Piccolo® AmLyte 13

For In Vitro Diagnostic Use and Professional Use Only
Customer and Technical Service: 1-800-822-2947
Customers outside the US: +49 6155 780 210

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

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

1. Intended Use

The Piccolo® AmLyte 13 , used with the Piccolo Xpress® chemistry analyzer, is intended to be used for the *in vitro* quantitative determination of alanine aminotransferase (ALT), albumin, amylase, aspartate aminotransferase (AST), calcium, c-reactive protein (CRP), creatine kinase, creatinine, glucose, potassium, sodium, total bilirubin and blood urea nitrogen (BUN) 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 AmLyte 13 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.
Amylase:	Pancreatitis.
Aspartate aminotransferase (AST):	Liver disease including hepatitis and viral jaundice, shock.
Calcium:	Parathyroid, bone and chronic renal diseases; tetany.
C-Reactive Protein (CRP):	Infection, tissue injury, and inflammatory disorders.
Creatine Kinase:	Myocardial infarction, progressive muscular dystrophy, dermatomyositis, rhabdomyolysis due to drug ingestion, hyperosmolality, autoimmune disease, delirium tremens, convulsions, Crush syndrome, hypothyroidism, surgery, severe exercise, intramuscular injection, physical inactivity, decreased muscle mass.
Creatinine:	Renal disease and monitoring of renal dialysis.
Glucose:	Carbohydrate metabolism disorders, including adult and juvenile diabetes mellitus and hypoglycemia.
Potassium:	Renal glomerular or tubular disease, adrenocortical insufficiency, diabetic ketacidosis, excessive intravenous potassium therapy, sepsis, panhypopituitarism, *in vitro* hemolysis, hyperaldosteronism, malnutrition, hyperinsulinism, metabolic alkalosis and gastrointestinal loss.
Sodium:	Dehydration, diabetes insipidus, loss of hypotonic gastrointestinal fluids, salt poisoning, selective depression of sense of thirst, skin losses, burns, sweating, hyperaldosteronism, CNS disorders, dilutional, depletional and delusional hyponatremia and syndrome of inappropriate ADH secretion.
Total bilirubin:	Liver disorders, including hepatitis and gall bladder obstruction; jaundice.
Blood Urea Nitrogen (BUN):	Renal and metabolic diseases.

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

3. Principle of Procedure

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.

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[15], while turbidimetric and iodometric methods are difficult to standardize.[16,17] Commonly used are saccharogenic and chromolytic methods. The "classic" amylase measurement technique is a saccharogenic method [18], but is difficult and time-consuming.[19] Chromolytic methods using *p*-nitrophenyl-glycosides as substrates have been recently developed.[20] These assays have a higher specificity for pancreatic amylase than for salivary amylase and are easily monitored.[20]

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[21] as modified by Bergmeyer.[22] 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.[22,23] 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.

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

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

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.[24,25,26] 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.[27] Spectrophotometric methods using either *o*-cresolphthalein complexone or arsenazo III metallochromic indicators are most commonly used.[28,29,30] 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.

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.

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

Creatine Kinase (CK)
Creatine kinase catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP). The phosphorylation reaction is favored by alkaline conditions (optimum at pH 9.0) and the dephosphorylation reaction is favored by acidic conditions (optimum at pH 6.5 at 37ºC). Early CK measurement methods were based on the "forward reaction" with creatine phosphate and adenosine diphosphate (ADP) as the products.[34,35,36] The sensitivity of these tests was shown to be low due to problems with interferences. The procedure of choice utilizes the "reverse reaction" coupled with a reaction to produce NADPH, which is directly related to CK levels.[37,38,39]

The CK measurement procedure used by Abaxis is a modified version of the International Federation of Clinical Chemistry (IFCC) method.[40] Key modifications are sample volume fraction, buffer, and temperature. N-acetyl cysteine (NAC) has been added to reactivate the CK.[41] Magnesium is used as a cofactor for both CK and hexokinase. EDTA has been added as a stabilizer for NAC and for the removal of various cations, such as calcium and iron, that inhibit CK. P^1, P^5-di (adenosine-5')penta phosphate and adenosine monophosphate (AMP) have also been added to inhibit adenylate kinase, another skeletal muscle and erythrocyte enzyme that reacts with the substrates used to measure CK.

