Piccolo® Basic Metabolic Panel Plus

For In Vitro Diagnostic Use and For Professional Use Only
Customer and Technical Service : 800-822-2947
Customers outside the US should contact their local
Abaxis representative for customer service

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1. Intended Use

The Piccolo® Basic Metabolic Panel Plus, used with the Piccolo blood chemistry analyzer or Piccolo Xpress® chemistry analyzer, is intended to be used for the *in vitro* quantitative determination of calcium, chloride, creatinine, glucose, lactate dehydrogenase, magnesium, potassium, sodium, total carbon dioxide, and blood urea nitrogen in a clinical laboratory setting or point-of-care location. **<u>This disc is for testing heparinized plasma and serum, only</u>**.

2. Summary and Explanation of Tests

The Piccolo Basic Metabolic Panel Plus reagent disc and the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer comprise an *in vitro* diagnostic system that aids the physician in the diagnosis and treatment of the following disorders :

Calcium:	Hyperparathyroidism, hypothyroidism, bone and chronic renal diseases, tetany.
Chloride:	Dehydration, prolonged diarrhea and vomiting, renal tubular disease, hyperparathyroidism, burns, salt-losing renal diseases, overhydration and thiazide therapy.
Creatinine:	Renal disease and monitoring of renal dialysis.
Glucose:	Carbohydrate metabolism disorders, including adult and juvenile diabetes mellitus and hypoglycemia, hypopituitarism, pancreatitis & renal disease.
Lactate Dehydrogenase:	Liver diseases such as acute viral hepatitis and cirrhosis; cardiac diseases such as myocardial infarction; and tissue alterations of the heart, kidney, liver, and muscle.
Magnesium:	Hypomagnesemia and hypermagnesemia.
Potassium:	Renal glomerular or tubular disease, adrenocortical insufficiency, diabetic ketacidosis, excessive intravenous potassium therapy, sepsis, panhypopituitarism, hyperaldosteronism, malnutrition, hyperinsulinism, metabolic alkalosis and gastrointestinal loss.
Sodium:	Dehydration, diabetes insipidus, loss of hypotonic gastrointestinal fluids, salt poisoning, selective depression of sense of thirst, skin losses, burns, sweating, hyperaldosteronism, CNS disorders, dilutional, depletional and delusional hyponatremia and syndrome of inappropriate ADH secretion.
Total Carbon Dioxide:	Primary metabolic alkalosis and acidosis and primary respiratory alkalosis and acidosis.
Blood Urea Nitrogen:	Renal and metabolic diseases.

As with any diagnostic test procedure, all other test procedures including the clinical status of the patient, should be considered prior to final diagnosis.

3. Principle of Procedure

Calcium (CA)
The first methods used to analyze calcium involved precipitating calcium with an excess of anions.[1,2,3] Precipitation methods are laborious and often imprecise. The reference method for calcium is atomic absorption spectroscopy; however, this method is not suited for routine use.[4] Spectrophotometric methods using either *o*-cresolphthalein complexone (CPC) or arsenazo III

metallochromic indicators are most commonly used.[5,6,7] Arsenazo III has a high affinity for calcium and is not temperature dependent as is CPC.

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

$$Ca^{2+} + \text{Arsenazo III} \longrightarrow Ca^{2+}\text{-Arsenazo III Complex}$$

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.[8]

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

Creatinine (CRE)

The Jaffe method, first introduced in 1886, is still a commonly used method of determining creatinine levels in blood. The current reference method combines the use of Fuller's earth (floridin) with the Jaffe technique to increase the specificity of the reaction.[9,10] Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.[11,12,13] Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohyrolase.[14]

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

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

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{Sarcosine Oxidase}} \text{Glycine} + \text{Formaldehyde} + H_2O_2$$

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

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 630 nm.

eGFR (calculated)

Serum creatinine is routinely measured as an indicator of renal function. Because creatinine is influenced by age, gender and race, chronic kidney disease (CKD) may not be detected using serum creatinine alone. Thus, the National Kidney Disease Education Program strongly recommends that laboratories routinely report an estimated Glomerular Filtration Rate (eGFR) when serum creatinine is measured for patients 18 and older. Routinely reporting the eGFR with all serum creatinine determinations allows laboratories to help identify individuals with reduced kidney function and help facilitate the detection of CKD. Calculated eGFR values of <60 ml/min are generally associated with increased risk of adverse outcomes of CKD.

