Piccolo® General Chemistry 13

CE 2797

For In Vitro Diagnostic Use and Healthcare Professional Use Only
Customer and Technical Service: 1- 800-822-2947
Customers outside the US: +49 6155 780 210
AB-PiccoloTechSupport@zoetis.com

Applicable to US customers only
CLIA Waived: Use lithium heparin whole blood, only
Moderate Complexity: Use lithium heparin whole blood, lithium heparin plasma, or serum

Abaxis, Inc.
3240 Whipple Rd.
Union City, CA 94587
USA

CH REP
Tao of Excellence GmbH
Vorstadt 26,
8200 Schaffhausen
Switzerland

EC REP
ABAXIS Europe GmbH
Bunsenstr. 9-11
64347 Griesheim
Germany

1. Intended Use

The Piccolo® General Chemistry 13 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), 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 in a clinical laboratory setting or point-of-care location/ near-patient testing.

For US Customers Only

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).

2. Summary and Explanation of Tests

The Piccolo General Chemistry 13 reagent disc and the Piccolo Xpress chemistry analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders in adults:

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

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.

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

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.

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

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.

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

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

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 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. Calcu lated 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-carbo xy-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.

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

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.

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

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

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.

$$\text{Bilirubin} + O_2 \xrightarrow{\text{Bilirubin Oxidase}} \text{Biliverdin} + H_2O$$

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.

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

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^+.

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

$$NH_3 + \text{2-Oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + H_2O + NAD^+$$

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.[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 Operation

Refer to 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, monopotassium salt	4 µg
Adenosine-5'-triphosphate, disodium salt	12 µg
L-Alanine	874 µg
4-Aminoantipyrine	5.4 µg
4-Aminoantipyrine-HCl (4-AAP)	25 µg
Arsenazo III, sodium salt	4 µg
Ascorbate oxidase	0.4 U
L-Aspartic acid	426 µg
Bilirubin oxidase	0.1 U
Bromcresol purple	2 µg
2-Cloro-*p*-nitrophenyl-□-D-maltotrioside (NPG3)	36 µg
Creatine amidinohydrolase	2 U
Creatinine amidohydrolase	1 U
Cupric sulfate	134 µg
3,5-Dichloro-2-hydroxybenzenesulfonic acid (DHBSA)	72 µg
Ethylenediaminetetraacetic acid (EDTA), disodium salt	61 µg
Ethylenediaminetetraacetic acid (EDTA), Tetrasodium Salt	219 µg
Glucose-6-phosphate dehydrogenase	0.05 U
L-Glutamic acid dehydrogenase	0.01 U

L-Glutamic acid γ-(3-carboxy-4-nitroanilide), ammonium salt	32 µg
Glycylglycine	317 µg
Hexokinase	0.1 U
α-Ketoglutarate, disodium salt	60 µg
α -Ketoglutaric acid	54 µg
Lactate dehydrogenase	0.002 U
Lactate dehydrogenase (LDH)	0.02 U
Lactate dehydrogenase	0.1 U
Magnesium acetate, tetrahydrate	5 µg
Magnesium chloride, hexahydrate	2 µg
Malate dehydrogenase (MDH)	0.01 U
Nicotinamide adenine dinucleotide (NAD+)	20 µg
Nicotinamide adenine dinucleotide, reduced (NADH)	18 µg
Peroxidase	0.9 U
p-Nitrophenyl Phosphate (p-NPP)	56 µg
Potassium ferrocyanide	1 µg
Potassium iodide	56 µg
Sarcosine oxidase	0.7 U
Sodium ferrocyanide	1 µg
Sodium potassium tartrate	686 µg
2,4,6-Tribromo-3-hydroxybenzoic acid	188 µg
Urease	0.05 U
Uricase	0.05 U
Zinc sulfate	2 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 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.

- This mixture does not contain substances assessed to be vPvB / PBT according to Regulation (EC) No 1907/2006, Annex XIII. This product contains components considered to have endocrine disrupting properties for environment, according to REACH Article 57(f), Regulation (EU) 2018/605 or Regulation (EU) 2017/2100. Refer to the Safety Data Sheet (SDS) for specific details.