Creatine kinase catalyzes the formation of creatine and ATP from creatine phosphate and ADP at pH 6.7. With hexokinase as a catalyst, ATP reacts with D-glucose to form ADP and D-glucose-6-phosphate (G-6-P), which is reacted with nicotinamide adenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate dehydrogenase (G-6-PDH) to produce G-6-P and NADPH.

$$\text{Creatine phosphate + ADP} \xrightarrow[Mg^{2+}]{CK} \text{Creatine + ATP}$$

$$\text{ATP + D-glucose} \xrightarrow{\text{Hexokinase}} \text{ADP + G-6-P}$$

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

The formation of NADPH is measured as a change in absorbance at 340 nm relative to 405 nm. This absorbance change is directly proportional to creatine kinase activity in the sample.

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.[42,43] Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.[44,45,46] Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.[47]

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

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

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

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

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.

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.

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

Glucose (GLU)
Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu[48] and Somogyi-Nelson[49,50]). 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 AmLyte 13 is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.[51]

The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), procedures 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.

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

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

Potassium (K^+)
Spectrophotometric methods have been developed that allow the measurement of potassium concentration on standard clinical chemistry instrumentation. The Abaxis enzymatic method is based on the activation of pyruvate kinase with potassium and shows excellent linearity and negligible susceptibility to endogenous substances.[52,53,54] Interference from sodium and ammonium ion are minimized with the addition of Kryptofix and glutamine synthetase respectively.[52]

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

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

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

Sodium (NA^+)
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.[55,56,57] In the Abaxis enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyzes the reaction of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol and galactose.

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

Total Bilirubin (TBIL)
Total bilirubin levels have been typically measured by tests that employ diazotized sulfanilic acid.[58,59] A newer, more specific method has been developed using the enzyme bilirubin oxidase.[60,61,62] 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.

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.

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 AmLyte 13 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), amylase (AMY), aspartate aminotransferase (AST), calcium (CA), c-reactive protein (CRP), creatine kinase (CK), glucose (GLU), potassium (K+), sodium (NA+), and blood urea nitrogen (BUN). A dedicated sample blank is included in the disc to calculate concentrations of creatinine (CRE), and total bilirubin (TBIL). Each disc also contains a diluent consisting of surfactants and preservatives.

Table 1: Reagents

Component	Quantity/Disc
2, 4, 6-Tribomo-3-hydroxybenzoic acid (TBHBA)	188 µg
2-Chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3)	52.5 µg
4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]trisocosane (Kryptofix 221)	84 µg
4-Aminoantipyrine hydrochloride	13 µg
Adenosine-5'-diphosphate	38 µg
Adenosine-5'-monophosphate	33 µg
Adenosine-5'-triphosphate	11 µg
Amylase	0.0357 U
Anti-human CRP coated latex (mouse)	268.8 µg
Anti-human CRP (goat)	0.5 µg
Ascorbate oxidase (*Cucurbita spp.*)	0.3 U
Calcium acetate	25.2 µg
Citric acid, trisodium salt	567 µg
Creatine amidinohydrolase (*Actinobacillus spp*.)	3 U
Creatine phosphate	122 µg
Creatinine amidohydrolase (*Pseudomonas spp.)*	1 U
Ethylene glyco-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)	4 µg
Ethylenediaminetetraacetic acid (EDTA)	191.1 µg
Glucose	58 µg
Glucose-6-phosphate dehydrogenase (G6PDH) (yeast)	0.1 U
Glutamate dehydrogenase	0.1 U
Glutamine synthetase	0.2 U

Table 1: Reagents (cont.)

Component	Quantity/Disc
Hexokinase (yeast)	0.2 U
Imidazole	26 µg
Lactate dehydrogenase (chicken heart)	0.3 U
Magnesium acetate	60 µg
Magnesium sulfate	29 µg
Malate dehydrogenase (porcine heart)	0.1 U
N-Acetyl cysteine	60 µg
o-Nitrophenyl-ß-D galactopyranoside (ONPG)	22 µg
P1, P5di(adenosine-5')pentaphosphate	0.2 µg
Peroxidase (horseradish)	1 U
Phosphoenol pyruvate	23 µg
Phosphoenol pyruvate carboxylase	0.001 U
Potassium ferrocyanide	0.4 µg
Pyruvate kinase	0.01 U
Sarcosine oxidase (microorganism)	1 U
Sodium Cholate	58 µg
Sodium lauryl sulfate	145 µg
Sulfhydryl blocked BSA	420 µg
ß-Nicotinamide adenine dinucleotide, (NAD)	20 µg
ß-Nicotinamide adenine dinucleotide, reduced (NADH)	28 µg
ß-Nicotinamide adenine dinucleotide phosphate (NADP)	101 µg
Urease (jack bean)	0.05 U
α-Ketoglutaric acid	19 µg
β-Galactosidase	0.005 U
Buffers, surfactants, excipients and preservatives	

Warnings and Precautions

- For *In vitro* Diagnostic Use

- The diluent container in the reagent disc is automatically opened when the analyzer drawer closes. A disc with an opened diluent container can not 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.[39] 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.

