

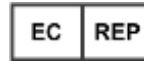
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1. Intended Use

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, <i>in vitro</i> hemolysis, hyperaldosteronism, malnutrition, hyperinsulinism, metabolic alkalosis and gastrointestinal loss.
Sodium:	Dehydration, diabetes insipidus, loss of hypotonic gastrointestinal fluids, salt poisoning, selective depression of the sense of thirst, skin loss, burns, sweating, hyperaldosteronism, CNS disorders, dilutional, depletion and delusional hyponatremia and ADH secretion syndrome.
Total Carbon Dioxide:	Primary metabolic alkalosis and acidosis and primary respiratory alkalosis and acidosis.
Blood urea nitrogen (BUN):	Renal and metabolic diseases.

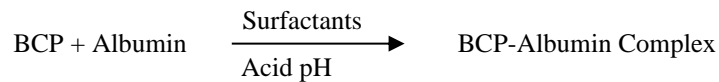
As with any diagnostic test procedure, all other test procedures including the clinical status of the patient, should be considered prior to final diagnosis.

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}

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

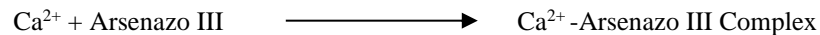


Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as absorbance at 600 nm.

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.

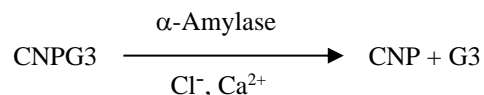
Calcium in the patient sample binds with arsenazo III to form a calcium-dye complex.



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

Chloride (CL⁻)

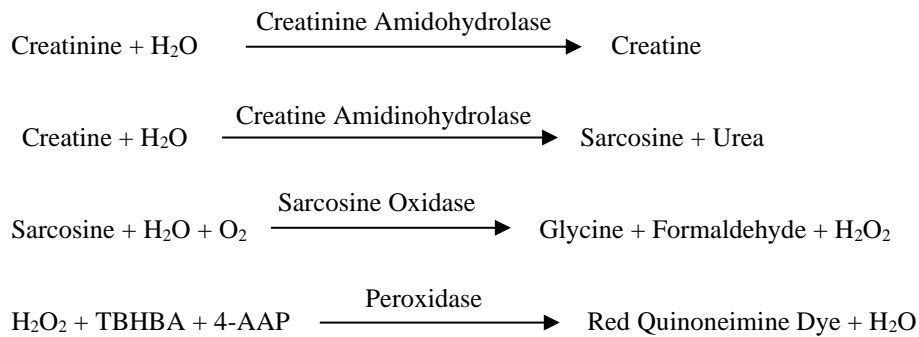
The Abaxis chloride method is based on the determination of chloride-dependent activation of α -amylase activity. Deactivated α -amylase is reactivated by addition of the chloride ion, allowing the calcium to re-associate with the enzyme. The reactivation of α -amylase activity is proportional to the concentration of chloride ions in the sample. The reactivated α -amylase converts the substrate, 2-chloro-*p*-nitrophenyl- α -D-maltotrioxide (CNP3) 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



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 (H₂O₂). In a Trinder reaction, peroxidase catalyzes the reaction among the hydrogen peroxide, 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) and 4-aminoantipyrine (4-AAP) into a red quinoneimine dye. Potassium ferrocyanide and ascorbate oxidase are added to the reaction mixture to minimize the potential interference of bilirubin and ascorbic acid, respectively.



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

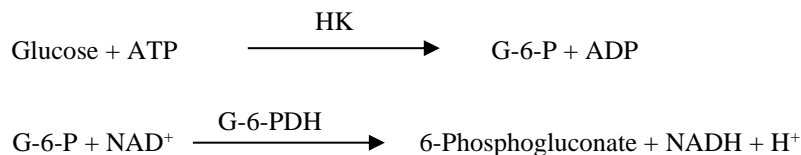
Calculation of the eGFR is performed by the Piccolo using the patient's age, gender and race. The Piccolo method for creatinine is traceable to the IDMS reference method for creatinine so that the following form of the MDRD equation for calculating the eGFR can be used.

