Regional Medical Center

POLICY TITLE: Chemistry Testing on the Piccolo Xpress Chemistry Analyzer	
DISTRIBUTION: Lab, ED, MS, OB, RFH **OWNER:** Laboratory: Lab	**LAST REVISED:** **ORIGINATION DATE:** November 2024 **LAB DIRECTOR:**

Purpose:

The Piccolo® Comprehensive Metabolic Panel, Biochem Plus Panel and MetLac 12 Panels are used with the Piccolo Xpress® chemistry analyzer. It will be utilized as a backup instrument when the main Chemistry analyzer is unavailable.The Piccolo is to be used for the in vitro quantitative determination of alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium, chloride, creatinine, glucose, potassium, sodium, total bilirubin, total carbon dioxide, total protein, blood urea nitrogen (BUN) Amylase (AMY), Gamma glutamyltransferase (GGT) uric acid (UA), C-reactive protein(CRP),lactate (LAC), magnesium (MG) and phosphorus (PHOS) in heparinized whole blood, heparinized plasma, or serum.

Principle:

ALT (ALT) catalyzes the transfer of an amino group from L-alanine to -ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+ , as illustrated in the following reaction scheme.

$$\text{L-Alanine} + \alpha\text{Ketoglutarate} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

The rate of change of the absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD^+ and is directly proportional to the amount of ALT present in the sample.

Albumin (ALB) Bromcresol purple (BCP), when bound with albumin, changes color from a yellow to blue color. The absorbance maximum changes with the color shift.

$$\text{BCP} + \text{Albumin} \xrightarrow[\text{Acid pH}]{\text{Surfactants}} \text{BCP-Albumin Complex}$$

Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as the difference in absorbance between 600 nm and 550 nm.

Alkaline Phosphatase (ALP) Alkaline phosphatase hydrolyzes p-NPP in a metal-ion buffer and forms p-nitrophenol and phosphate.

$$\text{p-Nitrophenyl Phosphate} \xrightarrow[Zn^{2+},\ Mg^{2+}]{ALP} \text{p-Nitrophenol} + \text{Phosphate}$$

The amount of ALP in the sample is proportional to the rate of increase in absorbance difference between 405 nm and 500 nm.

Aspartate Aminotransferase (AST) AST catalyzes the reaction of L-aspartate and -ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to NAD^+ by the catalyst MDH.

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{AST} \text{Oxaloacetate} + \text{L-glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{MDH} \text{Malate} + NAD^+$$

The rate of absorbance change at 340 nm/405 nm caused by the conversion of NADH to NAD^+ is directly proportional to the amount of AST present in the sample.

Calcium (CA) Calcium in the patient sample binds with arsenazo III to form a calcium-dye complex.

Ca^{2+} + Arsenazo III ⟶ Ca^{2+} -Arsenazo III Complex

The endpoint reaction is monitored at 405 nm, 467 nm and 600 nm. The amount of calcium in the sample is proportional to the absorbance.

Chloride (CL-) The method is based on the determination of chloride-dependent activation of α-amylase activity. Deactivated α-amylase is reactivated by addition of the chloride ion, allowing the calcium to re-associate with the enzyme. The reactivation of α-amylase activity is proportional to the concentration of chloride ions in the sample. The reactivated α-amylase converts the substrate, 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) to 2-chloro-p-nitrophenol (CNP) producing color and α-maltotriose (G3). The reaction is measured bichromatically and the increase in absorbance is directly proportional to the reactivated α-amylase activity and the concentration of chloride ion in the sample.

$$\text{CNPG3} \xrightarrow[Cl^-,\ Ca^{2+}]{\alpha\text{-Amylase}} \text{CNP} + \text{G3}$$

Creatinine (CRE) Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique. Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine Amidohydrolase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatine Amidinohydrolase}} \text{Sarcosine} + \text{Urea}$$

Amylase (AMY) In the Piccolo method, the substrate, 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3), reacts with α-amylase in the patient sample, releasing 2-chloro-p-nitrophenol (CNP). The release of CNP creates a change in color.

$$\text{CNPG3} \xrightarrow{\alpha\text{-Amylase}} \text{CNP} + \text{D-Maltotrioside}$$

The reaction is measured bichromatically at 405 nm and 500 nm. The change in absorbance due to the formation of CNP is directly proportional to α-amylase activity in the sample.

