Piccolo® Basic Metabolic Panel

CE 2797

For In Vitro Diagnostic Use and Healthcare Professional Use Only
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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® Basic Metabolic Panel, used with the Piccolo Xpress® chemistry analyzer, is intended to be used for the *in vitro* quantitative determination of calcium, chloride, creatinine, glucose, potassium, sodium, total carbon dioxide, and blood urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum in a clinical laboratory setting or point-of-care location/ near-patient testing.

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).

2. Summary and Explanation of Tests

The Piccolo Basic Metabolic Panel comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders in adults:

Calcium:	General metabolic, for example, Parathyroid, bone and chronic renal diseases; tetany.
Chloride:	General metabolic, for example, Dehydration, prolonged diarrhea and vomiting, renal tubular disease, hyperparathyroidism, burns, salt-losing renal diseases, overhydration and thiazide therapy.
Creatinine:	Renal disease and renal dialysis.
Glucose:	Carbohydrate metabolism disorders, for example, adult and juvenile diabetes mellitus and hypoglycemia.
Potassium:	Renal, endocrine, and metabolic disease as well as iatrogenic causes, for example, 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:	Metabolic diseases, for example, Dehydration, diabetes insipidus, loss of hypotonic gastrointestinal fluids, salt poisoning, selective depression of sense of thirst, skin losses, burns, sweating, hyperaldosteronism, CNS disorders, delusional, depletion and delusional hyponatremia and syndrome of inappropriate ADH secretion.
Total carbon dioxide:	Metabolic disorders, for example, metabolic alkalosis and acidosis and primary respiratory alkalosis and acidosis.
Blood Urea Nitrogen (BUN):	Renal diseases.

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As with any diagnostic test procedure, all other test procedures including the clinical status of the patient, should be considered prior to a 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 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 iminohydrolase.[14]

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

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

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

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

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 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.

GFR (mL/min/1.73 m^2) = 175 x $(S_{cr})^{-1.154}$ x $(Age)^{-0.203}$ x (0.742 if female) x (1.212 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 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), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

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

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. [19,20,21] Interference from sodium and ammonium ion are minimized with the addition of Kryptofix and glutamine synthetase, respectively.[21]

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^+.

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

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

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

Sodium (NA^+)
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation. [22,23,24] 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.

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

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. [25,26] 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 tCO_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. [27] Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests. [28] The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique[29,30] and coupled enzymatic reactions. [31,32] Catalyzed Berthelot procedures, however, are erratic when measuring ammonia. [33] 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. [34]

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

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

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

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

4. Principle of Operation

Refer to the Piccolo Xpress chemistry analyzer Operator's Manual, for the Principles and Limitations of the Procedure**.**

5. Description of Reagents

Reagents
Each Piccolo Basic 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 calcium (CA), chloride (CL^-), glucose (GLU), potassium (K^+), sodium (NA^+), total carbon dioxide (tCO_2), and blood urea nitrogen (BUN). 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	188µg
2-Chloro-4-nitrophenyl -alpha-maltotrioside (CNPG3)	52.5µg
4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]trisocosane	139µg
N-Acetyl cysteine	23.1µg
4-Aminoantipyrine	5.4µg
4-Aminoantipyrine *HCl	27µg
Adenosine-5'-triphosphate, disodium salt	12µg
Adenosine 5'-diphosphate, trilithium salt	3.7µg
Amylase	0.286U
Arsenazo III, sodium salt	3.5µg
Ascorbate oxidase	0.3U
Calcium acetate	50.4µg
Citric acid, trisodium salt	1134µg
Creatine amidinohydrolase	3U
Creatinine amidohydrolase	3U
3,5-Dichloro-2-hydroxybenzenesulfonic acid , Sodium salt (DHBSA)	34.8µg
Ethylene glycol-bis(b-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)	7µg
Ethylenediaminetetraacetic acid (EDTA)	11µg
Ethylenediaminetetraacetic acid (EDTA), disodium salt	356µg
Ethylenediaminetetraacetic acid (EDTA), Tetrasodium Salt	180µg
β-Galactosidase	0.009U
Glucose-6-phosphate dehydrogenase (G6PDH)	0U
L-glutamic acid	9µg
Glutamine synthetase	0.2U
Glutamate dehydrogenase	0.01U
Hexokinase	0.1U
Imidazole	57µg
α-Ketoglutaric acid	18µg
Lactate dehydrogenase	0.1U
Lactate dehydrogenase	0U
Lactate dehydrogenase (LDH)	0.1U
Magnesium acetate, tetrahydrate	22µg
Magnesium sulfate, heptahydrate	122µg
Malate dehydrogenase	0.1U
Manganese chloride	10µg
ß-Nicotinamide adenine dinucleotide (NAD)	20µg
ß-Nicotinamide adenine dinucleotide, reduced (NADH)	33µg
o-Nitrophenyl-ß-D galactopyranoside (ONPG)	44µg
Peroxidase	1U
Phosphoenol pyruvate	32µg
Phosphoenol pyruvate carboxylase	0.002U
Potassium ferrocyanide	0.6µg
Pyruvate kinase	0.01U
Sarcosine oxidase	1U
Uricase	0U
Urease	0.05U
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. [35] See 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.

