Piccolo® BioChemistry Panel Plus

CE

For In Vitro Diagnostic Use and Professional Use Only
Customer and Technical Service: 800-822-2947
Customers outside the US should contact their local
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1. Intended Use

The Piccolo® BioChemistry Panel Plus, used with 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), c-reactive protein (CRP), calcium, creatinine, gamma glutamyltransferase (GGT), glucose, total protein, blood urea nitrogen (BUN), and uric acid in lithium heparinized whole blood, lithium heparinized plasma, or serum in a clinical laboratory setting or point-of-care location. The Abaxis CRP method is not intended for high sensitivity CRP measurement.

2. Summary and Explanation of Tests

The Piccolo BioChemistry Panel Plus and 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.
C-Reactive Protein (CRP):	Infection, tissue injury, and inflammatory disorders.
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 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. Principle of Procedure

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.

ALT

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

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

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 absorbance at 600 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.

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

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.

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

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.

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

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

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.

C-Reactive Protein (CRP)
Original assays for quantifying CRP were primarily for research and based on ELISA methodology.[31] More recently, latex-enhanced immunonephelometric methods have been used.[32] However; this requires a nephelometer to measure the light scattering. Now, several automated immunoturbidimetric and immunoluminometric assays have been developed that can be run on conventional clinical chemistry analyzers.[33]

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

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

Calcium (CA)
The first methods used to analyze calcium involved precipitating calcium with an excess of anions.[34,35,36] 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.[37] Spectrophotometric methods using either *o*-cresolphthalein complexone (CPC) or arsenazo III metallochromic indicators are most commonly used.[38,39,40] 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.[41,42] Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.[43,44,45] Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.[46]

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. Sodium 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 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.[47,48,49]

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.[50,51] The change to L-γ-glutamyl-*p*-nitroanilide as the substrate in the reaction eliminated the dye-formation step.[52] 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.[53] The International Federation of Clinical Chemistry (IFCC) recommended GGT method is based on the latter substrate, with glycylglycine as the other substrate.[54]

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[55] and Somogyi-Nelson[56,57]). 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 BioChemistry Panel Plus reagent disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.[58]

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.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

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

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 Protein (TP)
The total protein method is a modification of the biuret reaction, noted for its precision, accuracy, and specificity.[59] Originally developed by Riegler[60] and modified by Weichselbaum[61], Doumas, et al.[62] 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.[61] The Cu(II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-protein complex.

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

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.[63] Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.[64] The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique. [65,66] and coupled enzymatic reactions.[67,68] Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.[69] 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.[70]

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^+.

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

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

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

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.[71,72] 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.[73]

The uricase method is coupled through a Trinder peroxidase finish.[74] 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. 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 BioChemistry Panel Plus 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), c-reactive protein (CRP), calcium (CA), gamma glutamyltransferase (GGT), glucose (GLU), total protein (TP), blood urea nitrogen (BUN) and uric acid (UA). A dedicated sample blank is included in the disc to calculate concentrations of creatinine (CRE). Each disc also contains a diluent consisting of surfactants and preservatives.