- Hemolysis may cause erroneously high results in **potassium** assays. This problem may go undetected when analyzing whole blood (release of potassium from as few as 0.5% of the erythrocytes can increase the potassium serum level by 0.5 mmol/L). In addition, even unhemolyzed specimens that are not promptly processed may have increased potassium levels due to intracellular potassium leakage.[71]

- Whole blood venipuncture samples should be run within 60 minutes of collection.[72] **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.[73]

- Refrigerating whole blood samples can cause significant changes in concentration of **aspartate aminotransferase**, **creatinine** and **glucose**.[74] The sample may be separated into plasma or serum and stored in capped sample tubes at 2-8°C (36-46°F) if the sample cannot be run within 60 minutes.

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

- **Total bilirubin** results may be adversely affected by photodegradation.[75] 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.[76]

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

8. Procedure

Materials Provided
- One Piccolo AmLyte 13 PN: 400-1041 (a box of discs PN 400-0041)

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 AmLyte 13 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 paper rolls available from Abaxis. The paper rolls have 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**. 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 AmLyte 13 .
- 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[77]. 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 EP7-A.[78]

Effects of Endogenous Substances

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

- The Piccolo 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 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.

- The potassium assay in the Piccolo system is a coupled pyruvate kinase (PK) / lactate dehydrogenase (LDH) assay. Therefore, in cases of extreme muscle trauma or highly elevated levels of creatine kinase (CK), the Piccolo may recover a falsely elevated potassium (K+) value. In such cases, unexpected high potassium recoveries need to be confirmed utilizing a different methodology.

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

Effects of Exogenous and Therapeutic Substances

Thirty-five exogenous and therapeutic substances were selected as potential interferents for Abaxis test methods based on recommendations by Young. [79] 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 (mg/dL unless otherwise specified)
Acetaminophen	100
Acetoacetate	102
Acetylsalicylic Acid	50
Ampicillin	30
Ascorbic acid	20
Bromide*	30
Caffeine	10
Calcium Chloride	20
Cephalothin (Keflin)	400
Chloramphenicol	100
Cimetidine	16
Digoxin*	5
D-lactate*	45
Dopamine	19
Epinephrine	1
Erythromycin	10
Glucose*	700
Glutathione	30
Glycolic acid*	7.6
Hydrochlorothiazide	7.5
Hydroxyurea*	0.7
Ibuprofen	50
Isoniazide	4
α-Ketoglutarate	5
Ketoprofen	50
L-dopa	5
Lidocaine	1
Lithium Lactate	84
Methicillin	100

Table 2: Exogenous and Therapeutic Substances Evaluated (continued)

Potential Interferent	Highest Concentration Tested (mg/dL unless otherwise specified)
Methotrexate	0.5
Metronidazole	5
Nafcillin	1
Nitrofurantoin	20
Oxacillin	1
Oxaloacetate	132
Penicillin G	100
Phenytoin (5,5-Diphenylhydantion)	3
Proline	4
Pyruvate	44
Rifampin	0.5
Salicylic Acid	50
Sulfadiazine	150
Sulfanilamide	50
Theophylline	20

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

	Concentration Which Produces > 10% Interference	% Interference[A] Observed
Alanine Aminotransferase (ALT)		
Ascorbic acid	20	11% inc*
Oxaloacetate	132	843% inc
Albumin (ALB)		
Acetoacetate	102	18% dec*
Ampicillin	30	12% dec
Caffeine	10	14% dec
Calcium chloride	20	17% dec
Cephalothin (Keflin)	400	13% inc
Ibuprofen	50	28% inc
α-Ketoglutarate	5	11% dec
Nitrofurantoin	20	13% dec
Proline	4	12% inc
Sulfalazine	10	14% dec
Sulfanilamide	50	12% dec
Theophylline	20	11% dec
C-Reactive Protein		
Glutathione	30	13% dec.
Isoniazide	4	16% dec.
L-dopa	5	28% dec.
Oxaloacetate	132	57% dec.
Creatine Kinase		
Cephalothin	400	43% dec
Dopamine	15	46% dec
L-dopa	5	13% dec
Methotextrate	0.5	16 % dec
Nitrofurantoin	20	18 % dec

Creatinine		
Ascorbic acid	20	11% dec.
Dopamine	19	80% dec.
L-dopa	5	71% dec.
Epinephrine	1	45% dec.
Glutathione	30	13% dec.