- This mixture does not contain substances assessed to be vPvB / PBT according to Regulation (EC) No 1907/2006, Annex XIII. This product contains components considered to have endocrine disrupting properties for environment, according to REACH Article 57(f), Regulation (EU) 2018/605 or Regulation (EU) 2017/2100. Refer to the Safety Data Sheet (SDS) for specific details.

- Materials incorporated into the device may consist of substances derived from other microbial and animal sourced materials.

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 at 20-25°C (68-77°F) 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 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 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 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 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.

- Whole blood samples should only be obtained via venipuncture, not from capillary blood.

- 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. [36]

- Whole blood venipuncture samples should be run within 60 minutes of collection. [37, 48] **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 0.28-0.67 mmol/L in 1 hour in uncentrifuged samples stored at room temperature.[71]

- Use only lithium heparin evacuated specimen collection tubes for whole blood or plasma samples. Use no-additive evacuated specimen collection tubes or serum separator tubes 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. [38]

- Refrigerating whole blood samples can cause significant changes in concentration of **creatinine** and **glucose**[45]. 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.

8. Procedure

Materials Provided

- One Piccolo Basic Metabolic Panel PN: 400-1024 (a box of discs PN: 400-0024)

Materials Required but not Provided

- Piccolo Xpress chemistry analyzer
- Sample transfer pipettes (fixed volume approximately 100 µL) and tips are provided with each 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 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 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 Xpress chemistry analyzer Operator's Manual.

Calibration
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 Xpress chemistry analyzer Operator's Manual.

The Piccolo Xpress chemistry analyzer is calibrated using internal calibrators or reference materials. The methods and materials used in the procedures for the calibration and control value assignments are traceable either to the comparative method and/or to the standards listed below:

- IFCC – International Federation of Clinical Chemistry
- NIST – National Institute of Standards and Technology
- CRMLN – Cholesterol Reference Method Laboratory Network
- SRM – Standard Reference Material
- CRM – Certified Reference Material

The calibration and value assignment processes are in compliance with EN ISO 17511, Metrological Traceability of Values Assigned to Calibrators and Control Materials.

The measured analytes in the Basic Metabolic Panel are traceable to the following reference materials or methods. The Piccolo Xpress Analysis System controls and calibration verification materials are validated for use only with the Piccolo Xpress Analysis System and assigned values may not be commutable with other methods. For specific analyte reference standard methods, refer to Section 3. Principles of Method.

Analyte	Calibration Standard	Method
CA	Correlation to Beckman LX-20 / DX-20	Arsenazo III Dye
BUN	NIST SRM #912A	Enzymatic
CL	Correlation to Beckman LX-20 / DX-20	Enzymatic
CRE	NIST SRM #967	Enzymatic
GLU	NIST SRM #909	Enzymatic
K+	NIST SRM #909	Enzymatic
NA+	Correlation to Beckman LX-20 / DX-20	Enzymatic
tCO2	Correlation to Beckman LX-20 / DX-20	Enzymatic

Quality Control
See Section 6 (Calibration and Quality Control) of the Piccolo Xpress chemistry analyzer Operator's Manual. Performance of 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 Xpress chemistry analyzer Operator's Manual for a detailed discussion on running, recording, interpreting, and plotting control results.

Waived Laboratories (US only): 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 (US only): Abaxis recommends control testing to follow federal, state, and local guidelines.

And the non-waived ones will use the below:

- Performance of 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 Xpress chemistry analyzer Operator's Manual for a detailed discussion on running, recording, interpreting, and plotting control results.

9. Results

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 Xpress chemistry analyzer Operator's Manual.

