

Piccolo[®] Basic Metabolic Reagent Disc



Customer and Technical Service: 800-822-2947

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

December 2009

PN: 400-7128 Rev. G

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1. Intended Use

The Piccolo[®] Basic Metabolic Reagent Disc, 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 calcium, chloride, creatinine, glucose, potassium, sodium, total carbon dioxide, and blood urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum.

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 Basic Metabolic Reagent Disc and the Piccolo Blood Chemistry Analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders:

| | |
|----------------------------|--|
| 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, <i>in vitro</i> 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 delusional hyponatremia and syndrome of inappropriate ADH secretion. |
| Total carbon dioxide: | Primary metabolic alkalosis and acidosis and primary respiratory alkalosis and acidosis. |
| Blood Urea Nitrogen (BUN): | Renal and metabolic diseases. |

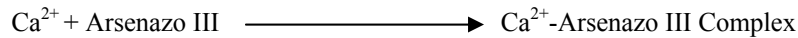
As with any diagnostic test procedure, all other test procedures including the clinical status of the patient, should be considered prior to final diagnosis.

3. Principle of Procedure

Calcium (CA)

The first methods used to analyze calcium involved precipitating calcium with an excess of anions.^{1,2,3} Precipitation methods are laborious and often imprecise. The reference method for calcium is atomic absorption spectroscopy; however, this method is not suited for routine use.⁴ Spectrophotometric methods using either *o*-cresolphthalein complexone 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.

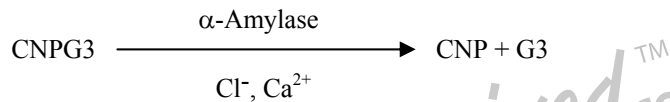
Calcium in the patient sample binds with arsenazo III to form a calcium-dye complex.



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.

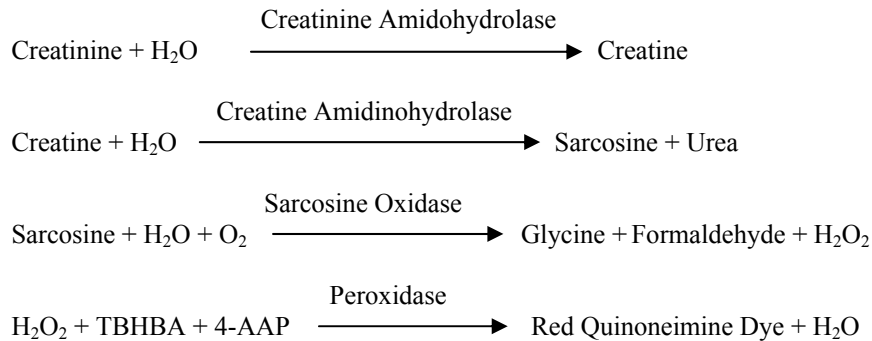
Chloride (CL)

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-maltotrioxide (CNP3) 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



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



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.

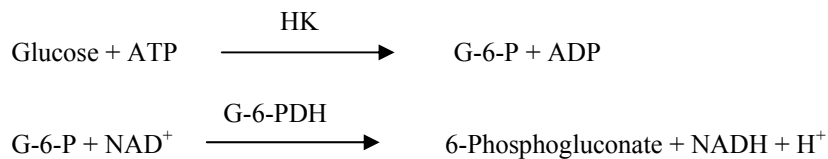
Calculation of the eGFR is performed by the Piccolo using the patient's age, gender and race. The Piccolo method for creatinine is traceable to the IDMS reference method for creatinine so that the following form of the MDRD equation for calculating the eGFR can be used.