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.

$$\text{GFR (mL/min/1.73 m}^2\text{)} = 175 \times (S_{cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})$$

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu [15] and Somogyi-Nelson [16,17]). The lack of specificity in copper-reduction techniques led to the development of quantitative procedures using the enzymes hexokinase and glucose oxidase. The glucose test incorporated into the Piccolo Basic Metabolic Panel Plus reagent disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.[18] The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), procedures 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.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-phosphate + ADP}$$

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

Lactate dehydrogenase (LD)

Lactate dehydrogenase (LD) catalyzes the reversible oxidation of L-lactate to pyruvate with the concurrent reduction of nicotinamide adenine dinucleotide (NAD^+) to reduced nicotinamide adenine dinucleotide (NADH). The method is based on the lactate-to-pyruvate reaction of Wacker et al.[19] NADH is subsequently oxidized with the simultaneous reduction of p-Iodonitrotetrazolium Violet (INT) to a highly colored formazan dye in a reaction catalyzed by diaphorase.

$$\text{L-Lactate} + NAD^+ \xrightarrow{\text{LD}} \text{Pyruvate + NADH} + H^+$$

$$\text{NADH} + H^+ + \text{INT} \xrightarrow{\text{Diaphorase}} NAD^+ + \text{Formazan}$$

The rate of formation of formazan is measured bichromatically at 500 nm and 630 nm. The rate is directly proportional to the LD activity of the sample.

Magnesium (MG)

The hexokinase (HK) activation method for magnesium is the best-fit method for the Piccolo system in terms of sensitivity, precision, and accuracy.[20] The enzymatic magnesium method can be described as :

$$\text{Glucose + ATP} \xrightarrow[Mg^{2+}]{\text{HK}} \text{G-6-P + ADP}$$

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

The rate limiting reaction is the HK reaction. Magnesium from the sample activates HK, which in turn catalyzes the break down 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.

Potassium (K^+)

Spectrophotometric methods have been developed that allow the measurement of potassium concentration on standard clinical chemistry instrumentation. The Abaxis enzymatic method is based on the activation of pyruvate kinase with potassium and shows excellent linearity and negligible susceptibility to endogenous substances.[21,22,23] Interference from sodium and ammonium ion are minimized with the addition of Kryptofix and glutamate dehydrogenase, respectively.[23]

In the coupled-enzyme reaction, pyruvate kinase (PK) dephosphorylates phosphoenolpyruvate (PEP) to form pyruvate. Lactate dehydrogenase (LD) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+. The rate of change in absorbance due to the conversion of NADH to NAD^+ is directly proportional to the amount of potassium in the sample.

$$ADP + PEP \xrightarrow{K^+,\ PK} Pyruvate + ATP$$

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

Sodium (NA^+)

Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.[24,25,26] In the Abaxis enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyzes the reaction of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol and galactose.

$$ONPG \xrightarrow[\beta\text{-Galactosidase}]{Na^+} o\text{-Nitrophenol} + Galactose$$

Total Carbon Dioxide (tCO_2)

Total carbon dioxide in serum or plasma exists as dissolved carbon dioxide, carbamino derivatives of proteins, bicarbonate and carbonate ions and carbonic acid. Total carbon dioxide can be measured by pH indicator, CO_2 electrode and spectrophotometric enzymatic methods, which all produce accurate and precise results.[27,28] The enzymatic method is well suited for use on a routine blood chemistry analyzer without adding complexity.

In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide (CO_2) toward bicarbonate (HCO_3^-). 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 the CO_2 in the sample.

$$PEP + HCO_3^- \xrightarrow{PEPC} Oxaloacetate + Phosphate$$

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

Blood Urea Nitrogen (BUN)

Urea can be measured both directly and indirectly. The diacetyl monoxime reaction, the only direct method to measure urea, is commonly used but employs dangerous reagents.[29] Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.[30] The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique[31,32] and coupled enzymatic reactions.[33,34] Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.[35] Coupled-enzyme reactions are rapid, have a high specificity for ammonia, and are commonly used. One such reaction has been proposed as a candidate reference method.[36]

In the coupled-enzyme reaction, urease hydrolyzes urea into ammonia and carbon dioxide. Upon combining ammonia with α-ketoglutarate and NADH, the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD^+.

