Piccolo® MetLyte Plus CRP

For In Vitro Diagnostic Use and Professional Use Only
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1. Intended Use

The Piccolo® MetLyte Plus CRP reagent disc, used with the Piccolo Xpress® chemistry analyzer, is intended to be used for the *in vitro* quantitative determination of c-reactive protein (CRP), chloride, creatine kinase, creatinine, glucose, potassium, sodium, total carbon dioxide and blood urea nitrogen (BUN) in lithium heparinized whole blood, lithium heparinized plasma, or serum in a clinical laboratory setting or point-of-care location. The Abaxis CRP method is not intended for high sensitivity CRP measurement.

2. Summary and Explanation of Tests

The Piccolo® MetLyte Plus CRP Reagent Disc and the Piccolo Xpress® chemistry analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders.

C-Reactive Protein (CRP):	Infection, tissue injury, and inflammatory 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.
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

C-Reactive Protein (CRP)
Original assays for quantifying CRP were primarily for research and based on ELISA methodology.[1] More recently, latex-enhanced immunonephelometric methods have been used.[2] However; this requires a nephelometer to measure the light

scattering. Now, several automated immunoturbidimetric and immunoluminometric assays have been developed that can be run on conventional clinical chemistry analyzers.[3]

The method used by Abaxis is an enhanced latex-agglutination turbidimetric immunoassay. Sample is mixed with a suspension of mouse anti-human CRP monoclonal antibody that is bound to latex. CRP in the sample binds to the antibody-latex particles and agglutinates creating turbidity. Light scattering from the turbidity is used as a measure of CRP. Turbidity is measured as a change in absorbance at 630 nm. This absorbance change is directly proportional to the CRP in the sample.

$$\text{Anti-CRP latex particles + CRP} \longrightarrow \text{Agglutinated CRP-Anti-CRP latex particles}$$

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-4-nitrophenyl-α-D-maltotrioside (CNPG3) to 2-chloro-4-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.[4]

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

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 37ºC). Early CK measurement methods were based on the "forward reaction" with creatine phosphate and adenosine diphosphate (ADP) as the products.[5,6,7] 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.[8,9,10]

The CK measurement procedure used by Abaxis is a modified version of the International Federation of Clinical Chemistry (IFCC) method.[11] Key modifications are sample volume fraction, buffer, and temperature. N-acetyl cysteine (NAC) has been added to reactivate the CK.[12] 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.

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

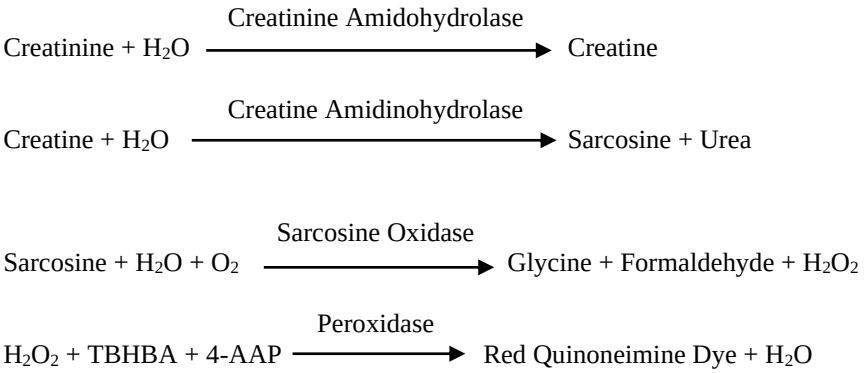
$$\text{ATP + D-glucose} \xrightarrow{\text{Hexokinase}} \text{ADP + G-6-P}$$

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

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.[13,14] Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.[15,16,17] Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.[18]



Two cuvettes are used to determine the concentration of creatinine in the sample. Endogenous creatine is measured in the blank cuvette, which is subtracted from the combined endogenous creatine and the creatine formed from the enzyme reactions in the test cuvette. Once the endogenous creatine is eliminated from the calculations, the concentration of creatinine is proportional to the intensity of the red color produced. The endpoint reaction is measured as the difference in absorbance between 550 nm and 600 nm.

eGFR (calculated)

Serum creatinine is routinely measured as an indicator of renal function. Because creatinine is influenced by age, gender and race, chronic kidney disease (CKD) may not be detected using serum creatinine alone. Thus, the National Kidney Disease Education Program strongly recommends that laboratories routinely report an estimated Glomerular Filtration Rate (eGFR) when serum creatinine is measured for patients 18 and older. Routinely reporting the eGFR with all serum creatinine determinations allows laboratories to help identify individuals with reduced kidney function and help facilitate the detection of CKD. Calculated eGFR values of <60 ml/min are generally associated with increased risk of adverse outcomes of CKD.