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

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu²⁷ 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

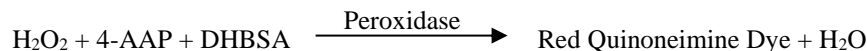
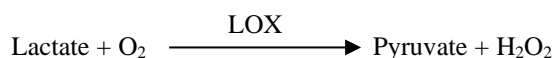
The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine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

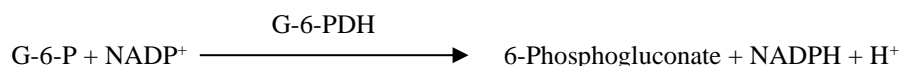
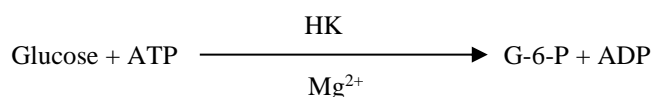
In the Abaxis method, lactate (LAC) is oxidized by lactate oxidase (LOX) to pyruvate and hydrogen peroxide (H₂O₂). Peroxidase catalyzes the reaction of H₂O₂, 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBSA) to form a red quinoneimine dye.



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)

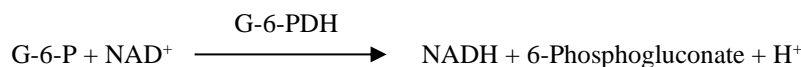
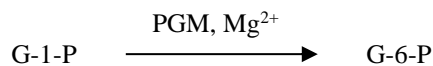
The hexokinase (HK) activation method for magnesium is the best-fit method for the Piccolo system in terms of sensitivity, precision, and accuracy.³¹ The enzymatic magnesium method can be described as :



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)

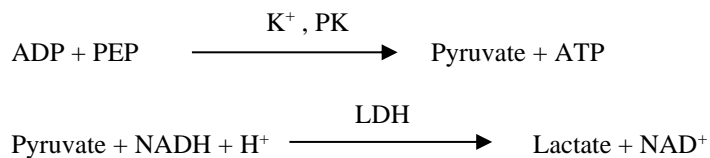
The most applicable enzymatic method for the Abaxis system uses sucrose phosphorylase (SP) coupled through phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6PDH).^{32,33} Using the enzymatic system for each mole of phosphorus present in the sample, one mole of NADH is formed. The amount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



Potassium (K⁺)

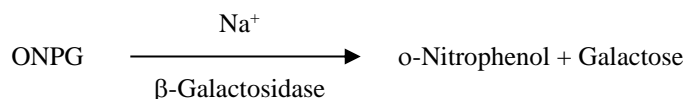
Spectrophotometric methods have been developed that allow the measurement of potassium concentration on standard clinical chemistry instrumentation. The Abaxis enzymatic method is based on the activation of pyruvate kinase with potassium and shows excellent linearity and negligible susceptibility to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



Sodium (Na⁺)

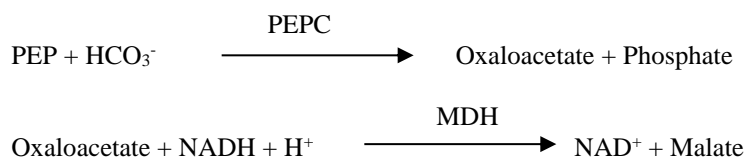
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.^{37,38,39} In the Abaxis enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyses the reaction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



Total Carbon Dioxide (tCO₂)

Total carbon dioxide in serum or plasma exists as dissolved carbon dioxide, carbamino derivatives of proteins, bicarbonate and carbonate ions and carbonic acid. Total carbon dioxide can be measured by pH indicator, CO₂ electrode and spectrophotometric enzymatic methods, which all produce accurate and precise results.^{40,41} The enzymatic method is well suited for use on a routine blood chemistry analyzer without adding complexity.

