**ALT/GPT 4+1 SL**

*In vitro* diagnostic reagent, for professional use only

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**CLINICAL SIGNIFICANCE (1-4)**

Alanine aminotransferase (ALT) also known as glutamate pyruvate transaminase (GPT) is a transaminase. ALT catalyses the transfer of the amino group of L-alanine to α-ketoglutarate to give L-glutamate. The highest levels are found in the liver and the kidneys, and in smaller amounts in heart and skeletal muscle.

ALT concentration is increased when hepatic cells are damaged (liver cell necrosis or injury of any cause). Indeed, viral and toxic hepatitis induce a marked elevation of ALT activity in serum. Intake of alcohol, delirium tremens, and administration of various drug induce slight or moderate elevation of ALT. ALT concentration in serum is also slightly increased in various conditions such as: muscular dystrophy, hemolytic disease, myocardial infarction...

ALT is more liver specific than AST (Aspartate aminotransferase). Measurement of both AST and ALT has some value in distinguishing hepatitis from other parenchymal lesions.

**METHOD (5)**

IFCC method without pyridoxal phosphate (P-5’-P).

Kinetic. UV.

**PRINCIPLE (5)**

Kinetic determination of the alanine aminotransferase (ALT) activity:

\[
\text{ALT} \quad \text{L-Alanine + α-Ketoglutarate} \quad \text{Pyruvate + L-Glutamate} \\
\]

\[
\text{LDH} \quad \text{Pyruvate + NADH + H+} \quad \text{L-Lactate + NAD+} \\
\]

**REAGENTS COMPOSITION**

**Reagent 1: R1**

- Tris buffer, pH 7.50 (30°C) 125 mmol/L
- L-alanine 680 mmol/L
- LDH ≥ 2000 U/L

**Reagent 2: R2**

- α-Ketoglutarate 97 mmol/L
- NADH 1.1 mmol/L

**MATERIAL REQUIRED BUT NOT PROVIDE**

- CONT-0060 ELITROL I 10 × 5 mL
- CONT-0160 ELITROL II 10 × 5 mL

**PRECAUTIONS**

- The reagents contain less than 0.1% sodium azide. Sodium azide can react with copper and lead plumbing to form explosive metal azides. If discharge in the canalisations, rinse with plenty of water.
- Use clean or single use laboratory equipment only to avoid contaminations.
- High ALT values may induce falsely low results due to the depletion of the substrate (total consumption of NADH before reading of the result). If an analyser is used, verify the presence of a depletion factor on the application.

**WASTE MANAGEMENT**

Disposal of all waste material should be in accordance with local and legal requirements.

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**STABILITY OF REAGENTS**

To store at 2-8 °C and protected from light.

The reagents are stable until the expiry date stated on the label.

**On-board stability:**

The stability is specific for each analyser (for Selectra refer to § PERFORMANCE DATA).

**PREPARATION AND STABILITY OF WORKING REAGENT**

- **One-reagent procedure:**
  - Mix 4 volumes of the reagent R1 with 1 volume of the reagent R2.
  - Stability: 5 days at 20 - 25 °C
  - 2 weeks at 2 - 8 °C

- **Two-reagent procedure:**
  - The reagents are ready to use.

**SAMPLES (2,3,6)**

- **Specimen**
  - Serum free from hemolysis.
  - Lithium heparinizied or EDTA plasma free of hemolysis.

- **Storage**
  - ALT is stable in serum for 3 days at room temperature or 7 days at 2-8 °C. Freezing is not recommended.

**REFERENCE VALUES (2)**

Serum, plasma (37 °C): < 40 U/L

Note: It is recommended for each laboratory to establish and maintain its own reference values. The data given here are only an indication.

**PROCEDURE**

These reagents can be used on most analysers, semi-automated analysers and manual method.

The applications are available on request.

**Wavelength:** 340 nm

**Temperature:** 37 °C

Read against distilled water.

- **One-reagent procedure:**
  - Working reagent: 200 µL
  - Sample: 20 µL
  - Mix and after a 50 second incubation, measure the change of absorbance per minute (ΔA/min.) during 175 seconds.

- **Two-reagent procedure:**
  - Reagent R1: 200 µL
  - Reagent R2: 50 µL
  - Mix, wait 25 seconds and add:
  - Sample: 25 µL
  - Mix and after a 50 second incubation, measure the change of absorbance per minute (ΔA/min.) during 150 seconds.