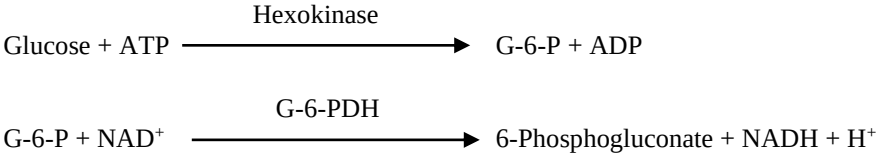
Calculation of the eGFR is performed by the Piccolo using the patient's age, gender and race. The Piccolo method for creatinine is traceable to the IDMS reference method for creatinine so that the following form of the MDRD equation for calculating the eGFR can be used.

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

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu[19] and Somogyi-Nelson[20,21]). 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 Plus CRP reagent disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.[22]

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.



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.[23,24,25] Interference from sodium and ammonium ion are minimized with the addition of Kryptofix and glutamine synthetase respectively.[23]

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

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

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

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

Sodium (NA^+)

Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.[26,27,28] 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.

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

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.[29,30] The enzymatic method is well suited for use on a routine blood chemistry analyzer without adding complexity.

In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide (CO_2) toward bicarbonate (HCO_3^-). Phosphoenolpyruvate (PEP) and HCO_3^- then react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MDH) catalyzes the reaction of oxaloacetate and reduced nicotinamide adenine dinucleotide (NADH) to NAD^+ and malate. The rate of change in absorbance due to the conversion of NADH to NAD^+ is directly proportional to the amount of tCO_2 in the sample.

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

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

Blood Urea Nitrogen (BUN)

Urea can be measured both directly and indirectly. The diacetyl monoxime reaction, the only direct method to measure urea, is commonly used but employs dangerous reagents.[31] Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.[32] The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique.[33,34] and coupled enzymatic reactions.[35,36] Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.[37] 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.[38]

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

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

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

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

4. Principle of Operation

See the Piccolo Xpress® chemistry analyzer Operator's Manual, for the Principles and Limitations of the Procedure.

5. Description of Reagents

Reagents
Each Piccolo® MetLyte Plus CRP 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 c-reactive protein (CRP), 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 (TBHBA)	188 µg
2-Chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3)	52.5 µg
4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]trisocosane (Kryptofix 221)	84 µg
4-Aminoantipyrine hydrochloride	13 µg
Adenosine-5'-diphosphate	38 µg
Adenosine-5'-monophosphate	33 µg
Adenosine-5'-triphosphate	11 µg
Amylase	0.0357 U
Anti-human CRP coated latex (mouse)	67.2 µg
Anti-human CRP (goat)	0.3 µg
Ascorbate oxidase (*Cucurbita spp.*)	0.3 U
Calcium acetate	25.2 µg
Citric acid, trisodium salt	567 µg
Creatine amidinohydrolase (*Actinobacillus spp*.)	3 U
Creatine phosphate	122 µg
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) (yeast)	0.1 U
Glutamate dehydrogenase	0.1 U
Glutamine synthetase	0.2 U
Hexokinase (yeast)	0.2 U
Imidazole	26 µg
Lactate dehydrogenase (chicken heart)	0.3 U
Magnesium acetate	60 µg
Magnesium sulfate	29 µg
Malate dehydrogenase (porcine heart)	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-Nicotinamide adenine dinucleotide, (NAD)	20 µg

Table 1: Reagents (continued)

Component	Quantity/Disc
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.[39] See 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 Xpress® chemistry analyzer Operator's Manual. A disc not used within 20 minutes of opening the pouch should be discarded.

Storage

Store reagent discs in their sealed pouches at 2-8°C (36-46°F). Do not expose opened or unopened discs to direct sunlight or temperatures above 32°C (90°F). Reagent discs may be used until the expiration date included on the package. The expiration date is also encoded in the bar code printed on the bar code ring. An error message will appear on the Piccolo Xpress® chemistry analyzer Display if the reagents have expired.