Table 1: Reagents

Component	Quantity/Disc
Adenosine-5'-triphosphate	12 µg
L-Alanine	874 µg
4-Aminoantipyrine	27 µg
4-Aminoantipyrine hydrochloride	6 µg
Anti-human CRP (goat)	0.3 µg
Anti-human CRP coated latex (mouse)	67 µg
Arsenazo III, sodium salt	3 µg
Ascorbate oxidase (Cucurbita spp.)	0.4 U
L-Aspartic acid	426 µg
Bromcresol purple	2 µg
2-Chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3)	36 µg
Creatine amidinohydrolase (Actinobacillus spp.)	3 U
Creatinine amidohydrolase (Pseudomonas spp.)	1 U
Cupric sulfate	210 µg
3,5-Dichloro-2-hydroxybenzenesulfonic acid (DHBSA)	37 µg
Glucose-6-phosphate dehydrogenase (yeast)	0.05 U
Glutamate dehydrogenase (bovine liver)	0.01 U
L-Glutamic acid γ-(3-carboxy-4-nitroanilide), ammonium salt	32 µg
Glycylglycine	317 µg
Hexokinase (yeast)	0.1 U
α-Ketoglutarate, disodium salt	37 µg
α-Ketoglutaric acid	72 µg
Lactate dehydrogenase (chicken heart)	0.002 U
Lactate dehydrogenase (Staphylococcus epidermidis)	0.1 U
Magnesium acetate	7 µg
Magnesium chloride	5 µg
Malate dehydrogenase (MDH) (porcine heart)	0.01 U
ß-Nicotinamide adenine dinucleotide (NAD)	20 µg
ß-Nicotinamide adenine dinucleotide, reduced (NADH)	19 µg
p-Nitrophenyl Phosphate (p-NPP)	67 µg
Peroxidase (horseradish)	0.9 U
Potassium iodide	28 µg
Sarcosine oxidase (mircoorganism)	0.7 U
Sodium ferrocyanide	1 µg
Sodium potassium tartrate	343 µg
2,4,6-Tribomo-3-hydroxybenzoic acid (TBHBA)	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.[75] 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 sealed in their foil pouches to remain at room temperature longer than 48 hours prior to use. Open the sealed foil pouch, remove the disc and use according to the instructions provided in the Piccolo Xpress chemistry analyzer Operator's Manual. A disc not used within 20 minutes of opening the pouch should be discarded.

Storage

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

Indications of Reagent Disc Instability/Deterioration

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

6. Instrument

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

7. Sample Collection and Preparation

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

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

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

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

- Whole blood venipuncture samples should be run within 60 minutes of collection.[76] **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.[77]

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

- Refrigerating whole blood samples can cause significant changes in concentration of **aspartate aminotransferase**, **creatinine** and **glucose**.[78] 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.

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

8. Procedure

Materials Provided
- One Piccolo BioChemistry Panel Plus PN: 400-1035 (a box of discs PN 400-0035)

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

Test Parameters
The Piccolo Xpress chemistry analyzer operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each Piccolo BioChemistry Panel Plus reagent disc is less than 14 minutes. The analyzer maintains the reagent disc at a temperature of 37°C (98.6°F) over the measurement interval.

Test Procedure
The complete sample collection and step-by-step operating procedures are detailed in the Piccolo Xpress chemistry analyzer Operator's Manual.

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

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

9. Results

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

Interpretation of results is detailed in the Operator's Manual. Results are printed onto result tape supplied by Abaxis. The result tape 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 Xpress chemistry analyzer 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 PiccoloBioChemistry Panel Plus reagent disc.

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

- CRP is an "acute-phase" protein and rises non-specifically in response to inflammation. Intra-individual variation of c-reactive protein are significant (30 to 60%) and should be taken into account when interpreting values.[31] Serial measurements may be required to estimate the true mean of c-reactive protein in any specific individual.

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

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

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

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

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

Interference
Substances were tested as interferents with the analytes. Human serum pools were prepared. The concentration at which each potential interferent was tested was based on the testing levels in CLSI (formerly NCCLS) EP7-P[79] (except CRP) and CLSI EP7-A[80] (CRP).

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 result tape in place of the result.

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

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

Effects of Exogenous and Therapeutic Substances
Exogenous and therapeutic substances were selected as potential interferents for Abaxis test methods based on recommendations by Young.[81] Significant interference is defined as greater than ± 10% shift in the result for a normal range specimen. Human serum pools were supplemented with known concentrations of the drugs or chemicals and then analyzed. Please see Table 2 for a list of exogenous and therapeutic substances evaluated. **Please see TABLE 3 for a list of analytes where interference was observed.**

Table 2: Exogenous and Therapeutic Substances Evaluated

Potential Interferent	Highest Concentration Tested[A] (mg/dL unless otherwise specified)
Acetaminophen	100
Acetoacetate	102
Acetylsalicylic acid	50
Ampicillin	30
Ascorbic Acid (except CRP)	20
Ascorbic Acid (CRP only)	3
Caffeine	10
Calcium Chloride (except CRP)	20
Calcium Chloride (CRP only)	ND
Cephalothin (Keflin)	400
Chloramphenicol	100
Cimetidine	16