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

	Concentration Which Produces > 10% Interference	% Interference[A] Observed
Glucose		
Oxaloacetate	132	11% dec.
Pyruvate	44	13% dec.
Potassium		
Penicillin G	100	17% inc.
Sulfadiazine	150	12% dec.
Sodium		
Cephalothin	400	12% inc.
Methotrexate	0.5	11% inc.
Penicillin G	100	10% inc.
Alanine Aminotransferase (ALT)		
Ascorbic acid	20	11% inc*
Oxaloacetate	132	843% inc
Total Bilirubin (TBIL)		
Dopamine	19	55% dec
L-dopa	5	17% dec

[A] 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, amylase, calcium, creatinine, glucose, total bilirubin, and BUN. Samples from a total of 186 adult males and females were used to determine the reference range for AST . Samples from 125-150 adult males and females were analyzed for electrolytes on the Piccolo blood chemistry analyzer and 69 adult males and females were analyzed on the Piccolo Xpress chemistry analyzer for CRP to determine the reference intervals. The electrolyte ranges were calculated based on the 95% reference interval estimated from the combined (overall) values obtained from the reference subjects and the CRP range was based on demonstrated transferability of the reference intervals from the Beckman.[80] These intervals 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
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
C-Reactive Protein	< 7.5 mg/L	< 7.5 mg/L

Creatine Kinase (Female)	30-190 U/L	30-190 U/L
Creatine Kinase (Male)	39-380 U/L	39-380 U/L
Creatinine	0.6-1.2 mg/dL	53-106 µmol/L
Glucose	73-118 mg/dL	4.1-6.6 mmol/L
Potassium	3.6-5.1 mmol/L	3.6-5.1 mmol/L
Sodium	128-145 mmol/L	128-145 mmol/L
Total Bilirubin (TBIL)	0.2-1.6 mg/dL	3.4-27.4 µmol/L

Table 4: Piccolo Reference Intervals (continued)

Analyte	Common Units	SI Units
Blood Urea Nitrogen (BUN)	7-22 mg/dL	2.5-7.9 mmol/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
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
C-Reactive Protein	5.0-200.0 mg/L	5.0-200.0 mg/L
Creatine Kinase	5-5,000 U/L	5-5,000 U/L
Creatinine	0.2-20 mg/dL	18-1768 µmol/L
Glucose	10-700 mg/dL	0.6-38.9 mmol/L
Potassium	1.5-8.5 mmol/L	1.5-8.5 mmol/L
Sodium	110-170 mmol/L	110-170 mmol/L
Total Bilirubin (TBIL)	0.1-30 mg/dL	1.7-513 µmol/L
Blood Urea Nitrogen (BUN)	2-180 mg/dL	0.7-64.3 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. GLU >700* U/L. If lower than the dynamic range, a "<" will be printed with an asterisk, e.g. GLU <10* 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 Technical Support.

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); amylase 5 U/L; aspartate aminotransferase 5 U/L; calcium 4.0 mg/dL (1.0 mmol/L); c-reactive protein 5.0 mg/L; creatine kinase 5 U/L; creatinine 0.2 mg/dL (18 µmol/L); glucose 10 mg/dL (0.6 mmol/L); potassium 1.5 mmol/L; sodium 110 mmol/L; total bilirubin 0.1 mg/dL (1.7 µmol/L); and blood urea nitrogen 2.0 mg/dL (0.7 mmol/L).

Precision
Precision studies were conducted using CLSI EP5-A guidelines[81] with modifications based on CLSI EP18-A[82] 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. Two reagent disc lots were used for the electrolytes and one for c-reactive protein. Creatine kinase, creatinine, glucose, sodium and urea nitrogen testing was performed at one site; potassium testing was performed at two sites over 20 days; c-reactive protein (serum 1, control 1 and control 2) testing was done at two sites over a period of five days. C-reactive protein serums 2 and 3 and plasmas 1 and 2 were done at one site over a period of five days.

