

March 2007

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1. Intended Use

The Piccolo[®] Renal Function Panel Reagent Disc, used with the Piccolo Blood Chemistry Analyzer is intended to be used for the *in vitro* quantitative determination of albumin, calcium, chloride, creatinine, glucose, phosphorus, potassium, sodium, total carbon dioxide and urea nitrogen in heparinized whole blood, heparinized plasma, or serum in a clinical laboratory setting

2. Summary and Explanation of Tests

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

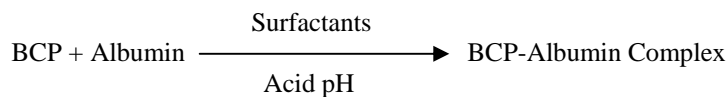
Albumin:	Dehydration, renal disease, liver insufficiency with decreased albumin synthesis, severe malnutrition, acute inflammation, chronic inflammation, malignancy, pregnancy and burns.
Calcium:	Parathyroid, bone and chronic renal diseases; tetany.
Chloride:	Dehydration, parathyroidism, and renal disease.
Creatinine:	Renal diseases and monitoring of renal dialysis.
Glucose:	Carbohydrate metabolism disorders, including adult and juvenile diabetes mellitus and hypoglycemia.
Phosphorus:	Dehydration, diabetes, parathyroidism, and renal disease.
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 the sense of thirst, skin loss, burns, sweating, hyperaldosteronism, CNS disorders, dilutional, depletion and delusional hyponatremia and ADH secretion syndrome.
Total Carbon Dioxide:	Primary metabolic alkalosis and acidosis and primary respiratory alkalosis and acidosis.
Urea nitrogen:	Renal and metabolic diseases.

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

3. Principle of Procedure

Albumin (ALB)

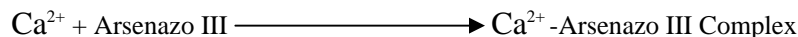
Early methods used to measure albumin include fractionation techniques and tryptophan content of globulins.¹⁻⁵ These methods were unwieldy to perform and did not have a high specificity. Two immunochemical techniques are considered as reference methods, but are expensive and time consuming.⁶ Dye binding 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.⁷ Bromocresol purple (BCP) is the most specific of the dyes in use.^{8,9}



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.

Calcium (CA⁺⁺)

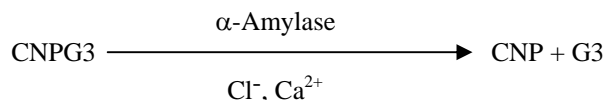
The reference method for calcium is atomic absorption spectroscopy; however, this method is not suited for routine use.¹⁰ Spectrophotometric methods using either *o*-cresolphthalein complexone (CPC) or arsenazo III metallochromic indicators are most commonly used.^{11,12,13} Arsenazo III has a high affinity 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 total calcium in the sample is proportional to the absorbance.

Chloride (CL⁻)

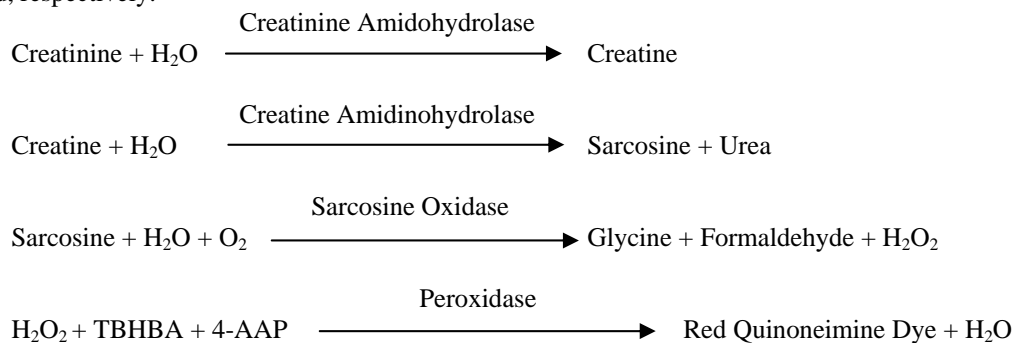
The Abaxis chloride method is based on the determination of chloride-dependent activation of α -amylase activity. Deactivated α -amylase is reactivated by addition of the chloride ion, allowing the calcium to re-associate with the enzyme. The reactivation of α -amylase activity is proportional to the concentration of chloride ions in the sample. The reactivated α -amylase converts the substrate, 2-chloro-*p*-nitrophenyl- α -D-maltotrioxide (CNPG3) to 2-chloro-*p*-nitrophenyl (CNP) producing color and α -maltotriose (G3). The reaction is measured bichromatically and the increase absorbance is directly proportional to the reactivated α -amylase activity and the concentration of chloride in the sample.¹⁴



