Piccolo® Metlyte 8 Reagent Disc

For In Vitro Diagnostic Use and Professional Use Only
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: 400-7122 Rev: G

1. Intended Use

The Piccolo® Metlyte 8 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 chloride, creatine kinase, 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 Metlyte 8 Reagent Disc and the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders.

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.
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

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]

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

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.

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow[Mg^{2+}]{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{D-glucose} \xrightarrow{\text{Hexokinase}} \text{ADP} + \text{G-6-P}$$

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{6-Phosphogluconate} + \text{NADPH} + H^+$$

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 iminohydrolase.[15]

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

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

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

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

Two cuvettes are used to determine the concentration of creatinine in the sample. Endogenous creatine is measured in the blank cuvette, which is subtracted from the combined endogenous creatine and the creatine formed from the enzyme reactions in the test cuvette. Once the endogenous creatine is eliminated from the calculations, the concentration of creatinine is proportional to the intensity of the red color produced. The endpoint reaction is measured as the difference in absorbance between 550 nm and 630 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.

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

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu[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 triphosphate (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.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

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

Potassium (K^+)

Spectrophotometric methods have been developed that allow the measurement of potassium concentration on standard clinical chemistry instrumentation. The Abaxis enzymatic method is based on the activation of pyruvate kinase with potassium and shows excellent linearity and negligible susceptibility to endogenous substances.[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.

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

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

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.

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

Total Carbon Dioxide (tCO_2)

Total carbon dioxide in serum or plasma exists as dissolved carbon dioxide, carbamino derivatives of proteins, bicarbonate and carbonate ions and carbonic acid. Total carbon dioxide can be measured by pH indicator, CO_2 electrode and spectrophotometric enzymatic methods, which all produce accurate and precise results.[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.

$$\text{PEP} + HCO_3^- \xrightarrow{\text{PEPC}} \text{Oxaloacetate} + \text{Phosphate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} NAD^+ + \text{Malate}$$

Blood Urea Nitrogen (BUN)

Urea can be measured both directly and indirectly. The diacetyl monoxime reaction, the only direct method to measure urea, is commonly used but employs dangerous reagents.[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 erratic when 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^+.

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

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

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 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 (CL^-), creatine kinase (CK), glucose (GLU), potassium (K+), sodium (NA+), total carbon dioxide (tCO_2) and blood urea nitrogen (BUN). A dedicated sample blank is included in the disc to calculate concentrations of creatinine (CRE). Each disc also contains a diluent consisting of surfactants and preservatives.

Table 1: Reagents

Component	Quantity/Disc
2, 4, 6-Tribomo-3-hydroxybenzoic acid	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 (*Cucurbita spp.*)	0.3 U
Calcium acetate	25.2 µg
Citric acid, trisodium salt	567 µg
Creatine amidinohydrolase (*Actinobacillus spp.*)	3 U
Creatinine amidohydrolase (*Pseudomonas spp.)*	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 (chicken heart)	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 (horseradish)	1 U
Phosphoenol pyruvate	23 µg
Phosphoenol pyruvate carboxylase	0.001 U
Potassium ferrocyanide	0.4 µg
Pyruvate kinase	0.01 U
Sarcosine oxidase (microorganism)	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 (jack bean)	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 Analyzer or the Piccolo xpress Chemistry Analyzer 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 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.[37]

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

- Refrigerating whole blood samples can cause significant changes in concentration of **creatinine**[39]. 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.[40]

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

8. Procedure

Materials Provided
- One Piccolo Metlyte 8 Reagent Disc PN: 400-1023 (a box of discs PN 400-0023)

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 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 Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

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

- The only anticoagulant **recommended for use** with the Piccolo Blood Chemistry or the Piccolo xpress Chemistry Analyzer 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 62-65% 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 Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer 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.[41]

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.[42] Significant interference is defined as greater than ± 10% shift in the result for a normal range specimen. Human serum pools were supplemented with known concentrations of the drugs or chemicals and then analyzed. Please see Table 2 for a list of exogenous and therapeutic substances evaluated. **Please see TABLE 3 for a list of analytes where interference was observed.**

Table 2: Exogenous and Therapeutic Substances Evaluated

Potential Interferent	Highest Concentration Tested (mg/dL unless otherwise specified)
Acetaminophen	100
Acetoacetate	102
Acetylsalicylic Acid	50
Ampicillin	30
Ascorbic acid	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 ± 10 % shift in the result for a normal range specimen.

