Piccolo® Metlyte 8 Reagent Disc

CE

Customer and Technical Service: 800-822-2947

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1. Intended Use

The Piccolo® Metlyte 8 Reagent Disc, used with the Piccolo Blood Chemistry Analzyer, is intended to be used for the *in vitro* quantitative determination of chloride, creatine kinase, creatinine, glucose, 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

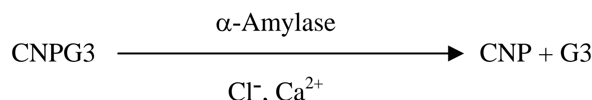
The Piccolo Metlyte 8 Reagent Disc and the Piccolo Blood Chemistry Analzyer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders.

Chloride:	Dehydration, prolonged diarrhea and vomiting, renal tubular disease, hyperparathyroidism, burns, salt-losing renal diseases, overhydration and thiazide therapy.
Creatine Kinase:	Myocardial infarction, progressive muscular dystrophy, dermatomyositis, rhabdomyolysis due to drug ingestion, hyperosmolality, autoimmune disease, delirium tremens, convulsions, Crush syndrome, hypothyroidism, surgery, severe exercise, intramuscular injection, physical inactivity, decreased muscle mass.
Creatinine:	Renal disease and monitoring of renal dialysis.
Glucose:	Carbohydrate metabolism disorders, including adult and juvenile diabetes mellitus and hypoglycemia, hypopituitarism, pancreatitis and renal disease.
Potassium:	Renal glomerular or tubular disease, adrenocortical insufficiency, diabetic ketacidosis, excessive intravenous potassium therapy, sepsis, panhypopituitarism, *in vitro* hemolysis, hyperaldosteronism, malnutrition, hyperinsulinism, metabolic alkalosis and gastrointestinal loss.
Sodium:	Dehydration, diabetes insipidus, loss of hypotonic gastrointestinal fluids, salt poisoning, selective depression of sense of thirst, skin losses, burns, sweating, hyperaldosteronism, CNS disorders, dilutional, depletional and delusional hyponatremia and syndrome of inappropriate ADH secretion.
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

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.[1]



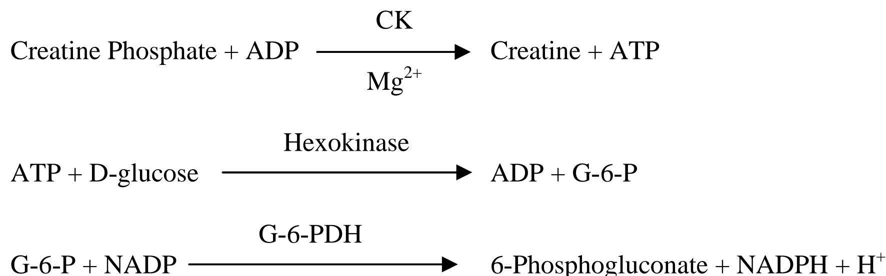
$$\text{CNPG3} \xrightarrow[\text{Cl}^-,\ \text{Ca}^{2+}]{\alpha\text{-Amylase}} \text{CNP} + \text{G3}$$

Creatine Kinase (CK)

Creatine kinase catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP). The phosphorylation reaction is favored by alkaline conditions (optimum at pH 9.0) and the dephosphorylation reaction is favored by acidic conditions (optimum at pH 6.5 at 37°C). Early CK measurement methods were based on the "forward reaction" with creatine phosphate and adenosine diphosphate (ADP) as the products.[2,3,4] The sensitivity of these tests was shown to be low due to problems with interferences. The procedure of choice utilizes the "reverse reaction" coupled with a reaction to produce NADPH, which is directly related to CK levels.[5,6,7]

The CK measurement procedure used by Abaxis is a modified version of the International Federation of Clinical Chemistry (IFCC) method.[8] Key modifications are sample volume fraction, buffer, and temperature. N-acetyl cysteine (NAC) has been added to reactivate the CK.[9] Magnesium is used as a cofactor for both CK and hexokinase. EDTA has been added as a stabilizer for NAC and for the removal of various cations, such as calcium and iron, that inhibit CK. P^1, P^5-di (adenosine-5')penta phosphate and adenosine monophosphate (AMP) have also been added to inhibit adenylate kinase, another skeletal muscle and erythrocyte enzyme that reacts with the substrates used to measure CK.