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.^{15,16} Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.^{17,18,19} Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.²⁰

In the coupled enzyme reactions, creatinine amidohydrolase hydrolyzes creatinine to creatine. A second enzyme, creatine amidinohydrolase, catalyzes the formation of sarcosine from creatine. Sarcosine oxidase causes the oxidation of sarcosine to glycine, formaldehyde and hydrogen peroxide (H₂O₂). In a Trinder reaction, peroxidase catalyzes the reaction among the hydrogen peroxide, 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) and 4-aminoantipyrine (4-AAP) into a red quinoneimine dye. Potassium ferrocyanide and ascorbate oxidase are added to the reaction mixture to minimize the potential interference of bilirubin and ascorbic acid, respectively.



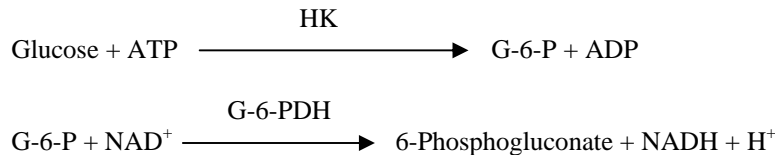
Two cuvettes are used to determine the concentration of creatinine in the sample. Endogenous creatine is measured in the blank cuvette, which is subtracted from the combined endogenous creatine and the creatine formed from the enzyme reactions in the test cuvette. Once the endogenous creatine is eliminated from the calculations, the concentration of creatinine is proportional to

the intensity of the red color produced. The endpoint reaction is measured as the difference in absorbance between 550 nm and 630 nm.

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu²¹ and Somogyi-Nelson^{22,23}). 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 Renal Function Panel Reagent Disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.²⁴

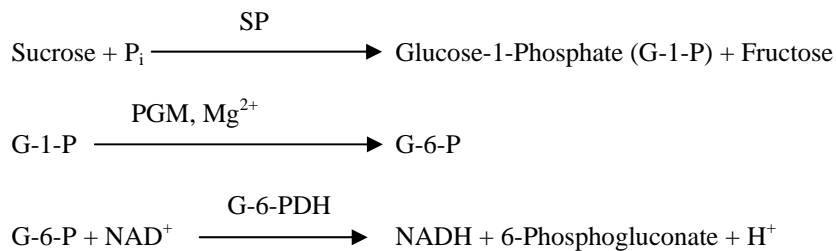
The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine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



The absorbance is measured bichromatically at 340 nm and 850 nm. The production of NADH is directly proportional to the amount of glucose present in the sample.

Phosphorus (PHOS)

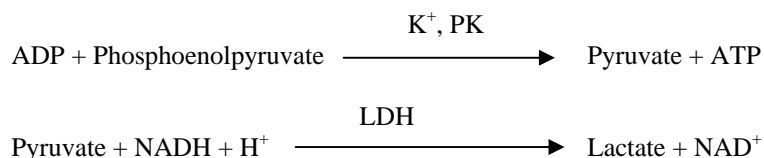
The most applicable enzymatic method for the Abaxis system uses sucrose phosphorylase (SP) coupled through phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6PDH).^{25,26} Using the enzymatic system for each mole of phosphorus present in the sample, one mole of NADH is formed. The amount of NADH formed can be measured as an endpoint at 340 nm.



