Piccolo® General Chemistry 13

For In Vitro Diagnostic Use and For Professional Use Only
Customer and Technical Service: 800-822-2947

CLIA Waived: Use lithium heparin whole blood, only
Moderate Complexity: Use lithium heparin whole blood lithium heparin plasma, or serum

December 2009
PN: 400-7149-1 Rev: H

1. Intended Use

The Piccolo® General Chemistry 13 reagent disc, used with the Piccolo Blood Chemistry Analyzer or the Piccolo xpress™ Chemistry Analyzer, is intended to be used for the *in vitro* quantitative determination of alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), amylase, aspartate aminotransferase (AST), calcium, creatinine, gamma glutamyltransferase (GGT), glucose, total bilirubin, total protein, blood urea nitrogen (BUN), and uric acid in heparinized whole blood, heparinized plasma, or serum.

The tests on this panel are waived under CLIA '88 regulations. If a laboratory modifies the test system instructions, then the tests are considered high complexity and subject to all CLIA requirements. For CLIA waived labs, only lithium heparin whole blood may be tested. For use in moderate complexity labs, lithium heparinized whole blood, lithium heparinized plasma, or serum may be used.

A CLIA Certificate of Waiver is needed to perform CLIA waived testing. A Certificate of Waiver can be obtained from the Centers for Medicare & Medicaid Services (CMS). Please contact the Commission on Laboratory Accreditation (COLA) at 1-800-981-9883 for assistance in obtaining one.

2. Summary and Explanation of Tests

The Piccolo Gen Chem 13 Reagent Disc and the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders:

Alanine aminotransferase (ALT):	Liver diseases, including viral hepatitis and cirrhosis.
Albumin:	Liver and kidney diseases.
Alkaline phosphatase (ALP):	Liver, bone, parathyroid, and intestinal diseases.
Amylase:	Pancreatitis.
Aspartate aminotransferase (AST):	Liver disease including hepatitis and viral jaundice, shock.
Calcium:	Parathyroid, bone and chronic renal diseases; tetany.
Creatinine:	Renal disease and monitoring of renal dialysis.
Gamma glutamyltransferase (GGT):	Liver diseases, including alcoholic cirrhosis and primary and secondary liver tumors.
Glucose:	Carbohydrate metabolism disorders, including adult and juvenile diabetes mellitus and hypoglycemia.
Total bilirubin:	Liver disorders, including hepatitis and gall bladder obstruction; jaundice.
Total protein:	Liver, kidney, bone marrow diseases; metabolic and nutritional disorders.
Blood urea nitrogen (BUN):	Renal and metabolic diseases.
Uric Acid:	Renal and metabolic disorders, including renal failure and gout.

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. Test Principles

Alanine Aminotransferase (ALT)
Alanine aminotransferase (ALT) has been measured by three methods. Two of these methods—the colorimetric dinitrophenylhydrazine coupling technique [1,2] and the fluorescent enzymatic assay—are rarely used.[3] An enzymatic method based on the work of Wróblewski and LaDue[4] is the most common technique for determining ALT concentrations in serum. A modified Wróblewski and LaDue procedure has been proposed as the recommended procedure of the International Federation of Clinical Chemistry (IFCC).[5]

The method developed for use on the Piccolo Analyzers is a modification of the IFCC-recommended procedure. In this reaction, ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+, as illustrated in the following reaction scheme.

$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

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

The rate of change of the 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 ALT present in the sample.

Albumin (ALB)
Early methods used to measure albumin include fractionation techniques[6,7,8] and tryptophan content of globulins.[9,10] These methods are unwieldy to perform and do not have a high specificity. Two immunochemical techniques are considered as reference methods, but are expensive and time consuming.[11] Dye binding techniques are the most frequently used methods for measuring albumin. Bromcresol 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.[12] Bromcresol purple (BCP) is the most specific of the dyes in use.[13,14]

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

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

Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as the difference in absorbance between 600 nm and 550 nm.

Alkaline Phosphatase (ALP)
Techniques to measure alkaline phosphatase were first developed over 60 years ago. Several of these endpoint or two-point spectrophotometric methods[15,16] are now considered obsolete or too cumbersome. The use of *p*-nitrophenyl phosphate (*p*-NPP) increased the speed of the reaction.[17,18] The reliability of this technique was greatly increased by the use of a metal-ion buffer to maintain the concentration of magnesium and zinc ions in the reaction.[19] The American Association for Clinical Chemistry (AACC) reference method[20] uses *p*-NPP as a substrate and a metal-ion buffer.

The Piccolo procedure is modified from the AACC and IFCC[21] methods. Alkaline phosphatase hydrolyzes *p*-NPP in a metal-ion buffer and forms *p*-nitrophenol and phosphate.

$$\textit{p}\text{-Nitrophenyl Phosphate} \xrightarrow[Zn^{2+},\ Mg^{2+}]{\text{ALP}} \textit{p}\text{-Nitrophenol} + \text{Phosphate}$$

The amount of ALP in the sample is proportional to the rate of increase in absorbance difference between 405 nm and 500 nm.

Amylase (AMY)
About 200 different tests have been developed to measure amylase. Most procedures use a buffered polysaccharide solution but employ different detection techniques. Viscosimetric methods are lacking in precision and accuracy[22], while turbidimetric and iodometric methods are difficult to standardize.[23,24] Commonly used are saccharogenic and chromolytic methods. The "classic" amylase measurement technique is a saccharogenic method [25], but is difficult and time-consuming.[26] Chromolytic methods using

p-nitrophenyl-glycosides as substrates have been recently developed.[27] These assays have a higher specificity for pancreatic amylase than for salivary amylase and are easily monitored.[27]

In the Piccolo method, the substrate, 2-chloro-*p*-nitrophenyl-α-D-maltotrioside (CNPG3), reacts with α-amylase in the patient sample, releasing 2-chloro-*p*-nitrophenol (CNP). The release of CNP creates a change in color.

$$\text{CNPG3} \xrightarrow{\alpha\text{-Amylase}} \text{CNP + D-Maltotrioside}$$

The reaction is measured bichromatically at 405 nm and 500 nm. The change in absorbance due to the formation of CNP is directly proportional to α-amylase activity in the sample.

Aspartate Aminotransferase (AST)
The aspartate aminotransferase (AST) test is based on the Karmen rate method[28] as modified by Bergmeyer.[29] The current International Federation of Clinical Chemistry (IFCC) reference method utilizes the Karmen/Bergmeyer technique of coupling malate dehydrogenase (MDH) and reduced nicotinamide dinucleotide (NADH) in the detection of AST in serum.[29,30] Lactate dehydrogenase (LDH) is added to the reaction to decrease interference caused by endogenous pyruvate.