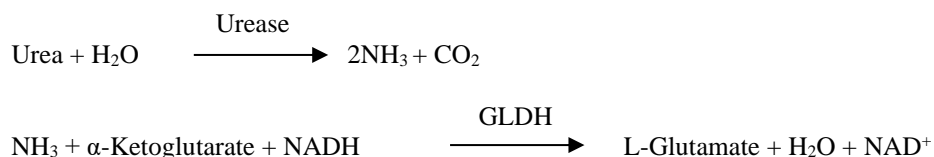
In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide (CO₂) to bicarbonate (HCO₃⁻). Phosphoenolpyruvate (PEP) and HCO₃⁻ then react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MDH) catalyzes the reaction of oxaloacetate and reduced nicotinamide adenine dinucleotide (NADH) to NAD⁺ and malate. The rate of change in absorbance due to the conversion of NADH to NAD⁺ is directly proportional to the amount of tCO₂ in the sample.



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⁺.



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 disc contains dry test-specific reagent beads (described below). A dry sample blank reagent (comprised of buffer, surfactants, excipients, and preservatives) is included in each disc for use in calculating concentrations of albumin (ALB), chloride (CL⁻), calcium (CA), glucose (GLU), lactate (LAC), magnesium (MG), phosphorus (PHOS), potassium (K⁺), sodium (NA⁺), total carbon dioxide (tCO₂) 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 (<i>Cucurbita spp.</i>)	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-maltotrioxide (CNPG3)	53 µg
Creatine amidinohydrolase (<i>Actinobacillus spp.</i>)	3 U
Creatinine amidohydrolase (<i>Pseudomonas spp.</i>)	1 U
3,5-Dichloro-2-hydroxy-benzenesulfonic 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-isothiazolin-3-one hydrochloride (MIT)	4.2 µg
β-Nicotinamide Adenine Dinucleotide (NAD)	83 µg
β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt (NADP)	30 µg
β-Nicotinamide adenine dinucleotide, reduced (NADH)	36 µg
<i>o</i> -Nitrophenyl-β-D-galactopyranoside (ONPG)	22 µg
<i>n</i> -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 μ L) 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 Operator'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-P⁵⁹ 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 $\pm 10\%$ 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	20
Bromide*	30
Caffeine	10
Calcium Chloride	20
Cephalothin (Keflin)	400
Chloramphenicol	100
Cimetidine	16
Digoxin*	5
D-lactate*	45
Dopamine	19
Epinephrine	1
Erythromycin	10
Glucose*	700
Glutathione	30
Glycolic acid*	7.6
Hydrochlorothiazide	7.5
Hydroxyurea*	0.7

Table 2: Exogenous and Therapeutic Substances Evaluated (continued)

Potential Interferent	Highest Concentration Tested (mg/dL unless otherwise specified)
Ibuprofen	50
Isoniazide	4
α -Ketoglutarate	5
Ketoprofen	50
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

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

(continued)

	Concentration Which Produces > 10% Interference	% Interference^A Observed
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.
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

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 and in the case of potassium two levels of plasma pools. 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. 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 the precision studies are shown in Table 6.

Table 6: Precision

Analyte	Sample Size	Within-Run	Total
Albumin (g/dL)	N=80		
<u>Control 1</u>			
Mean		5.6	5.6
SD		0.09	0.11
%CV		1.7	2.1
<u>Control 2</u>			
Mean		3.7	3.7
SD		0.07	0.11
%CV		2.0	2.9
Calcium (mg/dL)	N=80		
<u>Control 1</u>			
Mean		8.6	8.6
SD		0.21	0.25
%CV		2.4	2.9
<u>Control 2</u>			
Mean		11.8	11.8
SD		0.39	0.40
%CV		3.3	3.4
Chloride (mmol/L)	N=160		
<u>Control 1</u>			
Mean		97.8	97.8
SD		1.63	1.74
%CV		1.7	1.7
<u>Control 2</u>			
Mean		113.6	113.6
SD		1.97	2.22
%CV		1.7	2.0
Creatinine (mg/dL)	N=80		
<u>Control 1</u>			
Mean		1.1	1.1
SD		0.14	0.14
%CV		12.5	13.1