Table 2: Exogenous & Therapeutic Substances Evaluated (continued)

Potential Interferent	Highest Concentration Tested[A] (mg/dL unless otherwise specified)
L-Dopa	5
Dopamine (except CRP)	19
Dopamine (CRP only)	13
Epinephrine	1
Erythromycin	10
Glutathione	30
Hydrochlorothiazide	7.5
Ibuprofen	50
Isoniazide	4
α-Ketoglutarate (except CRP)	5
α-Ketoglutarate (CRP only)	ND
Ketoprofen	50
Lidocaine	1
Lactate (except CRP)	230
Lithium Lactate (CRP only)	84
Methicillin	100
Methotrexate	0.5
Methyldopa (AST, GLU, TP & UA only)	0.5
Methyldopa (all others)	ND
Metronidazole	5
Nafcillin	1
Nitrofurantoin	20
Oxacillin	1
Oxaloacetate	132
Penicillin G	100
Phenytoin (5,5-Diphenylhydantion)	3
Proline	4
Pyruvate (except CRP)	44
Pyruvate (CRP only)	ND
Rifampin (AST, GLU, TP & UA only)	1.5
Rifampin (all others)	0.5
Salicylic Acid (except CRP)	25
Salicylic Acid (CRP only)	50
Sulfasalazine (except CRP)	10
Sulfasalazine (CRP only)	ND
Sulfadiazine (except CRP)	ND
Sulfadiazine (CRP only)	150
Sulfanilamide	50
Theophylline	20

[A] ND = not done

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

	Concentration Which Produces > 10% Interference	% Interference[B] Observed
Alanine Aminotransferase (ALT)		
Ascorbic acid	20	11% inc
Oxaloacetate	132	843% inc
Albumin (ALB)		
Acetoacetate	102	18% dec

Table 3: The following substances showed greater than ± >10% shift (continued)

	Concentration Which Produces > 10% Interference	% Interference[B] Observed
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
Sulfasalazine	10	14% dec
Sulfanilamide	50	12% dec
Theophylline	20	11% dec
Alkaline Phosphatase (ALP)		
Theophylline	20	42% dec
C-Reactive Protein		
Glutathione	30	13% dec.
Isoniazide	4	16% dec.
L-dopa	5	28% dec.
Oxaloacetate	132	57% dec.
Creatinine (CRE)		
Ascorbic acid	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	44	13% dec
Uric Acid		
Ascorbic acid	20	13% dec
Epinephrine	1	14% dec
L-dopa	5	78% dec
Methyldopa	0.5	12% dec
Rifampin	1.5	14% dec
Salicylic acid	25	20% dec

[B] dec. = decreased concentration of the specified analyte; inc. = increased concentration of the specified analyte

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 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. The ranges below for these analytes were calculated based on the 95% reference interval estimated from the combined (overall) values obtained from the reference subjects. Samples from a total of 69 adult males and females were analyzed on the Piccolo Xpress chemistry analyzer for CRP. The CRP range was based on demonstrated transferability of the reference intervals from the Beckman.[82] 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
C-Reactive Protein	< 7.5 mg/L	< 7.5 mg/L
Calcium (CA)	8.0-10.3 mg/dL	2.00-2.58 mmol/L
Creatinine	0.6-1.2 mg/dL	53-106 µmol/L
Gamma Glutamyltransferase (GGT)	5-65 U/L	5-65 U/L
Glucose	73-118 mg/dL	4.1-6.6 mmol/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/L
Uric Acid (UA)		
Female	2.2-6.6 mg/dL	131-393 µmol/L
Male	3.6-8.0 mg/dL	214-476 µmol/L