Indications of Reagent Disc Instability/Deterioration

A torn or otherwise damaged pouch may allow moisture to reach the unused disc and adversely affect reagent performance. Do not use a disc from a damaged pouch.

6. Instrument

See the Piccolo Xpress® chemistry analyzer Operator's Manual for complete information on use of the analyzer.

7. Sample Collection and Preparation

Sample collection techniques are described in the "Sample Collection" section of the Piccolo Xpress® chemistry analyzer Operator's Manual.

- The minimum required sample size is ~100 µL of lithium heparinized whole blood, lithium heparinized plasma, serum or control material. The reagent disc sample chamber can contain up to 120 µL of sample.

- Whole blood samples obtained by venipuncture must be homogeneous before transferring a sample to the reagent disc. Gently invert the collection tube several times just prior to sample transfer. Do not shake the collection tube; shaking may cause hemolysis.

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

- Hemolysis may cause erroneously high results in **potassium** assays. This problem may go undetected when analyzing whole blood (release of potassium from as few as 0.5% of the erythrocytes can increase the potassium serum level by 0.5 mmol/L). In addition, even unhemolyzed specimens that are not promptly processed may have increased potassium levels due to intracellular potassium leakage.[40]

- Whole blood venipuncture samples should be run within 60 minutes of collection.[41] **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.[42]

- Refrigerating whole blood samples can cause significant changes in concentration of **creatinine** and **glucose**.[43] 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/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.[44]

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

8. Procedure

Materials Provided
- One Piccolo® MetLyte Plus CRP PN: 400-1034 (a box of discs PN 400-0034)

Materials Required but not Provided
- Piccolo Xpress® chemistry analyzer
- Sample transfer pipettes (fixed volume approximately 100 µL) and tips are provided with each Piccolo Xpress® chemistry analyzer and may be reordered from Abaxis.
- Commercially available control reagents recommended by Abaxis (contact Abaxis Technical Support for approved control materials and expected values).
- Timer

Test Parameters
The Piccolo Xpress® chemistry analyzer operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each Piccolo® MetLyte Plus CRP 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 Xpress® chemistry analyzer Operator's Manual.

Calibration
The Piccolo Xpress® chemistry analyzer is calibrated by the manufacturer before shipment. The bar code printed on the bar code ring provides the analyzer with disc-specific calibration data. See the Piccolo Xpress® chemistry analyzer Operator's Manual.

Quality Control
See the Piccolo Xpress® chemistry analyzer Operator's Manual, for a detailed discussion on running, recording, interpreting, and plotting control results.

9. Results

The Piccolo Xpress® chemistry analyzer automatically calculates and prints the analyte concentrations in the sample. Details of the endpoint and rate reaction calculations are found in the Piccolo Xpress® chemistry analyzer Operator's Manual.

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

10. Limitations of Procedure

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

- The only anticoagulant **recommended for use** with the Piccolo Xpress® chemistry analyzer 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 Plus CRP 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.

- CRP is an "acute-phase" protein and rises non-specifically in response to inflammation. Intra-individual variation of c-reactive protein are significant (30 to 60%) and should be taken into account when interpreting values.[1] Serial measurements may be required to estimate the true mean of c-reactive protein in any specific individual.

- HAMA (human anti-mouse antibodies) up to 115 ng/mL do not interfere. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain HAMA.

- Rheumatoid factor up to a concentration of 644 U/mL do not interfere.

- No high dose hook effect (prozone effect) was observed in this assay at CRP concentrations up to 1,000 mg/L.

- **Any result for a particular test that exceeds the assay range should be analyzed by another approved test method or sent to a referral laboratory. Do not dilute the sample and run it again on the Piccolo Xpress® chemistry analyzer.**

Warning: Extensive testing of the Piccolo Xpress® chemistry analyzer 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 CLSI EP7-A.[45]

Effects of Endogenous Substances
- Physiological interferents (hemolysis, icterus and lipemia) cause changes in the reported concentrations of some analytes. The sample indices are printed on the bottom of each printout to inform the operator about the levels of interferents present in each sample.