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

The reaction for each analyte occurs at 37°C (98.6°F).

10. Limitations of Procedure

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

- The only anticoagulant **recommended for use** with 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 Basic Metabolic Panel reagent disc. Do not use sodium heparin.

- Samples with hematocrits in excess of 62% packed red cell volume (a volume fraction of 0.62) 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 Xpress chemistry analyzer.**

  Warning: Extensive testing of 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.

  Note: Operators shall report any serious incident that has occurred in relation to the device to the manufacturer.

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. [39]

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 printout to inform the operator about the levels of interferents present in each sample.

- 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 printout 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^+) 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. [40] Significant interference is defined as greater than ± 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
α-Ketoglutarate	5
Ketoprofen	50
L-dopa	5
Lidocaine	1
Lithium Lactate	84
Methicillin	100
Methotrexate	0.5
Metronidazole	5
Nafcillin	1
Nitrofurantoin	20
Oxacillin	1
Oxaloacetate	132
Penicillin G	100
Phenytoin (5,5-Diphenylhydantion)	3
Proline	4
Pyruvate	44
Rifampin	0.5
Salicylic Acid	50

Sulfasalazine	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 ± 10 % shift in the result for a normal range specimen.

	Concentration Which Produces > 10% Interference
Creatinine	
Ascorbic acid	20
Dopamine	19
L-dopa	5
Epinephrine	1
Glutathione	30
Glucose	
Oxaloacetate	132
Pyruvate	44
Potassium	
Penicillin G	100
Sulfasalazine	150
Sodium	
Cephalothin	400
Methotrexate	0.5
Penicillin G	100
Total Carbon Dioxide	
Acetaminophen	100
Ascorbic Acid	20
Cephalothin	400
Cimetidine	16
Erythromycin	10
Lidocaine	1
Methotrexate	0.5
Nitrofurantoin	20
Salicylic Acid	50
Sulfasalazine	150

- 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 60-140 adult males and females were analyzed 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.[41] These intervals are provided as a guideline only. 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
Calcium (CA)	8.0-10.3 mg/dL	2.0-2.58 mmol/L
Chloride (CL^-)	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^+)	3.6-5.1 mmol/L	3.6-5.1 mmol/L
Sodium (NA^+)	128-145 mmol/L	128-145 mmol/L
Total Carbon Dioxide (tCO_2)	18-33 mmol/L	18-33 mmol/L
Blood Urea Nitrogen (BUN)	7-22 mg/dL	2.5-7.9 mmol urea/L

*Data was generated using equivalence device Piccolo Blood Chemistry Analyzer.

12. Performance Characteristics

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

Table 5: Piccolo Dynamic Ranges

Analyte	Common Units	SI Units
Calcium (CA)	4.0-16.0 mg/dL	1.0-4.0 mmol/L
Chloride (CL^-)	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^+)	1.5-8.5 mmol/L	1.5-8.5 mmol/L
Sodium (NA^+)	110-170 mmol/L	110-170 mmol/L
Total Carbon Dioxide (tCO_2)	5-40 mmol/L	5-40 mmol/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. CA >16.0* mg/dL. If lower than the dynamic range, a "<" will be printed with an asterisk, e.g. CA <4.0* mg/dL. 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: 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 carbon dioxide 5 mmol/L; and blood urea nitrogen 2.0mg/dL (0.7 mmol urea/L).

Precision
Precision studies were conducted using CLSI EP5-A guidelines[42] with modifications based on CLSI EP18-P[43] for unit-use devices. Results for within-run and total precision were determined using two levels of commercially available control materials and in case of potassium two levels of plasma pools. 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. Potassium testing was conducted at a CLIA waived site making use of three analyzers, one lot of reagent discs, and two operators over five days.

The results of precision studies are shown in Table 6.

