Piccolo® MetLac 12 Panel

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

The Piccolo® MetLac 12 Panel reagent disc, used with the Piccolo Xpress® chemistry analyzer, is intended to be used for the *in vitro* quantitative determination of albumin, calcium, chloride, creatinine, glucose, lactate, magnesium, phosphorus, potassium, sodium, total carbon dioxide and blood urea nitrogen (BUN) in lithium heparinized whole blood or lithium heparinized plasma in a clinical laboratory setting or point-of-care location.

2. Summary and Explanation of Tests

The Piccolo MetLac 12 Panel reagent disc and the Piccolo Xpress chemistry analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders:

Albumin:	Liver and kidney disease.
Calcium:	Parathyroid, bone and chronic renal diseases; tetany.
Chloride:	Dehydration, prolonged diarrhea and vomiting, renal tubular disease, hyperparathyroidism, burns, salt-losing renal diseases, overhydration and thiazide therapy.
Creatinine:	Renal diseases and monitoring of renal dialysis.
Glucose:	Carbohydrate metabolism disorders, including adult and juvenile diabetes mellitus and hypoglycemia.
Lactate:	Lactate measurements are used in the diagnosis and treatment of lactate acidosis, monitoring tissue hypoxia, and diagnosis of hyperlactatemia.
Magnesium:	Hypomagnesemia and hypermagnesemia.
Phosphorus:	Dehydration, diabetes, parathyroidism, and renal disease.
Potassium:	Renal glomerular or tubular disease, adrenocortical insufficiency, diabetic ketoacidosis, 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 the sense of thirst, skin loss, burns, sweating, hyperaldosteronism, CNS disorders, dilutional, depletional and delusional hyponatremia and ADH secretion syndrome.
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

Albumin (ALB)
Early methods used to measure albumin include fractionation techniques[1,2,3] and tryptophan content of globulins.[4,5] These methods were unwieldy to perform and did not have a high specificity. Two immunochemical techniques are considered as reference methods, but are expensive and time consuming.[6] Dye binding techniques are the most frequently used methods for measuring albumin. Bromocresol green (BCG) is the most commonly used of the dye binding methods but may over-estimate albumin concentration, especially at the low end of the normal range.[7] Bromocresol purple (BCP) is the most specific of the dyes in use.[8,9]

Bromcresol purple (BCP), when bound with albumin, changes color from a yellow to blue color. The absorbance maximum changes with the color shift.

$$\text{BCP + Albumin} \xrightarrow[\text{Acid pH}]{\text{Surfactants}} \text{BCP-Albumin Complex}$$

Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as absorbance at 600 nm.

Calcium (CA)
The first methods used to analyze calcium involved precipitating calcium with an excess of anions.[10,11,12] Precipitation methods are laborious and often imprecise. The reference method for calcium is atomic absorption spectroscopy; however, this method is not suited for routine use.[13] Spectrophotometric methods using either *o*-cresolphthalein complexone (CPC) or arsenazo III metallochromic indicators are most commonly used.[14,15,16] Arsenazo III has a high affinity for calcium and is not temperature dependent as is CPC.

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

$$Ca^{2+} + \text{Arsenazo III} \longrightarrow Ca^{2+}\text{-Arsenazo III Complex}$$

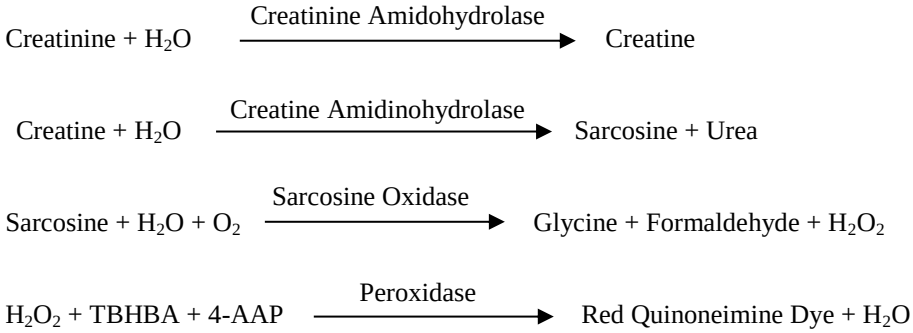
The endpoint reaction is monitored at 405 nm, 467 nm, and 600 nm. The amount of total calcium in the sample is proportional to the absorbance.