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

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

4. Principle of Operation

See the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual, for the Principles and Limitations of the Procedure**.** A detailed description of the Piccolo analyzer and reagent disc has been described by Schembri et al.[37]

5. Description of Reagents

Reagents

Each Piccolo Basic Metabolic Panel Plus reagentdisc contains dry test-specific reagent beads (described below). A dry sample blank reagent (comprised of buffer, surfactants, excipients, and preservatives) is included in each disc for use in calculating concentrations of calcium, chloride, glucose, lactate dehydrogenase, magnesium, potassium, sodium, total carbon dioxide, and blood urea nitrogen. A dedicated sample blank is included in the disc for creatinine (CRE). Each disc also contains a diluent consisting of surfactants and preservatives.

Table 1 : Reagents

Component	Quantity/Disc
2, 4, 6-Tribomo-3-hydroxybenzoic acid	188 µg
2-Chloro-4-nitrophenyl -alpha-maltotrioside (CNPG3)	52.5 µg
4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 222)	0.3 µg
4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]trisocosane (Kryptofix 221)	84 µg
4-Aminoantipyrine*HCl	13 µg
N-Acetyl cysteine	15.3 µg
Adenosine-5'-triphosphate	27 µg
Amylase	0.0357 U
Arsenazo III, sodium salt	1.7 µg
Ascorbate oxidase	0.3 U
Bovuminar reagent, pure powder	164 µg
Calcium acetate	25.2 µg
Citric acid, trisodium salt	567 µg
Creatine amidinohydrolase	3 U
Creatinine amidohydrolase	1 U
Dextran, Low Fraction	224 µg
Diaphorase	0.084 U
Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)	18.8 µg
Ethylenediaminetetraacetic acid (EDTA)	178.42 µg
β-Galactosidase	0.005 U
Glucose	64 µg
Glucose-6-phosphate dehydrogenase	0.022 U
Glutamate dehydrogenase	0.1 U
Hexokinase	0.112 U
p-Iodonitrotetrazolium violet (INT)	5.082 µg
Imidazole	29 µg
myo-Inositol	160 µg
α-Ketoglutaric acid	19 µg
Lactate dehydrogenase	0.3 U
Lithium lactate	96.77 µg
Magnesium sulfate	29 µg
Malate dehydrogenase	0.1 U
D-Mannitol	420 µg
β-Nicotinamide adenine dinucleotide (NAD+)	89.2 µg
β-Nicotinamide adenine dinucleotide, reduced (NADH)	28 µg
β-Nicotinamide adenine dinucleotide phosphate (NADP+), sodium salt	29.6 µg
o-Nitrophenyl-ß-D-galactopyranoside (ONPG)	22 µg
n-Octylglucoside	21 µg
Peroxidase	1 U
Phosphoenolpyruvate	23 µg
Phosphoenolpyruvate carboxylase	0.001 U

Table 1 : Reagents (Continued)

Component	Quantity/Disc
Polyethylene glycol, 3400	168 µg
Polyvinylpyrrolidone (K 29-32)	4 µg
Potassium chloride	47.59 µg
Potassium ferrocyanide	0.4 µg
Pyruvate kinase	0.01 U
Sarcosine oxidase	1 U
Sodium chloride	12 µg
D(+) Trehalose, dihydrate	650 µg
Triethanolamine-hydrochloride	19.16 µg
Tris(hydroxymethyl)aminomethane (free base)	296.44 µg
Tris(hydroxymethyl)aminomethane*HCl	40.91 µg
Triton X-100	1.72 µg
Urease	0.05 U
Buffers, surfactants, excipients and preservatives	

Warnings and Precautions

- For *In vitro* Diagnostic Use
- The diluent container in the reagent disc is automatically opened when the analyzer drawer closes. A disc with an opened diluent container cannot be re-used. Ensure that the sample or control has been placed into the disc before closing the drawer.
- Used reagent discs contain human body fluids. Follow good laboratory safety practices when handling and disposing of used discs.[38] See the Piccolo Blood Chemistry Analyzer or Piccolo Xpress chemistry analyzer Operator's Manual for instructions on cleaning biohazardous spills.
- The reagent discs are plastic and may crack or chip if dropped. Never use a dropped disc as it may spray biohazardous material throughout the interior of the analyzer.
- Reagent beads may contain acids or caustic substances. The operator does not come into contact with the reagent beads when following the recommended procedures. In the event that the beads are handled (e.g., cleaning up after dropping and cracking a reagent disc), avoid ingestion, skin contact, or inhalation of the reagent beads.