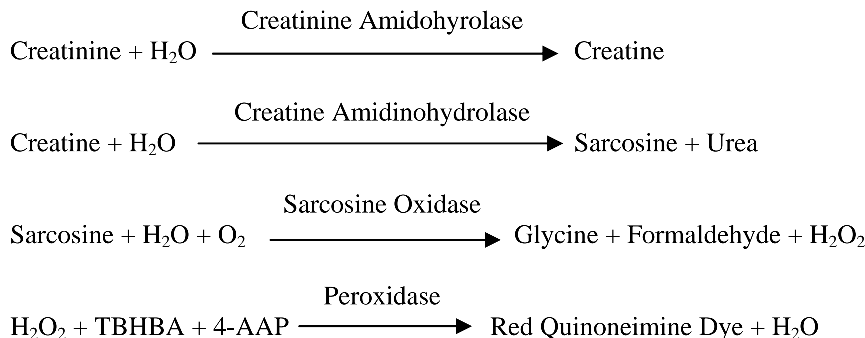
Creatine kinase catalyzes the formation of creatine and ATP from creatine phosphate and ADP at pH 6.7. With hexokinase as a catalyst, ATP reacts with D-glucose to form ADP and D-glucose-6-phosphate (G-6-P), which is reacted with nicotinamide adenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate dehydrogenase (G-6-PDH) to produce G-6-P and NADPH.



The formation of NADPH is measured as a change in absorbance at 340 nm relative to 405 nm. This absorbance change is directly proportional to creatine kinase activity in the sample.

Creatinine (CRE)

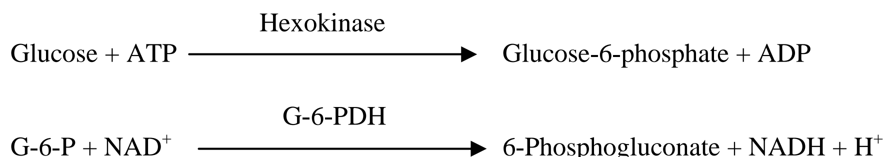
The Jaffe method, first introduced in 1886, is still a commonly used method of determining creatinine levels in blood. The current reference method combines the use of Fuller's earth (floridin) with the Jaffe technique to increase the specificity of the reaction.[10,11] Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.[12,13,14] Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohyrolase.[15]



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 creatin 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.

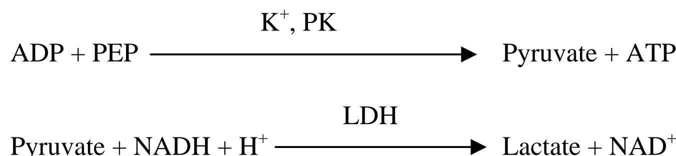
Glucose (GLU)
Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu[16] and Somogyi-Nelson[17,18]). The lack of specificity in copper-reduction techniques led to the development of quantitative procedures using the enzymes hexokinase and glucose oxidase. The glucose test incorporated into the Metlyte 8 Reagent Disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.[18,19]

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



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.[20,21,22] Interference from sodium and ammonium ion are minimized with the addition of Kryptofix and glutamate dehydrogenase respectively.[20]

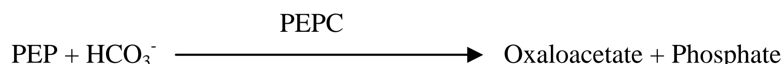
In the coupled-enzyme reaction, pyruvate kinase (PK) dephosphorylates phosphoenolpyruvate (PEP) to form pyruvate. Lactate dehydrogenase (LDH) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+. The rate of change in absorbance due to the conversion of NADH to NAD^+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

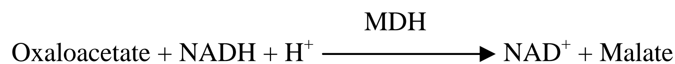


Sodium (Na^+)
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.[23,24,25] In the Abaxis enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyzes the reaction of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol and galactose.