Gamma Glutamyltransferase (GGT) Piccolo has modified the IFCC method to react at 37°C. The addition of sample containing gamma glutamyltranferase to the substrates L-γ-glutamyl-3-carboxy-4-nitroanilide and glycylglycine (gly-gly) causes the formation of L-γ-glutamylglycylglycine (glu-gly-gly) and 3-carboxy-4-nitroaniline.

$$\text{L-}\gamma\text{-glutamyl-3-carboxy 4-nitroanilide} + \text{Gly-gly} \xrightarrow{\text{GGT}} \text{Glu-gly-gly} + \text{3-carboxy-4-nitroaniline}$$

The absorbance of this rate reaction is measured at 405 nm. The production of 3-carboxy-4-nitroaniline is directly proportional to the GGT activity in the sample.

Uric Acid (UA) The uricase method is coupled through a Trinder peroxidase finish. In this method, uricase catalyzes the oxidation of uric acid to allantoin and hydrogen peroxide. Peroxidase catalyzes the reaction among the hydrogen peroxide (H_2O_2), 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBSA) into a red quinoneimine dye. Sodium ferrocyanide and ascorbate oxidase are added to the reaction mixture to minimize the potential interference of bilirubin and ascorbic acid.

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{DHBSA} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + H_2O$$

The amount of uric acid in the sample is directly proportional to the absorbance of the quinoneimine dye. The final absorbance of this endpoint reaction is measured bichromatically at 500 nm and 600 nm.

C-Reactive Protein (CRP) The method is an enhanced latex-agglutination turbidimetric immunoassay. The sample is mixed with a suspension of mouse anti-human CRP monoclonal antibody that is bound to latex. CRP in the sample binds to the antibody-latex particles and agglutinates creating turbidity. Light scattering from the turbidity is used as a measure of CRP. Turbidity is measured as a change in absorbance at 630 nm. This absorbance change is directly proportional to the CRP in the sample.

$$\text{Anti-CRP latex particles} + \text{CRP} \longrightarrow \text{Agglutinated CRP-Anti-CRP latex particles}$$

Lactate (LAC) Lactate (LAC) is oxidized by lactate oxidase (LOX) to pyruvate and hydrogen peroxide (H_2O_2). Peroxidase catalyzes the reaction of H_2O_2, 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBSA) to form a red quinoneimine dye.

$$\text{Lactate} + O_2 \xrightarrow{\text{LOX}} \text{Pyruvate} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{DHBSA} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine Dye} + H_2O$$

The rate of formation of the red dye is proportional to the LAC concentration in the sample. The reaction is measured bichromatically at 515 nm and 600 nm.

Magnesium (MG) The enzymatic magnesium method can be described as :

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg2^+]{\text{HK}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NADP} \longrightarrow \text{6-Phosphogluconate} + \text{NADPH} + H^+$$

The rate limiting reaction is the HK reaction. Magnesium from the sample activates HK, which in turn catalyzes the breakdown of glucose to form glucose-6-phosphate (G-6-P) and ADP. G-6-P reacts with nicotinamide adenine dinucleotide phosphate ($NADP^+$) to form reduced nicotinamide adenine dinucleotide phosphate (NADPH) and 6-phosphogluconate in the presence of glucose-6-phosphate-dehydrogenase (G-6-PDH). This is a first-order rate reaction. The rate of production of NADPH is directly proportional to the amount of magnesium present in the sample. Absorbance is measured bichromatically at 340 nm and 405 nm.