Instructions for Reagent Handling

Reagent discs may be used directly from the refrigerator without warming. Do not allow discs sealed in their foil pouches to remain at room temperature longer than 48 hours prior to use. Open the sealed foil pouch, remove the disc and use according to the instructions provided in the Piccolo Blood Chemistry Analyzer or Piccolo Xpress chemistry analyzer Operator's Manual. A disc not used within 20 minutes of opening the pouch should be discarded.

Storage

Store reagent discs in their sealed pouches at 2-8°C (36-46°F). Do not expose opened or unopened discs to direct sunlight or temperatures above 32°C (90°F). Reagent discs may be used until the expiration date included on the package. The expiration date is also encoded in the bar code printed on the bar code ring. An error message will appear on the Piccolo Blood Chemistry Analyzer or Piccolo Xpress chemistry analyzer display if the reagents have expired.

Indications of Reagent Disc Instability/Deterioration

A torn or otherwise damaged pouch may allow moisture to reach the unused disc and adversely affect reagent performance. Do not use a disc from a damaged pouch.

6. Instrument

See the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual for complete information on use of the analyzer.

7. Sample Collection and Preparation

Sample collection techniques are described in the "Sample Collection" section of the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual.

- The minimum required sample size is ~100 µL of heparinized plasma, serum, or control material. The reagent disc sample chamber can contain up to 120 µL of sample.

- Do not shake the collection tube; shaking may cause hemolysis. Hemolysis will cause erroneously high results in potassium and lactate dehydrogenase assays.

- In addition, even unhemolyzed specimens that are not promptly processed may have increased potassium levels due to intracellular potassium leakage.[39]

- Use only lithium heparin (green stopper) evacuated specimen collection tubes for plasma samples. Use no-additive (red stopper) evacuated specimen collection tubes or serum separator tubes (red or red/black stopper) for serum samples.

- Start the test within 10 minutes of transferring the sample into the reagent disc.

- The concentration of total carbon dioxide is most accurately determined when the assay is done immediately after opening the tube and as promptly as possible after collection and processing of the blood in the unopened tube. Ambient air contains far less carbon dioxide than does plasma, and gaseous dissolved carbon dioxide will escape from the specimen into the air, with a consequent decrease in carbon dioxide value of up to 6 mmol/L in the course of 1 hour.[40]

8. Procedure

Materials Provided
- One Piccolo Basic Metabolic Panel Plus PN : 400-1031 (a box of discs PN : 400-0031)

Materials Required but not Provided
- Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer

- Commercially available control reagents recommended by Abaxis (refer to Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual)

Test Parameters
The Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each Piccolo Basic Metabolic Panel Plus is less than 14 minutes. The analyzer maintains the reagent disc at a temperature of 37°C (98.6°F) over the measurement interval.

Test Procedure
The complete sample collection and step-by-step operating procedures are detailed in the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual.

Calibration
The Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer is calibrated by the manufacturer before shipment. The bar code printed on the bar code ring provides the analyzer with disc-specific calibration data. See the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual.

Quality Control
Performance of the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer can be verified by running controls. Controls recommended by Abaxis are listed in the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual. Other human serum or plasma-based controls may not be compatible.

See the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual, for a detailed discussion on running, recording, interpreting, and plotting control results.

9. Results

The Piccolo Blood Chemistry Analyzer or Piccolo Xpress chemistry analyzer automatically calculates and prints the analyte concentrations in the sample. Details of the endpoint and rate reaction calculations are found in the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual.

Interpretation of results is detailed in the Operator's Manual. Results are printed onto result cards supplied by Abaxis. The result cards have an adhesive backing for easy placement in the patient's files.

10. Limitations of Procedure

General procedural limitations are discussed in the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual.

- Only heparinized plasma or serum may be used with this disc due to the susceptibility of falsely high LD values from ruptured blood cells.

