

# Piccolo® Comprehensive Metabolic Panel



For In Vitro Diagnostic Use and For Professional Use Only

Customer and Technical Service: 1-800-822-2947

Customers outside the US: +49 6155 780 210

Applicable to US customers only

**CLIA Waived: Use lithium heparin whole blood, only**  
**Moderate Complexity: Use lithium heparin whole blood, lithium heparin plasma , or serum**



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## 1. Intended Use

The Piccolo® Comprehensive Metabolic Panel, used with the Piccolo® blood chemistry analyzer or the Piccolo Xpress® chemistry analyzer, is intended 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, and blood urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum in a clinical laboratory setting or point-of-care location.

### For US Customers Only

The tests on this panel are waived under CLIA '88 regulations. If a laboratory modifies the test system instructions, then the tests are considered high complexity and subject to all CLIA requirements. For CLIA waived labs, only lithium heparin whole blood may be tested. For use in moderate complexity labs, lithium heparinized whole blood, lithium heparinized plasma, or serum may be used.

A CLIA Certificate of Waiver is needed to perform CLIA waived testing. A Certificate of Waiver can be obtained from the Centers for Medicare & Medicaid Services (CMS). Please contact the Commission on Laboratory Accreditation (COLA) at 1-800-981-9883 for assistance in obtaining one.

## 2. Summary and Explanation of Tests

The Piccolo Comprehensive Metabolic Panel and the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders:

Alanine aminotransferase (ALT):	Liver diseases; including viral hepatitis and cirrhosis.
Albumin:	Liver and kidney disease.
Alkaline phosphatase (ALP):	Liver, bone, parathyroid and intestinal diseases.
Aspartate aminotransferase (AST):	Liver disease including hepatitis and viral jaundice; shock.
Calcium:	Parathyroid, 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.
Potassium:	Renal glomerular or tubular disease, adrenocortical insufficiency, diabetic ketacidosis, excessive intravenous potassium therapy, sepsis, panhypopituitarism, in vitro hemolysis, 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, depletion and

Total bilirubin:	delusional hyponatremia and syndrome of inappropriate ADH secretion.
Total carbon dioxide:	Liver disorders, including hepatitis and gall bladder obstruction; jaundice.
Total protein:	Primary metabolic alkalosis and acidosis and primary respiratory alkalosis and acidosis.
Blood Urea Nitrogen (BUN):	Liver, kidney, bone marrow diseases; metabolic and nutritional disorders.
	Renal and metabolic diseases.

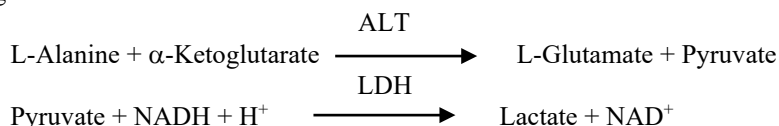
**As with any diagnostic test procedure, all other test procedures including the clinical status of the patient, should be considered prior to final diagnosis.**

### 3. Principle of Procedure

#### Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT) has been measured by three methods. Two of these methods—the colorimetric dinitrophenylhydrazine coupling technique<sup>1,2</sup> and the fluorescent enzymatic assay—are rarely used.<sup>3</sup> An enzymatic method based on the work of Wróblewski and LaDue<sup>4</sup> is the most common technique for determining ALT concentrations in serum. A modified Wróblewski and LaDue procedure has been proposed as the recommended procedure of the International Federation of Clinical Chemistry (IFCC).<sup>5</sup>

The method developed for use on the Piccolo analyzers is a modification of the IFCC-recommended procedure. In this reaction, ALT catalyzes the transfer of an amino group from L-alanine to  $\alpha$ -ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to  $\text{NAD}^+$ , as illustrated in the following reaction scheme.

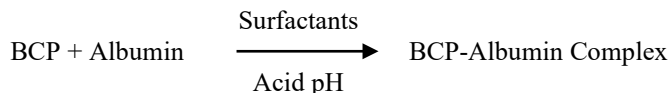


The rate of change of the absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to  $\text{NAD}^+$  and is directly proportional to the amount of ALT present in the sample.

#### Albumin (ALB)

Early methods used to measure albumin include fractionation techniques<sup>6,7,8</sup> and tryptophan content of globulins.<sup>9,10</sup> These methods are unwieldy to perform and do not have a high specificity. Two immunochemical techniques are considered as reference methods, but are expensive and time consuming.<sup>11</sup> Dye binding techniques are the most frequently used methods for measuring albumin. Bromocresol green (BCG) is the most commonly used of the dye binding methods but may over-estimate albumin concentration, especially at the low end of the normal range.<sup>12</sup> Bromocresol purple (BCP) is the most specific of the dyes in use.<sup>13,14</sup>

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

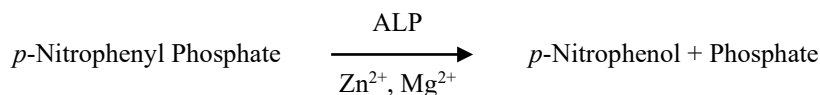


Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as the difference in absorbance between 600 nm and 550 nm.

#### Alkaline Phosphatase (ALP)

Techniques to measure alkaline phosphatase were first developed over 60 years ago. Several of these endpoint or two-point spectrophotometric methods<sup>15,16</sup> are now considered obsolete or too cumbersome. The use of *p*-nitrophenyl phosphate (*p*-NPP) increased the speed of the reaction.<sup>17,18</sup> The reliability of this technique was greatly increased by the use of a metal-ion buffer to maintain the concentration of magnesium and zinc ions in the reaction.<sup>19</sup> The American Association for Clinical Chemistry (AACC) reference method<sup>20</sup> uses *p*-NPP as a substrate and a metal-ion buffer.

The Piccolo procedure is modified from the AACC and IFCC<sup>21</sup> methods. Alkaline phosphatase hydrolyzes *p*-NPP in a metal-ion buffer and forms *p*-nitrophenol and phosphate.