<u>Control 2</u>			
Mean		5.2	5.2
SD		0.23	0.27
%CV		4.4	5.2
Glucose (mg/dL)			
<u>Control 1</u>		N=80	
Mean		66	66
SD		0.76	1.03
%CV		1.1	1.6
<u>Control 2</u>			
Mean		278	278
SD		2.47	3.84
%CV	N =80	0.9	1.4
Lactate (mmol/L)			
<u>Control 1</u>			
Mean		1.62	1.62
SD		0.03	0.04
%CV	N =80	1.8	2.2
<u>Control 2</u>			
Mean		3.63	3.63
SD		0.05	0.08
%CV	N =80	1.5	2.3
<u>Control 3</u>			
Mean		6.99	6.99
SD		0.18	0.36
%CV	N =40	2.6	5.2
<u>Pooled Plasma 1</u>			
Mean		0.86	0.86
SD		0.02	0.02
%CV	N =40	1.9	1.9
<u>Pooled Plasma 2</u>			
Mean		6.22	6.22
SD		0.20	0.20
%CV		3.2	3.2
		Operator 1 (N = 10)	Operator 2 (N = 10)
<u>Whole Blood 1 - Site 1*</u>			Combined (N = 20)
Mean		1.52	1.51
SD		0.03	0.03
%CV		2.1	2.0
<u>Whole Blood 2 - Site 1</u>			
Mean		0.72	0.71
SD		0.02	0.02
%CV		2.2	2.7
<u>Whole Blood 3 - Site 1</u>			
Mean		4.67	4.63
SD		0.17	0.15
%CV		3.7	3.1
<u>Whole Blood 4 - Site 1</u>			
Mean		4.13	4.15
SD		0.12	0.15
		4.1	3.5

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

Phosphorus (mg/dL)	N = 80		
<u>Control 1</u>			
Mean		3.1	3.1
SD		0.12	0.14
%CV		3.7	4.7
<u>Control 2</u>			
Mean		7.3	7.3
SD		0.09	0.15
%CV		1.3	2.0
Potassium (mmol/L)	N = 150		
<u>Control 1</u>			
Mean		3.2	3.2
SD		0.09	0.11
%CV		2.8	3.3
<u>Control 2</u>	N = 149		
Mean		6.2	6.2
SD		0.09	0.10
%CV		1.4	1.7
<u>Plasma Pool 1</u>	N = 150	3.2	3.2
Mean		0.07	0.09
SD		2.3	2.9
CV			
<u>Plasma Pool 2</u>	N = 150	5.4	5.4
Mean		0.09	0.10
SD		1.6	1.9
CV			
Sodium (mmol/L)	N = 80		
<u>Control 1</u>			
Mean		143.5	143.5
SD		2.28	2.28
%CV		1.6	1.6
<u>Control 2</u>			
Mean		120.0	120.0
SD		2.13	2.13
%CV		1.8	1.8
Total Carbon Dioxide (mmol/L)	N=120		
<u>Control 1</u>			
Mean		21.4	21.4
SD		2.29	2.29
%CV		10.7	10.7
<u>Control 2</u>			
Mean		10.5	10.5
SD		0.90	0.90
%CV		8.6	8.6
Urea Nitrogen (mg/dL)	N = 80		
<u>Control 1</u>			
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

For lactate, lithium heparinized whole blood samples were collected and assayed on the Piccolo Xpress chemistry analyzer and by a comparative method(s). For all other methods, serum samples were collected and tested.

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) 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	2.0	0.84	60	6 – 39	Cobas® Fara
Blood Urea Nitrogen (mg/dL)	0.983	0.946	0.0	0.66	92	6 – 38	Beckman

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 and is dependent on a number of factors. The primary effect is dependent upon the number of blood cells present in the patient sample.⁸²

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

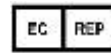


Caution



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).