AST catalyzes the reaction of L-aspartate and α-ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to NAD^+ by the catalyst MDH.

$$\text{L-aspartate + α-ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate + L-glutamate}$$

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

The rate of absorbance change at 340 nm/405 nm caused by the conversion of NADH to NAD^+ is directly proportional to the amount of AST present in the sample.

Calcium (CA)
The first methods used to analyze calcium involved precipitating calcium with an excess of anions.[31,32,33] 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.[34] Spectrophotometric methods using either *o*-cresolphthalein complexone or arsenazo III metallochromic indicators are most commonly used.[35,36,37] 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.

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.[38,39] Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.[40,41,42] Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.[43]

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 finish, peroxidase catalyzes the reaction among the hydrogen peroxide, 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) and 4-aminoantipyrine (4-AAAP) 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.

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

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

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

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

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

eGFR (calculated)

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

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

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

Gamma Glutamyltransferase (GGT)

The first quantitative methods developed to measure gamma glutamyltransferase (GGT) involved a second reaction to form an azo dye that combined with a chromophore.[44,45] The change to L-γ-glutamyl-*p*-nitroanilide as the substrate in the reaction eliminated the dye-formation step.[46] Due to the poor solubility and stability of L-γ-glutamyl-*p*-nitroanilide, this procedure was modified to use the substrate L-γ-glutamyl-3-carboxy-4-nitroanilide.[47] The International Federation of Clinical Chemistry (IFCC) recommended GGT method is based on the latter substrate, with glycylglycine as the other substrate.[48]

Abaxis has modified the IFCC method to react at 37°C. The addition of sample containing gamma glutamyltranferase to the substrates L-γ-glutamyl-3-carboxy-4-nitroanilide and glycylglycine (gly-gly) causes the formation of L-γ-glutamyl-glycylglycine (glu-gly-gly) and 3-carboxy-4-nitroaniline.

$$\text{L-γ-glutamyl-3-carboxy 4-nitroanilide} + \text{Gly-gly} \xrightarrow{\text{GGT}} \text{Glu-gly-gly} + \text{3-carboxy-4-nitroaniline}$$

The absorbance of this rate reaction is measured at 405 nm. The production of 3-carboxy-4-nitroaniline is directly proportional to the GGT activity in the sample.

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu[49] and Somogyi-Nelson[50,51]). 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 General Chemistry 13 reagent disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.[52]

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.

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

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

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.

Total Bilirubin (TBIL)
Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.[53,54] A newer, more specific method has been developed using the enzyme bilirubin oxidase.[55,56,57] In addition to using the more specific total bilirubin test method, photodegradation of the analyte is minimized on the Piccolo Analyzers because the sample can be tested immediately after collection.

In the enzyme procedure, bilirubin is oxidized by bilirubin oxidase into biliverdin.

$$\text{Bilirubin + O}_2 \xrightarrow{\text{Bilirubin Oxidase}} \text{Biliverdin + H}_2\text{O}$$

Bilirubin is quantitated as the difference in absorbance between 467 nm and 550 nm. The initial absorbance of this endpoint reaction is determined from the bilirubin blank cuvette and the final absorbance is obtained from the bilirubin test cuvette. The amount of bilirubin in the sample is proportional to the difference between the initial and final absorbance measurements.

Total Protein (TP)
The total protein method is a modification of the biuret reaction, noted for its precision, accuracy, and specificity.[58] Originally developed by Riegler[59] and modified by Weichselbaum[60], Doumas, et al.[61] proposed a biuret reaction as a candidate total protein reference method.

In the biuret reaction, the protein solution is treated with cupric [Cu(II)] ions in a strong alkaline medium. Sodium potassium tartrate and potassium iodide are added to prevent the precipitation of copper hydroxide and the auto-reduction of copper, respectively.[60] The Cu(II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-protein complex.

$$\text{Total Protein + Cu(II)} \xrightarrow{\text{OH}} \text{Cu-Protein Complex}$$

The amount of total protein present in the sample is directly proportional to the absorbance of the Cu-protein complex. The total protein test is an endpoint reaction and the absorbance is measured as the difference in absorbance between 550 nm and 850 nm.

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.[62] Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.[63] The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique[64,65] and coupled enzymatic reactions.[66,67] Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.[68] 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.[69]

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

$$\text{Urea + H}_2\text{O} \xrightarrow{\text{Urease}} \text{NH}_3 + \text{CO}_2$$

$$\text{NH}_3 + \text{2-Oxoglutarate + NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate + H}_2\text{O + NAD}^{+}$$

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.

Uric Acid (UA)
Early quantitative methods to determine uric acid concentrations in blood were based on the reduction of phosphotungstic acid to tungsten blue in alkaline solutions of uric acid.[70,71] A uric acid test, with improved specificity, was developed using the uric acid-specific enzyme uricase. This method has since become the standard clinical chemistry technique for uric acid.[72]

The uricase method is coupled through a Trinder peroxidase finish.[73] In this method, uricase catalyzes the oxidation of uric acid to allantoin and hydrogen peroxide. Peroxidase catalyzes the reaction among the hydrogen peroxide (H_2O_2), 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBSA) into a red quinoneimine dye. Sodium ferrocyanide and ascorbate oxidase are added to the reaction mixture to minimize the potential interference of bilirubin and ascorbic acid.

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

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

The amount of uric acid in the sample is directly proportional to the absorbance of the quinoneimine dye. The final absorbance of this endpoint reaction is measured bichromatically at 500 nm and 600 nm.

4. Principles of Procedure

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

5. Description of Reagents

Reagents
Each Piccolo General Chemistry 13 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 alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), amylase (AMY), aspartate aminotransferase (AST), calcium (CA), gamma glutamyltransferase (GGT), glucose (GLU), urea nitrogen (BUN), and uric acid (UA). Dedicated sample blanks are included in the disc for creatinine (CRE), total bilirubin (TBIL), and total protein (TP). Each reagent disc also contains a diluent consisting of surfactants, excipients, and preservatives.