Chloride (CL^-)
The Abaxis chloride method is based on the determination of chloride-dependent activation of α-amylase activity. Deactivated α-amylase is reactivated by addition of the chloride ion, allowing the calcium to re-associate with the enzyme. The reactivation of α-amylase activity is proportional to the concentration of chloride ions in the sample. The reactivated α-amylase converts the substrate, 2-chloro-*p*-nitrophenyl-α-D-maltotrioside (CNPG3) to 2-chloro-*p*-nitrophenyl (CNP) producing color and α-maltotriose (G3). The reaction is measured bichromatically and the increase absorbance is directly proportional to the reactivated α-amylase activity and the concentration of chloride in the sample.[17]

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

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.[18,19] Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.[20,21,22] Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.[23]

In the coupled enzyme reactions, creatinine amidohydrolase hydrolyzes creatinine to creatine. A second enzyme, creatine amidinohydrolase, catalyzes the formation of sarcosine from creatine. Sarcosine oxidase causes the oxidation of sarcosine to glycine, formaldehyde and hydrogen peroxide (H_2O_2). In a Trinder reaction, peroxidase catalyzes the reaction among the hydrogen peroxide, 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) and 4-aminoantipyrine (4-AAP) into a red quinoneimine dye. Potassium ferrocyanide and ascorbate oxidase are added to the reaction mixture to minimize the potential interference of bilirubin and ascorbic acid, respectively.



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

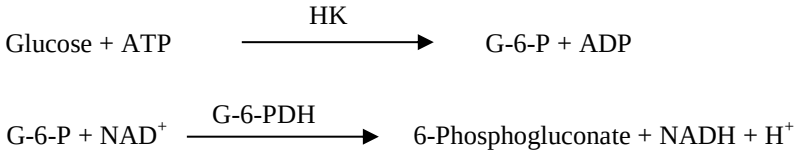
eGFR (calculated) Serum creatinine is routinely measured as an indicator of renal function. Because creatinine is influenced by age, gender and race, chronic kidney disease (CKD) may not be detected using serum creatinine alone. Thus, the National Kidney Disease Education Program strongly recommends 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.[24,25,26]

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[27] and Somogyi-Nelson[28,29]). The lack of specificity in copper-reduction techniques led to the development of quantitative procedures using the enzymes hexokinase and glucose oxidase. The glucose test incorporated into the Piccolo MetLac 12 Panel reagent disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.[30]

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



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

Lactate (LAC)
In the Abaxis method, lactate (LAC) is oxidized by lactate oxidase (LOX) to pyruvate and hydrogen peroxide (H_2O_2). Peroxidase catalyzes the reaction of H_2O_2, 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBSA) to form a red quinoneimine dye.

$$\text{Lactate} + O_2 \xrightarrow{\text{LOX}} \text{Pyruvate} + H_2O_2$$

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

The rate of formation of the red dye is proportional to the LAC concentration in the sample. The reaction is measured bichromatically at 515 nm and 600 nm.

Magnesium (MG)
The hexokinase (HK) activation method for magnesium is the best-fit method for the Piccolo system in terms of sensitivity, precision, and accuracy.[31] The enzymatic magnesium method can be described as :

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{HK}} \text{G-6-P} + \text{ADP}$$

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

The rate limiting reaction is the HK reaction. Magnesium from the sample activates HK, which in turn catalyzes the break down of glucose to form glucose-6-phosphate (G-6-P) and ADP. G-6-P reacts with nicotinamide adenine dinucleotide phosphate ($NADP^+$) to form reduced nicotinamide adenine dinucleotide phosphate (NADPH) and 6-phosphogluconate in the presence of glucose-6-phosphate-dehydrogenase (G-6-PDH). This is a first-order rate reaction. The rate of production of NADPH is directly proportional to the amount of magnesium present in the sample. Absorbance is measured bichromatically at 340 nm and 405 nm.