- The Piccolo Xpress® chemistry analyzer suppresses any results that are affected by >10% interference from hemolysis, lipemia or icterus. "HEM", "LIP", or "ICT" respectively, is printed on the printout in place of the result.

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

- For the CRP method, the levels of endogenous substances that trip the HEM, LIP, or ICT suppressions are 750 mg/dL for hemoglobin, 750 mg/dL for lipemia, and 35 mg/dL for bilirubin.

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

Table 2: Exogenous and Therapeutic Substances Evaluated

Potential Interferent	Highest Concentration Tested (mg/dL unless otherwise specified)
Acetaminophen	100
Acetoacetate	102
Acetylsalicylic Acid	50
Ampicillin	30
Ascorbic acid	20
Caffeine	10
Calcium Chloride	20
Cephalothin (Keflin)	400
Chloramphenicol	100
Cimetidine	16
Dopamine	19
Epinephrine	1
Erythromycin	10
Glutathione	30
Hydrochlorothiazide	7.5
Ibuprofen	50
Isoniazide	4
α-Ketoglutarate	5
Ketoprofen	50
L-dopa	5
Lidocaine	1
Lithium Lactate	84
Methicillin	100
Methotrexate	0.5
Metronidazole	5
Nafcillin	1
Nitrofurantoin	20
Oxacillin	1
Oxaloacetate	132
Penicillin G	100
Phenytoin (5,5-Diphenylhydantion)	3
Proline	4
Pyruvate	44
Rifampin	0.5
Salicylic Acid	50
Sulfadiazine	150
Sulfanilamide	50
Theophylline	20

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
C-Reactive Protein		
Glutathione	30	13% dec.
Isoniazide	4	16% dec.
L-dopa	5	28% dec.
Oxaloacetate	132	57% dec.
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 for electrolytes on the Piccolo Blood Chemistry Analyzer and 69 adult males and females were analyzed on the Piccolo Xpress® chemistry analyzer for CRP to determine the reference intervals. The electrolyte ranges were calculated based on the 95% reference interval estimated from the combined (overall) values obtained from the reference subjects and the CRP range was based on demonstrated transferability of the reference intervals from the Beckman.[47] 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
C-Reactive Protein	< 7.5 mg/L	< 7.5 mg/L
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/L

12. Performance Characteristics

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

Table 5: Piccolo Dynamic Ranges

Analyte	Common Units	SI Units
C-Reactive Protein	5.0-200.0 mg/L	5.0-200.0 mg/L
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/L

If the analyte concentration is above the measuring range (dynamic range), but less than the system range, the data tape will indicate a ">" sign at the upper limit and an asterisk after the number, e.g. GLU >700* U/L. If lower than the dynamic range, a "<" will be printed with an asterisk, e.g. GLU <10* 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 data tape, collect a new sample and rerun the test. If results for the second sample are suppressed again, please call Abaxis Technical Support.

Sensitivity
The lower limit of the reportable (dynamic) range for each analyte is: c-reactive protein 5.0 mg/L; 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/L).

Precision

Precision studies were conducted using CLSI EP5-A guidelines[48] with modifications based on CLSI EP18-A[49] for unit-use devices. Results for within-run and total precision were determined using two levels of commercially available control materials and in the case of potassium two levels of plasma pools. The studies made use of multiple instruments. Two reagent disc lots were used for the electrolytes and one for c-reactive protein. 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; c-reactive protein (serum 1, control 1 and control 2) and chloride testing was done at two sites over a period of five days. C-reactive protein serums 2 and 3 and plasmas 1 and 2 were done at one site over a period of five days. Potassium testing was conducted at a CLIA waived site making use of three analyzers, one lot of reagent discs, and two operators over five days.

Results of precision studies are shown in Table 6.