Table 1: Reagents

Component	Quantity/Disc
Adenosine-5'-diphosphate	4 µg
Adenosine-5'-triphosphate	11 µg
L-Alanine	874 µg
4-Aminoantipyrine-HCl (4-AAP)	20 µg
Arsenazo III, sodium salt	3 µg
Ascorbate oxidase (*Cucurbita spp.*)	0.5 U
L-Aspartic acid	426 µg
Bilirubin oxidase	0.1 U
Bromcresol purple	2 µg
2-Cloro-*p*-nitrophenyl-α-D-maltotrioside (NPG3)	40 µg
Creatine amidinohydrolase (*Actinobacillus spp.*)	2 U
Creatinine amidohydrolase (Pseudomonas spp.)	1 U
Cupric sulfate	134 µg
3,5-Dichloro-2-hydroxybenzenesulfonic acid (DHBSA)	37 µg
Glucose-6-phosphate dehydrogenase (yeast)	0.05 U
L-Glutamic acid dehydrogenase (bovine liver)	0.01 U
L-Glutamic acid γ-(3-carboxy-4-nitroanilide), ammonium salt	30 µg
Glycylglycine	317 µg

Table 1: Reagents (cont.)

Component	Quantity/Disc
Hexokinase (yeast)	0.1 U
α-Ketoglutarate, disodium salt	28 µg
α -Ketoglutaric acid	72 µg
Lactate dehydrogenase (chicken heart)	0.002 U
Lactate dehydrogenase (LDH) (microbial)	0.03 U
Lactate dehydrogenase (*Staphylococcus epidermidis*)	0.1 U
Magnesium acetate	7 µg
Magnesium chloride	3 µg
Malate dehydrogenase (MDH) (porcine heart)	0.01 U
Nicotinamide adenine dinucleotide (NAD+)	20 µg
Nicotinamide adenine dinucleotide, reduced (NADH)	18 µg
Peroxidase (horseradish)	0.8 U
p-Nitrophenyl Phosphate (p-NPP)	56 µg
Potassium ferrocyanide	0.4 µg
Potassium iodide	56 µg
Sarcosine oxidase (microorganism)	0.6 U
Sodium ferrocyanide	1 µg
Sodium potassium tartrate	686 µg
2,4,6-Tribromo-3-hydroxybenzoic acid	188 µg
Urease (jack bean)	0.05 U
Uricase (microbial)	0.04 U
Zinc sulfate	3 µg
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.[74] See the Piccolo Blood Chemistry Analyzer or 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 Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual. A disc not used within 20 minutes of opening the pouch should be discarded.

Storage

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

Indications of Reagent Disc Instability/Deterioration
A torn or otherwise damaged pouch may allow moisture to reach the unused rotor and adversely affect reagent performance. Do not use a rotor from a damaged pouch.

6. Instrument

See the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual for complete information on use of the analyzer.

7. Sample Collection and Preparation

Sample collection techniques are described in the "Sample Collection" section of the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

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

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

- Whole blood venipuncture samples should be run within 60 minutes of collection.[75] **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.[76]

- Refrigerating whole blood samples can cause significant changes in concentrations of **aspartate aminotransferase**, **creatinine** and **glucose**.[77] 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.

- **Total bilirubin** results may be adversely affected by photodegradation.[78] Whole blood samples not run immediately should be stored in the dark for no longer than 60 minutes. If the sample can not be analyzed within that period, it should be separated into plasma or serum and stored in a capped sample tube in the dark at low temperatures.[79]

- Use only lithium heparin (green stopper) evacuated specimen collection tubes for whole blood or plasma samples. Use no-additive (red stopper) evacuated specimen collection tubes or serum separator tubes (red or red/black stopper) for serum samples.

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

8. Procedure

Materials Provided

- One Piccolo General Chemistry 13 Reagent Disc PN: 400-1029 (a box of discs PN: 400-0029)

Materials Required but not Provided

- Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer
- Sample transfer pipettes (fixed volume approximately 100 µL) and tips are provided with each Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer and may be reordered from Abaxis.
- Commercially available control reagents recommended by Abaxis (contact Abaxis Technical Service for approved control materials and expected values).
- Timer

Test Parameters
The Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each Piccolo General Chemistry 13 Reagent Disc is less than 14 minutes. The analyzer maintains the reagent disc at a temperature of 37°C (98.6°F) over the measurement interval.

Test Procedure
The complete sample collection and step-by-step operating procedures are detailed in the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

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

Quality Control
See Section 2.4 of the Piccolo Operator's Manual or Section 6 (Calibration and Quality Control) of the Piccolo xpress Operator's Manual. Performance of the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer can be verified by running controls. For a list of approved quality control materials with acceptance ranges, please contact Abaxis Technical Support. Other human serum or plasma-based controls may not be compatible. Quality control materials should be stored as per the package-insert included with the controls.

If control results are out of range, repeat one time. If still out of range, call Technical Support. Do not report results if controls are outside their labeled limits. See the Piccolo or Piccolo xpress Operator's Manual for a detailed discussion on running, recording, interpreting, and plotting control results.

Waived Laboratories: Abaxis recommends control testing as follows:

- at least every 30 days
- whenever the laboratory conditions have changed significantly, e.g. Piccolo moved to a new location or changes in temperature control
- when training or retraining of personnel is indicated
- with each new lot (CLIA waived tests in waived status labs)

Non-Waived Laboratories: Abaxis recommends control testing to follow federal, state, and local guidelines.

9. Results

The Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer automatically calculates and prints the analyte concentrations in the sample. Details of the endpoint and rate reaction calculations are found in the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual.

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

10. Limitations of Procedure

General procedural limitations are discussed in the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual**.**

- The only anticoagulant **recommended for use** with the Piccolo Blood Chemistry System or the Piccolo xpress Chemistry System is **lithium heparin**. Do not use sodium 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 General Chemistry 13 Reagent Disc.

- Samples with hematocrits in excess of 62-65% packed red cell volume (a volume fraction of 0.62-0.65) may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down to get plasma then re-run in a new reagent disc.

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

  Warning: Extensive testing of the Piccolo Blood Chemistry System or 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. Human serum pools were prepared. The concentration at which each potential interferent was tested was based on the testing levels in NCCLS EP7-P.[80]

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

- The Piccolo Blood Chemistry System or the Piccolo xpress Chemistry Analyzer suppresses any results that are affected by >10% interference from hemolysis, lipemia or icterus. "HEM", "LIP", or "ICT" respectively, is printed on the result card in place of the result.

- For maximum levels of endogenous substances contact Abaxis Technical Support.

Effects of Exogenous and Therapeutic Substances
- Thirty-five exogenous and therapeutic substances were selected as potential interferents for Abaxis test methods based on recommendations by Young.[81] Significant interference is defined as a >10% shift in the result for a normal range specimen. Human serum pools were supplemented with a known concentration of the drugs or chemicals and then analyzed.

