 120)</td>
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<tr>
<td>(♂)</td>
<td>(2 — 119)</td>
<td>(4 — 145)</td>
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<tr>
<td>Salivary amylase (U/l)</td>
<td>2 — 119</td>
<td>4 — 145</td>
</tr>
<tr>
<td>(♀)</td>
<td>(7 — 115)</td>
<td>(14 — 150)</td>
</tr>
</tbody>
</table>

Discussion

A fully automated application for isoamylase determination on the Hitachi 705 analyser based on the salivary isoenzyme specific Inhibitor is described. In contrast to earlier published inhibition methods (3, 8, 9, 13, 14, 16), no extra preincubation is needed, but the assay procedure is exactly the same as for the total amylase assay. The inhibition curve is linear and therefore only two standard points are needed. The reagents are so stable that standardisation is necessary only when the inhibitor solution is changed.

Because the precision is also excellent the method is very suitable for routine isoamylase determination. However, due to matrix effects or isoenzyme patterns including P3, there may be a very few cases in which R/T ratios exceed those for the pure pancreatic standard, thereby giving anomalous results (17).

References