Phosphorus (PHOS) The most applicable enzymatic method for the Piccolo system uses sucrose phosphorylase (SP) coupled through phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6PDH) Using the enzymatic system for each mole of phosphorus present in the sample, one mole of NADH is formed. The amount of NADH formed can be measured as an endpoint at 340 nm.

$$\text{Sucrose} + Pi \xrightarrow{\text{SP}} \text{Glucose-1-Phosphate (G-1-P)} + \text{Fructose}$$

$$\text{G-1-P} \xrightarrow{\text{PGM, } Mg^{2+}} \text{G-6-P}$$

$$\text{G-6-P} + NAD^+ \xrightarrow{\text{G-6-PDH}} \text{NADH} + \text{6-Phosphogluconate} + H^+$$

Sample: The minimum required sample size is ~100 µL of heparinized whole blood, heparinized plasma or serum. The MetLac 12 Panel uses only ~100 µL of heparinized whole blood, heparinized plasma. Specimens must be free of hemolysis, icterus and turbidity.

Sarcosine Oxidase

Sarcosine + H_2O + O_2 ——→ Glycine + Formaldehyde + H_2O_2

Peroxidase

H_2O_2 + TBHBA + 4-AAP ——→ Red Quinoneimine Dye + H_2O

Two cuvettes are used to determine the concentration of creatinine in the sample. Endogenous creatine is measured in the blank cuvette, which is subtracted from the combined endogenous creatine and the creatine formed from the enzyme reactions in the test cuvette. Once the endogenous creatine is eliminated from the calculations, the concentration of creatinine is proportional to the intensity of the red color produced. The endpoint reaction is measured as the difference in absorbance between 550 nm and 600 nm.

eGFR (calculated) Calculation of the eGFR is performed by the Piccolo using the patient's age, gender and race. The Piccolo method for creatinine is traceable to the IDMS reference method for creatinine so that the following form of the MDRD equation for calculating the eGFR can be used. GFR (mL/min/1.73 m2) = 175 x (Scr) -1.154 x (Age)-0.203 x (0.742 if female) x (1.212 if African American)

Glucose (GLU) The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH.

HK

Glucose + ATP ——→ G-6-P + ADP

G-6-PDH

G-6-P + NAD^+ ——→ 6-Phosphogluconate + NADH + H^+

Potassium (K^+) An enzymatic method is used based on the activation of pyruvate kinase with potassium in the coupled-enzyme reaction, pyruvate kinase (PK) dephosphorylates phosphoenolpyruvate (PEP) to form pyruvate. Lactate dehydrogenase (LDH) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+ .

K+ , PK

ADP + PEP ——→ Pyruvate + ATP

LDH

Pyruvate + NADH + H^+ ——→ Lactate + NAD^+

The rate of change in absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD^+ and is directly proportional to the amount of potassium in the sample.

Sodium (NA^+) Galactosidase is activated by the sodium in the sample. The activated enzyme catalyzes the reaction of -nitrophenyl--D-galactopyranoside (ONPG) to -nitrophenol and galactose.

Na+

ONPG ——→ Nitrophenol + Galactose

B-Galactosidase

Total Bilirubin (TBIL) The enzymatic procedure, bilirubin is oxidized by bilirubin oxidase into biliverdin.

$$\text{Bilirubin} + O2 \xrightarrow{\text{Bilirubin Oxidase}} \text{Biliverdin} + H2O$$

Bilirubin is quantitated as the difference in absorbance between 467 nm and 550 nm. The initial absorbance of this endpoint reaction is determined from the bilirubin blank cuvette and the final absorbance is obtained from the bilirubin test cuvette. The amount of bilirubin in the sample is proportional to the difference between the initial and final absorbance measurements.

Total Carbon Dioxide (tCO_2) In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide (CO_2) toward bicarbonate (HCO3 -) Phosphoenolpyruvate (PEP) and HCO_3 - then react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MDH) catalyzes the reaction of oxaloacetate and reduced nicotinamide adenine dinucleotide (NADH) to NAD^+ and malate. The rate of change in absorbance due to the conversion of NADH to NAD^+ is directly proportional to the amount of tCO_2 in the sample.

$$PEP + HCO_3 \xrightarrow{PEPC} \text{Oxaloacetate} + \text{Phosphate}$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightarrow{MDH} NAD^+ + \text{Malate}$$

Total Protein (TP) The biuret reaction is a candidate total protein reference method. In the biuret reaction, the protein solution is treated with cupric [Cu(II)] ions in a strong alkaline medium. Sodium potassium tartrate and potassium iodide are added to prevent the precipitation of copper hydroxide and the auto-reduction of copper, respectively.The Cu(II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-Protein complex.

$$\text{Total Protein} + Cu(II) \xrightarrow{OH} \text{Cu-Protein Complex}$$

The amount of total protein present in the sample is directly proportional to the absorbance of the Cu-protein complex. The total protein test is an endpoint reaction and the absorbance is measured as the difference in absorbance between 550 nm and 850 nm.