	Concentration Which Produces > 10% Interference	% Interference[A] Observed
Creatine Kinase		
Cephalothin	400	43% dec
Dopamine	15	46% dec
L-dopa	5	13% dec
Methotextrate	0.5	16 % dec
Nitrofurantoin	20	18 % dec
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 125-150 adult males and females were analyzed on the Piccolo Blood Chemistry Analyzer 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.[43] 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
Chloride	98-108 mmol/L	98-108 mmol/L
Creatine Kinase (Female)	30-190 U/L	30-190 U/L
Creatine Kinase (Male)	39-380 U/L	39-380 U/L
Creatinine	0.6-1.2 mg/dL	53-106 µmol/L
Glucose	73-118 mg/dL	4.1-6.6 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
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
Chloride	80-135 mmol/L	80-135 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
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. ALT >2000* U/L. If lower than the dynamic range, a "<" will be printed with an asterisk, e.g. ALT <5* U/L. For values that are grossly beyond the measurement range (system range), "~~~" will be printed instead of a result. Any time "~~~" appears on a 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: 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 blood urea nitrogen 2.0 mg/dL (0.7 mmol urea/L).

Precision
Precision studies were conducted using NCCLS EP5-A guidelines[44] with modifications based on NCCLS EP18-P[45] 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 6.

Table 6: 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		0.23	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 6: 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
Blood 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 Analyzer 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 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.[46]

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
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
Blood Urea Nitrogen	0.964	0.923	0.5	1.08	251	6-52	Paramax®
(mg/dL)	0.983	0.946	0.0	0.66	92	6-38	Beckman

Figure 1. CK Piccolo xpress (whole blood) vs IFCC (plasma)
40 samples in duplicate by each method; all data points included

