Biochemistry Analyses – Method Details

Albumin

Albumin measurements were determined using a commercial serum albumin kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on either a Cobas Fara or Mira analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). The measurement of serum albumin is based on its quantitative binding to bromocresol green (BCG). The albumin-BCG-complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration in the sample. Intra-assay precision was CV < 2.5% while inter-assay precision was CV < 5%.

Kit = Albumin colorimetric bromocresol green assay, Sentinel Diagnostics, 17600H

Suitable samples = serum, plasma (heparin or EDTA)

ALP (Alkaline Phosphatase)

ALP was determined by a commercial kit (Randox Laboratories Ltd., UK) following manufacturer provided guidelines for use on a Cobas Mira analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). Intra-assay precision was CV < 2% while inter-assay precision was CV < 5%.

Kit = Alkaline Phosphatase assay, Randox Laboratories, Ltd., AP307

Suitable samples = serum, plasma (heparin)

ALT (Alanine Aminotransferase)

ALT was measured using the method described by Bergmeyer *et al.* (1978), utilising a commercial kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on either a Cobas Fara or Mira analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). Intra-assay precision was CV < 4% while inter-assay precision was CV < 8%.

Bergmeyer HU, Scheibe P, and Wahlefeld AW (1978) Optimization of Methods for Aspartate Aminotransferase and Alanine Aminotransferase. *Clin. Chem.* **24/1** 58-73 https://doi.org/10.1093/clinchem/24.1.58

Kit = Alanine Aminotransferase ALT GPT Kinetic Assay, Sentinel Diagnostics, 17234H

Suitable samples = serum, plasma (heparin or EDTA)

Amylase

Amylase was determined by a commercial kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on a Cobas Fara or Mira analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). The method utilises 2-chloro-pnitrophenyl-α-D-maltotrioside as the substrate. α-Amylase hydrolyzes the 2-chloro-p-nitrophenyl-α-D-maltotrioside to release 2-chloro-p-nitrophenol and produce 2-chloro-p-nitrophenyl-α-D-maltoside, maltotriose, and glucose. The rate of formation of the 2-chloro-p-nitrophenol can be detected spectrophotometrically at 405 nm to give a direct measurement of α-amylase activity in the sample. Intra-assay precision was CV < 4% while inter-assay precision was CV < 7%.

Kit = Alpha Amylase Liquid Enzymatic Colourimetric Assay, Sentinel Diagnostics, 17632H

Suitable samples = serum, plasma (heparin or EDTA), urine

AST (Aspartate Aminotransferase)

AST was determined by a commercial kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on either a Cobas Fara or Mira analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). α-oxogluterate reacts with L-aspartate in the presence of AST to form L-glutamate plus oxaloacetate. The indicator reaction utilises the oxaloacetate for a kinetic determination of NADH consumption. Intra-assay precision was CV < 4% while inter-assay precision was CV < 5%.

Kit = Aspartate Aminotransferase AST GOT Kinetic Assay, Sentinel Diagnostics, 17224H

Suitable samples = serum, plasma (heparin or EDTA)

Bilirubin

Total bilirubin was determined by the acid diazo method described by Pearlman and Lee (1974), using a commercial kit (Randox Laboratories Ltd, County Antrim, UK) adapted for use on a Cobas Fara centrifugal analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). This method uses a surfactant as a solubiliser. Conjugated and solubilised unconjugated bilirubin react with diazotised sulphanilic acid, producing an acid azobilirubin. The absorbance of this is proportional to the concentration of bilirubin in the sample and can be measured at 550nm. Intra-assay precision was CV < 4% while inter-assay precision was CV < 5%.

Pearlman FC and Lee RTY (1974) Detection and Measurement of Total Bilirubin in Serum, with Use of Surfactants as Solubilising Agents. *Clin. Chem.* **20:4** 447-453. https://doi.org/10.1093/clinchem/20.4.447

Kit = Bilirubin (Total) assay, Randox Laboratories, BR 243

Suitable samples = serum, plasma (heparin or EDTA)

Cholesterol

Total cholesterol measurements were determined using a commercial kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on a Cobas Mira centrifugal analyser (Roche Diagnostics Ltd., Welwyn Garden City, UK) following the manufacturers guidelines. Intra-assay precision was CV <3% while inter-assay precision was CV <4%.