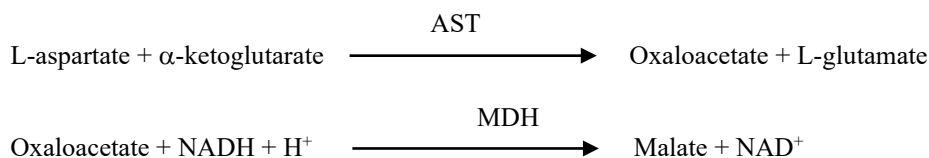


The amount of ALP in the sample is proportional to the rate of increase in absorbance difference between 405 nm and 500 nm.

### Aspartate Aminotransferase (AST)

The aspartate aminotransferase (AST) test is based on the Karmen rate method<sup>22</sup> as modified by Bergmeyer.<sup>23</sup> The current International Federation of Clinical Chemistry (IFCC) reference method utilizes the Karmen/Bergmeyer technique of coupling malate dehydrogenase (MDH) and reduced nicotinamide dinucleotide (NADH) in the detection of AST in serum.<sup>23,24</sup> Lactate dehydrogenase (LDH) is added to the reaction to decrease interference caused by endogenous pyruvate.

AST catalyzes the reaction of L-aspartate and  $\alpha$ -ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to  $\text{NAD}^+$  by the catalyst MDH.

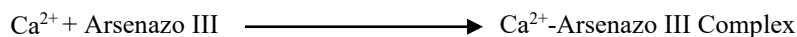


The rate of absorbance change at 340 nm/405 nm caused by the conversion of NADH to  $\text{NAD}^+$  is directly proportional to the amount of AST present in the sample.

### Calcium (CA)

The first methods used to analyze calcium involved precipitating calcium with an excess of anions.<sup>25,26,27</sup> 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.<sup>28</sup> Spectrophotometric methods using either *o*-cresolphthalein complexone or arsenazo III metallochromic indicators are most commonly used.<sup>29,30,31</sup> Arsenazo III has a high affinity for calcium and is not temperature dependent as is CPC.

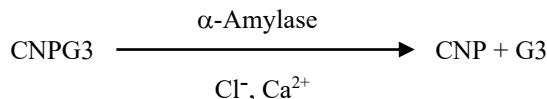
Calcium in the patient sample binds with arsenazo III to form a calcium-dye complex.



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.

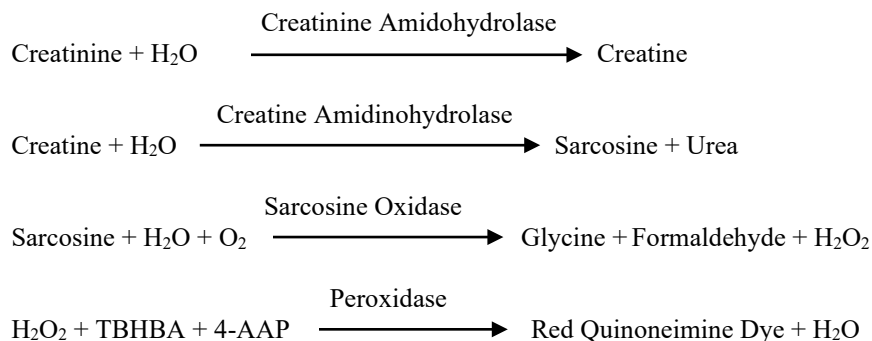
### Chloride (CL<sup>-</sup>)

The method is based on the determination of chloride-dependent activation of  $\alpha$ -amylase activity. Deactivated  $\alpha$ -amylase is reactivated by addition of the chloride ion, allowing the calcium to re-associate with the enzyme. The reactivation of  $\alpha$ -amylase activity is proportional to the concentration of chloride ions in the sample. The reactivated  $\alpha$ -amylase converts the substrate, 2-chloro-*p*-nitrophenyl- $\alpha$ -D-maltotriose (CNP3) to 2-chloro-*p*-nitrophenol (CNP) producing color and  $\alpha$ -maltotriose (G3). The reaction is measured bichromatically and the increase in absorbance is directly proportional to the reactivated  $\alpha$ -amylase activity and the concentration of chloride ion in the sample.<sup>32</sup>



### 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.<sup>33,34</sup> Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.<sup>35,36,37</sup> Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.<sup>38</sup>



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)

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.

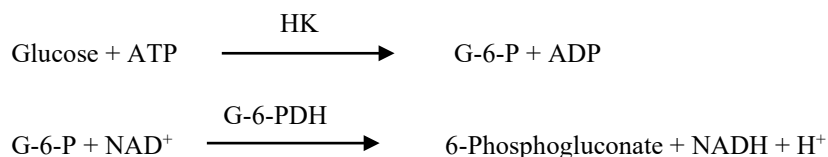
Calculation of the eGFR is performed by the Piccolo using the patient's age, gender and race. The Piccolo method for creatinine is traceable to the IDMS reference method for creatinine so that the following form of the MDRD equation for calculating the eGFR can be used.

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

### Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu<sup>39</sup> and Somogyi-Nelson<sup>40,41</sup>) 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 Comprehensive Metabolic Panel is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.<sup>42</sup>

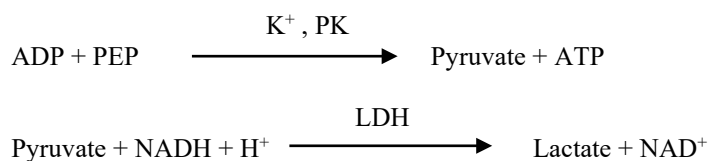
The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine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



### Potassium (K<sup>+</sup>)

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.<sup>43,44,45</sup> Interference from sodium and ammonium ion are minimized with the addition of Kryptofix and glutamine synthetase, respectively.<sup>43</sup>

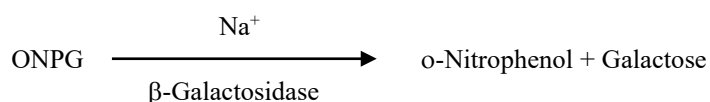
In the coupled-enzyme reaction, pyruvate kinase (PK) dephosphorylates phosphoenolpyruvate (PEP) to form pyruvate. Lactate dehydrogenase (LDH) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD<sup>+</sup>.