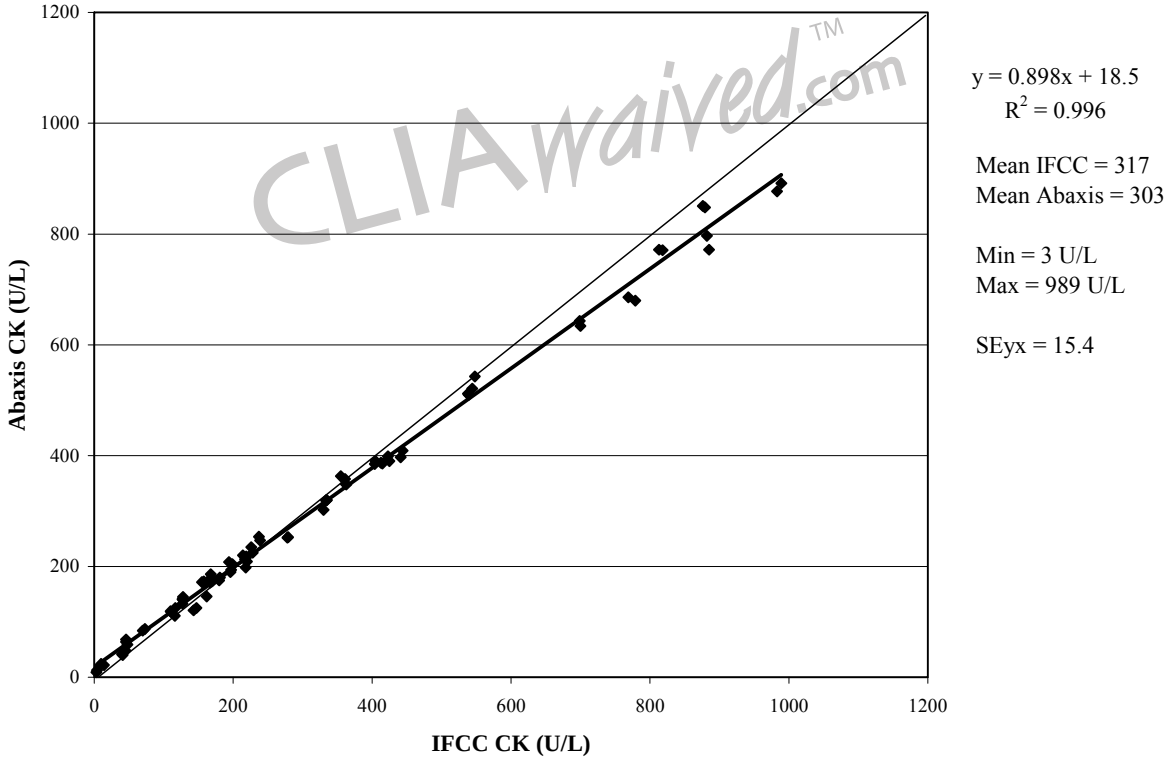

Table 8. Bias Evaluation for Abaxis (whole blood) vs IFCC (plasma)

	Bias	95% CI	SE	p
Constant (Intercept)	18.5	13.1 to 23.9	2.72	< 0.0001
Proportional (Slope)	0.898	0.885 to 0.912	0.007	< 0.0001

Table 9. Abaxis Bias vs IFCC CK as Calculated from Linear Regression

Abaxis CK (U/L)	**IFCC CK** (U/L)	**Abaxis Bias** (U/L)
30	13	17
39	23	16
110	102	8
190	191	-1
210	213	-3
380	402	-22

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, chloride, creatine kinase, 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 or instrument. A total of 62 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.

Chloride (CL^-)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	93	105	115
Mean value By Piccolo (mmol/L)	94.6	106	115.5
SD	1.66	1.5	1.74
%CV	1.8	1.4	1.5
Observed Range	90 – 100	102 - 108	110 - 119

Creatine Kinase (CK)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	121	308	746
Mean value By Piccolo (U/L)	119.0	308.0	745.6
SD	4.9	6.2	11.2
%CV	4.1	2.0	1.5
Observed Range	110 – 131	291 – 234	718 – 771

Creatinine (CRE)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	0.9	2.1	6.9
Mean value By Piccolo (mg/dL)	0.89	2.07	6.89
SD	0.10	0.10	0.11
%CV	11.2%	4.8%	1.6%
Observed Range	0.7 – 1.2	1.8 – 2.3	6.5 – 7.2

Glucose

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	96	131	363
Mean value By Piccolo (mg/dL)	95.2	130.3	365.8
SD	1.08	1.33	2.85
%CV	1.1%	1.0%	0.8%
Observed Range	93 – 98	125 – 133	351 – 373

Potassium (K^+)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	3.4	5.6	7.2
Mean value By Piccolo (mmol/L)	3.42	5.66	7.19
SD	0.11	0.14	0.14
%CV	3.3	2.5	1.9
Observed Range	3.2 – 3.7	5.2 – 5.9	6.7 – 7.5

Sodium (NA^+)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	122	141	158
Mean value By Piccolo (mmol/L)	122.1	140.8	157.5
SD	1.25	1.15	1.63
%CV	1.0	0.8	1.0
Observed Range	118 - 127	138 - 143	154 - 162

Total Carbon Dioxide (tCO_2)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	21	28	33
Mean value By Piccolo (mmol/L)	20.3	27.6	34.4
SD	1.03	1.26	1.27
%CV	5.1	4.6	3.7
Observed Range	18 – 23	23 - 30	32 - 38