Total Carbon Dioxide (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.[26,27] The enzymatic method is well suited for use on a routine blood chemistry analyzer without adding complexity.

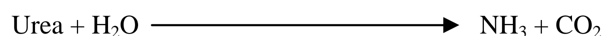
In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide (CO_2) toward bicarbonate (HCO_3^-). Phosphoenolpyruvate (PEP) and HCO_3^- then react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (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.





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.[28] Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.[29] The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique[30,31] and coupled enzymatic reactions.[32,33] Catalyzed Berthelot procedures, however, are erraticwhen measuring ammonia.[34] 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.[35]

In the coupled-enzyme reaction, urease hydrolyzes urea into ammonia and carbon dioxide. Upon combining ammonia with 2-oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD^+.



4. Principle of Operation

See the Piccolo Blood Chemistry Analzyer Operator's Manual, for the Principles and Limitations of the Procedure.

5. Description of Reagents

Reagents
Each Piccolo Metlyte 8 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 chloride, creatine kinase, creatinine, glucose, potassium, sodium, total carbon dioxide and urea nitrogen. Each disc also contains a diluent consisting of surfactants and preservatives.

Table 1: Reagents

Component	Quantity/Disc
2, 4, 6-Tribomo-3-hydroxybenzoic acid	188 µg
2-Chloro-4-nitrophenyl-alpha-maltotrioside (CNPG3)	52.5 µg
4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 222)	0.3 µg
4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]trisocosane (Kryptofix 221)	84 µg
4-Aminoantipyrine *HCl	13 µg
Adenosine-5'-diphosphate	38 µg
Adenosine-5'-monophosphate	33 µg
Adenosine-5'-triphosphate	11 µg
Amylase	0.0357 U
Ascorbate oxidase	0.3 U
Calcium acetate	25.2 µg
Citric acid, trisodium salt	567 µg
Creatine amidinohydrolase	3 U
Creatinine amidohydrolase	1 U
Ethylene glyco-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)	4 µg
Ethylenediaminetetraacetic acid (EDTA)	191.1 µg
Glucose	58 µg
Glucose-6-phosphate dehydrogenase (G6PDH)	0.1 U
Glutamate dehydrogenase	0.1 U
Hexokinase	0.2 U
Imidazole	26 µg
Lactate dehydrogenase	0.3 U
Magnesium acetate	60 µg
Magnesium sulfate	29 µg
Malate dehydrogenase	0.1 U
N-Acetyl cysteine	60 µg
o-Nitrophenyl-ß-D galactopyranoside (ONPG)	22 µg
P1, P5di(adenosine-5')pentaphosphate	0.2 µg
Peroxidase	1 U
Phosphoenol pyruvate	23 µg
Phosphoenol pyruvate carboxylase	0.001 U
Potassium ferrocyanide	0.4 µg
Pyruvate kinase	0.01 U
Sarcosine oxidase	1 U
ß-Nico-tinamide adenine dinucleotide, (NAD)	20 µg
ß-Nicotinamide adenine dinucleotide, reduced (NADH)	28 µg
ß-Nicotinamide adenine dinucleotide phosphate (NADP)	101 µg
Urease	0.05 U
α-Ketoglutaric acid	19 µg
β-Galactosidase	0.005 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 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.[36] See the Piccolo Blood Chemistry Analzyer Operator's Manual for instructions on cleaning biohazardous spills.