Table 2: Exogenous & Therapeutic Substances Evaluated

	Physiologic or Therapeutic Range[80-85] (mg/dL)	Highest Concentration Tested (mg/dL)
Acetaminophen	1-2	100
Acetoacetate	0.05-3.60	102
Acetylsalicylic acid	2-10	50
Ampicillin	0.5	30
Ascorbic Acid	0.8-1.2	20
Caffeine	0.3-1.5	10
Calcium Chloride	—	20
Cephalothin (Keflin)	10	400
Chloramphenicol	1-2.5	100
Cimetidine	0.1-1	16
L-Dopa	—	5
Dopamine	—	19
Epinephrine	—	1
Erythromycin	0.2-2.0	10
Glutathione	—	30
Ibuprofen	0.5-4.2	50
Isoniazide	0.1-0.7	4
α-Ketoglutarate	—	5
Ketoprofen	—	50
Methicillin	—	100
Methotrexate	0.1	0.5
Metyldopa	0.1-0.5	0.5
Metronidazole	0.1	5
Nafcillin	—	1
Nitrofurantoin	0.2	20

Table 2: Exogenous & Therapeutic Substances Evaluated (continued)

	Physiologic or Therapeutic Range[80-85] (mg/dL)	Highest Concentration Tested (mg/dL)
Oxacillin	—	1
Oxaloacetate	—	132
Phenytoin	1-2	3
Proline	—	4
Pyruvate	0.3-0.9	44
Rifampin	0.4-3	1.5
Salicylic Acid	15-30	25
Sulfalazine	2-4	10
Sulfanilamide	10-15	50
Theophylline	1-2	20

- The following substances showed greater than 10% interference. Significant interference is defined as >10% shift in the result for a normal range specimen. Human serum pools were supplemented with known concentrations of the drugs or chemicals and then analyzed.

Table 3: Substances With Significant Interference >10%

	Physiologic/ Therapeutic Range[80-85] (mg/dL)	Concentration with > 10% Interference (mg/dL)	% Interference
Alanine Aminotransferase (ALT)			
Ascorbic acid	0.8-1.2	20	11% inc*
Oxaloacetate	—	132	843% inc
Albumin (ALB)			
Acetoacetate	0.05-3.60	102	18% dec*
Ampicillin	0.5	30	12% dec
Caffeine	0.3-1.5	10	14% dec
Calcium chloride	—	20	17% dec
Cephalothin (Keflin)	10	400	13% inc
Ibuprofen	0.5-4.2	50	28% inc
α-Ketoglutarate	—	5	11% dec
Nitrofurantoin	0.2	20	13% dec
Proline	—	4	12% inc
Sulfalazine	2-4	10	14% dec
Sulfanilamide	10-15	50	12% dec
Theophylline	1-2	20	11% dec
Alkaline Phosphatase (ALP)			
Theophylline	1-2	20	42% dec
Creatinine (CRE)			
Ascorbic acid	0.8-1.2	20	11% dec
Dopamine	—	19	80% dec
L-dopa	—	5	71% dec
Epinephrine	—	1	45% dec
Glutathione	—	30	13% dec
Glucose (GLU)			
Oxaloacetate	—	132	11% dec
Pyruvate	0.3-0.9	44	13% dec

Table 3: Substances With Significant Interference >10% (continued)

	Physiologic/ Therapeutic Range[80-85] (mg/dL)	Concentration with > 10% Interference (mg/dL)	% Interference
Total Bilirubin (TBIL)			
Dopamine	—	19	55% dec
L-dopa	—	5	17% dec
Uric Acid			
Ascorbic acid	0.8–1.2	20	13% dec
Epinephrine	—	1	14% dec
L-dopa	—	5	78% dec
Methyldopa	0.1–0.5	0.5	12% dec
Rifampin	0.4–3	1.5	14% dec
Salicylic acid	15–30	25	20% dec

* inc=increase; dec=decrease.

For additional information on potential chemical interferents, see the Bibliography.

11. Expected Values

Samples from a total of 193 adult males and females, analyzed on the Piccolo Blood Chemistry Analyzer, were used to determine the reference ranges for ALT, albumin, ALP, amylase, calcium, creatinine, glucose, total bilirubin, total protein, and BUN. Samples from a total of 186 adult males and females were used to determine the reference range for AST and uric acid. Samples from a total of 131 adult males and females were used to determine the reference range for GGT. These ranges are provided as a guideline only. It is recommended that your office or institution establish normal ranges for your particular patient population.

Table 4: Piccolo Reference Intervals

Analyte	Common Units	SI Units
Alanine Aminotransferase (ALT)	10-47 U/L	10-47 U/L
Albumin (ALB)	3.3-5.5 g/dL	33-55 g/L
Alkaline Phosphatase (ALP)		
Female	42-141 U/L	42-141 U/L
Male	53-128 U/L	53-128 U/L
Amylase (AMY)	14-97 U/l	14-97 U/L
Aspartate Aminotransferase (AST)	11-38 U/L	11-38 U/L
Calcium (CA)	8.0-10.3 mg/dL	2.00-2.58 mmol/L
Creatinine (CRE)	0.6-1.2 mg/dL	53-106 µmol/L
Gamma Glutamyltransferase (GGT)	5-65 U/L	5-65 U/L
Glucose (GLU)	73-118 mg/dL	4.05-6.55 mmol/L
Total Bilirubin (TBIL)	0.2-1.6 mg/dL	3.4-27.4 µmol/L
Total Protein (TP)	6.4-8.1 g/dL	64-81 g/L
Blood Urea Nitrogen (BUN)	7-22 mg/dL	2.5-7.9 mmol urea/L
Uric Acid (UA)		
Female	2.2-6.6 mg/dL	0.13-0.39 mmol/L
Male	3.6-8.0 mg/dL	0.21-0.47 mmol/L

12. Performance Characteristics

Linearity
The chemistry for each analyte is linear over the dynamic range listed below when the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer is operated according to the recommended procedure (refer to the Piccolo Blood Chemistry Analyzer or the Piccolo xpress Chemistry Analyzer Operator's Manual).