- Materials incorporated into the device may consist of substances derived from other microbial and animal sourced materials.

Instructions for Reagent Handling

Reagent discs may be used directly from the refrigerator without warming. Do not allow discs to remain at room temperature at 20-25°C (68-77°F) longer than 48 hours prior to use. Open the sealed foil pouch and remove the disc, being careful not to touch the bar code ring located on the top of the disc. Use according to the instructions provided in the Piccolo Xpress chemistry analyzer Operator's Manual. A disc not used within 20 minutes of opening the pouch should be discarded.

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

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

6. Instrument

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

7. Sample Collection and Preparation

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

- The minimum required sample size is ~100 µL of heparinized whole blood, heparinized plasma, 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.[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 0.28-0.67 mmol/L 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, 87] Whole blood samples not run immediately should be stored in the dark for no longer than 60 minutes. If the sample cannot 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 evacuated specimen collection tubes for whole blood or plasma samples. Use no - additive evacuated specimen collection tubes or serum separator tubes 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 PN: 400-1029 (a box of discs PN: 400-0029)

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 Service 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 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 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.

The Piccolo Xpress chemistry analyzer is calibrated using internal calibrators or reference materials. The methods and materials used in the procedures for the calibration and control value assignments are traceable either to the comparative method and/or to the standards listed below:

- IFCC – International Federation of Clinical Chemistry
- NIST – National Institute of Standards and Technology
- CRMLN – Cholesterol Reference Method Laboratory Network
- SRM – Standard Reference Material
- CRM – Certified Reference Material

The calibration and value assignment processes are in compliance with EN ISO 17511, Metrological Traceability of Values Assigned to Calibrators and Control Materials.

The measured analytes in the General Chemistry 13 are traceable to the following reference materials or methods. The Piccolo Xpress Analysis System controls and calibration verification materials are validated for use only with the Piccolo Xpress Analysis System and assigned values may not be commutable with other methods. For specific analyte reference standard methods, refer to Section 3. Principles of Method.

Analyte	Calibration Standard	Method
ALT	IFCC	Colorimetric
ALB (BCP)	Correlation to Beckman LX-20 / DX-20	Dye Binding Bromocresol Purple
ALP	IFCC	Colorimetric
AMY	IFCC	Colorimetric
AST	IFCC	Colorimetric
CA	Correlation to Beckman LX-20 / DX-20	Arsenazo III Dye
CRE	NIST SRM #967	Enzymatic
GGT	IFCC	Colorimetric
GLU	NIST SRM #909	Enzymatic
TBIL	NIST SRM #916A	Enzymatic
TP	NIST SRM #909	Biuret (Copper II)
BUN	NIST SRM #912A	Enzymatic
UA	Correlation to Beckman LX-20 / DX-20	Enzymatic

Quality Control
See Section 6 (Calibration and Quality Control) of the Piccolo Xpress® Operator's Manual. Performance of 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 Abaxis Technical Support. Do not report results if controls are outside their labeled limits. See the Piccolo Xpress chemistry analyzer Operator's Manual for a detailed discussion on running, recording, interpreting, and plotting control results.

Waived Laboratories (US only): 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 (US only): Abaxis recommends control testing to follow federal, state, and local guidelines.

And the non-waived ones will use the below:

- Performance of 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 Abaxis Technical Support. Do not report results if controls are outside their labeled limits. See the Piccolo Xpress chemistry analyzer Operator's Manual for a detailed discussion on running, recording, interpreting, and plotting control results.

9. Results

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

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

The reaction for each analyte occurs at 37°C (98.6°F).

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 or the Piccolo Xpress chemistry analyzer 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% packed red cell volume (a volume fraction of 0.62) 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 Xpress chemistry analyzer.**

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

Note: Operators shall report any serious incident that has occurred in relation to the device to the manufacturer.

Interference

Substances were tested as interferents with the analytes. Human serum pools were prepared. The concentration at which each potential interferent was tested was based on the testing levels in CLSI EP7-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 printout to inform the operator about the levels of interferents present in each sample.