Blood Urea Nitrogen (BUN)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	15	42	72
Mean value By Piccolo (mg/dL)	15.1	41.0	72.2
SD	0.35	1.0	1.3
%CV	2.3%	2.5%	1.8%
Observed Range	14 – 16	37 – 43	68 – 75

13. Bibliography

1. Ono T, et al. A new enzymatic assay of chloride in serum. Clin Chem 1988;34:552-3.
2. Kuby SA, Noda, L and Lardy HA. Adenosinetriphosphate-Creatine Transphosphorylase. J. Biol Chem 1954; 209: 191-201.
3. Tanzer Ml And Gilvarg C. Creatine And Creatine Kinase Measurement. J Biol Chem 1959; 234:3201-3204.
4. Nuttall FQ And Wedin DS. A Simple Rapid Colorimetric Method For Determination Of Creatine Kinase Activity. J Lab Clin Med 1966;68:324-332.
5. Oliver IT. 1955 A Spectrophotometric Method For The Determination Of Creatine Phosphokinase And Myokinase. Biochem J 1955;61:116-122.
6. Rosalki SB.. An Improved Procedure Or Serum Creatine Phosphokinase Determination. J Lab Clin Med 1967;69:696-705.
7. Szasz G, Gruber W And Bernt E. Creatine Kinase In Serum: I. Determination Of Optimum Reaction Conditions. Clin Chem 1976;22: 650-656.
8. Expert Panel On Enzymes, Committee Of Standards (IFCC). 1979 Approval Recommendations Of IFCC Methods For The Measurement Of Catalytic Concentrations Of Enzymes, Part 1. General Considerations. Clin Chim Acta, IFCC Sections: 98: 163-174.
9. Committee On Enzymes Of The Scandinavian Society For Clinical Chemistry And Clinical Physiology. 1976. Recommended Method For The Determination Of Creatine Kinase In Blood. Scand J. Clin Lab Invest 36: 711-723.
10. Knoll VE, et al. Spezifische Kreatininbetimmung Im Serum. Z Klin Chemi Clin Biochem. 1970;8:582-587.
11. Haeckel R, et al. Simplified Determinations of the "True" Creatinine Concentration In Serum And Urine. J Cklin Chem Clin Biochem. 1980;18:385-394.
12. Moss GA, et al. Kinetic Enzymatic Method For Determining Serum Creatinine. 1975;21:1422-1426.
13. Jaynes PK, et al. An Enzymatic, Reaction-Rate Assay For Serum Creatinine With a Centrifugal Analyzer. 1982; 28:114-117.
14. Fossati P, et al. Enzymatic Creatinine Assay: A New Colorimetric Method Based on Hydrogen Peroxide Measurement. 1983;29:1494-1496.
15. Whelton A, et al. Nitrogen Metabolites and Renal Function. In:CA Burtis and ER Ashwood, Eds., Tietz Textbook of Clinical Chemistry, 2nd Ed. Philadelphia: W.B. Saunders Company. 1994; 1513-1575.
16. Folin O, et al. A system of blood analysis. J Biol Chem. 1919;38:81-110.
17. Somogyi M. A reagent for the copper-iodometric determination of very small amounts of sugar. J Biol Chem. 1937;117:771-776.
18. Nelson N, et al. A photometric adaption of the Somogyi method for the determination of glucose. J Biol. 1944; 153:375-380.
19. Kaplan LA. Glucose. In:LA Kaplan and AJ Pesce, eds., Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed St. Louis: The C.V. Mosby Company;1989;pp.850-856.
20. Berry MN, et al. Enzymatic determination of potassium in serum. Clin Chem 1989;35:817-20.
21. Van Pelt J. Enzymatic determination of sodium, potassium and chloride in serum compared with determination by flame photometry, coulometry and ion selective electrodes. Clin Chem 1994;40:846-7.
22. Hubl W, et al. Enzymatic determination of sodium, potassium and chloride in abnormal (hemolyzed, icteric, lipemic, paraprteinemic, or uremic) serum samples compared with indirect determination with ion selective electrodes. Clin Chem 1994;40:1528-31.
23. Helgerson RC, et al. Host-guest Complexation. 50. Potassium and sodium ion-selective chromogenic ionophores. J Amer Chem Soc 1989;111:6339-50.
24. Kumar A, et al. Chromogenic ionophere-based methods for spectrophotometric assay of sodium and potassium in serum and plasma. Clin Chem 1988;34:1709-12.
25. Berry MN, et al. Enzymatic determination of sodium in serum. Clin Chem 1988;34:2295-8.