Potassium (K⁺)

Spectrophotometric methods have been developed that allow the measurement of potassium concentration on standard clinical chemistry instrumentation. An enzymatic method based on the activation of pyruvate kinase with potassium show excellent linearity and negligible susceptibility to endogenous substances.^{27,28,29} Interference from sodium and ammonium ion are minimized with the addition of Kryptofix and glutamine synthetase, respectively.²⁷

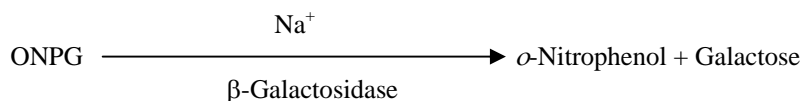
In the coupled-enzyme reaction, pyruvate kinase (PK) dephosphorylates phosphoenolpyruvate to form pyruvate. Lactate dehydrogenase (LDH) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD⁺.



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⁺)

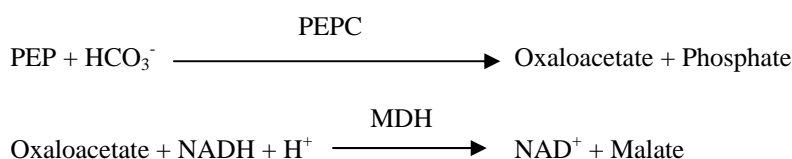
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.^{30,31,32} In the Abaxis enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyses the reaction o-nitrophenyl-β-galactopyranoside (ONPG) to o-nitrophenyl 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.^{33,34} The enzymatic method is well suited for use on a routine blood chemistry analyzer without adding complexity.

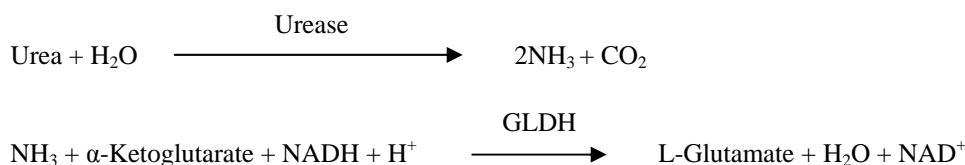
In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide (CO₂) to bicarbonate (HCO₃⁻). Phosphoenolpyruvate (PEP) and HCO₃⁻ then react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MDH) catalyzes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



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.³⁵ Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.³⁶ The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique^{37,38} and coupled enzymatic reactions.^{39,40} Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.⁴¹ 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.⁴²

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 Operator's Manual, for the Principles and Limitations of the Procedure.

5. Description of Reagents

Reagents

Each Piccolo Renal Function Panel 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 albumin, calcium, glucose, phosphorus, and urea nitrogen. A dedicated sample blank is included in the disc to calculate concentrations of creatinine. Each disc also contains a diluent consisting of surfactants and preservatives.

Table 1: Reagents

Components	Quantity/Disc
N-Acetyl cysteine	60 µg
Adenosine 5'-diphosphate	36 µg
Adenosine 5'-triphosphate	22µg
α-Ketoglutaric acid	19 µg
4-Aminoantipyrine hydrochloride	13 µg
Amylase	0.036 U
Arsenazo III, sodium salt	1.7 µg
Ascorbate oxidase	0.3 U
Brij	3 µg
Bromocresol purple, sodium salt	0.2 µg
Calcium acetate	25 µg
Citric acid, trisodium salt	567 µg
2-Chloro-4-nitrophenyl-α-maltotrioxide (CNPG3)	53 µg
Creatine amidinohydrolase	3 U
Creatinine amidohydrolase	1 U
Ethylenediaminetetraacetic acid (EDTA)	182 µg
Ethylenediaminetetraacetic acid (EDTA), disodium salt	15 µg
Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)	4 µg
β-Galactosidase	0.005 U
Glucose-1,6-diphosphate	1 µg
L-Glutamic acid	9.2 µg
Glutamine synthetase	0.17 U
Hexokinase	0.1 U
Imidazole	29 µg
Lactate dehydrogenase	0.13 U
Lithium hydroxide, monohydrate	23 µg
Magnesium acetate, tetrahydrate	67 µg
Magnesium sulfate	33 µg
Malate dehydrogenase	0.1 U
Manganese chloride	10 µg
D-Mannitol	675 µg
2-Methyl-4-isothiazolin-3-one hydrochloride (MIT)	4.2 µg
β-Nicotinamide adenine dinucleotide (NAD)	83 µg
β-Nicotinamide adenine dinucleotide, reduced (NADH)	36 µg
o-Nitrophenyl-β-D-galactopyranoside (ONPG)	22 µg
4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]tricosane (Kryptofix 221)	86 µg
Peroxidase	1 U
Phosphoenol pyruvate	57 µg
Phosphoenol pyruvate carboxylase	0.001 U
Phosphoglucomutase	0.035 U
Pluronic F68	1 µg
Polyethylene glycol, 8000	4 µg
Potassium ferrocyanide	0.4 µg
Pyruvate kinase	0.01 U
Sarcosine oxidase	1 U
Sucrose	11 µg
Sucrose phosphorylase	0.07 U
Sodium chloride	57 µg
2,4,6-Tribromo-3-hydroxybenzoic acid	188 µg
Triethanolamine hydrochloride	195 µg
Triton X-100	24 µg
Urease	0.05 U
Buffers, surfactants, excipients and preservatives	