Table 5: Piccolo Dynamic Ranges

Analyte	Common Units	SI Units
Alanine Aminotransferase (ALT)	5-2000 U/L	5-2000 U/L
Albumin (ALB)	1-6.5 g/dL	10-65 g/L
Alkaline Phosphatase (ALP)	5-2400 U/L	5-2400 U/L
Amylase (AMY)	5-4000 U/L	5-4000 U/L
Aspartate Aminotransferase (AST)	5-2000 U/L	5-2000 U/L
Calcium	4.0-16.0 mg/dL	1.0-4.0 mmol/L
Creatinine	0.2-20 mg/dL	18-1768 µmol/L
Gamma Glutamyltransferase (GGT)	5-3000 U/L	5-3000 U/L
Glucose	10-700 mg/dL	0.56-38.9 mmol/L
Total Bilirubin (TBIL)	0.1-30 mg/dL	1.7-513 µmol/L
Total Protein (TP)	2-14 g/dL	20-140 g/L
Urea Nitrogen (BUN)	2-180 mg/dL	0.7-64.3 mmol/urea/L
Uric Acid	1-15 mg/dL	0.1-0.9 mmol/L

If the analyte concentration is above the measuring range (dynamic range), but less than the system range, the print card will indicate a ">" sign at the upper limit and an asterisk after the number, e.g. ALT >2000* U/L. If lower than the dynamic range, a "<" will be printed with an asterisk, e.g. ALT <5* U/L. For values that are grossly beyond the measurement range (system range), "~~~" will be printed instead of a result. Any time "~~~" appears on a print card, collect a new sample and rerun the test. If results for the second sample are suppressed again, please call Abaxis Customer Service.

Sensitivity (Limits of Detection)
The lower limit of the reportable (dynamic) range for each analyte is: alanine aminotransferase 5 U/L; albumin 1 g/dL (10 g/L); alkaline phosphatase 5 U/L; amylase 5 U/L; aspartate aminotransferase 5 U/L; calcium 4.0 mg/dL (1.0 mmol/L); creatinine 0.2 mg/dL (18 µmol/L); gamma glutamyltransferase 5 U/L; glucose 10 mg/dL (0.56 mmol/L) total bilirubin 0.1 mg/dL (1.7 µmol/L); total protein 2 g/dL (20 g/L); urea nitrogen 2.0 mg/dL (0.7 mmol urea/L); and uric acid 1 mg/dL (0.1 mmol/L).

Precision
Precision studies were conducted using NCCLS EP5-T2 guidelines.[86] Results for within-run and total precision were determined by testing two levels of control material. Controls were run in duplicate twice each day for 20 days over a four-week period. Results of the precision studies are shown in Table 6.

Table 6: Precision (N=80)

Analyte	Within-Run	Total
Alanine Aminotranferase (U/L)		
Control Level 1		
Mean	21	21
SD	2.76	2.79
%CV	13.4	13.5
Control Level 2		
Mean	52	52
SD	2.70	3.25
%CV	5.2	6.2
Albumin (g/dL)		
Control Level 1		
Mean	5.6	5.6
SD	0.09	0.11
%CV	1.7	2.1

Table 6 Precision (N=80) (cont.)

Analyte	Within-Run	Total
Control Level 2		
Mean	3.7	3.7
SD	0.07	0.11
%CV	2.0	2.9
Alkaline Phosphatase (U/L)		
Control Level 1		
Mean	39	39
SD	1.81	2.29
%CV	4.6	5.8
Control Level 2		
Mean	281	281
SD	4.08	8.75
%CV	1.5	3.1
Amylase (U/L)		
Control Level 1		
Mean	46	46
SD	2.40	2.63
%CV	5.2	5.7
Control Level 2		
Mean	300	300
SD	11.15	11.50
%CV	3.7	3.8
Aspartate Aminotransferase (U/L)		
Control Level 1		
Mean	47	49
SD	0.98	0.92
%CV	2.1	1.9
Control Level 2		
Mean	145	147
SD	1.83	1.70
%CV	1.3	1.2
Calcium (mg/dL)		
Control Level 1		
Mean	8.6	8.6
SD	0.21	0.25
%CV	2.4	2.9
Control Level 2		
Mean	11.8	11.8
SD	0.39	0.40
%CV	3.3	3.4
Creatinine (mg/dL)		
Control Level 1		
Mean	1.1	1.1
SD	0.14	0.14
%CV	12.5	13.1
Control Level 2		
Mean	5.2	5.2
SD	0.23	0.27
%CV	4.4	5.2

Table 6 Precision (N=80) (cont.)

Analyte	Within-Run	Total
Gamma Glutamyltransferase (U/L)		
Control Level 1		
Mean	25	25
SD	0.59	0.74
%CV	2.34	2.94
Control Level 2		
Mean	106	106
SD	1.52	2.29
%CV	1.43	2.15
Glucose (mg/dL)		
Control Level 1		
Mean	66	66
SD	0.76	1.03
%CV	1.1	1.6
Control Level 2		
Mean	278	278
SD	2.47	3.84
%CV	0.9	1.4
Total Bilirubin (mg/dL)		
Control Level 1		
Mean	0.8	0.8
SD	0.06	0.07
%CV	8.0	9.3
Control Level 2		
Mean	5.2	5.2
SD	0.09	0.15
%CV	1.7	2.8
Total Protein (g/dL)		
Control Level 1		
Mean	6.8	6.8
SD	0.05	0.08
%CV	0.8	1.2
Control Level 2		
Mean	4.7	4.7
SD	0.09	0.09
%CV	2.0	2.0
Blood Urea Nitrogen (mg/dL)		
Control Level 1		
Mean	19	19
SD	0.35	0.40
%CV	1.9	2.1
Control Level 2		
Mean	65	65
SD	1.06	1.18
%CV	1.6	1.8
Uric Acid (mg/dL)		
Control Level 1		
Mean	3.8	3.8
SD	0.15	0.18

Table 6 Precision (N=80) (cont.)

Analyte	Within-Run	Total
%CV	4.0	4.8
Control Level 2		
Mean	7.5	7.5
SD	0.24	0.29
%CV	3.2	3.9

Correlation

Heparinized whole blood and serum samples were collected from patients at two sites. The whole blood samples were analyzed by the Piccolo Blood Chemistry Analyzer at the field sites and the serum samples were analyzed by comparative methods. In two cases, the results of testing serum samples by the Piccolo were used and these are indicated appropriately in the table. In some cases, high and low supplemented samples were used to cover the dynamic range. All samples were run in singlicate on the same day. Representative correlation statistics are shown in Table 7.

