APPENDIX

APPENDIX A BLOOD BIOCHEMICAL ANALYTES PRINCIPLE

1. Glucose

Glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphophate (ATP) and magnesium ions to produce glucose-6-phosphate and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G6P-DH) specifically oxidises glucose-6-phosphate to gluconate-6-phosphate with the concurrent reduction of NAD^+ to NADH. The increase in absorbance at 340 nm is proportional to the glucose concentration in the sample.

$$\text{Glucose ATP} \xrightarrow{HK,\ Mg^{2+}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phospate} + NAD^+ \xrightarrow{G6P\text{-}DH} \text{Gluconate-6-phosphate} + NADH + H^+$$

2. Total cholesterol (TC)

Cholesterol reagent utilizes an enzymatic method to measure cholesterol in human serum and plasma. Cholesterol esters in sample are hydrolyzed by cholesterol esterase (CHE). The free cholesterol produced is oxidized by cholesterol oxidase (CHO) to cholestene-3-one with the simultaneous production of hydrogen peroxide (H_2O_2), which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to yield a chromophore. The red quinoneimine dye formed can be measured spectrophotometrically at 540/600 nm as an increase in absorbance.

$$2 \text{ Cholesrerol esters} + 2\ H_2O \xrightarrow{CHE} 2 \text{ Cholesterol} + 2 \text{ Fatty acids}$$

$$2 \text{ Cholesrerol} + 2\ O_2 \xrightarrow{CHO} 2 \text{ Cholestene-3-one} + 2\ H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{POD} \text{Quinoneimine} + 4\ H_2O$$

3. Triglyceride (TG)

Triglyceride measurement is based on a series of coupled enzymatic reactions. The triglycerides in the sample are hydrolyzed by a combination of microbial lipases to give glycerol and fatty acids. The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to produce glycerol-3-phosphate. The glycerol-3-phosphate is oxidised by molecular oxygen in

the presence of 4-aminophenazone and N,N-bis (4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) in the presence of peroxidase (POD) to produce a chromophore, which is read at 660/800 nm. The increase in absorbance at 660/800 nm is proportional to the triglyceride content of the sample.

$$\text{Triglyceride} + 3\,H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + 3\,\text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK, Mg 2+}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{MADB} \xrightarrow{\text{POD}} \text{Blue dye} + OH^- + 3\,H_2O$$

4. High density lipoprotein cholesterol (HDL-C)

Anti human-β-lipoprotein antibody (AH- β -LA) in R1 binds to lipoproteins other than HDL (LDL, VLDL and chylomicrons). The antigen-antibody complexes formed block enzyme reactions when R2 is added. HDL-cholesterol is quantified by the presence of an enzyme chromogen system.

$$\text{LDL, VLDL and chylomicrons} \xrightarrow{\text{AH-}\beta\text{-LA}} \text{Antigen-Antibody complexes}$$

$$\text{HDL-cholesterol} + H_2O + O_2 \xrightarrow{\text{CHE and CHO}} \text{Cholest-4-en-3-one} + \text{Fatty acids} + H_2O_2$$

$$H_2O_2 + \text{4-AA} + \text{F-DAOS} \xrightarrow{\text{POD}} \text{Blue dye}^+ + F + 2\,H_2O$$

5. Low density lipoprotein cholesterol (LDL-C)

A protecting reagent in R1 protects LDL from enzymatic reactions. All non-LDL lipoproteins (LDL, VLDL, Chylomicrons) are broken down by reaction with cholesterol esterase (CHE) and cholesterol oxidase (CHO). Hydrogen peroxide (POD) produced by this reaction is decomposed by catalase in R1. When R2 is added, the protecting reagent is released from LDL and catalase inactivated by sodium azide. LDL can now be quantified by the CHO/PAP system.

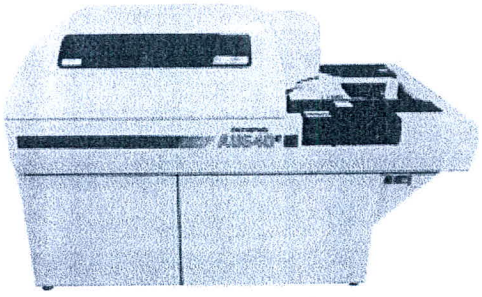
$$2\text{LDL-cholesterol} + 2H_2O + 2O_2 \xrightarrow[\text{Deprotecting Reagent}]{\text{CHE and CHO}} 2\text{Cholest-4-en-3-one} + 2\text{Fatty acids} + 2H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{HDAOS} \xrightarrow{\text{POD}} \text{Blue dye}^+ + OH^- + 3H_2O$$

6. High sensitivity C-reactive protein (hs-CRP)

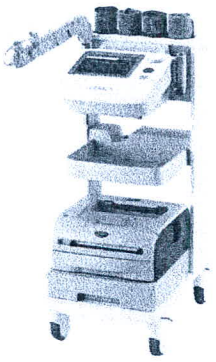
When a sample is mixed with R1 buffer and R2 latex suspension, CRP reacts specifically with anti-human CRP antibodies coated on the latex particles to yield insoluble aggregates. The absorbance of these aggregates is proportional to the CRP concentration in the sample.

APPENDIX B AUTOMATED CLINICAL CHEMISTRY, OLYMPUS AU 640



Analytical system	Fully automate, for routine, STAT, urine and homogeneous immunoassays
Analytical methods	Clinical chemistry and immunological parameters, End point, Kinetic assays, fixed –time-kinetics, optionally ISE
Throughput	800 photometric tests/hour; maximum of 1,200 with ISE .
Sample feeder	Racks with 10 samples each; capacity of 150 sample; continuous loading
STAT samples	Upto 22 position for STAT samples
Sample volume	2-50 ul in 1ul steps (1-50 ul for repeats)
Reagent supply	48 position for R1 and R2; refrigerated (4-12 °C)
Reagent volume	1. Reagent; 25-300 ul; 2. Reagent: 25-300 ul
Total reaction volume	150-550 ul
Reaction cuvette	Quartz cuvettes
Reaction time	Up to 8 minutes, 40 seconds
Wavelength	13 different wavelengths between 340-800 nm
Safety	Clot detection and crash prevention for sample and reagent dispenser
On-line	Full uni-and bidirectional communication possible
Software	Windowns-NT
Dimensions (WxHxD) mm	ANL 1,850 x 1,210 x 800; DPR 700 x 1,430 x 700

APPENDIX C VASCULAR INDEX, NON-INVASIVE MEASUREMENTS: VP-1000 ANALYZER



Fast	10 minutes to complete from start to finish
Simple	Once the cuffs, ECG & PCG sensors are in place, the VP-1000 automatically completes the assessment with no further input from the clinician.
Accurate & Reproducible	The 'Sensor Cuffs' measure arterial pressure and waveform, eliminating the need for hand-held probes, and hence eliminating investigator variability.
Simultaneous Measurement of all limbs	Simultaneously measures arterial pressures and waveform in all 4 limbs facilitating a highly accurate Ankle-Brachial Index calculation that isn't compromised by the passing of time between individual limb measurements done sequentially
Touch Screen Interface	Touch screen interface and logical menu system simplifies data entry and navigation
Multiple Report	Select printed reports available
Portable	Main unit can operate as a 'Bench Top' unit, seperate from the roller stand and printer, and can be transported in a small case making it portable enough to take between clinics or consultation rooms
Toe Brachial Index (TBI) function	'TBI' function is available using plethysmographic toe cuffs that can detect arterial pulses in the toes