Warnings and Precautions

- For In vitro Diagnostic Use
- The diluent container in the reagent disc is automatically opened when the analyzer drawer closes. A disc with an opened diluent container cannot be re-used. Ensure that the sample or control has been placed into the disc before closing the drawer.
- Used reagent discs contain human body fluids. Follow good laboratory safety practices when handling and disposing of used discs.⁴³ See the Piccolo Blood 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 to remain at room temperature longer than 48 hours prior to use. Open the sealed foil pouch and remove the disc, being careful not to touch the bar code ring located on the top of the disc. Use according to the instructions provided in the Piccolo Blood 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 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 rotor and adversely affect reagent performance. Do not use a rotor from a damaged pouch.

6. Instrument

See the Piccolo Blood 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 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 venipuncture samples should be run within 60 minutes of collection.⁴⁴ **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.⁴⁵
- Refrigerating whole blood samples can cause significant changes in concentrations of **creatinine** and **glucose**.⁴⁶ 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.

- 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 leakage.⁴⁷
- 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.⁴⁸

8. Procedure

Materials Provided

- One Piccolo Renal Function Panel Reagent Disc

Materials Required but Not Provided

- Piccolo Blood Chemistry Analyzer
- Commercially available control reagents recommended by Abaxis (refer to the Piccolo Blood Chemistry Analyzer Operator's Manual)

Test Parameters

The Piccolo Blood Chemistry Analyzer operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each Piccolo Renal Function Panel Reagent Disc is less than 14 minutes. The analyzer maintains the reagent disc at a temperature of 37°C (98.6°F) over the measurement interval.

Test Procedure

The complete sample collection and step-by-step operating procedures are detailed in the Piccolo Blood Chemistry Analyzer Operator's Manual.

Calibration

The Piccolo Blood Chemistry Analyzer is calibrated by the manufacturer before shipment. The bar code printed on the reagent disc bar code ring provides the analyzer with disc-specific calibration data. See the Piccolo Blood Chemistry Analyzer Operator's Manual.

Quality Control

Performance of the Piccolo Blood Chemistry Analyzer can be verified by running controls. Controls recommended by Abaxis are listed in the Piccolo Blood Chemistry Analyzer Operator's Manual. Other human serum or plasma-based controls may not be compatible.

See the Piccolo Blood Chemistry Analyzer Operator's Manual, for a detailed discussion on running, recording, interpreting, and plotting control results.

9. Results

The Piccolo Blood 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 Operator's Manual.

Interpretation of results is detailed in the Piccolo Blood Chemistry Analyzer 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.

- The only anticoagulant **recommended for use** with the Piccolo Blood Chemistry Analyzer Blood Chemistry System 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 Renal Function Panel Reagent Disc.
- Samples with hematocrits in excess of 60% packed red cell volume may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down to get plasma. The plasma can then be 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.**

Warning: Extensive testing of the Piccolo Blood 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.⁴⁹

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 suppresses any result affected by >10% interference from hemolysis, lipemia or icterus. “HEM”, “LIP”, or “ICT” is printed on the result card in place of the result.
- 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.