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

### Sodium (Na<sup>+</sup>)

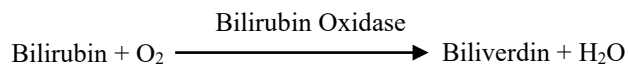
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.<sup>46,47,48</sup> In the Abaxis enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyzes the reaction of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol and galactose.



### Total Bilirubin (TBIL)

Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.<sup>49,50</sup> A newer, more specific method has been developed using the enzyme bilirubin oxidase.<sup>51,52,53</sup> In addition to using the more specific total bilirubin test method, photodegradation of the analyte is minimized on the Piccolo analyzers because the sample can be tested immediately after collection.

In the enzymatic procedure, bilirubin is oxidized by bilirubin oxidase into biliverdin.

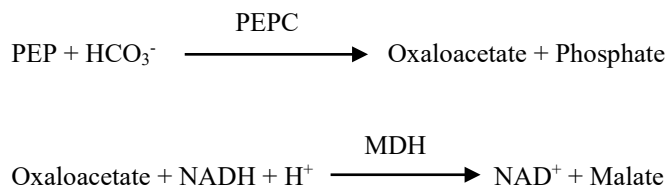


Bilirubin is quantitated as the difference in absorbance between 467 nm and 550 nm. The initial absorbance of this endpoint reaction is determined from the bilirubin blank cuvette and the final absorbance is obtained from the bilirubin test cuvette. The amount of bilirubin in the sample is proportional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

### Total Carbon Dioxide (tCO<sub>2</sub>)

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<sub>2</sub> electrode and spectrophotometric enzymatic methods, which all produce accurate and precise results.<sup>54,55</sup> The enzymatic method is well suited for use on a routine blood chemistry analyzer without adding complexity.

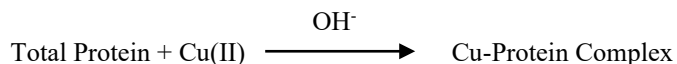
In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide (CO<sub>2</sub>) toward bicarbonate (HCO<sub>3</sub><sup>-</sup>). Phosphoenolpyruvate (PEP) and HCO<sub>3</sub><sup>-</sup> then react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MDH) catalyzes the reaction of oxaloacetate and reduced nicotinamide adenine dinucleotide (NADH) to NAD<sup>+</sup> and malate. The rate of change in absorbance due to the conversion of NADH to NAD<sup>+</sup> is directly proportional to the amount of tCO<sub>2</sub> in the sample.



### Total Protein (TP)

The total protein method is a modification of the biuret reaction, noted for its precision, accuracy, and specificity.<sup>56</sup> It was originally developed by Riegler and modified by Weichselbaum, Doumas, et al. The biuret reaction is a candidate total protein reference method.<sup>57,58,59</sup>

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.<sup>58</sup> The Cu(II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-Protein complex.

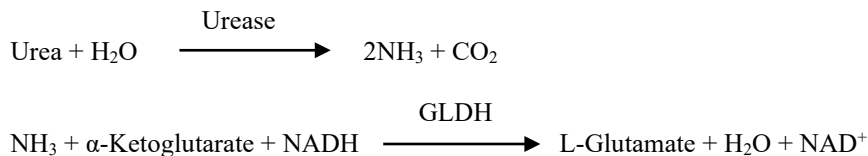


The amount of total protein present in the sample is directly proportional to the absorbance of the Cu-protein complex. The total protein test is an endpoint reaction and the absorbance is measured as the difference in absorbance between 550 nm and 850 nm.

### Blood Urea Nitrogen (BUN)

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.<sup>60</sup> Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.<sup>61</sup> The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique<sup>62,63</sup> and coupled enzymatic reactions.<sup>64,65</sup> Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.<sup>66</sup> 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.<sup>67</sup>

In the coupled-enzyme reaction, urease hydrolyzes urea into ammonia and carbon dioxide. Upon combining ammonia with  $\alpha$ -ketoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD<sup>+</sup>.



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

## 4. Principle of Operation

See the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer Operator's Manual, for the Principles and Limitations of the Procedure.

## 5. Description of Reagents

### Reagents

Each Piccolo Comprehensive Metabolic Panel 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 alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium (CA), chloride (CL<sup>-</sup>), glucose (GLU), potassium (K<sup>+</sup>), sodium (NA<sup>+</sup>), total carbon dioxide (tCO<sub>2</sub>), total protein (TP), and blood urea nitrogen (BUN). Dedicated sample blanks are included in the disc for creatinine (CRE), total bilirubin (TBIL). Each disc also contains a diluent consisting of surfactants and preservatives.