Table 6: Precision

Analyte	Sample Size	Within-Run	Total
C-Reactive Protein (mg/L)			
Serum 1	N = 80		
Mean		8.3	8.3
SD		0.70	0.81
%CV		8.4	9.8
Serum 2	N = 40		
Mean		8.1	8.1
SD		0.49	0.51
%CV		6.1	6.3
Serum 3	N = 40		
Mean		8.8	8.8
SD		0.54	0.54
%CV		6.2	6.2
Plasma 1	N = 40		
Mean		34.5	34.5
SD		1.04	1.09
%CV		3.0	3.2
Plasma 2	N = 40		
Mean		105.5	105.5
SD		2.06	2.30
%CV		1.9	2.2
Control 1	N = 80		
Mean		33.0	33.0
SD		1.21	2.12
%CV		3.7	6.4
Control 2	N = 80		
Mean		108.0	108.0
SD		1.88	3.14
%CV		1.7	2.9
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

Table 6: Precision (continued)

Analyte	Sample Size	Within-Run	Total
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 = 150		
Control 1			
Mean		3.2	3.2
SD		0.09	0.11
%CV		2.8	3.3
Control 2	N = 149		
Mean		6.2	6.2
SD		0.09	0.10
%CV		1.4	1.7
Plasma Pool 1	N= 150		
Mean		3.2	3.2
SD		0.07	0.09
CV		2.3	2.9
Plasma Pool 2	N= 150		
Mean		5.4	5.4
SD		0.09	0.10
CV		1.6	1.9
Sodium (mmol/L)	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

Table 6: Precision (continued)

Analyte	Sample Size	Within-Run	Total
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

Whole Blood Precision for Potassium

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

Table 7: Whole Blood Precision for Potassium

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

Lithium heparinized whole blood and serum samples were collected and assayed on the Piccolo Blood Chemistry Analyzer and by comparative methods for chloride, 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. For CRP, lithium heparinized plasma samples were tested by the Piccolo Xpress® chemistry analyzer and a comparative method.

Representative correlation statistics are shown in Table 7.

Table 7: Correlation of Piccolo Blood Chemistry Analyzer or Piccolo Xpress® chemistry analyzer (for CRP) with Comparative Method(s)

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
C-Reactive Protein (mg/L)	0.998	0.990	-0.4	4.6	113	5.4-198.6	Beckman
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) Whole Blood (waived laboratory)	0.984	0.99	0.13	0.10	130	1.3-9.5	Siemens VISTA Plasma
Potassium (mmol/L) Whole Blood (moderately complex laboratory)	0.984	0.98	0.12	0.18	178	1.5-8.6	Siemens VISTA Plasma
Potassium (mmol/L) Serum (moderately complex laboratory	0.99	0.98	0.06	0.14	178	1.4-8.5	Siemens VISTA Serum
Sodium (mmol/L)	0.937	0.782	27.7	3.79	113	116-154	Radiometer KNA™ 2
Total Carbon Dioxide (mmol/L)	0.947	0.903	2.4	0.84	60	6-39	Cobas Fara
Blood Urea Nitrogen (mg/dL)	0.964	0.923	0.5	1.08	251	6-52	Paramax®
	0.983	0.946	0.0	0.66	92	6-38	Beckman

It should be noted that serum will typically give higher results for K+ compared to whole blood or plasma for physiological reasons. The variation can range from approximately 0.2 to 0.9mmol/L ans is dependent on a number of factors. The primary effect is dependent upon the number of blood cells present in the patient sample.[82]

Table 8: Sample-type Correlation for CRP
For the CRP test method the Deming regression analysis for sample-type gave the following results for n = 21.

Y Axis	X Axis	R^2	Slope	Intercept
Lithium Heparinized Plasma	Lithium Heparinized Whole Blood	1.000	0.995	0.2
Serum	Lithium Heparinized Whole Blood	0.999	1.005	0.5
Serum	Lithium Heparinized Plasma	0.999	1.010	0.3

No significant differences between lithium heparinized whole blood, lithium heparinized plasma, and serum results were observed for CRP.

13. Symbols

Use By

Catalog Number

Batch Code

In Vitro Diagnostic
Medical Device

Consult Instructions
For Use

Manufacturer

Do Not Reuse

X Number of Test
Devices in Kit

Manufacturing
Sequence

Serial Number

Caution

Temperature
Limitation

PN:
Part Number

Authorized
Representative
In the European
Community

Denotes conformity to specified
European directives

UDI Barcode structure in
Health Industry Bar Code
(HIBC) standard format

Unique Device Identifier (UDI) in
human and machine-readable
form used to adequately identify
medical devices through their
distribution and use

Separate waste collection for this
electronic item indicated; Equipment
manufactured / placed on the market
after 13 August 2005; Indicates
compliance with Article 14(4) of
Directive 2012/19/EU (WEEE) for the
European Union (EU).

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