- The only anticoagulant **recommended for use** with the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer is **lithium heparin**. Abaxis has performed studies demonstrating that EDTA, fluoride, oxalate, and any anticoagulant containing ammonium ions will interfere with at least one chemistry contained in the Piccolo Basic Metabolic Panel Plus reagent disc.

- **Any result for a particular test that exceeds the assay range should be analyzed by another approved test method or sent to a referral laboratory. Do not dilute the sample and run it again on the Piccolo blood chemistry analyzer.**

  Warning : Extensive testing of the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer has shown that, in very rare instances, sample dispensed into the reagent disc may not flow smoothly into the sample chamber. Due to the uneven flow, an inadequate quantity of sample may be analyzed and several results may fall outside the reference ranges. The sample may be re-run using a new reagent disc.

Interference

Substances were tested as interferents with the analytes. Human serum pools were prepared. The concentration at which each potential interferent was tested was based on the testing levels in CLSI EP7-A.[41]

Effects of Endogenous Substances

- Physiological interferents (hemolysis, icterus and lipemia) cause changes in the reported concentrations of some analytes. The sample indices are printed on the bottom of each result card to inform the operator about the levels of interferents present in each sample. The Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer suppresses any results that are affected by >10% interference from hemolysis, lipemia or icterus. "HEM", "LIP", or "ICT" respectively, is printed on the result card in place of the result, with the exception of LD. See an explanation of the effects on LD in the next bullet point. For the endogenous limits, please contact Abaxis Technical Service.

- Significant levels of LD are found in blood cells. Rupture of these cells can lead to increased levels of LD. Hence; all LD assays are sensitive to hemolysis due to release of LD from the red blood cells. There was no significant interference in LD (> 10%) when HEM values up to 50 mg/dL were tested. For the LD assay, only, if HEM is greater than 50 and less than or equal to 100 mg/dL, the LD value will be printed followed by an "H" indicating some additional influence from hemolysis. If the HEM is greater than 100 mg/dL and less than or equal to 150 mg/dL, the LD value will be preceded by "<" and followed by an "H." Thus, indicating that the true LD recovery is less than that reported. The purpose of these annotations is to help interpret LD activity in the presence of small amounts of hemolysis. For values of HEM above 150, no result will be indicated and only "HEM" will be printed.

- Extremely elevated amylase levels (>9,000 U/L) will have a significant effect, >10% increase, on the chloride result. The concentration of amylase is not evaluated by the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer for each specimen.

- The potassium assay in the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer is a coupled pyruvate kinase (PK) / lactate dehydrogenase (LD or LDH) assay. Therefore, in cases of extreme muscle trauma or highly elevated levels of creatine kinase (CK), the Piccolo may recover a falsely elevated potassium (K+) value. In such cases, unexpected high potassium recoveries need to be confirmed utilizing a different methodology.

Effects of Therapeutic Substances and Metabolites

Thirty-eight drugs and metabolites were selected as potential interferents with chloride, calcium, creatinine, glucose, magnesium, potassium, sodium, total carbon dioxide and blood urea nitrogen methods. The drugs and metabolites that were evaluated were chosen based on recommendations by Young.[42] Eleven of these were tested by the LD assay and are marked with *. Two additional substances (lactic acid and lithium citrate), marked with (LD only), were selected as potential interferents for the LD assay and tested with it alone. Significant interference is defined as a >10% shift in the result from control sample. Human serum pools were supplemented with a known concentration of the drugs or chemicals and then analyzed.