Phosphorus (PHOS)
The most applicable enzymatic method for the Abaxis system uses sucrose phosphorylase (SP) coupled through phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6PDH).[32,33] Using the enzymatic system for each mole of phosphorus present in the sample, one mole of NADH is formed. The amount of NADH formed can be measured as an endpoint at 340 nm.

$$\text{Sucrose} + P_i \xrightarrow{\text{SP}} \text{Glucose-1-Phosphate (G-1-P)} + \text{Fructose}$$

$$\text{G-1-P} \xrightarrow{\text{PGM, } Mg^{2+}} \text{G-6-P}$$

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

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.[34,35,36] Interference from sodium and ammonium ions are minimized with the addition of Kryptofix and glutamine synthetase respectively.[34]

In the coupled-enzyme reaction, pyruvate kinase (PK) dephosphorylates phosphoenolpyruvate to form pyruvate. Lactate dehydrogenase (LDH) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+. The rate of change in absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD^+ and is directly proportional to the amount of potassium in the sample.

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

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

Sodium (NA^+)
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.[37,38,39] In the Abaxis enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyses the reaction o-nitrophenyl-β-galactopyranoside (ONPG) to o-nitrophenyl and galactose.

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

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.[40,41] 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) to 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.[42] Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.[43] The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique [44,45] and coupled enzymatic reactions.[46,47] Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.[48] 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.[49]

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 MetLac 12 Panel reagent d isc contains dry test-specific reagent beads (described below). A dry sample blank reagent (comprised of buffer, surfactants, excipients, and preservatives) is included in each disc for use in calculating concentrations of albumin (ALB), chloride (CL^-), calcium (CA), glucose (GLU), lactate (LAC), magnesium (MG), phosphorus (PHOS), 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

Components	Quantity/Disc
N-Acetyl cysteine	60 µg
Adenosine 5'-diphosphate	36 µg
Adenosine-5'-diphosphate, Lithium salt	40 µg
Adenosine 5'-triphosphate	22 µg
Adenosine 5'- Triphosphate, Disodium Salt	28 µg
α-Ketoglutaric acid	19 µg
4-Aminoantipyrine	27 µg
4-Aminoantipyrine hydrochloride	4 µg
Amylase	0.036 U
Arsenazo III, sodium salt	1.7 µg
Ascorbate oxidase (*Cucurbita spp.*)	0.3 U
Brij	3 µg
Bromocresol purple, sodium salt	0.2 µg
Calcium acetate	25 µg
Citric acid	735 µg
Citric acid, trisodium salt	567 µg
2-Chloro-4-nitrophenyl-α-maltotrioside (CNPG3)	53 µg
Creatine amidinohydrolase (*Actinobacillus spp.*)	3 U
Creatinine amidohydrolase (*Pseudomonas spp.*)	1 U
3,5-Dichloro-2-hydroxy-benzenesufonic acid, sodium salt (DHBSA)	31 µg
Ethylenediaminetetraacetic acid (EDTA)	182 µg
Ethylenediaminetetraacetic acid (EDTA), disodium salt	17 µg
Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)	19 µg
β-Galactosidase	0.005 U
Glucose-1,6-diphosphate	1 µg
L-Glutamic acid	9.2 µg
Glucose-6-Phosphate Dehydrogenase	0.1 U
Glutamate dehydrogenase	0.1 U
Glutamine synthetase	0.17 U
Hexokinase	0.1 U
Imidazole	29 µg
Lactate dehydrogenase (chicken heart)	0.13 U
Lactate oxidase	0.002 U
Lithium hydroxide, monohydrate	23 µg
Magnesium acetate, tetrahydrate	67 µg
Magnesium Chloride, hexahydrate	2 µg
Magnesium sulfate	33 µg
Malate dehydrogenase	0.1 U

Table 1: Reagents (continued)

Components	Quantity/Disc
Manganese chloride	10 µg
D-Mannitol	1051 µg
Methylated Cyclodextrin	314 µg
2-Methyl-4-isothizolin-3-one hydrochloride (MIT)	4.2 µg
β-Nicotinamide Adenine Dinucleotide (NAD)	83 µg
β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt (NADP)	30 µg
ß-Nicotinamide adenine dinucleotide, reduced (NADH)	36 µg
o-Nitrophenyl-β-D-galactopyranoside (ONPG)	22 µg
n-Octylglucoside	31 µg
4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]tricosane (Kryptofix 221)	86 µg
Peroxidase (horseradish)	1 U
Phosphoenol pyruvate	57 µg
Phosphoenol pyruvate carboxylase	0.001 U
Phosphoglucomutase	0.035 U
Pluronic F68	1 µg
Polyethylene glycol, 8000	4 µg
Potassium ferrocyanide	0.7 µg
Pyruvate kinase	0.01 U
Sarcosine oxidase (microorganism)	1 U
Sucrose	74 µg
Sucrose phosphorylase	0.07 U
Sodium chloride	69 µg
2,4,6-Tribromo-3-hydroxybenzoic acid (TBHBA)	188 µg
Triethanolamine hydrochloride	214 µg
Triton X-100	26 µg
Urease (jack bean)	0.05 U
Buffers, surfactants, excipients and preservatives	