The following table lists the levels of substances that will cause results not to be printed on the result card.

Table 2: Maximum Levels of Endogenous Substances*

Analyte	Hemolysis (Hemoglobin mg/dL)	Lipemia (Triglycerides mg/dL)	Icterus (Bilirubin mg/dL)
ALB	2580	4200	44
CA ⁺⁺	2000	1500	31
CL ⁻	1300	2000	40
CRE	688	3000	8
GLU	1790	1950	44
PHOS	400	1100	50
K ⁺	300	2000	20
NA ⁺	2000	2600	30
TCO2	1300	2200	20
BUN	2580	4200	44

*Above these levels individual assay results will be suppressed.

- Extremely elevated amylase levels (>9,000 U/L) will have a significant effect, >10% increase, on the **chloride** results. The concentration of amylase is not evaluated by the Piccolo Blood Chemistry Analyzer for each specimen.

Effects of Therapeutic Substances

Thirty-five therapeutic substances were selected as potential interferents with albumin, calcium, chloride, creatinine, glucose, phosphorus, potassium, sodium, total carbon dioxide and blood urea nitrogen methods based on recommendations by Young.⁵⁰ Significant interference is defined as > 10% shift in the result for a normal range specimen. Human serum pools were supplemented with a known concentration of the drugs or chemicals and then analyzed. The following compounds do not significantly interfere with the chemistries in the Piccolo Renal Function Panel Reagent Disc.

Table 3: Therapeutic Substances Evaluated with No Significant Interference <10%

	Physiologic or Therapeutic Range ⁴⁹⁻⁵³ (mg/dL)	Concentration with No Significant Interference (mg/dL)
Acetylsalicylic acid	1 - 2	50
Ampicillin	0.5	30
Caffeine	0.3 - 1.5	10
Chloramphenicol	1 - 2.5	100
Hydrochlorothiazide	--	7.5
Ibuprofen	0.5 - 4.2	50
Isoniazide	0.1 - 0.7	4
Ketoprofen	--	50
Methicillin	--	100
Metronidazole	0.1	5
Nafcillin	--	1
Oxacillin	--	1
Phenytoin	1 - 2	3
Rifampin	0.4 - 3	0.5
Sulfanilamide	10 - 15	50
Theophylline	1 - 2	20
Heparin	--	28,000

The following substances showed greater than 10% interference. Significant interference is defined as >10% shift in the result for a normal range specimen. Human serum pools were supplemented with a known concentration of the drugs or chemicals and then analyzed.

Table 4: Substances With Significant Interference >10%

	Physiologic/ Therapeutic Range ⁴⁹⁻⁵³ (mg/dL)	Concentration with > 10% Interference (mg/dL)	% Interference ^A
Albumin			
Acetoacetate	0.05-3.60	102	18% dec
Ampicillin	0.5	30	12% dec
Caffeine	0.3-1.5	10	14% dec
Calcium chloride	--	20	17% dec
Cephalothin (Keflin)	10	400	13% inc
Ibuprofen	0.5-4.2	50	28% inc
α -Ketoglutarate	--	5	11% dec
Nitrofurantoin	0.2	20	13% dec
Proline	--	4	12% inc
Sulfadiazine	2-4	10	14% dec
Sulfanilamide	10-15	50	12% dec
Theophylline	1-2	20	11% dec

Table 4: Substances With Significant Interference >10% (continued)