Kit = Cholesterol Liquid, Sentinel Diagnostics, 17644H

Suitable samples = serum, plasma (heparin or EDTA)

Creatinine

Creatinine measurements were determined using a creatininase/creatinase enzymatic method as described by Bömer *et al.* (1979) making use of a commercial kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on either a Cobas Fara or Mira analyser (Roche

Diagnostics Ltd, Welwyn Garden City, UK). Intra-assay precision was < 3% while inter-assay precision was CV < 5%.

Bömer U., Szaz, G. *et al.* (1979) A specific fully enzymatic method for creatinine: reference values in serum. *J Clin Chem Clin Biochem* **17** 679-882

Kit = Creatinine enzymatic colourimetric assay serum plasma urine dual liquid reagent system, Sentinel Diagnostics, 17654H

Suitable samples = serum, plasma (Li/Na heparin), urine

Glucose

Glucose measurements were determined using a commercial kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on either a Cobas Fara or Mira analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). Glucose is oxidised by glucose oxidase (GOD) into gluconicacid and hydrogen peroxide, which in the presence of peroxidase, reacts with 4-aminoantipyrine and hydroxybenzoic acid, forming a red compound with colour intensity proportional to the concentration of glucose, measured at 505 nm. Intra-assay precision was CV < 4% while inter-assay precision was CV < 10%.

Kit = Glucose Enzymatic Colourimetric Assay, Sentinel Diagnostics, 17630H

Suitable samples = serum, plasma (heparin or EDTA), urine, CSF

HDL-C (High density lipoprotein-cholesterol)

HDL-C was determined by a commercial kit (Wako Chemicals GmbH via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on a Cobas Mira centrifugal analyser following the manufacturers guidelines. Intra-assay precision was CV <4% while inter-assay precision was CV <5%.

Kit = HDL-C L-Type (R1+R2), Wako Chemicals GmBH, 412-72395 and 412-72495

Suitable samples = serum

LDL-C (Low density lipoprotein-cholesterol)

LDL-C was calculated through the following formula:

$$LDL = total\ cholesterol - HDL - (\frac{Triglycerides}{2.2})$$

Magnesium

Magnesium was measured using a commercial kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) following the manufacturers guidelines, adapted for use on a Cobas Mira analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). Intra-assay precision was CV < 8% while inter-assay precision was CV < 6%.

Kit = Magnesium Liquid, Sentinel Diagnostics, 17637H

Suitable samples = serum, plasma (heparin), urine

Microalbumin

Microalbumin measurements were determined using a commercial kit (Randox Laboratories Ltd., UK) following manufacturer provided guidelines for use on a Cobas Mira analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). Intra-assay precision was < 5% while inter-assay precision was < 7%.

Kit = Microalbumin Assay, Randox Laboratories Ltd., UK, #MA2426

Suitable samples = urine

Triglycerides

Triglyceride were determined using a commercial kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on a Cobas Mira centrifugal analyser following the manufacturers guidelines. Intra-assay precision was CV <3% while inter-assay precision was CV <5%.

Kit = Triglycerides Liquid, Sentinel Diagnostics, 17624H

Suitable samples = serum, plasma (heparin or EDTA)

Urea

Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia produced combines with α-oxogluterate and NADH in the presence of glutamate-dehydrogenase to yield glutamate and NAD. The production of NAD is monitored at 340 nm. Urea measurements were determined using a commercial urea kit (Sentinel Diagnostics via Alpha Laboratories Ltd., Eastleigh, UK) adapted for use on either a Cobas Fara or Mira analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). Intra-assay precision was CV < 3% while inter-assay precision was CV < 5%.

Kit = Urea Liquid Enzymatic Assay, Sentinel Diagnostics, 17629H

Suitable samples = serum, plasma (heparin or EDTA), urine