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

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu¹⁵ 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 Basic Metabolic Reagent Disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.¹⁸

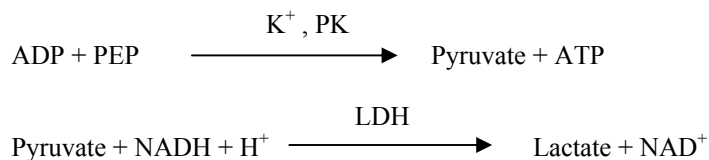
The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine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



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

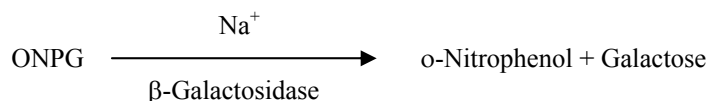
In the coupled-enzyme reaction, pyruvate kinase (PK) dephosphorylates phosphoenolpyruvate (PEP) to form pyruvate. Lactate dehydrogenase (LDH) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD⁺.



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.

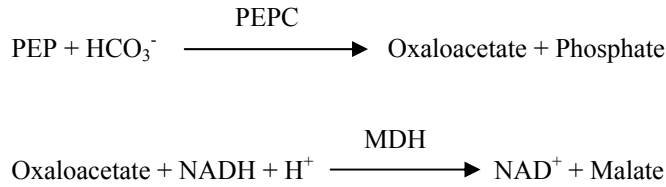


Total Carbon Dioxide (tCO₂)

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, CO₂ 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.

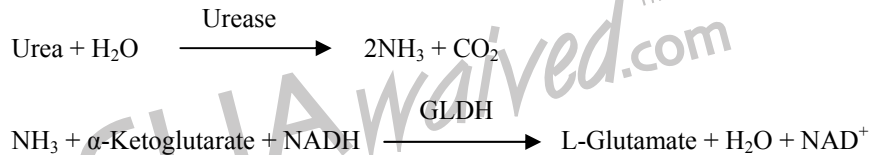
In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide (CO₂) toward bicarbonate (HCO₃⁻). Phosphoenolpyruvate (PEP) and HCO₃⁻ then react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MDH) 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 tCO₂ in the sample.



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⁺.



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

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 Basic Metabolic Reagent Disc 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 (tCO₂), 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-Tribromo-3-hydroxybenzoic acid | 188µg |
| 2-Chloro-4-nitrophenyl -alpha-maltotrioxide (CNPG3) | 52.5µg |
| 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 222) | 0.3µg |
| 4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]trisicosane (Kryptofix 221) | 84µg |
| N-Acetyl cysteine | 15.3µg |
| 4-Aminoantipyrine *HCl | 13µg |
| Adenosine-5'-triphosphate | 11µg |
| Amylase | 0.0357U |
| Arsenazo III, sodium salt | 1.7µg |
| Ascorbate oxidase (<i>Cucurbita spp.</i>) | 0.3U |
| Calcium acetate | 25.2µg |
| Citric acid, trisodium salt | 567µg |
| Creatine amidinohydrolase (<i>Actinobacillus spp.</i>) | 3U |
| Creatinine amidohydrolase (<i>Pseudomonas spp.</i>) | 1U |
| Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) | 4µg |
| Ethylenediaminetetraacetic acid (EDTA) | 178.1µg |
| β-Galactosidase | 0.005U |
| Glutamate dehydrogenase (bovine liver) | 0.01U |
| Hexokinase (yeast) | 0.1U |
| Imidazole | 29µg |
| α-Ketoglutaric acid | 19µg |
| Lactate dehydrogenase | 0.3U |
| Magnesium sulfate | 29µg |
| Malate dehydrogenase (porcine heart) | 0.1U |
| β-Nicotinamide adenine dinucleotide (NAD) | 20µg |
| β-Nicotinamide adenine dinucleotide, reduced (NADH) | 28µg |
| o-Nitrophenyl-β-D galactopyranoside (ONPG) | 22µg |
| Peroxidase (horseradish) | 1U |
| Phospho(enol)pyruvate | 4µg |
| Phosphoenol pyruvate | 19µg |
| Phosphoenol pyruvate carboxylase | 0.001U |
| Potassium ferrocyanide | 0.4µg |
| Pyruvate kinase | 0.01U |
| Sarcosine oxidase (microorganism) | 1U |
| Urease (jack bean) | 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 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.³⁵ 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.³⁶
- Whole blood venipuncture samples should be run within 60 minutes of collection.³⁷ **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.⁷¹
- 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.³⁸

8. Procedure

Materials Provided

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

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 Basic Metabolic Reagent Disc 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 Chemistry Analyzer Operator's Manual.

