the rate of formation of pNP (p-Nitrophenol), directly proportional to
E = Ethyliden, PNP = Paranitrophenol, G = Glucose

of creatinine, has been found useful as a diagnostic concept
clearance of amylase, as related to the reasonably constant clearance
elevation of alpha-amylase activity in urine remain disputed. Renal
P (pancreatic) or S (salivary). Diagnostic specificity and sensitivity of
identified by determining whether the major isoenzyme present is type
salivary glands disorders…). The organ source can sometimes be
diseases, diabetic ketoacidosis, severe glomerular dysfunction,
associated with other disorders (abdominal disorders, biliary tract
occurs within 2 to 12 h of the onset and maximal levels are attained 12
to 72 h later. However, elevation of α-amylase activity in serum is also
α-amylase is most frequently measured in the diagnostic of acute
pancreatitis. In this case, a transient rise in serum amylase activity
α-amylase activity in the specimen, is measured at 405 nm.

Several procedures are available to assay alpha-amylase activity
(Amyloclastic methods, saccharogenic methods). Both these methods
lake linearity, sensitivity and precision when compared to
E-PNPG7 method. Reaction scheme is as follows:

1) 5 E-PNP-G₇ + 5 H₂O
\[ \text{α-amylase} \]
E-G₃ + 1 pNP-G₄ + 2 E-G₄ + 2 pNP-G₃ + 2 E-G₅ + 2 pNP-G₂

2) 1 pNP-G₄ + 2 pNP-G₃ + 2 pNP-G₂ + 14 H₂O
\[ \text{α-glucosidase} \]
5 pNP + 14 G
E = Ethyliden, PNP = Paranitrophenol, G = Glucose

The rate of formation of pNP (p-Nitrophenol), directly proportional to
the α-amylase activity in the specimen, is measured at 405 nm.

Reagent for quantitative determination of alpha-amylase
[EC 3.2.1.1] in human serum and plasma, or urines

Reagent for quantitative determination of alpha-amylase
[EC 3.2.1.1] in human serum and plasma, or urines

<table>
<thead>
<tr>
<th>Vial R1</th>
<th>WORKING REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E-PNP₇ 1.1 mmol/L</td>
</tr>
<tr>
<td></td>
<td>α-Glucosidase 1500 IU/L</td>
</tr>
<tr>
<td></td>
<td>NaCl 51 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Buffer pH 7.0 at 20°C</td>
</tr>
<tr>
<td></td>
<td>Preservative</td>
</tr>
</tbody>
</table>

Before reconstitution: T, R25-R32 R38: Toxic if swallowed – irritant. Contact with acid liberates toxic gas – Irritating to skin. Once reconstituted: None
S22/S38: Do not breathe dust. After contact with skin, rinse with plenty of water

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic
use.

• Verify the integrity of the contents before use.
• Use adequate protections (overall, gloves, glasses). Do not pipette
with mouth.
• Avoid contact with skin and eyes. If spilt, thoroughly wash affected
areas with plenty of water.
• Reagents contain sodium azide (concentration < 0.1%) which may
react with copper and lead plumbing. Flush with plenty of water
when disposing.
• Material Safety Data Sheet is available upon request.
• Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in
accordance with good laboratory practices using appropriate
precautions. Respect legislation in force in the country.

REAGENT PREPARATION

REF 80023: Use a non-sharp instrument to remove aluminium cap.
Add promptly the amount of demineralised water stated on the label.
Avoid contamination with salivary amylase.
Mix gently and wait for complete dissolution before using reagents
(approximately 2 min.).

STABILITY AND STORAGE

Store away from light, well cap in the original vial at 2-8°C.

• Unopened, reagents are stable until expiry date stated on the label.
• Once reconstituted, working reagent is stable for 3 months when free
from contamination.
• Discard working reagent if cloudy or if absorbance measured at
405 nm is > 0.600.
• Don’t use working reagent after expiry date stated on the label of the
Kit.

SPECIMEN COLLECTION AND HANDLING

Unhemolysed serum or heparinised plasma.
α-amylase activity is stable in serum/plasma for:
• at least 7 days at room temperature.
• 1 month at 2-8°C.