Table 6: 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)			
Control 1	N = 160		
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
Creatinine (mg/dL)			
Control 1	N = 80		
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)			
Control 1	N = 80		
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
Potassium (mmol/L)			
Control 1	N = 150		
Mean		3.2	3.2
SD		0.09	0.11

CV		2.8	3.3
Control 2	N = 149		
Mean		6.2	6.2
SD		0.09	0.10
CV		1.4	1.7
Plasma Pool 1	N = 150		
Mean		3.2	3.2
SD		0.07	0.09
CV		2.3	2.9
Plasma Pool 2	N = 150		
Mean		5.4	5.4
SD		0.09	0.10
CV		1.6	1.9
Sodium (mmoL/L)			
Control 1	N = 80		
Mean		143.5	143.5
SD		2.28	2.28
CV		1.6	1.6
Control 1			
Mean		120.0	120.0
SD		2.13	2.13
CV		1.8	1.8
Total Carbon Dioxide (mmol/L)			
Control 1	N = 120		
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
Urea Nitrogen (mg/dL)			
Control 1	N = 80		
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

Whole Blood Precision for Potassium

Whole blood precision was tested at a CLIA waived site by two CLIA waiver operators. The study used four Piccolo Xpress Analyzers with 16 replicates per sample for four (4) fresh, lithium heparin whole blood samples.

Table 7: Whole Blood Precision for Potassium

Potassium (mmol/L)	Sample Size	Within-Run	Total
Whole Blood 1	N = 16		
Mean		3.9	3.9
SD		0.06	0.11
CV		1.6	2.8
Whole Blood 2	N = 16		
Mean		4.0	4.0
SD		0.11	0.14
CV		2.9	3.4
Whole Blood 3	N = 16		
Mean		4.0	4.0
SD		0.11	0.15
CV		2.8	3.9
Whole Blood 4	N = 16		
Mean		4.0	4.0
SD		0.11	0.13
CV		2.7	3.4

Correlation

Heparinized whole blood and serum samples were collected and assayed by a comparative method(s). The whole blood samples were analyzed at the field sites and the serum samples were analyzed by comparative methods. In some cases, high and low supplemented samples were used to cover the dynamic range.

Representative correlation statistics are shown in Table 8.

Table 8: Correlation with Comparative Method(s)**

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range (mmol/L)	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
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
Potassium (mmol/L) Whole Blood (waived laboratory)	0.984	0.98	0.13	0.10	130	1.3-9.5	Siemens VISTA Plasma
Potassium (mmol/L) Whole Blood (moderately complex laboratory)	0.984	0.98	0.12	0.18	178	1.5-8.6	Siemens VISTA Plasma
Potassium (mmol/L) Serum (moderately complex laboratory)	0.990	0.98	0.06	0.14	178	1.4-8.5	Siemens VISTA Serum
Sodium (mmol/L)	0.937	0.782	27.7	3.79	113	116-154	Radiometer KNA™ 2
Total Carbon Dioxide (mmol/L)	0.947	0.903	2.4	0.84	60	6-39	Cobas Fara
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.
**Data was generated using equivalence device Piccolo Blood Chemistry Analyzer.

It should be noted that serum will typically give higher results for K+ compared to whole blood or plasma for physiological reasons. The variation can range from approximately 0.2 to 0.9mmol/L and is dependent on a number of factors. The primary effect is dependent upon the number of blood cells present in the patient sample.[46]

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 eight analytes: calcium, chloride, creatinine, glucose, potassium, sodium, total carbon dioxide, 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.

Calcium (CA)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	2.0 mmol/L	2.62 mmol/L	3.27 mmol/L
%CV	1.7%	1.5%	1.4%
Observed Range	1.92 – 2.1	2.52 – 2.74	3.14 – 3.34
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%

* 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 (8.0 – 10.3 mg/dL) was considered.

Chloride (CL^-)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	94.6 mmol/L	106.0 mmol/L	115.5 mmol/L
%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	78.68 µmol/L	182.99 µmol/L	609.08 µmol/L
%CV	11.0	5.0	1.6
Observed Range	61.88 – 106.08	159.12 – 203.32	574.6 – 636.48
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	5.28 mmol/L	7.23 mmol/L	20.31 mmol/L
%CV	1.1%	1.0%	0.8%
Observed Range	5.16 – 5.44	6.94 – 7.38	19.48 – 20.71
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^+)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	3.4 mmol/L	5.7 mmol/L	7.2 mmol/L
%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^+)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	122.1 mmol/L	140.8 mmol/L	157.5 mmol/L
%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 Carbon Dioxide (tCO_2)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	20.3 mmol/L	27.6 mmol/L	34.4 mmol/L
%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%

Blood Urea Nitrogen (BUN)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	5.39 mmol/L	14.64 mmol/L	25.78 mmol/L
%CV	2.3	2.5	1.8
Observed Range	5.0 – 5.72	13.21 – 15.35	24.28 – 26.78
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


Authorized Representative In the European Community


Temperature Limitation


Caution

PN:
Part Number


denotes conformity to specified European directives

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