Warnings and Precautions

- For *in vitro* Diagnostic Use
- The diluent container in the reagent disc is automatically opened when the analyzer drawer closes. A disc with an opened diluent container cannot be re-used. Ensure that the sample or control has been placed into the disc before closing the drawer.
- Used reagent discs contain human body fluids. Follow good laboratory safety practices when handling and disposing of used discs.[50] See the Piccolo Xpress chemistry analyzer Operator's Manual for instructions on cleaning biohazardous spills.
- The reagent discs are plastic and may crack or chip if dropped. Never use a dropped disc as it may spray biohazardous material throughout the interior of the analyzer.
- Reagent beads may contain acids or caustic substances. The operator does not come into contact with the reagent beads when following the recommended procedures. In the event that the beads are handled (e.g., cleaning up after dropping and cracking a reagent disc), avoid ingestion, skin contact, or inhalation of the reagent beads.

Instructions for Reagent Handling

Reagent discs may be used directly from the refrigerator without warming. Do not allow discs to remain at room temperature longer than 48 hours prior to use. Open the sealed foil pouch and remove the disc, being careful not to touch the bar code ring located on the top of the disc. Use according to the instructions provided in the Piccolo 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 heparinized whole blood, heparinized plasma, 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 leakage.[51]

- Whole blood venipuncture samples for **lactate** determination require that the patient should be at rest for 2 hours, avoid any forearm exercise, and the blood should be obtained either without a tourniquet or immediately after the tourniquet is applied. Lactate should be analyzed or blood cells separated from the plasma as soon as possible after collection. Lactate in whole blood increases rapidly because of glycolysis.[52] Blood lactate increases by 0.01 to 0.02 mmol/L/minute at room temperature in the absence of antiglycolytic agents.[53]

- For all other methods, whole blood venipuncture samples should be run within 60 minutes of collection.[54,55] **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.[56]

- Refrigerating whole blood samples can cause significant changes in concentrations of **creatinine** and **glucose**.[57] 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.

- 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.[58]

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

8. Procedure

Materials Provided
- One Piccolo MetLac 12 Panel PN: 400-1037 (a box of discs PN 400-0037)

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 MetLac 12 Panel reagent disc is less than 14 minutes. The analyzer maintains the reagent disc at a temperature of 37°C (98.6°F) over the measurement interval.

Test Procedure
The complete sample collection and step-by-step operating procedures are detailed in the Piccolo 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 reagent disc 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. Abaxis recommends control testing to follow federal, state, and local guidelines.

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 analyzer Operator's Manual. Results are printed onto paper rolls supplied by Abaxis. The paper roll has 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 perator's Manual.
- The only anticoagulant **recommended for use** with the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer is **lithium heparin**. Abaxis has performed studies demonstrating that EDTA, fluoride, oxalate, and any anticoagulant containing ammonium ions will interfere with at least one chemistry contained in the Piccolo MetLac 12 Panel 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. The plasma can then be re-run in a new reagent disc.

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

  Warning: Extensive testing of the Piccolo Xpress chemistry analyzer has shown that, in very rare instances, sample dispensed into the reagent disc may not flow smoothly into the sample chamber. Due to the uneven flow, an inadequate quantity of sample may be analyzed and several results may fall outside the reference ranges. The sample may be re-run using a new reagent disc.

Interference
Substances were tested as interferents with the analytes. For lactate human plasma pools (for all other analytes human serum pools) were prepared. The concentration at which each potential interferent was tested was based on the testing levels in CLSI (formerly NCCLS) EP7-P[59] and CLSI EP7-A2.[60]

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 tape 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.