**CALCULATION**

At 340 nm, with the one-reagent procedure and the two-reagent procedure for a 1 cm light path cuvette:

\[
\text{Activity (U/L) = } \Delta A/\text{min.} \times 1746
\]
QUALITY CONTROL
To ensure adequate quality, control sera such as ELITROL I (normal control) and ELITROL II (abnormal control) are recommended.

PERFORMANCE DATA at 37 °C on Selectra
- **Analytical range**
The reagent is linear from 15 to 250 U/L.

- **Detection limit**
  Determined according to SFBC protocol, the detection limit is equal to 2 U/L for the one-reagent procedure and to 3 U/L for the two-reagent procedure.

- **Analytical Sensitivity**
The average variation of the analytical signal is 0.57 mAU/min per U/L of ALT for a light path of 1 cm.

- **Precision**

  **Within-run reproducibility**

<table>
<thead>
<tr>
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<th>One-reagent procedure</th>
<th>Two-reagent procedure</th>
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<tbody>
<tr>
<td></td>
<td>n Mean (U/L) CV%</td>
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</tr>
<tr>
<td>SH1</td>
<td>20 25 2.3</td>
<td>20 24 4.6</td>
</tr>
<tr>
<td>SH2</td>
<td>20 58 2.2</td>
<td>19 56 2.0</td>
</tr>
<tr>
<td>SH3</td>
<td>20 193 1.0</td>
<td>20 199 1.3</td>
</tr>
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</table>

  **Between-run reproducibility**

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<td>n Mean (U/L) CV%</td>
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</tr>
<tr>
<td>SH1</td>
<td>80 28 4.7</td>
<td>73 27 6.5</td>
</tr>
<tr>
<td>SH2</td>
<td>80 57 2.9</td>
<td>79 58 4.6</td>
</tr>
<tr>
<td>SH3</td>
<td>80 196 2.2</td>
<td>79 203 2.4</td>
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- **Correlation**
A comparative study has been performed between ELITech method and another commercial reagent (IFCC method without pyridoxal phosphate) on 96 human sera samples. The sample concentrations ranged from 2 to 288 U/L. The parameters of linear regressions are as follows:

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<tr>
<td></td>
<td>Correlation coefficient[(r)] 0.999</td>
<td>0.999</td>
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<tr>
<td></td>
<td>Linear regression [y = 0.94 x + 1.7 U/L]</td>
<td>[y = 0.95 x + 0.9 U/L]</td>
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- **Interferences**
According to SFBC recommendations, studies have been performed to determine the level of interference from different compounds:

  - **Glucose:** No significant interference up to 500 mg/dL (5 g/L, 28 mmol/L).
  - **Ascorbic acid:** No significant interference up to 40 mg/dL (400 mg/L, 2.3 mmol/L).
  - **Pyruvate:** No significant interference up to 2 mg/dL (20 mg/L, 0.23 mmol/L).
  - **Unconjugated Bilirubin:** Negative bias from 16 mg/dL (160 mg/L, 270 µmol/L).
  - **Conjugated Bilirubin:** Negative bias from 11.5 mg/dL (115 mg/L, 200 µmol/L).
  - **Turbidity:** No significant interference up to 600 mg/dL (6 g/L, 6.9 mmol/L).
  - **Methyl-dopa:** No significant interference up to 5 mg/dL (50 mg/L).

Other compounds may interfere. [7-11]

Note: Hemolysed sera should not be used since significant hemolysis may increase ALT concentration because of high levels of ALT in erythrocytes.

- **On board stability on Selectra (not refrigered)**
On board stability: 5 days for one-reagent procedure and 14 days for two-reagent procedure (capped vials and stored at 2-8 °C during the night).

**BIBLIOGRAPHY**

**SYMBOLS USED ON LABELS**

<table>
<thead>
<tr>
<th>IVD</th>
<th>: In vitro diagnostic medical device</th>
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<tbody>
<tr>
<td>REF</td>
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<tr>
<td>LOT</td>
<td>: LOT number</td>
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<tr>
<td>TCD</td>
<td>: Temperature limitation</td>
</tr>
<tr>
<td>EXD</td>
<td>: Expiration date</td>
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