**Table 1: Reagents**

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<b>Component</b>	<b>Quantity/Disc</b>
2,4,6-Tribromo-3-hydroxybenzoic acid (TBHBA)	188µg
2-Chloro-4-nitrophenyl- $\alpha$ -maltotrioxide (CNPG3)	53µg
2-Methyl-4-isothiazolin-3-one hydrochloride (MIT)	4.2µg
4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]tricosane (Kryptofix 221)	86µg
4-Aminoantipyrine hydrochloride	13µg
Adenosine 5'-diphosphate	36µg
Adenosine 5'-triphosphate	22µg
$\alpha$ -ketoglutaric acid	101µg
Amylase	0.036U
Arsenazo III, sodium salt	1.7µg
Ascorbate oxidase ( <i>Cucurbita spp.</i> )	0.3U
Bilirubin oxidase	0.1U
Bromcresol purple	2.2µg
Calcium acetate	25µg
Citric acid, trisodium salt	567µg
Creatine amidinohydrolase ( <i>Actinobacillus spp.</i> )	3U
Creatinine amidohydrolase ( <i>Pseudomonas spp.</i> )	1U
Cupric sulfate	134µg
Ethylene glycol-bis( $\beta$ -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)	4µg
Ethylenediaminetetraacetic acid (EDTA)	178µg
Ethylenediaminetetraacetic acid (EDTA), disodium salt	15µg
$\beta$ -Galactosidase	0.005U
Glucose-6-phosphate dehydrogenase (yeast)	0.05U
Glutamate Dehydrogenase (bovine liver)	0.01U
Glutamine synthetase	0.17U
Hexokinase (yeast)	0.1U
Imidazole	29µg
Lactate dehydrogenase	0.27U
L-alanine	874µg
L-aspartic acid	426µg
L-glutamic acid	9.2µg
Magnesium chloride	3µg
Magnesium sulfate	33µg
Malate dehydrogenase (porcine heart)	0.1U
Manganese chloride	10µg
N-Acetyl cysteine	60µg
$\beta$ -Nicotinamide adenine dinucleotide (NAD)	40µg
$\beta$ -Nicotinamide adenine dinucleotide, reduced (NADH)	48µg
o-Nitrophenyl- $\beta$ -D-galactopyranoside (ONPG)	22µg
Peroxidase (horseradish)	1U
Phosphoenol pyruvate	57µg
Phosphoenol pyruvate carboxylase	0.001U
p-NPP	56µg
Potassium ferrocyanide	0.4µg
Potassium iodide	28µg
Pyruvate kinase	0.01U
Sarcosine oxidase (microorganism)	1U
Sodium potassium tartrate	343µg
Urease (jack bean)	0.05U
Zinc sulfate	3µg
Buffers, surfactants, excipients and preservatives	

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## 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 can not 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.<sup>68</sup> See the Piccolo blood chemistry analyzer or the 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 the 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 the 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 the 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 the Piccolo Xpress chemistry analyzer Operator's Manual.

- The minimum required sample size is ~100 µL of heparinized whole blood, heparinized plasma, serum or control material. The reagent disc sample chamber can contain up to 120 µL of sample.
- Whole blood samples obtained by venipuncture must be homogeneous before transferring a sample to the reagent disc. Gently invert the collection tube several times just prior to sample transfer. Do not shake the collection tube; shaking may cause hemolysis.
- Hemolysis may cause erroneously high results in potassium assays. This problem may go undetected when analyzing whole blood (release of potassium from as few as 0.5% of the erythrocytes can increase the potassium serum level by 0.5 mmol/L). In addition, even unhemolyzed specimens that are not promptly processed may have increased potassium levels due to intracellular potassium leakage.<sup>69</sup>



- Whole blood venipuncture samples should be run within 60 minutes of collection.<sup>70</sup> **Glucose** concentrations are affected by the length of time since the patient has eaten and by the type of sample collected from the patient. To accurately determine glucose results, samples should be obtained from a patient who has been fasting for at least 12 hours. The glucose concentration decreases approximately 5-12 mg/dL in 1 hour in uncentrifuged samples stored at room temperature.<sup>71</sup>
- Refrigerating whole blood samples can cause significant changes in concentrations of **aspartate aminotransferase, creatinine** and **glucose**.<sup>72</sup> The sample may be separated into plasma or serum and stored in capped sample tubes at 2-8°C (36-46°F) if the sample cannot be run within 60 minutes.
- **Total bilirubin** results may be adversely affected by photodegradation.<sup>73</sup> Whole blood samples not run immediately should be stored in the dark for no longer than 60 minutes. If the sample can not be analyzed within that period, it should be separated into plasma or serum and stored in a capped sample tube in the dark at low temperatures.<sup>74</sup>
- Use only lithium heparin (green stopper) evacuated specimen collection tubes for whole blood or 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.<sup>75</sup>

## 8. Procedure

### Materials Provided

- One Piccolo Comprehensive Metabolic Panel PN: 400-1028 (a box of discs PN: 400-0028)

### Materials Required but not Provided

- Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer
- Sample transfer pipettes (fixed volume approximately 100 µL) and tips are provided with each Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer and may be reordered from Abaxis.
- Commercially available control reagents recommended by Abaxis (contact Abaxis Technical Support for approved control materials and expected values).
- Timer

### Test Parameters

The Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each Piccolo Comprehensive Metabolic Panel is less than 14 minutes. The analyzers maintain 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 the Piccolo Xpress chemistry analyzer Operator's Manual.

### Calibration

The Piccolo blood chemistry analyzer or the 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

See Section 2.4 of the Piccolo blood chemistry analyzer Operator's Manual or Section 6 (Calibration and Quality Control) of the Piccolo Xpress Operator's Manual. Performance of the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer can be verified by running controls. For a list of approved quality control materials with acceptance ranges, please

contact Abaxis Technical Support. Other human serum or plasma-based controls may not be compatible. Quality control materials should be stored as per the package-insert included with the controls.

If control results are out of range, repeat one time. If still out of range, call Technical Support. Do not report results if controls are outside their labeled limits. 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.

**Waived Laboratories:** Abaxis recommends control testing as follows:

- at least every 30 days
- whenever the laboratory conditions have changed significantly, e.g. Piccolo moved to a new location or changes in temperature control
- when training or retraining of personnel is indicated
- with each new lot (CLIA waived tests in waived status labs)

**Non-Waived Laboratories:** Abaxis recommends control testing to follow federal, state, and local guidelines.

## 9. Results

The Piccolo blood chemistry analyzer or the 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 the Piccolo Xpress chemistry analyzer Operator's Manual.