Results of precision studies are shown in Table 6.

Table 6: Precision

Analyte	Sample Size	Within-Run	Total
Alanine Aminotranferase (U/L)	N = 80		
Control Level 1			
Mean		21	21
SD		2.76	2.79
%CV		13.4	13.5
Control Level 2			
Mean		52	52
SD		2.70	3.25
%CV		5.2	6.2
Albumin (g/dL)	N = 80		
Control Level 1			
Mean		5.6	5.6
SD		0.09	0.11
%CV		1.7	2.1
Control Level 2			
Mean		3.7	3.7
SD		0.07	0.11
%CV		2.0	2.9
Amylase (U/L)	N = 80		
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)	N = 80		
Control Level 1			
Mean		47	47
SD		0.98	1.84
%CV		2.1	3.9
Control Level 2			
Mean		145	145
SD		1.83	4.62
%CV		1.3	3.2
Calcium (mg/dL)	N = 80		
Control Level 1			
Mean		8.6	8.6
SD		0.21	0.25
%CV		2.4	2.9
Control Level 2			
Mean		11.8	11.8
SD		0.39	0.40

%CV	3.3	3.4

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
C-Reactive Protein (mg/L)			
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
Creatine Kinase (U/L)	N = 120		
Control 1			
Mean		134	134
SD		2.7	2.7
%CV		2.0	2.0
Control 2			
Mean		526	526
SD		7.7	7.7
%CV		1.5	1.5
Creatinine (mg/dL)	N=80		
Control 1			
Mean		1.1	1.1
SD		0.14	0.14
%CV		12.5	13.1
Control 2			
Mean		5.2	5.2
SD		0.23	0.27
%CV		4.4	5.2

Table 6: Precision (continued)

Analyte	Sample Size	Within-Run	Total
Glucose (mg/dL)	N=80		
Control 1			
Mean		66	66
SD		0.76	1.03
%CV		1.1	1.6
Control 2			
Mean		278	278
SD		2.47	3.84
%CV		0.9	1.4
Potassium (mmol/L)	N = 120		
Control 1			
Mean		6.12	6.12
SD		0.32	0.32
%CV		5.2	5.7
Control 2			
Mean		4.10	4.10
SD		0.24	0.26
%CV		5.9	6.3
Sodium (mmol/L)	N = 80		
Control 1			
Mean		143.5	143.5
SD		2.28	2.28
%CV		1.6	1.6
Control 2			
Mean		120.0	120.0
SD		2.13	2.13
%CV		1.8	1.8
Total Bilirubin (mg/dL)	N = 80		
Control Level 1			
Mean		0.8	0.8
SD		0.06	0.07
%CV		8.0	9.3
Control Level 2			
Mean		5.2	5.2
SD		0.09	0.15
%CV		1.7	2.8
Blood Urea Nitrogen (mg/dL)	N = 80		
Control 1			
Mean		19	19
SD		0.35	0.40
%CV		1.9	2.1
Control 2			
Mean		65	65
SD		1.06	1.18
%CV		1.6	1.8

Correlation

Lithium heparinized whole blood and serum samples were collected and assayed on the Piccolo blood chemistry analyzer and by comparative methods for chloride, creatine kinase, creatinine, glucose, potassium, sodium, total carbon dioxide and urea nitrogen. The whole blood samples were analyzed by the Piccolo blood chemistry analyzer at the field sites and the serum samples were analyzed by the Piccolo blood chemistry analyzer and by comparative methods. In some cases, high and low supplemented samples were used to cover the dynamic range. For CRP, 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.[83]

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 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/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
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 (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
C-Reactive Protein (mg/L)	0.998	0.990	-0.4	4.6	113	5.4-198.6	Beckman
Creatine Kinase (U/L)	0.967	1.194	-25	9.05	47	6-813	Cobas Fara®
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
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
Potassium (mmol/L)	0.969	0.863	0.6	0.14	58	2.0-6.8	Radiometer KNA 2
Sodium (mmol/L)	0.937	0.782	27.7	3.79	113	116-154	Radiometer KNA 2
Total Bilirubin (mg/dL)	0.974	0.901	0.0	0.07	250	0.2-3.7	Paramax
	0.980	1.113	-0.4	0.09	91	0.1-6.4	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

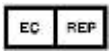
Table 8: Sample-type Correlation for CRP

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.