13. Bibliography (continued)

26. Skeggs LT Jr. An automatic method for the determination of carbon dioxide in blood plasma. Am J. Clin Pathol 1960;33:181-5.
27. Korzun WJ, Miller WG. Carbon Dioxide. In: Kaplan LA, Pesce AJ, eds. Clinical chemistry theory, analysis and correlation, 2nd ed. St. Louis: The CV Mosby Company, 1989:869-72.
28. Fales FW. Urea in serum, direct diacetyl monoxime method. *In*:WR Faulkner and S Meites, eds., Selected Methods of Clinical Chemistry, vol 9. Washington, DC.: American Association for Clinical Chemistry;1982:pp.365-373.
29. Van Slyke, et al. A permanent preparation of urease, and its use in the determination of urea. J Biol Chem, 1914; 19:211-228.
30. Fawcett JK, et al. A rapid and Precise method for the determination of urea. J Clin Pathol, 1960;13:156-159.
31. Chaney, et al. Urea and ammonia determinations. Clin Chem, 1962;8:130-132.
32. Talke H, et al. Enzymatische Harnstoffbestimmung in Blut and Serum im optischen Test nach Warburg. Klin Wochensch, 1965;43:174-175.
33. Hallett, et al. Reduced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. Clin Chim Acta, 1971;35:33-37.
34. Patton, et al. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal Chem, 1977;49:464-469.
35. Sampson EJ, et al. A coupled-enzyme equilibrium method for the measuring urea in serum: optimization and evaluation of the AACC study group on Urea Candidate reference method. Clin Chem, 1980;26:816-826.
36. National Committee For Clinical Laboratory Standards. Physician's office laboratory guidelines, tentative guideline, 2nd ed. NCCLS Document POL1-T2. Wayne, PA: NCCLS, 1992.
37. Scott, M.G. Electrolytes and Blood Gases. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders Company. 1999:1058-9.
38. National Committee For Clinical Laboratory Standards. Procedures for the handling and processing of blood specimens; tentative standard. NCCLS Document H18-T. Wayne, PA: NCCLS, 1984.
39. Rehak NN, Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. Clin Chem 1988; 34-2111-4.
40. Scott, M.G. Electrolytes and Blood Gases. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders Company,1999: 1065-6.
41. National Committee for Clinical Laboratory Standards. Interference testing in clinical chemistry; proposed guideline. NCCLS Document EP7-P. Wayne, PA: NCCLS, 1986.
42. Young DS. Effects of drugs on clinical laboratory tests, 3rd ed. Washington, DC: AACC Press, 1990.
43. National Committee for Clinical Laboratory Standards. How to define and determine reference intervals in the clinical laboratory, approved guidelines, 2nd ed. NCCLS Document C28-A2. Wayne, PA: NCCLS, 2000.
44. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices; approved guideline. NCCLS Document EP5-A. Wayne, PA: NCCLS, 1999.
45. National Committee for Clinical Laboratory Standards. Quality management for unit-use testing; proposed guideline. NCCLS Document EP18-P. Wayne, PA: NCCLS, 1999.
46. National Committee for Clinical Laboratory Standards. Method comparison and bias estimation using patient samples; approved guideline. NCCLS Document EP9-A. Wayne, PA: NCCLS, 1995.