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

- 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. **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 & Therapeutic Substances Evaluated

	Highest Concentration Tested (mg/dL)
Acetaminophen	100
Acetoacetate	102
Acetylsalicylic acid	50
Ampicillin	30
Ascorbic Acid	20
Caffeine	10
Calcium Chloride	20
Cephalothin (Keflin)	400
Chloramphenicol	100
Cimetidine	16
L-Dopa	5
Dopamine	19
Epinephrine	1
Erythromycin	10
Glutathione	30
Ibuprofen	50
Isoniazide	4
α-Ketoglutarate	5
Ketoprofen	50
Methicillin	100
Methotrexate	0.5
Metyldopa	0.5
Metronidazole	5
Nafcillin	1
Nitrofurantoin	20
Oxacillin	1
Oxaloacetate	132
Phenytoin	3
Proline	4
Pyruvate	44
Rifampin	1.5
Salicylic Acid	25
Sulfasalazine	10
Sulfanilamide	50
Theophylline	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%

	Concentration with > 10% Interference (mg/dL)
Alanine Aminotransferase (ALT)	
Ascorbic acid	20
Oxaloacetate	132
Albumin (ALB)	
Acetoacetate	102
Ampicillin	30
Caffeine	10
Calcium chloride	20
Cephalothin (Keflin)	400
Ibuprofen	50
α-Ketoglutarate	5
Nitrofurantoin	20
Proline	4
Sulfasalazine	10
Sulfanilamide	50
Theophylline	20
Alkaline Phosphatase (ALP)	
Theophylline	20
Creatinine (CRE)	
Ascorbic acid	20
Dopamine	19
L-dopa	5
Epinephrine	1
Glutathione	30
Glucose (GLU)	
Oxaloacetate	132
Pyruvate	44
Total Bilirubin (TBIL)	
Dopamine	19
L-dopa	5
Sulfasalazine	10
Uric Acid	
Ascorbic acid	20
Epinephrine	1
L-dopa	5
Methyldopa	0.5
Rifampin	1.5
Salicylic acid	25

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

11. Expected Values

Samples from a total of 193 adult males and females 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

*Data was generated using equivalence device Piccolo Blood Chemistry Analyzer.

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
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 printout 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 printout, 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 CLSI 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/L)		
Control Level 1		
Mean	56	56
SD	0.9	1.1
%CV	1.7	2.1

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

Analyte	Within-Run	Total
Control Level 2		
Mean	37	37
SD	0.7	1.1
%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 (mmol/L)		
Control Level 1		
Mean	2.15	2.15
SD	0.05	0.06
CV	2.4	2.9
Control 2		
Mean	2.94	2.94
SD	0.1	0.1
CV	3.3	3.4
Creatinine (µmol/L)		
Control Level 1		
Mean	97.24	97.24
SD	12.38	12.38
CV	12.5	13.1
Control 2		
Mean	459.68	459.68
SD	20.33	23.87
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 (mmol/L)		
Control 1		
Mean	3.66	3.66
SD	0.04	0.06
CV	1.1	1.6
Control 2		
Mean	15.43	15.43
SD	0.14	0.21
CV	0.9	1.4
Total Bilirubin (µmol/L)		
Control 1		
Mean	13.7	13.7
SD	1.03	1.2
CV	8.0	9.3
Control 2		
Mean	88.9	88.9
SD	1.54	2.57
CV	1.7	2.8
Total Protein (g/L)		
Control 1	N = 80	
Mean	68	68
SD	0.5	0.8
CV	0.8	1.2
Control 2		
Mean	47	47
SD	0.9	0.9
CV	2.0	2.0
Blood Urea Nitrogen (mmol/L)		
Control 1	N = 80	
Mean	6.78	6.78
SD	0.12	0.14
CV	1.9	2.1
Control 2		
Mean	23.2	23.2
SD	0.38	0.42
CV	1.6	1.8
Uric Acid (mmol/L)		
Control Level 1		
Mean	0.23	0.23
SD	0.01	0.01