13. Symbols

 Use By	 Catalog Number	 Batch Code
 In Vitro Diagnostic Medical Device	 Consult Instructions For Use	 Manufacturer
 Do Not Reuse	 X Number of Test Devices in Kit	 Manufacturing Sequence
 Serial Number	 Caution	 Temperature Limitation
PN: Part Number	 Authorized Representative In the European Community	 Denotes conformity to specified European directives
 UDI Barcode structure in Health Industry Bar Code (HIBC) standard format	 Unique Device Identifier (UDI) in human and machine-readable form used to adequately identify medical devices through their distribution and use	 Separate waste collection for this electronic item indicated; Equipment manufactured / placed on the market after 13 August 2005; Indicates compliance with Article 14(4) of Directive 2012/19/EU (WEEE) for the European Union (EU).

14. Bibliography

1. Tonhazy NE, NG White, WW Umbreit. A rapid method for the estimation of the glutamic-aspartic transaminase in tissues and its application to radiation sickness. Arch Biochem 1950; 28: 36-42.
2. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28: 56-63.
3. Murray RL. Alanine aminotransferase. In: Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. Kaplan LA,Pesce AJ, eds. St. Louis: The C.V. Mosby Company. 1989: 895-898.
4. Wróblewski F, LaDue JS. Serum glutamic-pyruvic transminase in cardiac and hepatic disease. Proc Soc Exp Biol Med 1956; 91: 569-571.
5. Bergmeyer HU, Horder M. IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem 1980; 18: 521-534.
6. Howe PE. The use of sodium sulfate as the globulin precipitant in the determination of proteins in blood. J Biol Chem 1921; 49: 93-107.
7. Howe PE. The determination of proteins in blood — a micro method. J Biol Chem 1921; 49: 109-113.
8. Wolfson WQ, et al. A rapid procedure for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin and gamma globulin in 10 ml of serum. Am J Clin Pathol 1948; 18: 723-730.
9. Saifer A, Gerstenfeld S, Vacsler F. Photometric microdetermination of total serum globulins by means of a tryptophan reaction. Clin Chem 1961; 7: 626-636.
10. Saifer A, Marven T. The photometric microdetermination of serum total globulins with a tryptophan reaction: a modified procedure. Clin Chem 1966; 12: 414-417.
11. Gendler SM, Albumin. In: Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. Kaplan LA, Pesce AJ, eds. St. Louis: The C.V. Mosby Company. 1989: 1029-1033.
12. Webster D, Bignell AHC, EC Attwood. An assessment of the suitability of bromocresol green for the determination of serum albumin. Clin Chim Acta 1974; 53: 101-108.
13. Louderback A, Mealey EH, NA Taylor. A new dye-binding technic using bromcresol purple for determination of albumin in serum. Clin Chem 1968; 14: 793-794. (Abstract)
14. Pinnell AE, Northam BE. New automated dye-binding method for serum albumin determination with bromcresol purple. Clin Chem 1978; 24: 80-86.
15. McNeely MDD. Amylase. In: Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. Kaplan LA, Pesce AJ, eds. St. Louis: The C.V. Mosby Company. 1989: 906-909.
16. Zinterhofer L, et al. Nephelometric determination of pancreatic enzymes. I. Amylase. Clin Chim Acta 1973; 43: 5-12.
17. Centers for Disease Control (CDC). Alpha-amylase methodology survey I. Atlanta: US Public Health Service; Nov, 1975.
18. Somogyi M. Modifications of two methods for the assay of amylase. Clin Chem 1960; 6: 23-35.
19. Gillard BK, Markman HC, Feig SA. Direct spectro-photometric determination of α-amylase activity in saliva, with p-nitrophenyl α-maltoside as substrate. Clin Chem 1977; 23: 2279-2282.
20. Wallenfels K,et al. The enzymic synthesis, by transglucosylation of a homologous series of glycosidically substituted malto-oligosaccharides, and their use as amylase substrates. Carbohydrate Res 1978; 61: 359-368.
21. Karmen A. A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood serum. J Clin Invest 1955; 34: 131-133.
22. Bergmeyer, HU, et al. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. IFCC method for aspartate aminotransferase. Clin Chem 1977; 23: 887-899.
23. Bergmeyer HU, Hørder M, Moss DW. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. Revised IFCC method for aspartate aminotransferase. Clin Chem 1978; 24: 720-721.
24. Kramer B, Tisdall FF. A simple technique for the determination of calcium and magnesium in small amounts of serum. J Biol Chem 1921; 47: 475-481.
25. Clark EP, Collips JB. A study of the Tisdall method for the determination of blood serum calcium with suggested modification. J Biol Chem 1925; 63: 461-464.
26. Katzman E, Jacobi M. The determination of serum calcium by titration with ceric sulfate. J Biol Chem 1937; 118: 539-544.
27. Cali JP, et al. A reference method for the determination of total calcium in serum. In: GR Cooper, ed., Selected Methods of Clinical Chemistry, vol 8. Washington, DC: American Association for Clinical Chemistry. 1997: 3-8.
28. Kessler G, M Wolfman. An automated procedure for the simultaneous determination of calcium and phosphorus. Clin Chem 1964; 10: 686-703.
29. Michaylova V, Ilkova P. Photometric determination of micro amounts of calcium with arsenazo III. Anal Chim Acta 1971; 53: 194-198.
30. Scarpa A, et al. Metallochromic indicators of ionized calcium. Ann NY Acad Sci 1978; 307: 86-112.
31. Macy E, Hayes T, Tracy R. Variability in the measurement of c-reactive protein in healthy subjects: implications for reference interval and epidemiological applications. Clin. Chem. 1997; 43: 52-58.