Interpretation of results is detailed in the Operator's Manual. Results are printed onto result tapes supplied by Abaxis. The result tape has 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 Operator's Manual or the Piccolo Xpress chemistry analyzer Operator's Manual.

- The only anticoagulant **recommended for use** with the Piccolo blood chemistry analyzer or the 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® Comprehensive Metabolic Panel reagent disc. Do not use sodium heparin.
- Samples with hematocrits in excess of 62-65% packed red cell volume (a volume fraction of 0.62-0.65) may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down to get plasma then re-run in a new 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 or the Piccolo Xpress chemistry analyzer.**

**Warning:** Extensive testing of the Piccolo blood chemistry analyzer or the 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-P.<sup>76</sup>

### 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 or paper roll to inform the operator about the levels of interferents present in each sample.
- The Piccolo blood chemistry analyzer or the 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 or paper roll in place of the result.
- 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 system for each specimen.
- The potassium assay in the Piccolo system is a coupled pyruvate kinase (PK) / lactate dehydrogenase (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<sup>+</sup>) value. In such cases, unexpected high potassium recoveries need to be confirmed utilizing a different methodology.
- For maximum levels of endogenous substances contact Abaxis Technical Support.

### Effects of Exogenous and Therapeutic Substances

- Thirty-five exogenous and therapeutic substances were selected as potential interferents for Abaxis test methods based on recommendations by Young.<sup>77</sup> Significant interference is defined as greater than  $\pm 10\%$  shift in the result for a normal range specimen. Human serum pools were supplemented with known concentrations of the drugs or chemicals and then analyzed. Please see Table 2 for a list of exogenous and therapeutic substances evaluated. **Please see TABLE 3 for a list of analytes where interference was observed.**

**Table 2: Exogenous and Therapeutic Substances Evaluated**

Potential Interferent	Highest Concentration Tested (mg/dL unless otherwise specified)
Acetaminophen	100
Acetoacetate	102
Acetylsalicylic Acid	50
Ampicillin	30
Ascorbic acid	20
Caffeine	10
Calcium Chloride	20
Cephalothin (Keflin)	400
Chloramphenicol	100
Cimetidine	16
Dopamine	19
Epinephrine	1
Erythromycin	10
Glutathione	30
Hydrochlorothiazide	7.5
Ibuprofen	50
Isoniazide	4
$\alpha$ -Ketoglutarate	5
Ketoprofen	50
L-dopa	5
Lidocaine	1
Lithium Lactate	84
Methicillin	100
Methotrexate	0.5
Metronidazole	5
Nafcillin	1
Nitrofurantoin	20

**Table 2: Exogenous and Therapeutic Substances Evaluated (continued)**

Potential Interferent	Highest Concentration Tested (mg/dL unless otherwise specified)
Oxacillin	1
Oxaloacetate	132
Penicillin G	100
Phenytoin (5,5-Diphenylhydantoin)	3
Proline	4
Pyruvate	44
Rifampin	0.5
Salicylic Acid	50
Sulfadiazine	150
Sulfanilamide	50
Theophylline	20

Please see Table 3 for a list of analytes where interference was observed.

**Table 3: The following substances showed greater than  $\pm 10\%$  shift in the result for a normal range specimen.**

	Concentration Which Produces > 10% Interference	% Interference <sup>A</sup> Observed
<b>Alanine Aminotransferase</b>		
Ascorbic acid	20	11% inc.
Oxaloacetate	132	843% inc.
<b>Albumin</b>		
Acetoacetate	102	18% dec.
Ampicillin	30	12% dec.
Caffeine	10	14% dec.
Calcium chloride	20	17% dec.
Cephalothin (Keflin)	400	13% inc.
Ibuprofen	50	28% inc.
$\alpha$ -Ketoglutarate	5	11% dec.
Nitrofurantoin	20	13% dec.
Proline	4	12% inc.
Sulfadiazine	10	14% dec.
Sulfanilamide	50	12% dec.
Theophylline	20	11% dec.
<b>Alkaline Phosphatase</b>		
Theophylline	20	42% dec.
<b>Creatinine</b>		
Ascorbic acid	20	11% dec.
Dopamine	19	80% dec.
L-dopa	5	71% dec.
Epinephrine	1	45% dec.
Glutathione	30	13% dec.
<b>Glucose</b>		
Oxaloacetate	132	11% dec.
Pyruvate	44	13% dec.
<b>Potassium</b>		
Penicillin G	100	17% inc.
Sulfadiazine	150	12% dec.

**Table 3: The following substances showed greater than  $\pm 10\%$  shift in the result for a normal range specimen.  
(continued)**

	<b>Concentration Which Produces &gt; 10% Interference</b>	<b>% Interference<sup>A</sup> Observed</b>
<b>Sodium</b>		
Cephalothin	400	12% inc.
Methotrexate	0.5	11% inc.
Penicillin G	100	10% inc.
<b>Total Bilirubin</b>		
Dopamine	19	55% dec.
L-dopa	5	17% dec.
<b>Total Carbon Dioxide</b>		
Acetaminophen	100	11% inc.
Ascorbic Acid	20	12% dec.
Cephalothin	400	13% inc.
Cimetidine	16	19% dec.
Erythromycin	10	21% dec.
Lidocaine	1	23% inc.
Methotrexate	0.5	80% dec.
Nitrofurantoin	20	13% inc.
Salicylic Acid	50	17% dec.
Sulfadiazine	150	25% dec.

<sup>A</sup> Dec. = decreased concentration of the specified analyte; Inc. = increased concentration of the specified analyte

- For the Chloride assay, bromide at toxic levels ( $\geq 15$  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 60-140 adult males and females were analyzed on the Piccolo blood chemistry analyzer to determine the reference interval. These ranges were calculated based on the 95% reference interval estimated from the combined (overall) values obtained from the reference subjects.<sup>78</sup> These intervals are provided as a guideline only. ALP levels in growing children are highly variable.<sup>79</sup> It is recommended that your office or institution establish normal ranges for your particular patient population.