Urines: Adjust pH to alkalin range before storage.
α-amylase activity is stable in urines for 7 days at 2-8°C.
In case of delay in transporting urines to the laboratory, use a
preservative as merthiolate (thimerosal) 0.24 mM or 0.1 g/L.

INTERFERENCES

Hemoglobin no interference up to 522 mg/dL
Free bilirubin no interference up to 15.5 mg/dL (265 µmol/L)
Conjugated bilirubin no interference up to 16.7 mg/dL (286 µmol/L)
Lipemia no interference from lipemia measured as an
absorbance at 630 nm up to 1.180 Abs.

For a more comprehensive review of factors affecting this assay refer
to the publication of Young D.S.
MATERIAL REQUIRED BUT NOT PROVIDED
1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera
3. Demineralised water for reagent preparation

CALIBRATION
Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.

• Use the theoretical calibration factor (§ CALCULATION)
• Or REF 95015 BIOLABO Multicalibrator (calibration value determined with validated statistical technics and metrologically controlled instrument)
• or a multiparametric calibrator traceable to a reference method or material.

QUALITY CONTROL
• BIOLABO EXATROL-N Level I REF 95010
• BIOLABO EXATROL-P Level II REF 95011
• Other assayed control sera referring to the same method.
• External quality control program.
It is recommended to control in the following cases:
• At least once a run.
• At least once within 24 hours.
• When changing vial of reagent.
• After maintenance operations on the instrument.
If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, verify analysis parameters: wavelength, temperature, specimen/reagent ratio, time counting, calibration factor.
4. If control is still out of range, use a new vial of reagent and reassy.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (1)

<table>
<thead>
<tr>
<th>Serum (37° C)</th>
<th>α-amylase (IU/L)</th>
<th>α-amylase (µKat/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-80</td>
<td>[0.38-1.36]</td>
<td></td>
</tr>
</tbody>
</table>

| Urines (37° C) | 24-408 IU/24 h [0.41-6.94/24 h |

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCE CHARACTERISTICS (5)

<table>
<thead>
<tr>
<th>Within-run</th>
<th>Normal level</th>
<th>High level</th>
<th>Run-to-run</th>
<th>Normal level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 40</td>
<td>Mean IU/L</td>
<td>80.3</td>
<td>185</td>
<td>Mean IU/L</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td>S.D. IU/L</td>
<td>3.4</td>
<td>4.6</td>
<td>S.D. IU/L</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>C.V. %</td>
<td>4.3</td>
<td>2.5</td>
<td>C.V. %</td>
<td>3.95</td>
</tr>
</tbody>
</table>

Detection limit: approximately 10 IU/L (0.2 µKat /L)
Sensitivity for 1 IU/L: 0.195 mAbs/min.
Comparison studies with commercially available reagent:
\[ y = 0.9987 \times - 1.8 \quad r = 0.9805 \]

LINEARITY
The assay is linear up to 2000 IU/L (33 µKat/L).
If ΔAbs./min > 0.390, dilute specimen with saline solution and reassy taking into account the dilution factor. Linearity depends on the specimen/reagent ratio.

MANUAL PROCEDURE
Let stand reagent and specimens at room temperature.

| Pipette into 1 cm path length cuvette: |
| Reagent 1 mL |
| Specimen 25 µL |

Mix. Start a timer. Record initial absorbance after 1 minute at 405 nm. Record the absorbance again every minute during 3 minutes.
Calculate absorbance change per minute (ΔAbs/min.).

Notes: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION
Calculate the result as follows:

With theoretical factor:

\[ \text{IU/L} = \left( \Delta \text{Abs/min} \right) \times 5140 \]

With seric multicalibrator

\[ \alpha \text{-amylase (IU/L)} = \left( \Delta \text{Abs/min} \right) \text{Assay x Calibrator Concentration} \]

\[ \left( \Delta \text{Abs/min} \right) \text{Calibrator} \]

REFERENCES

(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-43 to 3-46