- 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 Analzyer 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 Analzyer 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 Analzyer 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 Analzyer 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.[37]

- Whole blood venipuncture samples should be run within 60 minutes of collection.[38] 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.

- 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.

- 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.[39]

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

8. Procedure

Materials Provided
- One Piccolo Metlyte 8 Reagent Disc

Materials Required but not Provided
- Piccolo Blood Chemistry Analzyer
- Commercially available control reagents recommended by Abaxis (refer to Piccolo Blood Chemistry Analzyer Operator's Manual)

Test Parameters
The Piccolo Blood Chemistry Analzyer operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each Piccolo Metlyte 8 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 Analzyer Operator's Manual.

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

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

9. Results

The Piccolo Blood Chemistry Analzyer 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 Analzyer 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 Analzyer Operator's Manual**.**

- The only anticoagulant **recommended for use** with the Piccolo 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 Metlyte 8 Reagent Disc.
- Samples with hematocrits in excess of 62% 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 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 Analzyer.**

  Warning: Extensive testing of the Piccolo Blood Chemistry System 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.[40]

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 PiccoloBlood Chemistry System 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.

- 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 **no** results 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)
Cl^-	1400	2000	40
CK	1000	1600	50
Cre	688	3000	8
Gluc	1790	1950	44
Na^+	2000	2600	30
K^+	300	2000	20
tCO_2	1300	2500	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 result. The concentration of amylase is not evaluated by the Piccolo system for each specimen.

Effects of Therapeutic Substances

Thirty-five therapeutic substances were selected as potential interferents with chloride, potassium, sodium and total carbon dioxide methods based on recommendations by Young.[41] Significant interference is defined as a >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 3: Therapeutic Substances Evaluated

	Physiologic or Therapeutic Range[40-44] (mg/dL)	Highest Concentration Tested (mg/dL)
Acetaminophen	2-10	100
Acetoacetate	0.05-3.6	102
Acetylsalicylic Acid	1-2	50
Ampicillin	0.5	30
Ascorbic acid		3
Caffeine		10
Cephalothin (Keflin)	10	400
Chloramphenicol	1-2.5	100
Cimetidine	0.1-1	16
Dopamine		13
Epinephrine		1
Erythromycin	0.2-2.0	10
Glutathione		30
Hydrochlorothiazide		7.5
Ibuprofen	0.5-4.2	50
Isoniazide	0.1-0.7	4
Ketoprofen		50
L-dopa		5
Lidocaine	0.5–0.6	1
Lithium Lactate	6–12	84
Methicillin		100
Methotrexate	0.1	0.5
Metronidazole	0.1	5
Nafcillin		1
Nitrofurantoin	0.2	20
Oxacillin		1
Oxaloacetate		132
Penicillin G		100
Phenytoin (5,5-Diphenylhydantion)	1-2	3
Proline		4
Rifampin	0.4-3	0.5
Salicylic Acid		50
Sulfadiazine		150
Sulfanilamide	10-15	50
Theophylline	1-2	20

Table 4: Substances With Significant Interference >10%

	Physiologic/ Therapeutic Range [40-44] (mg/dL)	Concentration with > 10% Interference (mg/dL)	% Interference[A]
Chloride	None	None	None
Creatine Kinase			
Cephalothin	10	400	43% dec
Dopamine	0.3-1.5	15	46% dec
L-dopa	-	5	13% dec
Methotextrate	0.1	0.5	16 % dec
Nitrofurantoin	0.2	20	18 % dec
Creatinine			
Ascorbic Acid	20	0.8-1.2	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	0.3-0.9	13% 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	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
Chloride	93 mmol/L
Creatine Kinase	131.3 U/L
Creatinine	4.1 mg/dL
Glucose	96 mg/dL
Potassium	3.8 mmol/L
Sodium	124 mmol/L
Total Carbon Dioxide	6 mmol/L
Urea Nitrogen	26 mg/dL