14. Bibliography

1. Howe PE. The use of sodium sulfate as the globulin precipitant in the determination of proteins in blood. *J Biol Chem* 1921; 49:93-07.
2. Howe PE. The determination of proteins in blood - a micro method. *J Biol Chem* 1921; 49:109-13.
3. Wolfson WQ, et al. A rapid procedure for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin, and gamma globulin in 10 mL of serum. *Am J Clin Pathol* 1948; 18:723-30.
4. Saifer A, Gerstenfeld S, Vacsler F. Photometric microdetermination of total serum globulins by means of a tryptophan reaction. *Clin Chem* 1961; 7:626-36.
5. Saifer A, Marven T. The photometric microdetermination of serum total globulins with a tryptophan reaction: a modified procedure. *Clin Chem* 1966; 12:414-17.
6. Gendler SM. Albumin. *In: Clinical Chemistry: Theory, Analysis, and Correlation*, 2nd Ed. Kaplan LA, Pesce AJ, eds. St Louis: The C.V. Mosby Company. 1989:1029-33.
7. Webster D, Bignell AHC, Attwood EC. An assessment of the suitability of bromocresol green for the determination of serum albumin. *Clin Chim Acta* 1974; 53:101-8.
8. Louderback A, Mealey EH, Taylor NA. A new dye-binding technic using bromocresol purple for determination of albumin in serum. *Clin Chem* 1968; 14:793-794. (Abstract)
9. Pinnell AE, Northam BE. New automated dye-binding method for serum albumin determination with bromocresol purple. *Clin Chem* 1978; 24:80-86.
10. Kramer B, et al. A simple technique for the determination of calcium and magnesium in small amounts of serum. *J Biol Chem* 1921; 47:475-481.
11. Clark EP, et al. A study of the Tisdall method for the determination of blood serum calcium with suggested modification. *J Biol Chem* 1925; 63:461-464.
12. Katzman E, et al. The determination of serum calcium by titration with ceric sulfate. *J Biol Chem*.1937; 118:539-544.
13. Cali JP, et al. A reference method for the determination of total calcium in serum. *In: Selected Methods of Clinical Chemistry*, Vol 8. GR Cooper, ed. Washington, DC: American Association for Clinical Chemistry. 1997:3-8.
14. Kessler G, M Wolfman. An automated procedure for the simultaneous determination of calcium and phosphorus. *Clin Chem* 1964; 10:686-703.
15. Michaylova V, et al. Photometric determination of micro amounts of calcium with arsenazo III. *Anal Chim Acta* 1971; 53:194-198.
16. Scarpa A, et al. Metallochromic indicators of ionized calcium. *Ann NY Acad Sci* 1978; 307:86-112.
17. Ono T, et al. A new enzymatic assay of chloride in serum. *Clin Chem* 1988; 34:552-3.
18. Knoll VE, et al. Spezifische kreatininbestimmung im serum. *Z Klin Chem Klin Biochem* 1970; 8:582-587.
19. Haeckel R, et al. Simplified determinations of the "true" creatinine concentration in serum and urine. *J Clin Chem Clin Biochem* 1980; 18:385-394.
20. Moss GA, et al. Kinetic enzymatic method for determining serum creatinine. *Clin Chem* 1975; 21:1422-1426.
21. Jaynes PK, et al. An enzymatic, reaction-rate assay for serum creatinine with a centrifugal analyzer. *Clin Chem* 1982; 28:114-117.
22. Fossati P, et al. Enzymatic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. *Clin Chem* 1983; 29:1494-1496.
23. Whelton A, et al. Nitrogen metabolites and renal function. *In: Tietz Textbook of Clinical Chemistry*, 2nd Ed. Burtis CA, Ashwood ER, eds. Philadelphia: W.B. Saunders Company. 1994; 1513-1575.
24. National Kidney Disease Education Program (NKDEP). Creatinine Standardization. http://www.nkdep.nih.gov/labprofessionals/creatinine_standardization.htm (Accessed September 2010).
25. National Kidney Disease Education Program (NKDEP) Creatinine Standardization Recommendations. IVD Manufacturers. http://www.nkdep.nih.gov/labprofessionals/IVD_Manufacturers.htm (Accessed September 2010).
26. National Kidney Foundation. GFR Calculator. http://www.kidney.org/professionals/kls/gfr_calculator.cfm (Accessed September 2010).
27. Folin O, et al. A system of blood analysis. *J Biol Chem* 1919; 38:81-110.
28. Somogyi M. A reagent for the copper-iodometric determination of very small amounts of sugar. *J Biol Chem* 1937; 117:771-776.
29. Nelson N, et al. A photometric adaptation of the Somogyi method for the determination of glucose. *J Biol Chem* 1944; 153: 375-380.
30. Kaplan LA. Glucose. *In: Clinical Chemistry: Theory, Analysis, and Correlation*, 2nd Ed. Kaplan LA, Pesce AJ, eds. St Louis: The C.V. Mosby Company. 1989: 850-856.
31. Tabata M, et al. Direct Spectrophotometry of magnesium in serum after reaction with hexokinase and glucose-6-phosphate dehydrogenase. *Clin Chem* 1985; 31: 703-705.
32. Schultz DW, Passonneau JV, Lowry OH. An enzymic method for the measurement of inorganic phosphate determination. *Anal BioChem* 1967; 19:300-314.

