Clinical Acetoacetate Analysis

A fast enzymatic analysis for plasma and serum Acetoacetate.

Bulletin Reference	TB – Acetoacetate – Clinical – GMRD-180 – V.01
Order Code(s)	GMRD-180
Reagent Kit Size(s)	100 ml (140 analyser cycles)
Instruments	All GM7 Series analysers
Samples	Plasma (Heparinised), serum
Sample Volume	50 µl
Analysis Time	20 seconds (from injection)
Linearity	1000 µmol/L (100 µg/ml)
Detection Limit	10 µmol/L (1 µg/ml)
Precision (Within Run)	C.V. of 5 - 6 % @ 200 µmol/L (20 µg/ml) C.V. of 2 - 3 % @ 500 µmol/L (50 µg/ml)
Accuracy	Recovery data: 97 - 106 %
Reagent Stability	Shelf-life unopened: 9 months stored at 0 - 5°C. Shelf-life reconstituted: NADH reagent 10 days stored at 0 - 5°C.
Note	The number of tests can be doubled by using half volumes (micro-method). A sample blank allows for background interference by endogenous ammonia and other NADH consuming reactions.

Principle

i) In the presence of excess NADH and the enzyme 3-hydroxybutyrate dehydrogenase (3-HBDH), acetoacetate is reduced to 3-hydroxybutyrate in a brief pre-reaction,

$$\text{Acetoacetate} + \text{NADH} + \text{H}^+ \underset{}{\overset{\textit{3-Hydroxybutyrate Dehydrogenase (3-HBDH)}}{\rightleftharpoons}} \text{3-Hydroxybutyrate (3-HB)} + \text{NAD}^+$$

ii) Under the conditions of the assay, the rate of oxidation of excess NADH by peroxidase (POD) is inversely proportional to the plasma acetoacetate concentration.

$$\text{NADH} + \text{H}^+ + \tfrac{1}{2}\text{O}_2 \xrightarrow{\textit{Peroxidase (POD)}} \text{NAD}^+ + \text{H}_2\text{O}$$