**Table 4: Piccolo® Reference Intervals**

Analyte	Common Units	SI Units
Alanine Aminotransferase (ALT)	10-47 U/L	10-47 U/L
Albumin (ALB)	3.3-5.5 g/dL	33-55 g/L
Alkaline Phosphatase (ALP), Male	53-128 U/L	53-128 U/L
Alkaline Phosphatase (ALP), Female	42-141 U/L	42-141 U/L
Aspartate Aminotransferase (AST)	11-38 U/L	11-38 U/L
Calcium (CA)	8.0-10.3 mg/dL	2.0-2.58 mmol/L
Chloride (CL <sup>-</sup> )	98-108 mmol/L	98-108 mmol/L
Creatinine (CRE)	0.6-1.2 mg/dL	53-106 µmol/L
Glucose (GLU)	73-118 mg/dL	4.05-6.55 mmol/L
Potassium (K <sup>+</sup> )	3.6-5.1 mmol/L	3.6-5.1 mmol/L
Sodium (NA <sup>+</sup> )	128-145 mmol/L	128-145 mmol/L
Total Bilirubin (TBIL)	0.2-1.6 mg/dL	3.4-27.4 µmol/L
Total Carbon Dioxide (tCO <sub>2</sub> )	18-33 mmol/L	18-33 mmol/L
Total Protein (TP)	6.4-8.1 g/dL	64-81 g/L
Blood Urea Nitrogen (BUN)	7-22 mg/dL	2.5-7.9 mmol urea/L

## 12. Performance Characteristics

### Linearity

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

**Table 5: Piccolo® Dynamic Ranges**

Analyte	Common Units	SI Units
Alanine Aminotransferase (ALT)	5-2000 U/L	5-2000 U/L
Albumin (ALB)	1-6.5 g/dL	10-65 g/L
Alkaline Phosphatase (ALP)	5-2400 U/L	5-2400 U/L
Aspartate Aminotransferase (AST)	5-2000 U/L	5-2000 U/L
Calcium (CA)	4.0-16.0 mg/dL	1.0-4.0 mmol/L
Chloride (CL <sup>-</sup> )	80-135 mmol/L	80-135 mmol/L
Creatinine (CRE)	0.2-20 mg/dL	18-1768 µmol/L
Glucose (GLU)	10-700 mg/dL	0.56-38.9 mmol/L
Potassium (K <sup>+</sup> )	1.5-8.5 mmol/L	1.5-8.5 mmol/L
Sodium (NA <sup>+</sup> )	110-170 mmol/L	110-170 mmol/L
Total Bilirubin (TBIL)	0.1-30 mg/dL	1.7-513 µmol/L
Total Carbon Dioxide (tCO <sub>2</sub> )	5-40 mmol/L	5-40 mmol/L
Total Protein (TP)	2-14 g/dL	20-140 g/L
Blood Urea Nitrogen (BUN)	2-180 mg/dL	0.7-64.3 mmol urea/L

If the analyte concentration is above the measuring range (dynamic range), but less than the system range, the printout will indicate a ">" sign at the upper limit and an asterisk after the number, e.g. ALT >2000\* U/L. If lower than the dynamic range, a "<" will be printed with an asterisk, e.g. ALT <5\* U/L. For values that are grossly beyond the measurement range (system range), "~~~~" will be printed instead of a result. Any time "~~~~" appears on a printout, collect a new sample and rerun the test. If results for the second sample are suppressed again, please call Abaxis Technical Support.

### Sensitivity (Limits of Detection)

The lower limit of the reportable (dynamic) range for each analyte is: alanine aminotransferase 5 U/L; albumin 1 g/dL (10 g/L); alkaline phosphatase 5 U/L; aspartate aminotransferase 5 U/L; 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) potassium 1.5 mmol/L; sodium 110 mmol/L; total bilirubin 0.1 mg/dL (1.7 µmol/L); total carbon dioxide 5 mmol/L; total protein 2 g/dL (20 g/L) and blood urea nitrogen 2.0mg/dL (0.7 mmol urea/L).

## Precision

Precision studies were conducted using CLSI EP5-A guidelines<sup>80</sup> with modifications based on CLSI EP18-P<sup>81</sup> 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 and two reagent disc lots. Calcium, creatinine, glucose, sodium and urea nitrogen testing was performed at one site; potassium and total carbon dioxide testing was performed at two sites over 20 days; chloride testing was done at two sites over a period of five days.

Results of precision studies are shown in Table 6.

**Table 6: Precision**

Analyte	Sample Size	Within-Run	Total
<b>Alanine Aminotransferase (U/L)</b>			
<u>Control 1</u>	N = 80		
Mean		21	21
SD		2.76	2.79
CV		13.4	13.5
<u>Control 2</u>			
Mean		52	52
SD		2.7	3.25
CV		5.2	6.2
<b>Albumin (g/dL)</b>			
<u>Control 1</u>	N = 80		
Mean		5.6	5.6
SD		0.09	0.11
CV		1.7	2.1
<u>Control 2</u>			
Mean		3.7	3.7
SD		0.07	0.11
CV		2.0	2.9
<b>Alkaline Phosphatase (U/L)</b>			
<u>Control 1</u>	N = 80		
Mean		39	39
SD		1.81	2.29
CV		4.6	5.8
<u>Control 2</u>			
Mean		281	281
SD		4.08	8.75
CV		1.5	3.1
<b>Aspartate Aminotransferase (U/L)</b>			
<u>Control 1</u>	N = 80		
Mean		49	49
SD		0.98	0.98
CV		2.07	2.07
<u>Control 2</u>			
Mean		147	147
SD		1.83	1.83
CV		1.26	1.26