14. Bibliography (continued)

33. Tedokon M, et al. Enzymatic assay of inorganic phosphate with use of sucrose phosphorylase and phosphoglucomutase. *Clin Chem* 1992; 38:512-515.
34. Berry MN, et al. Enzymatic determination of potassium in serum. *Clin Chem*. 1989; 35:817-820.
35. 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; 40:846-847.
36. Hubl W, et al. Enzymatic determination of sodium, potassium and chloride in abnormal (hemolyzed, icteric, lipemic, paraproteinemic, or uremic) serum samples compared with indirect determination with ion selective electrodes. *Clin Chem* 1994; 40:1528-1531.
37. Helgerson RC, et al. Host-guest complexation. 50. Potassium and sodium ion-selective chromogenic ionophores. *J Amer Chem Soc* 1989; 111:6339-6350.
38. Kumar A, et al. Chromogenic ionophore-based methods for spectrophotometric assay of sodium and potassium in serum and plasma. *Clin Chem* 1988; 34:1709-1712.
39. Berry MN, et al. Enzymatic determination of sodium in serum. *Clin Chem* 1988; 34:2295-2298.
40. Skeggs LT Jr. An automatic method for the determination of carbon dioxide in blood plasma. *Am J Clin Pathol* 1960; 33:181-185.
41. Korzun WJ, Miller WG. Carbon dioxide. *In: Clinical Chemistry: Theory, Analysis, and Correlation*, 2nd ed. Kaplan LA, Pesce AJ, eds. St Louis: The C.V. Mosby Company. 1989: 869-872.
42. Fales FW. Urea in serum, direct diacetyl monoxime method. *In: Selected Methods of Clinical Chemistry, Vol 9*. Faulkner WR and Meites S, eds. Washington, DC: AACC Press. 1982: 365-373.
43. Van Slyke, et al. A permanent preparation of urease, and its use in the determination of urea. *J Biol Chem* 1914; 19:211-228.
44. Fawcett JK, et al. A rapid and precise method for the determination of urea. *J Clin Pathol* 1960; 13:156-159.
45. Chaney, et al. Urea and ammonia determinations. *Clin Chem* 1962; 8:130-132.
46. Talke H, et al. Enzymatische harnstoffbestimmung in blut and serum im optischen test nach Warburg. *Klin Wochensh* 1965; 43:174-175.
47. Hallett, et al. Reduced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. *Clin Chim Acta* 1971; 35:33-37.
48. Patton, et al. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal Chem* 1977; 49:464-469.
49. 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.
50. Clinical and Laboratory Standards Institute (CLSI (formerly NCCLS)). Physician's office laboratory guidelines; Tentative Guideline – Second Edition. NCCLS Document POL1-T2. Wayne, PA: NCCLS, 1992.
51. Scott, M.G. Electrolytes and blood gases. *In: Tietz Textbook of Clinical Chemistry*, 3rd Ed. Burtis CA, Ashwood ER, eds. Philadelphia: WB Saunders Company. 1999: 1058-1059.
52. D.B. Sacks, Carbohydrates, in *Tietz Textbook of Clinical Chemistry*, 3rd Ed. Burtis CA, Ashwood ER, eds. Philadelphia: WB Saunders Company. 1999.
53. Clinical and Laboratory Standards Institute (CLSI). *Blood Gas and pH Analysis and related measurements; Approved Guidelines – Second Edition*. Document C46-A2 Vol.29 No. 8. February 2009
54. Clinical and Laboratory Standards Institute. Procedures for the handling and processing of blood specimens; Approved Guidelines – Second Edition. CLSI Document H18-A2. Wayne, PA: CLSI, 1999.
55. Clinical and Laboratory Standards Institute. Procedures for the handling and processing of blood specimens; Approved Guidelines – Third Edition. CLSI Document H18-A3. Wayne, PA: CLSI, 2004.
56. Overfield CV, et al. Glycolysis: a re-evaluation of the effect on blood glucose. *Clin Chim Acta* 1972; 39: 35-40.
57. 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.
58. Scott, M.G. Electrolytes and blood gases. *In: Tietz Textbook of Clinical Chemistry*, 3rd ed. Burtis CA, Ashwood ER, eds. Philadelphia: WB Saunders Company. 1999: 1065-6.
59. Clinical and Laboratory Standards Institute. Interference testing in clinical chemistry; Proposed Guideline. CLSI Publication EP7-P. Wayne, PA: CLSI, 1986.
60. Clinical and Laboratory Standards Institute. Interference testing in clinical chemistry; Approved Guideline– Second Edition. CLSI Document EP7-A2. Wayne, PA: CLSI, 2005.
61. Young DS. *Effects of drugs on clinical laboratory tests*, 3rd Ed. Washington, DC: AACC Press, 1990.
62. National Committee for Clinical Laboratory Standards. How to define and determine reference intervals in the clinical laboratory, Approved Guidelines – Second Edition. NCCLS Document C28-A2. Wayne, PA: NCCLS, 2000.
63. Clinical and Laboratory Standards Institute. How to define and determine reference intervals in the clinical laboratory, Approved Guidelines – Third Edition. CLSI Document C28-A3c. Wayne, PA: CLSI, 2010.
64. Lum G, Gambino S. Serum vs plasma determinations in routine chemistry. *Clin Chem* 1972; 18(7);Abstr 134;710.