	Physiologic/ Therapeutic Range⁴⁹⁻⁵³ (mg/dL)	Concentration with > 10% Interference (mg/dL)	% Interference^A
Calcium		None	None
Chloride		None	None
Creatinine			
Ascorbic acid	0.8 - 1.2	20	11% dec.
Dopamine	0.3 - 1.5	19	80% dec.
L-dopa	--	5	71% dec.
Epinephrine	--	1	45% dec.
Glutathione	--	30	13% dec.
Glucose			
Oxaloacetate	--	132	11% dec.
Pyruvate	0.3 - 0.9	44	13% dec.
Phosphorus			
Nitrofurantoin	0.2	20	19% inc.
Oxaloacetate	--	132	14% dec.
Potassium			
Penicillin G	--	100	17% inc.
Sulfadiazine	2-4	150	12% dec.
Sodium			
Cephalothin	10	400	12% inc
Methotrexate	0.45 - 45	0.5	11% inc
Penicillin G	--	100	10% inc
Total Carbon Dioxide			
Acetaminophen	2 - 10	100	11% inc.
Ascorbic Acid	0.8 - 1.2	20	12% dec.
Cephalothin	10	400	13% inc.
Cimetidine	0.1 - 1	16	19% dec.
Erythromycin	0.2 - 2.0	10	21% dec.
Lidocaine	0.15 - 0.6	1	23% inc.
Methotrexate	0.1	0.5	80% dec.
Nitrofurantoin	0.2	20	13% inc.
Salicylic Acid	15 - 30	50	17% dec.
Sulfadiazine	2 - 4	150	25% dec.
Urea Nitrogen		None	None

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

Table 5: Concentration of Analytes in Serum Pool Used for Interference Studies

Analyte	Concentration
Albumin	4 mg/dL
Calcium	9.5 mg/dL
Chloride	93 mmol/L
Creatinine	4.1 mg/dL
Glucose	96 mg/dL
Phosphorus	5.6 mg/dL
Potassium	3.8 mmol/L
Sodium	124 mmol/L
Total Carbon Dioxide	6 mmol/L
Urea Nitrogen	26 mg/dL

11. Expected Values

Samples from approximately 90-140 adult males and females were analyzed on the Piccolo Blood Chemistry Analyzer to determine the reference intervals for the following assays. These intervals are provided as a guideline only. It is recommended that your office or institution establish normal ranges for your particular patient population.⁵⁴

Table 6: Piccolo Blood Chemistry Analyzer Reference Intervals

Analyte	Common Units	SI Units
Albumin	3.3 – 5.5 g/dL	33 – 55 g/L
Calcium	8.0 – 10.3 mg/dL	2.0 – 2.58 mmol/L
Chloride	98 – 108 mmol/L	98 – 108 mmol/L
Creatinine	0.6 – 1.2 mg/dL	53 – 106 µmol/L
Glucose	73 – 118 mg/dL	4.1 – 6.6 mmol/L
Phosphorus (plasma)	2.2 – 4.1 mg/dL	0.71 – 1.32 mmol/L
Phosphorus (serum)	2.5 – 4.4 mg/dL*	0.81 – 1.42 mmol/L*
Potassium	3.6-5.1 mmol/L	3.6-5.1 mmol/L
Sodium	128 – 145 mmol/L	128 – 145 mmol/L
Total Carbon Dioxide	18 – 33 mmol/L	18 – 33 mmol/L
Urea Nitrogen (BUN)	7 – 22 mg/dL	2.5 – 7.9 mmol/urea/L

* There is no observed difference between the concentration of Phosphorus measured in heparinized whole blood and heparinized plasma. However, a small increase (0.3 mg/dL) was observed in serum when compared to heparinized whole blood and heparinized plasma. This increase is consistent with the difference between Phosphorus in serum and plasma as described in the literature.^{55,56,57,58}

12. Performance Characteristics

Linearity

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

Table 7: Piccolo Blood Chemistry Analyzer Dynamic Ranges

Analyte	Dynamic Range	
	Common Units	SI Units
Albumin	1 – 6.5 g/dL	10 – 65 g/L
Calcium	4.0 – 16.0 mg/dL	1.0 – 4.0 mmol/L
Chloride	80 – 135 mmol/L	80 – 135 mmol/L
Creatinine	0.2 – 20 mg/dL	18 – 1768 µmol/L
Glucose	10 – 700 mg/dL	0.6 – 38.9 mmol/L
Phosphorus	0.2 - 20 mg/dL	0.06 –6.5 mmol/L
Potassium	1.5 – 8.5 mmol/L	1.5 – 8.5 mmol/L
Sodium	110 – 170 mmol/L	110 – 170 mmol/L
Total Carbon Dioxide	5 – 40 mmol/L	5 – 40 mmol/L
Urea Nitrogen (BUN)	2 – 180 mg/dL	0.7 – 64.3 mmol urea/L