**Table 6: Precision (continued)**

Analyte	Sample Size	Within-Run	Total
<b>Calcium (mg/dL)</b>			
<u>Control 1</u>	N = 80		
Mean		8.6	8.6
SD		0.21	0.25
CV		2.4	2.9
<u>Control 2</u>			
Mean		11.8	11.8
SD		0.39	0.40
CV		3.3	3.4
<b>Chloride (mmol/L)</b>			
<u>Control 1</u>	N = 160		
Mean		97.8	97.8
SD		1.63	1.74
CV		1.7	1.7
<u>Control 2</u>			
Mean		113.6	113.6
SD		1.97	2.22
CV		1.7	2.0
<b>Creatinine (mg/dL)</b>			
<u>Control 1</u>	N=80		
Mean		1.1	1.1
SD		0.14	0.14
CV		12.5	13.1
<u>Control 2</u>			
Mean		5.2	5.2
SD		0.23	0.27
CV		4.4	5.2
<b>Glucose (mg/dL)</b>			
<u>Control 1</u>	N=80		
Mean		66	66
SD		0.76	1.03
CV		1.1	1.6
<u>Control 2</u>			
Mean		278	278
SD		2.47	3.84
CV		0.9	1.4
<b>Potassium (mmol/L)</b>			
<u>Control 1</u>	N = 120		
Mean		6.12	6.12
SD		0.32	0.32
CV		5.2	5.7
<u>Control 2</u>			
Mean		4.10	4.10
SD		0.24	0.26
CV		5.9	6.3
<b>Sodium (mmol/L)</b>			
<u>Control 1</u>	N = 80		
Mean		143.5	143.5
SD		2.28	2.28
CV		1.6	1.6
<u>Control 2</u>			
Mean		120.0	120.0
SD		2.13	2.13
CV		1.8	1.8



**Table 6: Precision (continued)**

Analyte	Sample Size	Within-Run	Total
<b>Total Bilirubin (mg/dL)</b>			
<u>Control 1</u>	N = 80		
Mean		0.8	0.8
SD		0.06	0.07
CV		8.0	9.3
<u>Control 2</u>			
Mean		5.2	5.2
SD		0.09	0.15
CV		1.7	2.8
<b>Total Carbon Dioxide (mmol/L)</b>			
<u>Control 1</u>	N = 120		
Mean		21.4	21.4
SD		2.29	2.29
CV		10.7	10.7
<u>Control 2</u>			
Mean		10.5	10.5
SD		0.90	0.90
CV		8.6	8.6
<b>Total Protein (mg/dL)</b>			
<u>Control 1</u>	N = 80		
Mean		6.8	6.8
SD		0.05	0.08
CV		0.8	1.2
<u>Control 2</u>			
Mean		4.7	4.7
SD		0.09	0.09
CV		2.0	2.0
<b>Urea Nitrogen (mg/dL)</b>			
<u>Control 1</u>	N = 80		
Mean		19	19
SD		0.35	0.40
CV		1.9	2.1
<u>Control 2</u>			
Mean		65	65
SD		1.06	1.18
CV		1.6	1.8

**Correlation**

Heparinized whole blood and serum samples were collected and assayed on the Piccolo blood chemistry analyzer and by a comparative method(s). The whole blood samples were analyzed by the Piccolo blood chemistry analyzer at the field sites and the serum samples were analyzed by the Piccolo blood chemistry analyzer and by comparative methods. 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-A guideline.<sup>82</sup> Representative correlation statistics are shown in Table 7.

**Table 7: Correlation of Piccolo® Blood Chemistry Analyzer with Comparative Method(s)**

	<b>Correlation Coefficient</b>	<b>Slope</b>	<b>Intercept</b>	<b>SEE</b>	<b>N</b>	<b>Sample Range (mmol/L)</b>	<b>Comparative Method</b>
<b>Alanine Aminotransferase (U/L)</b>	0.981	0.905	1.3	3.21	86	10-174	Paramax
	0.985	0.946	-2.5	2.84	67	10-174	Technicon
<b>Albumin (g/dL)</b>	0.854	1.001	-0.3	0.22	261	1.1-5.3	Paramax
	0.896	0.877	-0.1	0.21	100	1.5-5.0	Beckman
<b>Alkaline Phosphatase (U/L)</b>	0.988	0.970	-5.9	3.97	99	27-368	Paramax
	0.929	1.136	-17.6	4.79	80	26-150	Technicon
<b>Aspartate Aminotransferase (U/L)</b>	0.93	0.87	5.3	2.76	159	13-111	Paramax
	1.0	0.97	3.0	1.9	46	13-252	DAX™
<b>Calcium (mg/dL)</b>	0.991*	0.990	-0.4	0.17	25	5.2-11.9	Paramax
	0.673	0.742	1.8	0.22	81	8.1-9.9	Beckman
<b>Chloride (mmol/L)</b>	0.978	0.982	-1.1	1.84	120	71-118	Vitros 950
<b>Creatinine (mg/dL)</b>	0.993	0.926	0.0	0.15	260	0.4-14.7	Paramax
	0.987	0.866	0.1	0.16	107	0.4-7.5	Beckman
<b>Glucose (mg/dL)</b>	0.987	1.009	-2.8	3.89	251	72-422	Paramax
	0.997	0.943	1.2	4.69	91	56-646	Beckman
<b>Potassium (mmol/L)</b>	0.969	0.863	0.6	0.14	58	2.0-6.8	Radiometer KNA™ 2
<b>Sodium (mmol/L)</b>	0.937	0.782	27.7	3.79	113	116-154	Radiometer KNA™ 2
	0.974	0.901	0.0	0.07	250	0.2-3.7	Paramax
<b>Total Bilirubin (mg/dL)</b>	0.98	1.113	-0.4	0.09	91	0.1-6.4	Beckman
	0.947	0.903	2.4	0.84	60	6-39	Cobas Fara
<b>Total Protein (g/dL)</b>	0.849	0.932	0.6	0.19	251	5.7-9.2	Paramax
	0.873	0.935	0.3	0.16	92	6.5-9.2	Beckman
<b>Blood Urea Nitrogen (mg/dL)</b>	0.964	0.923	0.5	1.08	251	6-52	Paramax
	0.983	0.946	0.0	0.66	92	6-38	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.