Table 2 : Therapeutic Substances Evaluated

	Physiologic or Therapeutic Range[41-45] (mg/dL)	**Highest Concentration Tested (mg/dL)**
Acetaminophen*	2 - 10	100
Acetoacetate*	0.05 - 3.6	102
Acetylsalicylic acid*	1 - 2	50
Ampicillin	0.5	30
Ascorbic acid	0.8-1.2	20
Ascorbic acid* (LD)	0.8-1.2	3
Caffeine*	0.3 - 1.5	10
Cephalothin (Keflin)	10	400
Chloramphenicol	1 - 2.5	100
Cimetidine	0.1 - 1	16
Dopamine	0.3 - 1.5	19
Epinephrine		1
Erythromycin	0.2 - 2.0	10
Glutathione		30
Hydrochlorothiazide		7.5
Ibuprofen*	0.5 - 4.2	50
Isoniazide	0.1 - 0.7	4
Ketoprofen		50
L-Dopa		5
Lactic acid (LD only)	4.5 - 19.8	60
Lidocaine*	0.15 - 0.60	1
Lithium citrate (LD only)	0.4 - 0.8	3.5
Lithium Lactate	6 - 12	84
Methicillin		100
Methotrexate	> 50.05 [A]	0.5
Methotrexate* (LD)	> 50.05	450
Metronidazole	0.1	5
Nafcillin		1
Nitrofurantoin	0.2	20
Oxacillin		1
Oxaloacetate*		132
Penicillin G		100
Phenytoin (5,5-Diphenylhydantion)*	1 - 2	3
Proline		4
Pyruvate*	0.3 - 0.9	44
Rifampin	0.4 - 3	0.5
Salicylic Acid		50
Sulfadiazine		150
Sulfanilamide	10 - 15	50
Theophylline	1 - 2	20

[A] Updated Methotrexate therapeutic concentration based on CLSI Vol. 22 No. 27 Guideline.

Table 3 : Substances With Significant Interference >10%

	Physiologic/ Therapeutic Range [41-45] (mg/dL)	Concentration with > 10% Interference (mg/dL)	% Interference[A]
Calcium	None	None	None
Chloride	None	None	None
Creatinine			
Ascorbic Acid	0.8 - 1.2	20	11% dec
Dopamine	0.3 - 1.5	19	80% dec
L-Dopa		5	71% dec
Epinephrine		1	45% dec
Glutathione		30	13% dec
Glucose			
Oxaloacetate		132	11% dec
Pyruvate	0.3 - 0.9	44	
Lactate dehydrogenase			
Oxaloacetate		99 (no effect at 66)	18% dec
Magnesium	None	None	None
Potassium			
Penicillin G		100	17% inc.
Sulfadiazine	2 - 4	150	12% dec.
Sodium			
Cephalothin	10	400	12% inc.
Methotrexate	> 50.05	0.5	11% inc.
Penicillin G		100	10% inc.
Total Carbon Dioxide			
Acetaminophen	2 - 10	100	11% inc.
Ascorbic Acid	0.8 - 1.2	20	12% dec.
Cephalothin	10	400	13% inc.
Cimetidine	0.1 - 1	16	19% dec.
Erythromycin	0.2 - 2.0	10	21% dec.
Lidocaine	0.15 - 0.60	1	23% inc.
Methotrexate	> 50.05	0.5	80% dec.
Nitrofurantoin	0.2	20	13% inc.
Salicylic Acid	15 - 30	50	17% dec.
Sulfadiazine	2 - 4	150	25% dec.
Blood Urea Nitrogen	None	None	None

[A] Dec. = decreased concentration of the specified analyte; Inc. = increased concentration of the specified analyte
[B]Updated Methotrexate therapeutic concentration based on CLSI Vol. 22 No. 27

Table 4 : Concentration of Analytes in Serum Pool Used for Interference Studies

Analyte	Concentration
Calcium	9.5 mg/dL
Chloride	93 mmol/L
Creatinine	4.1 mg/d/L
Glucose	96 mg/dL
Lactate Dehydrogenase	276 U/L and 703 U/L
Magnesium	4.3 mg/dL
Potassium	3.8 mmol/L
Sodium	124 mmol/L
Total Carbon Dioxide	6 mmol/L
Blood Urea Nitrogen	26 mg/dL

- For the Chloride assay, bromide at toxic levels (≥ 15 mmol/L) can cause a significant effect (> 10% increase), on the chloride result. Iodide at very high concentrations (30 mmol/L, highest level tested) has no effect. Normal physiological levels of bromide and iodide do not interfere with the Piccolo Chloride Test System.