14. Bibliography (continued)

32. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem 1999; 45: 2136-2141.
33. Roberts WL, Moulton L, Law TC, Farrow G, Cooper-Anderson M, Savory J, Rifai N. Evaluation of nine automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. Part 2. Clin Chem. 2001; 47: 418–425.
34. Kuby SA, Noda, L and Lardy HA. Adenosinetriphosphate-creatine transphosphorylase. J. Biol Chem. 1954; 209: 191-201.
35. Tanzer Ml And Gilvarg C. Creatine and creatine kinase measurement. J Biol Chem. 1959; 234: 3201-3204.
36. Nuttall FQ And Wedin DS. A simple rapid colorimetric method for determination of creatine kinase activity. J Lab Clin Med. 1966; 68: 324-332.
37. Oliver IT. A spectrophotometric method for the determination of creatine phosphokinase and myokinase. Biochem J. 1955; 61: 116-122.
38. Rosalki SB. An improved procedure or serum creatine phosphokinase determination. J Lab Clin Med. 1967; 69: 696-705.
39. Szasz G, Gruber W and Bernt E. Creatine kinase in serum: I. Determination of optimum reaction conditions. Clin Chem. 1976; 22: 650-656.
40. Expert Panel On Enzymes, Committee Of Standards (IFCC). 1979 Approval Recommendations Of IFCC Methods For The Measurement Of Catalytic Concentrations Of Enzymes, Part 1. General Considerations. Clin Chim Acta. IFCC Sections: 98: 163-174.
41. Committee On Enzymes Of The Scandinavian Society For Clinical Chemistry And Clinical Physiology. 1976. Recommended Method For The Determination Of Creatine Kinase In Blood. Scand J. Clin Lab Invest. 36: 711-723.
42. Knoll VE, et al. Spezifische kreatininbetimmung im serum. Z Klin Chem Klin Biochem. 1970; 8: 582-587.
43. Haeckel R, et al. Simplified determinations of the "true" creatinine concentration in serum and urine. J Clin Chem Clin Biochem. 1980; 18: 385-394.
44. Moss GA, et al. Kinetic enzymatic method for determining serum creatinine. Clin Chem. 1975; 21: 1422-1426.
45. Jaynes PK, et al. An enzymatic, reaction-rate assay for serum creatinine with a centrifugal analyzer. Clin Chem. 1982; 28: 114-117.
46. Fossati P, et al. Enzymatic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin Chem. 1983; 29: 1494-1496.
47. Whelton A, et al. Nitrogen metabolites and renal function. In: Tietz Textbook of Clinical Chemistry, 2nd Ed. Burtis CA, and Ashwood ER, Eds. Philadelphia: W.B. Saunders Company. 1994; 1513-1575.
48. Folin O, et al. A system of blood analysis. J Biol Chem. 1919; 38: 81-110.
49. Somogyi M. A reagent for the copper-iodometric determination of very small amounts of sugar. J Biol Chem. 1937; 117: 771-776.
50. Nelson N, et al. A photometric adaption of the Somogyi method for the determination of glucose. J Biol. 1944; 153: 375-380.
51. Kaplan LA. Glucose. In: Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. Kaplan LA, AJ Pesce AJ, eds. St. Louis: The C.V. Mosby Company. 1989; 850-856.
52. Berry MN, et al. Enzymatic determination of potassium in serum. Clin Chem. 1989; 35: 817-820.
53. Van Pelt J. Enzymatic determination of sodium, potassium and chloride in serum compared with determination by flame photometry, coulometry and ion selective electrodes. Clin Chem. 1994; 40: 846-847.
54. Hubl W, et al. Enzymatic determination of sodium, potassium and chloride in abnormal (hemolyzed, icteric, lipemic, paraprteinemic, or uremic) serum samples compared with indirect determination with ion selective electrodes. Clin Chem. 1994; 40: 1528-1531.
55. Helgerson RC, et al. Host-guest Complexation. 50. Potassium and sodium ion-selective chromogenic ionophores. J Amer Chem Soc. 1989; 111: 6339-6350.
56. Kumar A, et al. Chromogenic ionophere-based methods for spectrophotometric assay of sodium and potassium in serum and plasma. Clin Chem. 1988; 34: 1709-1712.
57. Berry MN, et al. Enzymatic determination of sodium in serum. Clin Chem. 1988; 34: 2295-2298.
58. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 1937; 119: 481-490.
59. Meites S. Bilirubin, direct reacting and total, modified Malloy-Evelyn method. In: Selected Methods of Clinical Chemistry, vol. 9. Faulkner WR, Meites S, eds. Washington, DC: American Association for Clinical Chemistry. 1982: 119-124.58.
60. Murao S Tanaka N. A new enzyme "bilirubin oxidase" produced by Myrothecium verrucaria MT-1. Agric Biol Chem 1981; 45: 2383-2384.

