Intended use. Amylase activity determination system for blood, urine and other biological fluids (duodenal, pleural and ascitic) by kinetic method of fixed-time.

Professional use.

[Only for in vitro diagnostic use.]

Test principle. The sample is incubated with a substrate of starch and the decrease of the blue color, after adding iodine is compared to a control, being proportional to the amylase in the sample.

Summary. Amylase measurement is considered medical urgency because it is an important data on diagnostic of pancreatitis, mainly acute pancreatitis that has high mortality index.

Labtest sought the resolution for two issues on amylase determination: speed and stability of substrate.

The proposed method is performed with the minimum of time and procedures, allowing a fast delivery of results. The technique has countless advantages on saccharogenic and iodometric methods by tittering, highlighting among those the speed and decreased operational steps.

Labtest Substrate has substances that provide excellent conditions for the action of amylase activity and a high power preservative that avoid bacterial or fungal contamination.

Methodology. Caraway modified

Reagents

1. **Enzyme** - Substrate - Store at 2 - 8 ºC.
Reagent label bears expiration date.
Starch 0.4 g/L; phosphate buffer pH 7.0 and stabilizer.

2. **Color Reagent (stock)** - Store at 2 - 8 ºC.
Reagent label bears expiration date.
Potassium iodide (16.7 mmol/L); potassium iodated (271 mmol/L) and chloridric acid (112 mmol/L).

Precautions and warnings

For in vitro diagnostic use.

Avoid pipetting the substrate with the mouth, using saliva contaminated materials, blow on the substrate and talk near the open bottle. These actions may contaminate the reagent with microscopic amounts of saliva or sweat and deteriorate the substrate.

A reduction of more than 10 % on the control absorbance indicates substrate contamination with saliva.

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling. Cares regarding reaction time, temperature of incubation and pipetting are extremely important in order to obtain correct results. One minute difference of incubation provides a 13.3 % error in the results.

The Color Reagent contains chloridric acid. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.

Storage and stability. Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label. During the use, microbial or chemical contamination may occur and may reduce the reagents stability.

Specimen collection and preparation

Use serum or plasma (heparin) and fluids (ascetic, duodenal or pleural). Samples with Citrate, EDTA or Oxalate should not be used because produce false decreased results.

Amylase activity is reportedly stable in serum, plasma or fluids for about 7 days at 15 - 25 ºC and several months at 2 - 8 ºC. Do not use samples with contamination signs.

Since there is no known test which can offer complete assurance that human blood samples will not transmit infectious diseases, all blood derivatives should be considered potentially infectious. Therefore, biosafety rules must be followed for handling the samples.

Interference

Bilirubin up to 5 mg/dL, hemoglobin up to 30 mg/dL and triglycerides up to 250 mg/dL do not interfere significantly. Values higher those cited above yield false decreased results.

Materials required not provided

1. Photometer capable of measuring absorbance in a range of 620 and 700 nm.
2. Pipettes to measure reagents and samples.
3. Water bath at constant temperature (37 ºC).
4. Timer.

Preparing the working reagent. Color Reagent for Using: transfer all the contents of the stock for the empty bottle supplied by the kit; add 45 mL of distilled or deionized water and mix. It is stable 6 months at 2 - 8 ºC.
Manual procedure

See notes 3 and 4.

For measurement in urine, collect in a determined range of time (2 hours, for example). Adjust the pH around 7.0 and 7.4 using solid sodium carbonate, when the pH is lower than 7.0, and solid monopotassium phosphate when higher than 7.4.

Set up two tubes and proceed as follows:

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Unknown</th>
<th>Control</th>
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</table>

Incubate in a water bath at 37 ºC during 2 minutes.

Mix and incubate in a water bath at 37 ºC for exactly 7 minutes and 30 seconds.

Mix, wait 5 minutes and determine the absorbance of the Unknown and control against distilled water at 660 nm or red filter (620 - 700 nm). The color is stable for 30 minutes.

Sample and reagent volume may be modified proportionally without affecting the test performance and the calculation procedure. In case of volume reduction is important to observe the minimum volume needed to the photometric reading. Volume of sample lower than 0.01 mL is critical in manual applications and should be avoided because it increases the measurement imprecision.

Quality control. For quality control use Qualitrol Level 1 and Qualitrol Level 2 or other suitable control material. The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective measures to be taken if values fall outside the control limits.