### Results of Untrained User Study

An “untrained user” study was conducted in which participants were given only the test instructions and asked to perform testing of 3 discs with blinded randomized samples. The samples consisted of serum pools prepared at three levels for each of the fourteen analytes: ALT, albumin, ALP, AST, calcium, chloride, creatinine, glucose, potassium, sodium, total bilirubin, total carbon dioxide, total protein, and blood urea nitrogen (BUN). The participants were not given any training on the use of the test. A total of approximately 60 participants were enrolled from 3 sites, representing a diverse demographic (educational, age, gender, etc) population.

Tables below present the summary of the performance for each analyte.

**Alanine Aminotransferase (ALT)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	45.4 U/L	98.9 U/L	184.3 U/L
%CV	3.7%	1.7%	1.5%
Observed Range	42 – 53	96 – 103	175 – 191
Percent of Results in the Range ± 15.0%*	98.4% 61/62 95%CI: 91.3% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

\* This percent is based on the premise that one cannot distinguish properly between normal and abnormal values when errors are greater than one-quarter of the normal range. The range of (10 U/L - 47 U/L) was considered.

**Albumin (ALB)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	3.0 g/dL	3.5 g/dL	4.2 g/dL
%CV	2.7%	2.5%	1.8%
Observed Range	2.9 – 3.2	3.3 – 3.7	4.0 – 4.4
Percent of Results in the Range ± 12.5%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Alkaline Phosphatase (ALP)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	94.5 U/L	171.5 U/L	337.5 U/L
%CV	5.2%	3.2%	2.4%
Observed Range	85 – 106	160-184	287 – 388
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Aspartate Aminotransferase (AST)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	56.0	120.4	276.3
%CV	2.4%	1.1%	1.0%
Observed Range	54 – 60	117 – 124	266 – 285
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Calcium (CA)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	8.0	10.5	13.1
%CV	1.7%	1.5%	1.4%
Observed Range	7.7 – 8.4	10.1 – 11.0	12.6 – 13.4
Percent of Results in the Range ± 6.3%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Chloride (CL<sup>-</sup>)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	94.6	106.0	115.5
%CV	1.8	1.4	1.5
Observed Range	90 – 100	102 – 108	110 – 119
Percent of Results in the Range ± 2.4%	91.9% 57/62 95%CI: 82.2% to 97.3%	96.8% 60/62 95%CI: 88.8% to 99.6%	95.2% 59/62 95%CI: 86.5% to 99.0%

**Creatinine (CRE)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	0.89	2.07	6.89
%CV	11.0	5.0	1.6
Observed Range	0.7 – 1.2	1.8 – 2.3	6.5 – 7.2
Percent of Results in the Range ± 15.0%	93.6 58/62 95%CI: 84.3% to 98.2%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Glucose (GLU)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	95.2	130.3	365.8
%CV	1.1%	1.0%	0.8%
Observed Range	93 – 98	125 – 133	351 – 373
Percent of Results in the Range ± 10.4%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Potassium (K<sup>+</sup>)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	3.4	5.7	7.2
%CV	3.3	2.5	2.0
Observed Range	3.2 – 3.7	5.2 – 5.9	6.7 – 7.5
Percent of Results in the Range ± 8.6%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Sodium (NA<sup>+</sup>)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	122.1	140.8	157.5
%CV	1.0	0.8	1.0
Observed Range	118 – 127	138 – 143	154 – 162
Percent of Results in the Range ± 3.1%	98.4% 61/62 95%CI: 91.3% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Total Bilirubin (TBIL)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	0.86 mg/dL	2.5 mg/dL	5.7 mg/dL
%CV	6.1%	2.6%	1.8%
Observed Range	0.8 – 1.0	2.3 – 2.6	5.4 – 5.9
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Total Carbon Dioxide (tCO<sub>2</sub>)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	20.3	27.6	34.4
%CV	5.1	4.6	3.7
Observed Range	18 – 23	23 – 30	32 – 38
Percent of Results in the Range ± 14.7%	100% 62/62 95%CI: 94.2% to 100%	98.4% 61/62 95%CI: 91.3% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Total Protein (TP)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	4.8 g/dL	5.7 g/dL	7.1 g/dL
%CV	2.0%	1.5%	1.5%
Observed Range	4.6 – 5.3	5.3 – 5.9	6.7 – 7.5
Percent of Results in the Range ± 5.9%	98.4% 61/62 95%CI: 91.3% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

**Blood Urea Nitrogen (BUN)**

	Level 1	Level 2	Level 3
N	62	62	62
Mean	15.1	41.0	72.2
%CV	2.3	2.5	1.8
Observed Range	14 – 16	37 – 43	68 – 75
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

### 13. Symbols



Use By



Catalog Number



Batch Code



In Vitro Diagnostic  
Medical Device



Consult Instructions  
For Use



Manufacturer



Do Not Reuse



X Number of Test  
Devices in Kit



Manufacturing  
Sequence



Serial Number



Caution



Temperature  
Limitation

PN:  
Part Number



Authorized  
Representative  
In the European  
Community



Denotes conformity to specified  
European directives



UDI Barcode structure  
in Health Industry Bar  
Code (HIBC) standard  
format



Unique Device Identifier  
(UDI) in human and  
machine-readable form  
used to adequately identify  
medical devices through  
their distribution and use



Separate waste collection for  
this electronic item indicated;  
Equipment manufactured /  
placed on the market after 13  
August 2005; Indicates  
compliance with Article 14(4) of  
Directive 2012/19/EU (WEEE)  
for the European Union (EU).

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