- 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

For lactate, 41 exogenous and therapeutic substances were selected as potential interferents for Abaxis test methods based on recommendations by Young.[61] For all other methods, 35 exogenous and therapeutic substances were selected and tested. Significant interference is defined as greater than ± 10% shift in the result for a normal range specimen. For lactate, human plasma pools were supplemented with known concentrations of the drugs or chemicals and then analyzed. For all other methods, human serum pools were used. 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
Bromide*	30
Caffeine	10
Cephalothin (Keflin)	400
Chloramphenicol	100
Cimetidine	16
Digoxin*	5
D-lactate*	45
Dopamine	13
Epinephrine	1
Erythromycin	10
Glucose*	700
Glutathione	30
Glycolic acid*	7.6
Hydrochlorothiazide	7.5
Hydroxyurea*	0.7
Ibuprofen	50
Isoniazide	4
Ketoprofen	50

Table 2: Exogenous and Therapeutic Substances Evaluated (continued)

Potential Interferent	Highest Concentration Tested (mg/dL unless otherwise specified)
L-dopa	5
Lidocaine	1
Lithium Lactate**	84
Metformin*	500
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
Uric Acid*	30

*Tested for lactate only
**Omitted for lactate only

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
Albumin		
Acetoacetate	102	18% dec
Ampicillin	30	12% dec
Caffeine	10	14% dec
Calcium chloride	20	17% dec
Cephalothin (Keflin)	400	13% inc
Ibuprofen	50	28% inc
α-Ketoglutarate	5	11% dec
Nitrofurantoin	20	13% dec
Proline	4	12% inc
Sulfadiazine	10	14% dec
Sulfanilamide	50	12% dec
Theophylline	20	11% dec
Creatinine		
Ascorbic acid	20	11% dec.
Dopamine	19	80% dec.
L-dopa	5	71% dec.
Epinephrine	1	45% dec.
Glutathione	30	13% dec.

Table 3: The following substances showed greater than ± 10 % shift in the result for a normal range specimen.

(continued)

	Concentration Which Produces > 10% Interference	% Interference[A] Observed
Glucose		
Oxaloacetate	132	11% dec.
Pyruvate	44	13% dec.
Lactate		
Dopamine	13	85% dec.
Dopamine	0.52	Not significant
L-dopa	5	49% dec.
L-dopa	0.50	Not significant
Magnesium	None	None
Phosphorus		
Nitrofurantoin	20	19% inc.
Oxaloacetate	132	14% 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 approximately 90 – 140 adult males and females were analyzed on the Piccolo Xpress chemistry analyzer to determine the reference intervals for the following assays. These intervals are provided as a guideline only. It is recommended that your office or institution establish normal ranges for your particular patient population.[62,63]

Table 4: Piccolo Xpress chemistry analyzer Reference Intervals

Analyte	Common Units	SI Units
Albumin	3.3-5.5 g/dL	33-55 g/L
Calcium	8.0-10.3 mg/dL	2.0-2.58 mmol/L
Chloride	98-108 mmol/L	98-108 mmol/L
Creatinine	0.6-1.2 mg/dL	53-106 µmol/L
Glucose	73-118 mg/dL	4.1-6.6 mmol/L
Lactate	4.8 – 18.9 mg/dL*	0.53 – 2.10 mmol/L
Magnesium	1.6 – 2.3 mg/dL	0.66 – 0.95 mmol/L
Phosphorus (plasma)	2.2-4.1 mg/dL	0.71-1.32 mmol/L
Phosphorus (serum)	2.5-4.4 mg/dL**	0.81-1.42 mmol/L
Potassium	3.6-5.1 mmol/L	3.6-5.1 mmol/L
Sodium	128-145 mmol/L	128-145 mmol/L
Total Carbon Dioxide	18-33 mmol/L	18-33 mmol/L
Blood Urea Nitrogen (BUN)	7-22 mg/dL	2.5-7.9 mmol urea/L

* In the United States, the SI units are used for reporting lactate. To convert values in "mmol/L" to "mg/dL" multiply the value in mmol/L by the factor 9.009.
** There is no observed difference between the concentration of Phosphorus measured in heparinized whole blood and heparinized plasma. However, a small increase (0.3 mg/dL) was observed in serum when compared to heparinized whole blood and heparinized plasma. This increase is consistent with the difference between Phosphorus in serum and plasma as described in the literature.[64, 65, 66, 67]

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 Xpress chemistry analyzer Dynamic Ranges