Calculations. See measurement/reportable range.

\[
\text{Amylase (Units/dL)} = \frac{\text{Ac} - \text{Au}}{\text{Ac}} \times 800
\]

\[
\text{Ac} = \text{Amylase of the Control}
\]

\[
\text{Au} = \text{Amylase of the Unknown}
\]

\[
\text{Urinary Amylase (Unit/hour)} = \frac{\text{Amylase (Units/dL)} \times V}{\text{H} \times 100}
\]

\[
V = \text{volume of the urine (mL)}
\]

\[
H = \text{number of hours in which the urine was collected}
\]

\[
\text{Ratio Amylase/Creatinine}
\]

\[
\text{Amylase/Creatinine (U/g)} = \frac{\text{Amylase (U/dL)} \times 1000}{\text{Creatinine (mg/dL)}}
\]

Measurement/reportable range

Up to 400 U/dL.

If Amylase activity exceeds 400 U/dL, the sample must be diluted with 0.85% NaCl. Multiply the result by the appropriate dilution factor. Dilute the sample so that the obtained value is around 80 and 320 U/dL.

Ratio amylase depuration/creatinine depuration. In most cases of acute pancreatitis, serum and urinary amylase increase concomitantly, but in some cases the increase of urinary amylase is not followed by the increase of serum amylase. Therefore, an evaluation of the ratio of amylase depuration / creatinine depuration, expressed in percentage, provides a great diagnostic value in cases of acute pancreatitis and recurrent pancreatitis.

Determine the amylase activity and the concentration of serum creatinine and in a urine sample and apply the results in the following formula:

\[
\text{Ratio} = \frac{\text{Urinary amylase (U/dL)} \times \text{serum Creatinine (mg/dL)}}{\text{Serum Amylase (U/dL)} \times \text{urinary Creatinine (mg/dL)}} \times 100
\]

Expected values. Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

All ages

Serum/Plasma

60 - 160 U/dL

Urine

50 - 140 U/h

Urinary amylase/urinary creatinine

Up to 400 U/g

Amylase Depuration/Creatinine Deputation

1.0 - 4.0 %

Unit definition: one unit is the amount of the enzyme that hydrolyzes totally 10 mg of starch in 30 minutes at 37 ºC.

Performance characteristics

Recovery studies. In two samples with Amylase activity of 84 and 303 U/dL were added different quantities of enzyme. Subsequent analyses provided recoveries ranging from 92 and 108 %. The mean proportional systematic error at a value of 120 U/dL was 3.6 U/dL or 3.0 %.

Method comparison. A group of 80 sera were assayed by the proposed method and the CNPG Labtest using serum with amylase values ranging from 28 - 517 U/dL. The comparisons yielded a correlation coefficient of 0.96 and regression equation was \[y = 28 + 0.6x\]. It's evident that there is an extremely positive correlation among the two methods. It was possible to observe a systematic difference of 16 % at the decision level of 50 U/dL what is explained by the difference of the substrate and methodologies used.
Analytical sensitivity. Detection limit: 8 U/dL. The detection limit represents the lowest measurable amylase activity that can be distinguished from zero. A sample without amylase was used to calculate the detection limit of the assay what was found to be 8 U/dL, equivalent to the average of 20 assays plus 2 standard deviations.

Matrix dilution effects. Two sample with values equal of 380 and 477 U/dL were used to evaluate the system response on the matrix dilutions with 150 mmol/L NaCl (0.85%). Recoveries were found a range of 97 and 112%, using dilution factors that vary from 2 to 8.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megohm.cm, or conductivity ≤1 microsiemens/cm and silicates concentration must be <0.1mg/L.

3. Since saliva is rich in amylase, it should be avoided the material and reagents contamination with saliva. Avoid using mouth to blow the water, reagent and samples away of the pipette. A decrease in the control indicates saliva contamination on the substrate.

4. In all enzymatic reaction, the rigorous observation of time and temperature of incubation is very important for the quality of the results. The difference of 1 minute on incubation time yields a error of 13.3% in the result.


References

5. Tonks DB Quality Control in Clinical Laboratories, Warner-Chilcot Laboratories, Diagnostic Reagents Division, Scarborough, Canada, 1972.
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<th>Símbolo</th>
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