Blood Urea Nitrogen (BUN) The diacetyl 7 In the coupled-enzyme reaction, urease hydrolyzes urea into ammonia and carbon dioxide. Upon combining ammonia with α-ketoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD^+ .

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-Ketoglutarate} + NADH \xrightarrow{GLDH} \text{L-Glutamate} + H_2O + NAD^+$$

The rate of change of the absorbance difference between 340 nm and 405 nm is caused by the conversion of NADH to NAD+ and is directly proportional to the amount of urea present in the sample.

Expected Values and Linearity:

Analyte	Expected Values	Linearity
CMP -alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium, chloride, creatinine, glucose, potassium, sodium, total bilirubin, total carbon dioxide, total protein, and blood urea nitrogen (BUN)		
Sodium (NA+)	128-145 mmol/L	110-170 mmol/L
Potassium (K+)	3.6-5.1 mmol/L	1.5-8.5 mmol/L
Total Carbon Dioxide (tCO2)	18-33 mmol/L	4-40 mmol/L
Chloride (CL-)	98-108 mmol/L	80-135 mmol/L
Glucose (GLU)	73-118 mg/dL	10-700 mg/dL
Calcium (CA)	8.0-10.3 mg/dL	4.0-16.0 mg/dL
Blood Urea Nitrogen (BUN)	7-22 mg/dL	2-180 mg/dL
Creatinine(CRE)	0.6-1.2 mg/dL	0.2-20 mg/dL
Alkaline Phosphatase (ALP)	Male 53-128 U/L	5-2400 U/L
Alkaline Phosphatase (ALP)	Female 42-141 U/L	5-2400 U/L
Alanine Aminotransferase (ALT)	10-47 U/L	5-2000 U/L
Aspartate Aminotransferase (AST)	11-38 U/L	5-2000 U/L
Total Bilirubin (TBIL)	0.2-1.6 mg/dL	0-1-30 mg/dL
Albumin (ALB)	3.3-5.5 g/dL	1-6.5 g/dL
Total Protein (TP)	6.4-8.1 g/dL	2-14 g/dL

BIOCHEM Plus- alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), amylase, aspartate aminotransferase (AST), c-reactive protein (CRP), calcium, creatinine, gamma glutamyltransferase (GGT), glucose, total protein, blood urea nitrogen (BUN), and uric acid. Same values as above.

AMY	14-97 U/L	5-4000 U/L
C-Reactive protein	<7.5 mg/L	5.0-200.0 mg/L
GGT	5-65 U/L	5-5000 U/L
Uric Acid (UA) Female	2.2-6.6 mg/dL	1.0-15 mg/dL
Uric Acid (UA) Male	3.6--8.0 mg/dL	1.0-15 mg/dL

MetLac 12 -Albumin, calcium, chloride, creatinine, glucose, lactate, magnesium, phosphorus, potassium, sodium, total carbon dioxide and blood urea nitrogen. Same values as above.

Phosphorus (plasma)	2.2-4.1 mg/dL	0.2-20 mg/dL
Magnesium	1.6 – 2.3 mg/dL	0.1-8.0mg/dL
Lactate	0.53 – 2.10 mmol/L	0.30-9.99 mmol/L

Quality Control:

Kits will have 2 levels of QC performed on arrival and monthly.
Calibration verification with 3 levels will be performed every 6 months.
Internal QC will be recorded with each day of use.

References:

Piccolo® Comprehensive Metabolic Panel Instructions for Use, July 2022, PN: 400-7139-1 Rev. R
Piccolo® BioChemistry Panel Plus January 2021 Instructions for Use,PN: 400-7182-1 Rev: F
Piccolo® MetLac 12 Panel Instructions for Use, July 2022 PN: 400-7192-1 Rev. K