14. Bibliography (continued)

65. Lum G, Gambino S. A comparison of serum vs heparinized plasma for routine chemistry tests. *Am J Clin Pathol* 1974; 61(1);108-13.
66. Carothers J, Kurtz N, Lehmann J, Jr. Error introduced by specimen handling before determination of inorganic phosphate concentrations in plasma and serum. *Clin Chem* 1976; 22(11);1909-12.
67. Ladenson J, et al. Serum vs heparinized plasma for routine chemistry tests. *Am J Clin Path* 1974; 62(4);545-52.
68. Clinical and Laboratory Standards Institute. Evaluation of precision performance of clinical chemistry devices; Approved Guideline. CLSI Document EP5-A. Wayne, PA: CLSI, 1999.
69. Clinical and Laboratory Standards Institute. Evaluation of precision performance of clinical chemistry devices; Approved Guideline– Second Edition. CLSI Document EP5-A2. Wayne, PA: CLSI, 2004.
70. Clinical and Laboratory Standards Institute. Quality management for unit-use testing; Proposed Guideline. CLSI Document EP18-A. Wayne, PA: CLSI, 2002.
71. Clinical and Laboratory Standards Institute. Quality management for unit-use testing; Approved Guideline– Second Edition. CLSI Document EP18-A2. Wayne, PA: CLSI, 2009
72. Clinical and Laboratory Standards Institute. Method comparison and bias estimation using patient samples; Approved Guideline. CLSI Document EP9-A. Wayne, PA: CLSI, 1995.
73. Clinical and Laboratory Standards Institute. Method comparison and bias estimation using patient samples; Approved Guideline– Second Edition. CLSI Document EP09-A2-IR. Wayne, PA: CLSI, 2010.