Table 7: Correlation of Piccolo Blood Chemistry Analyzer with Comparative Methods

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
Alanine Aminotransferase (U/L)	0.981 0.985	0.905 0.946	1.3 -2.5	3.21 2.84	86 67	10-174 10-174	Paramax® Technicon
Albumin (g/dL)	0.854 0.896	1.001 0.877	-0.3 -0.1	0.22 0.21	261 100	1.1-5.3 1.5-5.0	Paramax Beckman
Alkaline Phosphatase (U/L)	0.988 0.929	0.970 1.136	-5.9 -17.6	3.97 4.79	99 80	27-368 26-150	Paramax Technicon
Amylase (U/L)	0.979 0.963	0.692 1.065	-4.7 -4.1	3.11 3.47	99 80	11-92 19-118	Paramax Technicon
Aspartate Aminotransferase (U/L)	0.93 1.0	0.87 0.97	5.3 3.0	2.76 1.9	159 46	13-111 13-252	Paramax DAX™
Calcium (mg/dL)	0.991* 0.673	0.990 0.742	-0.4 1.8	0.17 0.22	25 81	5.2-11.9 8.1-9.9	Paramax Beckman
Creatinine (mg/dL)	0.993 0.987	0.926 0.866	0.0 0.1	0.15 0.16	260 107	0.4-14.7 0.4-7.5	Paramax Beckman
Gamma Glutamyl-transferase (U/L)	1.0 1.0**	0.98 1.60	-0.4 3.1	3.29 18.57	135 49	5-312 27-1848	Paramax Beckman
Glucose (mg/dL)	0.987 0.997	1.009 0.943	-2.8 1.2	3.89 4.69	251 91	72-422 56-646	Paramax Beckman
Total Bilirubin (mg/dL)	0.974 0.980	0.901 1.113	0.0 -0.4	0.07 0.09	250 91	0.2-3.7 0.1-6.4	Paramax Beckman
Total Protein (g/dL)	0.849 0.873	0.932 0.935	0.6 0.3	0.19 0.16	251 92	5.7-9.2 6.5-9.2	Paramax Beckman

Table 7: Correlation of Piccolo Blood Chemistry Analyzer with Comparative Methods (cont.)

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
Blood Urea Nitrogen (mg/dL)	0.964	0.923	0.5	1.08	251	6-52	Paramax
	0.983	0.946	0.0	0.66	92	6-38	Beckman
Uric Acid (mg/dL)	0.979	0.958	-0.3	0.20	159	1.4-7.6	Paramax
	0.975	0.908	-0.6	0.31	44	2.1-12.1	DAX

***** Serum samples from hospitalized patients provided a broader, and possibly more useful, sample range than did venous whole blood samples from out-patients. Correlation statistics for the Piccolo calcium test are from these serum samples.

** One site ran only serum on the Piccolo analyzer for the gamma glutamyltransferase correlation.

Results of Untrained User Study

An "untrained user" study was conducted in which participants were given only the test instructions and asked to perform testing of 3 discs with blinded randomized samples. The samples consisted of serum pools prepared at three levels for each of the thirteen analytes: ALT, albumin, ALP, AMY, AST, calcium, creatinine, GGT, glucose, total bilirubin, total protein, BUN, and UA. The participants were not given any training on the use of the test. A total of approximately 60 participants were enrolled from 3 sites, representing a diverse demographic (educational, age, gender, etc) population.

Tables below present the summary of the performance for each analyte.

Alanine Aminotransferase (ALT)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	45.4 U/L	98.9 U/L	184.3 U/L
%CV	3.7%	1.7%	1.5%
Observed Range	42 – 53	96 – 103	175 – 191
Percent of Results in the Range ± 15.0%*	98.4% 61/62 95%CI: 91.3% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

* This percent is based on the premise that one cannot distinguish properly between normal and abnormal values when errors are greater than one-quarter of the normal range. The range of (10 U/L - 47 U/L) was considered.

Albumin

	Level 1	Level 2	Level 3
N	62	62	62
Mean	3.0 g/dL	3.5 g/dL	4.2 g/dL
%CV	2.7%	2.5%	1.8%
Observed Range	2.9 – 3.2	3.3 – 3.7	4.0 – 4.4
Percent of Results in the Range ± 12.5%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Alkaline Phosphatase (ALP)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	94.5 U/L	171.5 U/L	337.5 U/L
%CV	5.2%	3.2%	2.4%
Observed Range	85 – 106	160-184	287 – 388
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Amylase (AMY)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	72.1 U/L	126.9 U/L	260.0 U/L
%CV	2.4%	2.1%	1.9%
Observed Range	67 – 75	120 – 133	248 – 273
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Asparate Aminotransferase (AST)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	56.0	120.4	276.3
%CV	2.4%	1.1%	1.0%
Observed Range	54 – 60	117 – 124	266 – 285
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Calcium

	Level 1	Level 2	Level 3
N	62	62	62
Mean	8.0	10.5	13.1
%CV	1.7	1.5	1.4
Observed Range	7.7 – 8.4	10.1 – 11.0	12.6 – 13.4
Percent of Results in the Range ± 6.3%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Creatinine

	Level 1	Level 2	Level 3
N	62	62	62
Mean	0.89	2.07	6.89
%CV	11.0	5.0	1.6
Observed Range	0.7 – 1.2	1.8 – 2.3	6.5 – 7.2
Percent of Results in the Range ± 15.0%	93.6 58/62 95%CI: 84.3% to 98.2%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Gamma Glutamyltransferase (GGT)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	35.0 U/L	86.2 U/L	131.3 U/L
%CV	2.8%	1.5%	1.5%
Observed Range	33 – 38	83 – 90	123 – 135
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Glucose

	Level 1	Level 2	Level 3
N	62	62	62
Mean	95.2	130.3	365.8
%CV	1.1%	1.0%	0.8%
Observed Range	93 – 98	125 – 133	351 – 373
Percent of Results in the Range ± 10.4%**	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

** The range of (65 mg/dL - 99 mg/dL) was considered.

Total Bilirubin (TBIL)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	0.86 mg/dL	2.5 mg/dL	5.7 mg/dL
%CV	6.1%	2.6%	1.8%
Observed Range	0.8 – 1.0	2.3 – 2.6	5.4 – 5.9
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Total Protein (TP)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	4.8 g/dL	5.7 g/dL	7.1 g/dL
%CV	2.0%	1.5%	1.5%
Observed Range	4.6 – 5.3	5.3 – 5.9	6.7 – 7.5
Percent of Results in the Range ± 5.9%	98.4% 61/62 95%CI: 91.3% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Blood Urea Nitrogen (BUN)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	15.1	41.0	72.2
%CV	2.3	2.5	1.8
Observed Range	14 – 16	37 – 43	68 – 75
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

Uric Acid

	Level 1	Level 2	Level 3
N	62	62	62
Mean	2.96	4.92	11.10
%CV	4.7	3.1	2.8
Observed Range	2.7 – 3.4	4.6 – 5.7	10.4 – 12.1
Percent of Results in the Range ± 15.0%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%	100% 62/62 95%CI: 94.2% to 100%