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

11. Expected Values

Samples from 125-150 adult males and females were analyzed on the Piccolo Blood Chemistry Analzyer to determine the reference interval for the electrolytes. These ranges were calculated based on the 95% reference interval estimated from the combined (overall) values obtained from the reference subjects.[45] 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 6: Piccolo Reference Intervals

Analyte	Sample Size	Common Units	SI Units
Chloride	N=150	98-108 mmol/L	98-108 mmol/L
Creatine Kinase (Female)	N=150	30-190 U/L	30-190 U/L
Creakine Kinase (Male)	N=150	39-380 U/L	39-380 U/L
Creatinine	N=125	0.6-1.2 mg/dL	53-106 µmol/L
Glucose	N=125	73-118 mg/dL	4.1-6.6 mmol/L
Potassium	N=150	3.6-5.1 mmol/L	3.6-5.1 mmol/L
Sodium	N=150	128-145 mmol/L	128-145 mmol/L
Total Carbon Dioxide	N=150	18-33 mmol/L	18-33 mmol/L
Urea Nitrogen (BUN)	N=125	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 Analzyer is operated according to the recommended procedure (refer to the Piccolo Blood Chemistry Analzyer Operator's Manual).

Table 7: Piccolo Dynamic Ranges

Analyte	Common Units	SI Units
Chloride	80-130 mmol/L	80-130 mmol/L
Creatine Kinase	5-5,000 U/L	5-5,000 U/L
Creatinine	0.2-20 mg/dL	18-1768 µmol/L
Glucose	10-700 mg/dL	0.6-38.9 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: chloride 80 mmol/L; creatine kinase 5 U/L; creatinine 0.2 mg/dL (18 µmol/L); glucose 10 mg/dL (0.6 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[45] with modifications based on NCCLS EP18-P[46] 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. Creatine kinase, 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 8.

Table 8: Precision

Analyte	Sample Size	Within-Run	Total
Chloride (mmol/L)	N = 160		
Control 1			
Mean		97.8	97.8
SD		1.63	1.74
CV		1.7	1.7
Control 2			
Mean		113.6	113.6
SD		1.97	2.22
CV		1.7	2.0
Creatine Kinase (U/L)	N = 120		
Control 1			
Mean		134	134
SD		2.7	2.7
CV		2.0	2.0
Control 2			
Mean		526	526
SD		7.7	7.7
CV		1.5	1.5
Creatinine (mg/dL)	N=80		
Control 1			
Mean		1.1	1.1
SD		0.14	0.14
CV		12.5	13.1
Control 2			
Mean		5.2	5.2
SD		0.09	0.27
CV		4.4	5.2
Glucose (mg/dL)	N=80		
Control 1			
Mean		66	66
SD		0.76	1.03
CV		1.1	1.6
Control 2			
Mean		278	278
SD		2.47	3.84
CV		0.9	1.4
Potassium (mmol/L)	N = 120		
Control 1			
Mean		6.12	6.12
SD		0.32	0.32
CV		5.2	5.7
Control 2			
Mean		4.10	4.10
SD		0.24	0.26
CV		5.9	6.3

Table 8: Precision (continued)

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

Correlation

Heparinized whole blood and serum samples were collected and assayed on the Piccolo Blood Chemistry Analzyer and by a comparative method(s) for creatine kinase, creatinine, glucose, potassium, sodium, total carbon dioxide and urea nitrogen. The whole blood samples were analyzed by the Piccolo Blood Chemistry Analzyer at the field sites and the serum samples were analyzed by the Piccolo Blood Chemistry Analzyer 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.[48]

Representative correlation statistics are shown in Table 9.

Table 9: Correlation of Piccolo Blood Chemistry Analzyer with Comparative Method(s)

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range (mmol/L)	Comparative Method
Chloride (mmol/L)	0.978	0.982	-1.1	1.84	120	71-118	Vitros 950
Creatine Kinase (U/L)	0.967	1.194	-25	9.05	47	6-813	Cobas Fara®
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	2.0	0.84	60	6-39	Cobas Fara
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

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