12. Performance Characteristics

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

Table 5: Piccolo Dynamic Ranges

Analyte	Common Units	SI Units
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
C-Reactive Protein	5.0-200.0 mg/L	5.0-200.0 mg/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	0.6-38.9 mmol/L
Total Protein (TP)	2-14 g/dL	20-140 g/L
Blood Urea Nitrogen (BUN)	2-180 mg/dL	0.7-64.3 mmol/L
Uric Acid	1-15 mg/dL	100-900 µmol/L

If the analyte concentration is above the measuring range (dynamic range), but less than the system range, the print tape 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 tape, 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; c-reactive protein 5.0 mg/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 (0.6 mmol/L); total protein 2 g/dL (20 g/L); blood urea nitrogen 2.0 mg/dL (0.7 mmol/L) and uric acid 1 mg/dL (100 µmol/L).

Precision

Precision studies were conducted using CLSI EP5-T2[83] guidelines (except CRP) and EP5-A guidelines[84] with modifications based on CLSI EP18-A[85] for unit-use devices. Results for within-run and total precision were determined using two levels of commercially available control materials. The studies made use of multiple instruments. ALT, AST, albumin, ALP, amylase, calcium, creatinine, GGT, glucose, total protein, BUN and uric acid testing was done at two sites with two reagent disc lots over 20 days. C-reactive protein testing was done with one reagent disc lot over five days; serum 1, control 1 and control 2 were done at two sites; serum 2, serum 3, plasma 1 and plasma 2 were done at one site.

Results of precision studies are shown in Table 6.

Table 6: Precision

Analyte	Sample Size	Within-Run	Total
Alanine Aminotranferase (U/L)			
Control 1	N = 80		
Mean		21	21
SD		2.76	2.79
%CV		13.4	13.5
Control 2	N = 80		
Mean		52	52
SD		2.70	3.25
%CV		5.2	6.2
Albumin (g/dL)			
Control 1	N = 80		
Mean		5.6	5.6
SD		0.09	0.11
%CV		1.7	2.1
Control 2	N = 80		
Mean		3.7	3.7
SD		0.07	0.11
%CV		2.0	2.9
Alkaline Phosphatase (U/L)			
Control 1	N = 80		
Mean		39	39
SD		1.81	2.29
%CV		4.6	5.8
Control 2	N = 80		
Mean		281	281
SD		4.08	8.75
%CV		1.5	3.1
Amylase (U/L)			
Control 1	N = 80		
Mean		46	46
SD		2.40	2.63
%CV		5.2	5.7
Control 2	N = 80		
Mean		300	300
SD		11.15	11.50
%CV		3.7	3.8
Aspartate Aminotransferase (U/L)			
Control 1	N = 80		
Mean		47	47
SD		0.98	1.84
%CV		2.1	3.9
Control 2	N = 80		
Mean		145	145
SD		1.83	4.62
%CV		1.3	3.2

Table 6: Precision (continued)

Analyte	Sample Size	Within-Run	Total
C-Reactive Protein (mg/L)			
Serum 1	N = 80		
Mean		8.3	8.3
SD		0.70	0.81
%CV		8.4	9.8
Serum 2	N = 40		
Mean		8.1	8.1
SD		0.49	0.51
%CV		6.1	6.3
Serum 3	N = 40		
Mean		8.8	8.8
SD		0.54	0.54
%CV		6.2	6.2
Plasma 1	N = 40		
Mean		34.5	34.5
SD		1.04	1.09
%CV		3.0	3.2
Plasma 2	N = 40		
Mean		105.5	105.5
SD		2.06	2.30
%CV		1.9	2.2
Control 1	N = 80		
Mean		33.0	33.0
SD		1.21	2.12
%CV		3.7	6.4
Control 2	N = 80		
Mean		108.0	108.0
SD		1.88	3.14
%CV		1.7	2.9
Calcium (mg/dL)			
Control 1	N = 80		
Mean		8.6	8.6
SD		0.21	0.25
%CV		2.4	2.9
Control 2	N = 80		
Mean		11.8	11.8
SD		0.39	0.40
%CV		3.3	3.4
Creatinine (mg/dL)			
Control 1	N = 80		
Mean		1.1	1.1
SD		0.14	0.14
%CV		12.5	13.1
Control 2	N = 80		
Mean		5.2	5.2
SD		0.23	0.27
%CV		4.4	5.2
Gamma Glutamyltransferase (U/L)			
Control 1	N = 80		
Mean		25	25
SD		0.59	0.74
%CV		2.3	2.9
Control 2	N = 80		
Mean		106	106
SD		1.52	2.29
%CV		1.4	2.2