13. Bibliography

1. Tonhazy NE, NG White, WW Umbreit. A rapid method for the estimation of the glutamic-aspartic transaminase in tissues and its application to radiation sickness. Arch Biochem 1950; 28: 36-42.
2. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28: 56-63.
3. Murray RL. Alanine aminotransferase. In: Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. Kaplan LA,Pesce AJ, eds. St. Louis: The C.V. Mosby Company. 1989: 895-898.
4. Wróblewski F, LaDue JS. Serum glutamic-pyruvic transminase in cardiac and hepatic disease. Proc Soc Exp Biol Med 1956; 91: 569-571.
5. Bergmeyer HU, Horder M. IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem 1980; 18: 521-534.
6. Howe PE. The use of sodium sulfate as the globulin precipitant in the determination of proteins in blood. J Biol Chem 1921; 49: 93-107.
7. Howe PE. The determination of proteins in blood — a micro method. J Biol Chem 1921; 49: 109-113.
8. 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: 723-730.
9. Saifer A, Gerstenfeld S, Vacsler F. Photometric microdetermination of total serum globulins by means of a tryptophan reaction. Clin Chem 1961; 7: 626-636.
10. Saifer A, Marven T. The photometric microdetermination of serum total globulins with a tryptophan reaction: a modified procedure. Clin Chem 1966; 12: 414-417.
11. 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: 1029-1033.
12. Webster D, Bignell AHC, EC Attwood. An assessment of the suitability of bromocresol green for the determination of serum albumin. Clin Chim Acta 1974; 53: 101-108.
13. Louderback A, Mealey EH, NA Taylor. A new dye-binding technic using bromcresol purple for determination of albumin in serum. Clin Chem 1968; 14: 793-794. (Abstract)
14. Pinnell AE, Northam BE. New automated dye-binding method for serum albumin determination with bromcresol purple. Clin Chem 1978; 24: 80-86.
15. King EJ, Armstrong AR. A convenient method for determining serum and bile phosphatase activity. Can Med Assoc J 1934; 31: 376-381.
16. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol 1954; 7: 322-326.
17. Ohmori Y. Uber die Phosphomonoesterase. Enzymologia 1937; 4: 217-231.
18. Fujita H. Uber die Mikrobestimmung der Blutphosphatase. J Biochem, Japan 1939; 30: 69-87.
19. Petitclerc C, et al. Mechanism of action of Mg^{2+} and Zn^{2+} on rat placental alkaline phosphatase. I. Studies on the soluble Zn^{2+} and Mg^{2+} alkaline phosphatase. Can J Biochem 1975; 53: 1089-1100.
20. Tietz NW, et al. A reference method for measurement of alkaline phosphatase activity in human serum. Clin Chem 1983; 29: 751-761.
21. Bowers GN, Jr, et al. IFCC methods for the measurement of catalytic concentration of enzymes. Part I. General considerations concerning the determination of the catalytic concentration of an enzyme in the blood serum or plasma of man. Clin Chim Acta 1979; 98: 163F-174F.
22. McNeely MDD. Amylase. In: Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. Kaplan LA, Pesce AJ, eds. St. Louis: The C.V. Mosby Company. 1989: 906-909.
23. Zinterhofer L, et al. Nephelometric determination of pancreatic enzymes. I. Amylase. Clin Chim Acta 1973; 43: 5-12.
24. Centers for Disease Control (CDC). Alpha-amylase methodology survey I. Atlanta: US Public Health Service; Nov, 1975.
25. Somogyi M. Modifications of two methods for the assay of amylase. Clin Chem 1960; 6: 23-35.

13. Bibliography (cont.)

26. Gillard BK, Markman HC, Feig SA. Direct spectro-photometric determination of α-amylase activity in saliva, with p-nitrophenyl α-maltoside as substrate. Clin Chem 1977; 23: 2279-2282.
27. Wallenfels K,et al. The enzymic synthesis, by transglucosylation of a homologous series of glycosidically substituted malto-oligosaccharides, and their use as amylase substrates. Carbohydrate Res 1978; 61: 359-368.
28. Karmen A. A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood serum. J Clin Invest 1955; 34: 131-133.
29. Bergmeyer, HU, et al. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. IFCC method for aspartate aminotransferase. Clin Chem 1977; 23: 887-899.
30. Bergmeyer HU, Hørder M, Moss DW. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. Revised IFCC method for aspartate aminotransferase. Clin Chem 1978; 24: 720-721.
31. Kramer B, Tisdall FF. A simple technique for the determination of calcium and magnesium in small amounts of serum. J Biol Chem 1921; 47: 475-481.
32. Clark EP, Collips JB. A study of the Tisdall method for the determination of blood serum calcium with suggested modification. J Biol Chem 1925; 63: 461-464.
33. Katzman E, Jacobi M. The determination of serum calcium by titration with ceric sulfate. J Biol Chem 1937; 118: 539-544.
34. Cali JP, et al. A reference method for the determination of total calcium in serum. In: GR Cooper, ed., Selected Methods of Clinical Chemistry, vol 8. Washington, DC: American Association for Clinical Chemistry. 1997: 3-8.
35. Kessler G, M Wolfman. An automated procedure for the simultaneous determination of calcium and phosphorus. Clin Chem 1964; 10: 686-703.
36. Michaylova V, Ilkova P. Photometric determination of micro amounts of calcium with arsenazo III. Anal Chim Acta 1971; 53: 194-198.
37. Scarpa A, et al. Metallochromic indicators of ionized calcium. Ann NY Acad Sci 1978; 307: 86-112.
38. Knoll VE, Stamm D. Spezifische kreatininbest-immung im serum. Z Klin Chem Klin Biochem 1970; 8: 582-587.
39. Haeckel R. Simplified determinations of the "true" creatinine concentration in serum and urine. J Clin Chem Clin Biochem 1980; 18: 385-394.
40. Moss GA, Bondar RJL, Buzzelli DM. Kinetic enzymatic method for determining serum creatinine. Clin Chem 1975; 21: 1422-1426.
41. Jaynes PK, Feld RD, Johnson GF. An enzymic, reaction-rate assay for serum creatinine with a centrifugal analyzer. Clin Chem 1982; 28: 114-117.
42. Fossati P, Prencipe L, and Berti G. Enzymic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin Chem 1983; 29: 1494-1496.
43. Whelton A, Watson AJ, Rock RC. 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.
44. Ball, EG, Revel JP, Cooper O. The quantitative measurement of γ-glutamyl transpeptidase activity. J Biol Chem 1956; 221: 895-908.
45. Goldbarg JA, et al. The colorimetric determination of γ-glutamyl transpeptidase with a synthetic substrate. Arch Biochem Biophys 1960; 91: 61-70.
46. Orlowski M, Meister A. γ-Glutamyl-*p*-nitroanilide: a new convenient substrate for determination and study of L- and D-γ-glutamyltranspeptidase activities. Biochim Biophys Acta 1963; 73: 679-681.
47. Persijn JP, van der Slik W. A new method for the determination of γ-glutamyltransferase in serum. J Clin Chem Clin Biochem 1976; 14: 421-427.
48. Shaw LM, et al. IFCC methods for the measurement of catalytic concentration of enzymes. Part 4. IFCC method for γ-glutamyltransferase. J Clin Chem Clin Biochem1983; 21: 633-646.
49. Folin O, Wu H. A system of blood analysis. J Biol Chem 1919; 38: 81-110.
50. Somogyi M. A reagent for the copper-iodometric determination of very small amounts of sugar. J Biol Chem 1937; 117: 771-776.
51. Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 1944; 153: 375-380.
52. 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.
53. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 1937; 119: 481-490.
54. Meites S. Bilirubin, direct reacting and total, modified Malloy-Evelyn method. In: Selected Methods of Clinical Chemistry, vol. 9. Faulkner WR, Meites S, eds. Washington, DC: American Association for Clinical Chemistry. 1982: 119-124.58.
55. Murao S Tanaka N. A new enzyme "bilirubin oxidase" produced by Myrothecium verrucaria MT-1. Agric Biol Chem 1981; 45: 2383-2384.
56. Osaki S, Anderson S. Enzymatic determination of bilirubin. Clin Chem 1984; 30: 971. (Abstract)