Analyte	Common Units	SI Units
Albumin	1-6.5 g/dL	10-65 g/L
Calcium	4.0-16.0 mg/dL	1.0-4.0 mmol/L
Chloride	80-135 mmol/L	80-135 mmol/L
Creatinine	0.2-20 mg/dL	18-1768 µmol/L
Glucose	10-700 mg/dL	0.6-38.9 mmol/L
Lactate	2.7 – 90.0 mg/dL	0.30 – 9.99 mmol/L
Magnesium	0.1 – 8.0 mg/dL	0.04 – 3.3 mmol/L
Phosphorus	0.2-20 mg/dL	0.06-6.5 mmol/L
Potassium	1.5-8.5 mmol/L	1.5-8.5 mmol/L
Sodium	110-170 mmol/L	110-170 mmol/L
Total Carbon Dioxide	5-40 mmol/L	5-40 mmol/L
Blood Urea Nitrogen (BUN)	2-180 mg/dL	0.7-64.3 mmol urea/L

Sensitivity (Limits of Detection)
The lower limit of the reportable (dynamic) range for each analyte is: albumin 1 g/dL (10 g/L); calcium 4.0 mg/dL (1.0 mmol/L); chloride 80 mmol/L; creatinine 0.2 mg/dL (18 µmol/L); glucose 10 mg/dL (0.56 mmol/L); lactate 0.07 mmol/L (0.6 mg/dL)); magnesium 0.1 mg/dL (0.04 mmol/L); phosphorus 0.2 mg/dL (0.06 mmol/L); potassium 1.5 mmol/L; sodium 110 mmol/L; total carbon dioxide 5 mmol/L; and blood urea nitrogen 2.0 mg/dL (0.7 mmol urea/L).

Precision

Precision studies were conducted using CLSI (formerly NCCLS) EP5-A and CLSI EP5-A2 guidelines[68,69], with modifications based on CLSI (formerly NCCLS) EP18-A and CLSI EP18-A2 for unit-use devices. [70, 71] Results for within-run and total precision were determined by testing levels of commercially available control materials. The studies made use of multiple instruments. Precision for albumin, calcium, creatinine, glucose, sodium and urea nitrogen was performed at one site; potassium and total carbon dioxide testing was performed at two sites over 20 days; and chloride, magnesium and phosphorus testing was conducted at two sites over a period of five days. Precision for lactate was performed on controls 1, 2, and 3 at two sites using two lots of disc over a period of five days; on plasma pools at Abaxis over a period of five days; and on whole blood at three point-of-care sites by two operators, each testing 10 replicates on 10 Abaxis analyzers. Results of the precision studies are shown in Table 6.

Table 6: Precision

Analyte	Sample Size	Within-Run	Total
Albumin (g/dL)	N = 80		
Control 1			
Mean		5.6	5.6
SD		0.09	0.11
%CV		1.7	2.1
Control 2			
Mean		3.7	3.7
SD		0.07	0.11
%CV		2.0	2.9
Calcium (mg/dL)	N = 80		
Control 1			
Mean		8.6	8.6
SD		0.21	0.25
%CV		2.4	2.9
Control 2			
Mean		11.8	11.8
SD		0.39	0.40
%CV		3.3	3.4
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
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

Table 6: Precision (continued)

Analyte	Sample Size	Within-Run		Total
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
Lactate (mmol/L)	N =80			
Control 1				
Mean		1.62		1.62
SD		0.03		0.04
%CV		1.8		2.2
Control 2	N =80			
Mean		3.63		3.63
SD		0.05		0.08
%CV		1.5		2.3
Control 3	N =80			
Mean		6.99		6.99
SD		0.18		0.36
%CV		2.6		5.2
Pooled Plasma 1	N =40			
Mean		0.86		0.86
SD		0.02		0.02
%CV		1.9		1.9
Pooled Plasma 2	N =40			
Mean		6.22		6.22
SD		0.20		0.20
%CV		3.2		3.2
		Operator 1 (N = 10)	**Operator** 2 (N = 10)	**Combined** (N = 20)
Whole Blood 1 - Site 1*				
Mean		1.52	1.50	1.51
SD		0.03	0.02	0.03
%CV		2.1	1.7	2.0
Whole Blood 2 - Site 1				
Mean		0.72	0.70	0.71
SD		0.02	0.02	0.02
%CV		2.2	2.5	2.7
Whole Blood 3 - Site 1				
Mean		4.67	4.59	4.63
SD		0.17	0.10	0.15
%CV		3.7	2.3	3.1
Whole Blood 4 - Site 1				
Mean		4.13	4.17	4.15
SD		0.12	0.17	0.15
%CV		3.0	4.1	3.5