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

Analyte	Within-Run	Total
%CV	4.0	4.8
Control Level 2		
Mean	0.45	0.45
SD	0.01	0.02
%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 Xpress 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 with Comparative Methods***

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
Alanine Aminotransferase (U/L)	0.981	0.905	1.3	3.21	86	10-174	Paramax®
	0.985	0.946	-2.5	2.84	67	10-174	Technicon
Albumin (g/L)	0.854	1.001	-0.3	0.22	261	11-53	Paramax
	0.896	0.877	-0.1	0.21	100	15-50	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	1.9	46	13-252	DAX™
Calcium (mmol/L)	0.991*	0.990	-0.4	0.17	25	1.29-2.97	Paramax
	0.673	0.742	1.8	0.22	81	2.02-2.47	Beckman
Creatinine (µmol/L)	0.993	0.926	0.0	0.15	260	35.36-1299.4	Paramax
	0.987	0.866	0.1	0.16	107	35.36-663	Beckman
Gamma Glutamyl-transferase (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 (mmol/L)	0.987	1.009	-2.8	3.89	251	4.0-23.43	Paramax
	0.997	0.943	1.2	4.69	91	3.11-35.86	Beckman
Total Bilirubin (µmol/L)	0.974	0.901	0.0	0.07	250	3.41-63.28	Paramax
	0.98	1.113	-0.4	0.09	91	1.71-109.47	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 (mmol/L)	0.964	0.923	0.5	1.08	251	2.14-18.56	Paramax
	0.983	0.946	0.0	0.66	92	2.14-13.57	Beckman
Uric Acid (mmol/L)	0.979	0.958	-0.3	0.20	159	0.08-0.45	Paramax
	0.975	0.908	-0.6	0.31	44	0.12-0.72	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.
***Data was generated using equivalence device Piccolo Blood Chemistry Analyzer

Results of Untrained User Study

An "untrained user" study was conducted in which participants were given only the test instructions and asked to perform test ing 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	30 g/L	35 g/L	42 g/L
%CV	2.7%	2.5%	1.8%
Observed Range	29 – 32	33 – 37	40 – 44
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%

Aspartate 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	2.0 mmol/L	2.62 mmol/L	3.27 mmol/L
%CV	1.7%	1.5%	1.4%
Observed Range	1.92 – 2.1	2.52 – 2.74	3.14 – 3.34
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	78.68 µmol/L	182.99 µmol/L	609.08 µmol/L
%CV	11.0	5.0	1.6
Observed Range	61.88 – 106.08	159.12 – 203.32	574.6 – 636.48
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	5.28 mmol/L	7.23 mmol/L	20.31 mmol/L
%CV	1.1%	1.0%	0.8%
Observed Range	5.16 – 5.44	6.94 – 7.38	19.48 – 20.71
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 (3.61 mmol/L – 5.5 mmol/L) was considered.

Total Bilirubin (TBIL)

	Level 1	Level 2	Level 3
N	62	62	62
Mean	14.71 mmol/L	42.76 mmol/L	97.49 mmol/L
%CV	6.1%	2.6%	1.8%
Observed Range	13.68 – 17.1	39.34 – 44.47	92.36 – 100.91
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	48 g/L	57 g/L	71 g/L
%CV	2.0%	1.5%	1.5%
Observed Range	46 – 53	53 – 59	67 – 75
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	5.39 mmol/L	14.64 mmol/L	25.78 mmol/L
%CV	2.3	2.5	1.8
Observed Range	5.0 – 5.72	13.21 – 15.35	24.28 – 26.78
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	0.18 mmol/L	0.29 mmol/L	0.66 mmol/L
%CV	4.7	3.1	2.8
Observed Range	0.16 – 0.26	0.27 – 0.34	0.62 – 0.72
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. Symbols

Use By

Catalog Number

Batch Code

In Vitro Diagnostic Medical Device

Consult Instructions for Use

Manufacturer

Do Not Reuse

X Number of Test Devices in Kit

Manufacturing Sequence

Serial Number

Authorized Representative in the European Community

Temperature Limitation

Caution

PN:
Part Number

denotes conformity to specified European directives

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