11. Expected Values

Samples from 60 - 150 adult males and females were analyzed on the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer to determine the reference interval for the analytes, with the exception of LD. For LD, the reference interval was established by applying linear regression statistics from a correlation study versus the Beckman Synchron LX20 to the LX20's published reference interval. These intervals are provided as a guideline only. It is recommended that your office or institution establish normal ranges for your particular patient population.[46]

Table 5 : Piccolo Reference Intervals

Analyte	Common Units	SI Units
Calcium	8.0 – 10.3 mg/dL	2.0 – 2.58 mmol/L
Chloride	98 – 108 mmol/L	98 – 108 mmol/L
Creatinine	0.6 – 1.2 mg/dL	53 – 106 µmol/L
Glucose	73 – 118 mg/dL	4.05 – 6.55 mmol/L
Lactate Dehydrogenase*	99 – 192 U/L	99 – 192 U/L
Magnesium	1.6 – 2.3 mg/dL	0.66 – 0.95 mmol/L
Potassium	3.6 – 5.1 mmol/L	3.6 – 5.1 mmol/L
Sodium	128 – 145 mmol/L	128 – 145 mmol/L
Total Carbon Dioxide	18 – 33 mmol/L	18 – 33 mmol/L
Blood Urea Nitrogen (BUN)	7 – 22 mg/dL	2.5 – 7.9 mmol urea/L

* A small increase (approximately 6 U/L) in lactate dehydrogenase was observed in serum when compared to heparinized plasma. This increase is consistent with the difference between serum and plasma for LD as described in the literature.[47,48] LD is released from blood cells during the coagulation process in the preparation of serum.

12. Performance Characteristics

Linearity

The chemistry for each analyte is linear over the dynamic range listed below when the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer is operated according to the recommended procedure (refer to the Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer Operator's Manual).

Table 6 : Piccolo Dynamic Ranges

Analyte	Common Units	SI Units
Calcium	4.0 – 16.0 mg/dL	1.0 – 4.0 mmol/L
Chloride	80 – 135 mmol/L	80 – 135 mmol/L
Creatinine	0.2 – 20 mg/dL	18 – 1768 µmol/L
Glucose	10 – 700 mg/dL	0.56 – 38.9 mmol/L
Lactate Dehydrogenase	50 – 1000 U/L	50 – 1000 U/L
Magnesium	0.1 – 8.0 mg/dL	0.04 – 3.3 mmol/L
Potassium	1.5 – 8.5 mmol/L	1.5 – 8.5 mmol/L
Sodium	110 – 170 mmol/L	110 – 170 mmol/L
Total Carbon Dioxide	5 – 40 mmol/L	5 – 40 mmol/L
Blood Urea Nitrogen (BUN)	2 – 180 mg/dL	0.7 – 64.3 mmol urea/L

Sensitivity (Limits of Detection)

The lower limit of the reportable (dynamic) range for each analyte is : calcium 4.0 mg/dL (1.0 mmol/L); chloride 80 mmol/L; creatinine 0.2 mg/dL (18 µmol/L); glucose 10 mg/dL (0.56 mmol/L); lactate dehydrogenase 50.0 U/L; magnesium 0.1 mg/dL (0.04 mmol/L); potassium 1.5 mmol/L; sodium 110 mmol/L; total carbon dioxide 5 mmol/L; and blood urea nitrogen 2.0 mg/dL (0.7 mmol urea/L).

Precision

Precision studies were conducted using CLSI EP5-A2 guidelines[49] with modifications based on CLSI EP18-A[50] for unit-use devices. Results for within-run and total precision were determined using two levels of commercially available control materials. The studies made use of multiple instruments. Calcium, creatinine, glucose, sodium and blood urea nitrogen testing was performed at one site; potassium and total carbon dioxide testing was performed at two sites over 20 days; chloride, lactate dehydrogenase and magnesium testing was done at two sites over a period of five days.

Results of precision studies are shown in Table 7.

Table 7 : Precision

Analyte	Sample Size	Within-Run	Total
Calcium (mg/dL)			
Control 1	N = 80		
Mean		8.6	8.6
SD		0.21	0.25
%CV		2.4	2.9
Control 2			
Mean		11.8	11.8
SD		0.39	0.40
%CV		3.3	3.4
Chloride (mmol/L)	N = 160		
Control 1			
Mean		97.8	97.8
SD		1.63	1.74
%CV		1.7	1.7
Control 2			
Mean		113.6	113.6
SD		1.97	2.22
%CV		1.7	2.0

Table 7 : Precision (Continued)