Table 6: Precision (continued)

Analyte	Sample Size	Within-Run		Total
Lactate (mmol/L)		**Operator 1** (N = 10)	**Operator 2** (N = 10)	**Combined** (N = 20)
Whole Blood 5 - Site 2*				
Mean		1.00	1.02	1.01
SD		0.03	0.02	0.03
%CV		2.8	1.7	2.5
Whole Blood 6 - Site 2				
Mean		1.09	1.12	1.10
SD		0.03	0.03	0.03
%CV		2.6	2.3	2.7
Whole Blood 7 - Site 2				
Mean		6.18	6.38	6.28
SD		0.21	0.25	0.24
%CV		3.4	3.9	3.9
Whole Blood 8 - Site 2	N =20			
Mean		5.88	5.90	5.89
SD		0.32	0.17	0.25
%CV		5.5	2.9	4.2
Whole Blood 9 - Site 3*	N =20			
Mean		0.88	0.88	0.88
SD		0.03	0.04	0.03
%CV		3.3	4.3	3.8
Whole Blood 10 - Site 3	N =20			
Mean		1.09	1.06	1.08
SD		0.03	0.03	0.03
%CV		3.0	2.9	3.2
Whole Blood 11 - Site 3	N =20			
Mean		5.93	5.86	5.89
SD		0.24	0.14	0.20
%CV		4.1	2.5	3.3
Whole Blood 12 - Site 3	N =20			
Mean		7.76	7.76	7.76
SD		0.28	0.28	0.27
%CV		3.7	3.5	3.5

* Whole Blood testing performed at three point-of-care sites by two operators with 10 repetitions per operator. Two normal samples and two elevated samples were tested by each operator.

Magnesium (mg/dL)	N =80			
Control 1				
Mean		1.9		1.9
SD		0.03		0.06
%CV		1.7		3.4
Control 2				
Mean		3.9		3.9
SD		0.04		0.10
%CV		1.0		2.6

Table 6: Precision (continued)

Analyte	Sample Size	Within-Run	Total
Phosphorus (mg/dL)	N = 80		
Control 1			
Mean		3.1	3.1
SD		0.12	0.14
%CV		3.7	4.7
Control 2			
Mean		7.3	7.3
SD		0.09	0.15
%CV		1.3	2.0
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
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

For lactate, lithium heparinized whole blood samples were collected and assayed on the Piccolo Xpress chemistry analyzer and by a comparative method. For all other methods, serum samples were collected and tested. The samples were chosen to meet the distribution values in CLSI (formerly NCCLS) EP9-A and CLSI EP09-A2-IR guidelines.[72,73]

Table 7: Correlation of Piccolo Xpress chemistry analyzer with Comparative Method

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
Albumin (g/dL)	0.854	1.001	-0.3	0.22	261	1.1-5.3	Paramax®
	0.896	0.877	-0.1	0.21	100	1.5-5.0	Beckman
Calcium (mg/dL)	0.980	0.98	-0.17	0.31	111	4.6-13.2	Beckman
Chloride (mmol/L)	0.978	0.982	-1.1	1.84	120	71-118	Vitros® 950
Creatinine (mg/dL)	0.993	0.926	0.0	0.15	260	0.4–14.7	Paramax®
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
Lactate (mmol/L)	0.996	1.02	0.08	0.19	126	0.30–9.88	i-STAT
Magnesium (mg/dL)	0.992	0.990	0.0	0.16	44	0.8 - 6.8	Inductively Coupled Plasma-Atomic Optical Emission Spectroscopy (ICP-OES)
Phosphorus (mg/dL)	0.993	1.017	-0.2	0.236	90	0.8 – 11.7	Vitros® 950
Potassium (mmol/L)	0.969	0.863	0.6	0.14	58	2.0 – 6.8	Radiometer KNA® 2
Sodium (mmol/L)	0.937	0.782	27.7	3.79	113	116 - 154	Radiometer KNA® 2
Total Carbon Dioxide (mmol/L)	0.947	0.903	2.0	0.84	60	6 – 39	Cobas® Fara
Blood Urea Nitrogen (mg/dL)	0.983	0.946	0.0	0.66	92	6 – 38	Beckman

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

Authorized Representative In the European Community

Temperature Limitation

Caution

PN:
Part Number

denotes conformity to specified European directives

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