Quality Control

See Section 2.4 of the Piccolo 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 or Piccolo xpress 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 cards supplied by Abaxis. The result cards have an adhesive backing for easy placement in the patient's files.

10. Limitations of Procedure

General procedural limitations are discussed in the Piccolo Blood Chemistry Analyzer Operator's Manual or the Piccolo xpress Chemistry Analyzer.

- The only anticoagulant **recommended for use** with the Piccolo Blood Chemistry System 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 Basic Metabolic 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 System 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 NCCLS 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 result card to inform the operator about the levels of interferents present in each sample.
- The Piccolo Blood Chemistry System 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 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 $\pm 10\%$ 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 | 3 |
| Caffeine | 10 |
| Cephalothin (Keflin) | 400 |
| Chloramphenicol | 100 |
| Cimetidine | 16 |
| Dopamine | 13 |
| Epinephrine | 1 |
| Erythromycin | 10 |
| Glutathione | 30 |
| Hydrochlorothiazide | 7.5 |
| Ibuprofen | 50 |
| Isoniazide | 4 |
| 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 |
| 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^A Observed |
|-----------------------------|---|--|
| Creatinine | | |
| Ascorbic acid | 20 | 11% dec. |
| Dopamine | 19 | 80% dec. |
| L-dopa | 5 | 71% dec. |
| Epinephrine | 1 | 45% dec. |
| Glutathione | 30 | 13% dec. |
| Glucose | | |
| Oxaloacetate | 132 | 11% dec. |
| Pyruvate | 44 | 13% dec. |
| Potassium | | |
| Penicillin G | 100 | 17% inc. |
| Sulfadiazine | 150 | 12% dec. |
| Sodium | | |
| Cephalothin | 400 | 12% inc. |
| Methotrexate | 0.5 | 11% inc. |
| Penicillin G | 100 | 10% inc. |
| Total Carbon Dioxide | | |
| 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. |

^A Dec. = decreased concentration of the specified analyte; Inc. = increased concentration of the specified analyte

- 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 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.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 |

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 |
|--|----------------|----------------------|
| 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 print card 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 print card, 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 NCCLS EP5-A guidelines⁴² with modifications based on NCCLS EP18-P⁴³ 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 |
|---------------------------|-------------|------------|-------|
| Calcium (mg/dL) | | | |
| <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 |
| Chloride (mmol/L) | | | |
| <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 |
| Creatinine (mg/dL) | | | |
| <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 |
| Glucose (mg/dL) | | | |
| <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 |
| Potassium (mmol/L) | | | |
| <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 |
| Sodium (mmol/L) | | | |
| <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 |
|--------------------------------------|-------------|------------|-------|
| Total Carbon Dioxide (mmol/L) | | | |
| <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 |
| Urea Nitrogen (mg/dL) | | | |
| <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 NCCLS EP9-A guideline.⁴⁴ Representative correlation statistics are shown in Table 7.

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

| | Correlation Coefficient | Slope | Intercept | SEE | N | Sample Range (mmol/L) | Comparative Method |
|-------------------------------|-------------------------|-------|-----------|------|-----|-----------------------|--------------------|
| Calcium (mg/dL) | 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 |
| Chloride (mmol/L) | 0.978 | 0.982 | -1.1 | 1.84 | 120 | 71-118 | Vitros 950 |
| Creatinine (mg/dL) | 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 |
| Glucose (mg/dL) | 0.987 | 1.009 | -2.8 | 3.89 | 251 | 72-422 | Paramax |
| | 0.997 | 0.943 | 1.2 | 4.69 | 91 | 56-646 | Beckman |
| Potassium (mmol/L) | 0.969 | 0.863 | 0.6 | 0.14 | 58 | 2.0-6.8 | Radiometer KNA™ 2 |
| 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 | 0.923 | 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 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 | 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% |

* 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 | 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⁺)

| | 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⁺)

| | 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 Carbon Dioxide (tCO₂)

| | 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% |

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% |

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