Analyte	Sample Size	Within-Run	Total
Creatinine (mg/dL)	N=80		
Control 1			
Mean		1.1	1.1
SD		0.14	0.14
%CV		12.5	13.1
Control 2			
Mean		5.2	5.2
SD		0.23	0.27
%CV		4.4	5.2
Glucose (mg/dL)	N=80		
Control 1			
Mean		66	66
SD		0.76	1.03
%CV		1.1	1.6
Control 2			
Mean		278	278
SD		2.47	3.84
%CV		0.9	1.4
Lactate Dehydrogenase (U/L)	N=80		
Control 1			
Mean		87	87
SD		3.0	4.4
%CV		3.4	5.0
Control 2			
Mean		350	350
SD		3.8	7.0
%CV		1.1	2.0
Magnesium (mg/dL)	N =80		
Control 1			
Mean		1.9	1.9
SD		0.03	0.06
%CV		1.7	3.4
Control 2			
Mean		3.9	3.9
SD		0.04	0.10
%CV		1.0	2.6
Potassium (mmol/L)	N = 120		
Control 1			
Mean		6.12	6.12
SD		0.32	0.32
%CV		5.2	5.7
Control 2			
Mean		4.10	4.10
SD		0.24	0.26
%CV		5.9	6.3

Table 7 : Precision (Continued)

Analyte	Sample Size	Within-Run	Total
Sodium (mmol/L)	N = 80		
Control 1			
Mean		143.5	143.5
SD		2.28	2.28
%CV		1.6	1.6
Control 2			
Mean		120.0	120.0
SD		2.13	2.13
%CV		1.8	1.8
Total Carbon Dioxide (mmol/L)	N = 120		
Control 1			
Mean		21.4	21.4
SD		2.29	2.29
%CV		10.7	10.7
Control 2			
Mean		10.5	10.5
SD		0.90	0.90
%CV		8.6	8.6
Blood Urea Nitrogen (mg/dL)	N = 80		
Control 1			
Mean		19	19
SD		0.35	0.40
%CV		1.9	2.1
Control 2			
Mean		65	65
SD		1.06	1.18
%CV		1.6	1.8

Correlation

Heparinized serum samples and plasma samples for LD assay were collected and assayed on the Piccolo blood chemistry analyzer and by a comparative method(s). In some cases, high and low supplemented samples were used to cover the dynamic range. The samples were chosen to meet the distribution values in CLSI EP9-A2 guideline.[51] Representative correlation statistics are shown in Table 8.

Table 8 : Correlation of Piccolo Blood Chemistry Analyzer with Comparative Method(s)

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
Calcium (mg/dL)	0.991* 0.673	0.990 0.742	-0.4 1.8	0.17 0.22	25 81	5.2-11.9 8.1-9.9	Paramax® Beckman
Chloride (mmol/L)	0.978	0.982	-1.1	1.84	120	71 - 118	Vitros 950® Ortho
Creatinine (mg/dL)	0.993 0.987	0.926 0.866	0.0 0.1	0.15 0.16	260 107	0.4-14.7 0.4-7.5	Paramax® Beckman
Glucose (mg/dL)	0.987 0.997	1.009 0.943	-2.8 1.2	3.89 4.69	251 91	72-422 56-646	Paramax® Beckman
Lactate Dehydrogenase (U/L)	0.994	0.983	3.8	26.3	60	44 – 1172	Synchron® LX20 Beckman
Magnesium (mg/dL)	0.992	0.990	0.0	0.16	44	0.8 - 6.8	Inductively Coupled Plasma-Atomic Optical Emission Spectroscopy (ICP-OES)
Potassium (mmol/L)	0.969	0.863	0.6	0.14	58	2.0 - 6.8	KNA™ 2 Radiometer
Sodium (mmol/L)	0.937	0.782	27.7	3.79	113	116 - 154	KNA™ 2 Radiometer
Total Carbon Dioxide (mmol/L)	0.947	0.903	2.0	0.84	60	6 – 39	Cobas Fara® Roche
Blood Urea Nitrogen (mg/dL)	0.964 0.983	0.923 0.946	0.5 0.0	1.08 0.66	251 92	6 –52 6-38	Paramax® Beckman

* Serum samples from hospitalized patients provided a broader, and possibly more useful, sample range than did venous whole blood samples from out-patients. Correlation statistics for the Piccolo calcium test are from these serum samples.

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