Table 6: Precision (continued)

Analyte	Sample Size	Within-Run	Total
Glucose (mg/dL)			
Control 1	N = 80		
Mean		66	66
SD		0.76	1.03
%CV		1.1	1.6
Control 2	N = 80		
Mean		278	278
SD		2.47	3.84
%CV		0.9	1.4
Total Protein (g/dL)			
Control 1	N = 80		
Mean		6.8	6.8
SD		0.05	0.08
%CV		0.8	1.2
Control 2	N = 80		
Mean		4.7	4.7
SD		0.09	0.09
%CV		2.0	2.0
Blood Urea Nitrogen (mg/dL)			
Control 1	N = 80		
Mean		19	19
SD		0.35	0.40
%CV		1.9	2.1
Control 2	N = 80		
Mean		65	65
SD		1.06	1.18
%CV		1.6	1.8
Uric Acid (mg/dL)			
Control 1	N = 80		
Mean		3.8	3.8
SD		0.15	0.18
%CV		4.0	4.8
Control 2	N = 80		
Mean		7.5	7.5
SD		0.24	0.29
%CV		3.2	3.9

Correlation

Lithium 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. For CRP, samples were collected from patients at one site. Lithium heparinized plasma samples were tested by the Piccolo Xpress chemistry analyzer and a comparative method. The samples were chosen to meet the distribution values in CLSI EP9-A2 guideline.[86] Samples were run in singlicate by the Abaxis and comparative methods on the same day. Representative correlation statistics are shown in Table 7.

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

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
Alanine	0.981	0.905	1.3	3.21	86	10-174	Paramax®
Aminotransferase (U/L)	0.985	0.946	-2.5	2.84	67	10-174	Technicon

Table 7: Correlation (continued)

	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
Alkaline Phosphatase (U/L)	0.988	0.970	-5.9	3.97	99	27-368	Paramax
	0.929	1.136	-17.6	4.79	80	26-150	Technicon
Amylase (U/L)	0.979	0.692	-4.7	3.11	99	11-92	Paramax
	0.963	1.065	-4.1	3.47	80	19-118	Technicon
Aspartate Aminotransferase (U/L)	0.93	0.87	5.3	2.76	159	13-111	Paramax
	1.0	0.97	3.0	1.90	46	13-252	DAX™
C-Reactive Protein (mg/L)	0.998	0.990	-0.4	4.60	113	5.4-198.6	Beckman
Calcium (mg/dL)	0.991*	0.990	-0.4	0.17	25	5.2-11.9	Paramax
	0.673	0.742	1.8	0.22	81	8.1-9.9	Beckman
Creatinine (mg/dL)	0.993	0.926	0.0	0.15	260	0.4-14.7	Paramax
	0.987	0.866	0.1	0.16	107	0.4-7.5	Beckman
Gamma Glutamyltransferase (U/L)	1.0	0.98	-0.4	3.29	135	5-312	Paramax
	1.0**	1.60	3.1	18.57	49	27-1848	Beckman
Glucose (mg/dL)	0.987	1.009	-2.8	3.89	251	72-422	Paramax
	0.997	0.943	1.2	4.69	91	56-646	Beckman
Total Protein (g/dL)	0.849	0.932	0.6	0.19	251	5.7-9.2	Paramax
	0.873	0.935	0.3	0.16	92	6.5-9.2	Beckman
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.

Table 8: Sample-type Correlation for CRP

For the CRP test method the Deming regression analysis for sample-type gave the following results for n = 21.

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

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

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