13. Bibliography (cont.)

57. Perry B, et al. of total bilirubin by use of bilirubin oxidase. Clin Chem 1986; 32: 329-332.
58. Koller A Kaplan LA. Total serum protein. In: Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. Kaplan LA, Pesce AJ, eds., St. Louis: The C.V. Mosby Company. 1989: 1057-1060.
59. Reigler E. Eine kolorimetrische Bestimmungsmethode des Eiweisses. Z Anal Chem 1914; 53: 242-245.
60. Weichselbaum TE. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am J Clin Path 1946; 16: 40-49.
61. Doumas BT, et al. A candidate reference method for determination of total protein in serum. I. Development and validation. Clin Chem 1981; 27: 1642-1650.
62. Fales FW. Urea in serum, direct diacetyl monoxime method. In: Selected Methods of Clinical Chemistry, vol 9. Faulkner WR, Meites S, eds. Washington, D.C.: American Association for Clinical Chemistry. 1982: 365-373.
63. Van Slyke DD, Cullen GE. A permanent preparation of urease, and its use in the determination of urea. J Biol Chem 1914; 19: 211-228.
64. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. J Clin Pathol 1960; 13: 156-159.
65. Chaney AL, Marbach EP. Urea and ammonia determinations. Clin Chem 1962; 8: 130-132.
66. Talke H, Schubert GE. Enzymatische Harnstoffbest-immung in Blut und Serum im optischen Test nach Warburg. Klin Wochensch 1965; 43: 174-175.
67. Hallett CJ, Cook JGH. Reduced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. Clin Chim Acta 1971; 35: 33-37.
68. Patton CJ, Crouch SR. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal Chem 1977; 49: 464-469.
69. Sampson EJ, et al. A coupled-enzyme equilibrium method for measuring urea in serum: optimization and evaluation of the AACC study group on urea candidate reference method. Clin Chem 1980; 26: 816-826.
70. Folin O, Denis W. A new (colorimetric) method for the determination of uric acid in blood. J Biol Chem 1912-1913; 13: 469-475.
71. Brown H. The determination of uric acid in human blood. J Biol Chem 1945; 158: 601-608.
72. Feichtmeir TV, Wrenn HT. Direct determination of uric acid using uricase. Am J Clin Pathol 1955; 25: 833-839.
73. Fossati P, Prencipe L, Berti G. Use of 3,5-dichloro-2-hydroxy-benzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem 1980; 26: 227-231.
74. National Committee for Clinical Laboratory Standards. Physician's office laboratory guidelines; tentative guideline – second edition. NCCLS Document POL1-T2 Wayne, PA: NCCLS, 1992.
75. National Committee for Clinical Laboratory Standards. Procedures for the handling and processing of blood specimens; approved guideline – second edition. NCCLS document H18-A2. Wayne, PA: NCCLS, 1999.
76. Overfield CV, Savory J, Heintges MG. Glycolysis: a re-evaluation of the effect on blood glucose. Clin Chim Acta 1972; 39: 35-40.
77. Rehak NN, Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. Clin Chem 1988; 34: 2111-2114.
78. Sherwin JE, Obernolte R. Bilirubin. In: Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. Kaplan LA, Pesce AJ, eds. St. Louis: The C.V. Mosby Company. 1989: 1009-1015.
79. Henry RJ, Cannon DC, Winkelman JW. Clinical Chemistry: Principles and Technics, 2nd ed. New York: Harper and Row. 1974: 417-421; 1058-1059
80. National Committee for Clinical Laboratory Standards. Interference testing in clinical chemistry; proposed guideline. NCCLS Publication EP7-P. Wayne, PA: NCCLS, 1986.
81. Young DS. Effects of drugs on clinical laboratory tests, 3rd ed. Washington, DC: AACC Press. 1990.
82. Benet LZ, Williams RL. Design and optimization of dosage regimens: pharmacokinetic data. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th ed. Gilman AG, et al, eds. New York: McGraw-Hill, Inc. 1990: 1650-1735.
83. Young DS. Effects of drugs on clinical laboratory tests. 1991 supplement to the third edition. Washington, DC: AACC Press. 1991.
84. Moss DW, Henderson AR. Enzymes. In: Tietz Textbook of Clinical Chemistry, 2nd ed. Burtis CA, Ashwood ER, eds. Philadelphia: W.B. Saunders Company. 1994: 735-896.
85. Painter PC, Cope JY, Smith JL. Appendix. In: Tietz Textbook of Clinical Chemistry, 2nd ed. Burtis CA, Ashwood ER, eds. Philadelphia: W.B. Saunders Company. 1994: 2161-2217.
86. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices; tentative guideline – second edition. NCCLS Document EP5-T2. Wayne, PA: NCCLS, 1992.