Sensitivity (Limits of Detection)

The lower limit of the reportable (dynamic) range for each analyte is: albumin 1 g/dL (10 g/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); phosphorus 0.2 mg/dL (0.06 mmol/L); potassium 1.5 mmol/L; sodium 110 mmol/L; total carbon dioxide 5 mmol/L and urea nitrogen 2.0 mg/dL (0.7 mmol urea/L).

Precision

Precision studies were conducted using NCCLS EP5-A guidelines, with modifications based on NCCLS EP18-P for unit-use devices.^{59,60} Results for within-run and total precision were determined by testing levels of commercially available control materials. The studies made use of multiple instruments. Precision for albumin, calcium, creatinine, glucose, sodium and urea nitrogen was performed at one site; potassium and total carbon dioxide testing was performed at two sites over 20 days; chloride and phosphorus testing was conducted at two sites over a period of 5 days. Results of the precision studies are shown in Table 8.

Table 8: Precision

Analyte	Sample Size	Within-Run	Total
Albumin (g/dL)	n = 80		
<u>Control 1</u>			
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
Calcium (mg/dL)	n = 80		
<u>Control 1</u>			
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)	n = 160		
<u>Control 1</u>			
Mean		97.8	97.8
SD		1.63	1.74
%CV		1.7	1.7

Table 8: Precision (continued)

Analyte	Sample Size	Within-Run	Total
<u>Control 2</u>			
Mean		113.6	113.6
SD		1.97	2.22
%CV		1.7	2.0
Creatinine (mg/dL)	n=80		
<u>Control 1</u>			
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)	n=80		
<u>Control 1</u>			
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
Phosphorus (mg/dL)	n =80		
<u>Control 1</u>			
Mean		3.1	3.1
SD		0.12	0.14
%CV		3.7	4.7
<u>Control 2</u>			
Mean		7.3	7.3
SD		0.09	0.15
%CV		1.3	2.0
Potassium (mmol/L)	n=120		
<u>Control 1</u>			
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)	n=80		
<u>Control 1</u>			
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
Total Carbon Dioxide (mmol/L)	n=120		
<u>Control 1</u>			
Mean		21.4	21.4
SD		2.29	2.29
%CV		10.7	10.7

Table 8: Precision (continued)

Analyte	Sample Size	Within-Run	Total
<u>Control 2</u>			
Mean		10.5	10.5
SD		0.90	0.90
%CV		8.6	8.6
Urea Nitrogen (mg/d/L)	n = 80		
<u>Control 1</u>			
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

Serum samples were collected and assayed on the Piccolo Blood Chemistry Analyzer and by a comparative method. The samples were chosen to meet the distribution values in NCCLS EP9-A guidelines.⁶¹

Table 9: Correlation of Piccolo Blood Chemistry Analyzer with Comparative Method

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
Albumin (g/dL)	0.854	1.001	-0.3	0.22	261	1.1-5.3	Paramax®
Calcium (mg/dL)	0.896	0.877	-0.1	0.21	100	1.5-5.0	Beckman
Chloride (mmol/L)	0.980	0.98	-0.17	0.31	111	4.6-13.2	Beckman
Creatinine (mg/dL)	0.978	0.982	-1.1	1.84	120	71 - 118	Vitros® 950
Glucose (mg/dL)	0.993	0.926	0.0	0.15	260	0.4 – 14.7	Paramax®
	0.987	1.009	-2.8	3.89	251	72-422	Paramax®
Phosphorus (mg/dL)	0.997	0.943	1.2	4.69	91	56-646	Beckman
	0.993	1.017	-0.2	0.236	90	0.8 – 11.7	Vitros® 950
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	2.0	0.84	60	6 – 39	Cobas® Fara
Urea Nitrogen (mg/dL)	0.983	0.946	0.0	0.66	92	6 – 38	Beckman

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