14. Bibliography (continued)

61. Osaki S, Anderson S. Enzymatic determination of bilirubin. Clin Chem 1984; 30: 971. (Abstract)
62. Perry B, et al. of total bilirubin by use of bilirubin oxidase. Clin Chem 1986; 32: 329-332.
63. Fales FW. Urea in serum, direct diacetyl monoxime method. In: Selected Methods of Clinical Chemistry, vol 9. Faulkner WR, Meites S, eds. Washington, DC.: American Association for Clinical Chemistry. 1982; 365-373.
64. Van Slyke, et al. A permanent preparation of urease, and its use in the determination of urea. J Biol Chem. 1914; 19: 211-228.
65. Fawcett JK, et al. A rapid and Precise method for the determination of urea. J Clin Pathol. 1960; 13: 156-159.
66. Chaney, et al. Urea and ammonia determinations. Clin Chem. 1962; 8: 130-132.
67. Talke H, et al. Enzymatische Harnstoffbestimmung in Blut and Serum im optischen Test nach Warburg. Klin Wochensch. 1965; 43: 174-175.
68. Hallett, et al. Reduced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. Clin Chim Acta. 1971; 35: 33-37.
69. Patton, et al. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal Chem. 1977; 49: 464-469.
70. Sampson EJ, et al. A coupled-enzyme equilibrium method for the measuring urea in serum: optimization and evaluation of the AACC study group on Urea Candidate reference method. Clin Chem. 1980; 26: 816-826.
71. Scott, M.G. Electrolytes and Blood Gases. In: Tietz Textbook of Clinical Chemistry. 3rd ed. Burtis CA, Ashwood ER, eds. Philadelphia: WB Saunders Company. 1999; 1058-1059.
72. Clinical and Laboratory Standards Institute. Procedures for the handling and processing of blood specimens; tentative standard. CLSI Document H18-A2. Wayne, PA: CLSI, 1999.
73. Overfield CV, et al. Glycolysis: a re-evaluation of the effect on blood glucose. Clin Chim Acta. 1972; 39: 35-40.
74. Rehak NN, Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. Clin Chem. 1988; 34-2111-4.
75. Sherwin JE, Obernolte R. Bilirubin. In: Clinical Chemistry: Theory, Analysis, and Correlation, 2nd ed. Kaplan LA, Pesce AJ, eds. St. Louis: The C.V. Mosby Company. 1989: 1009-1015.
76. Henry RJ, Cannon DC, Winkelman JW. Clinical Chemistry: Principles and Technics, 2nd ed. New York: Harper and Row. 1974: 417-421; 1058-1059.
77. Macy E, Hayes T, Tracy R. Variability in the measurement of c-reactive protein in healthy subjects: implications for reference interval and epidemiological applications. Clin. Chem. 1997; 43: 52-58.
78. Clinical and Laboratory Standards Institute. Interference testing in clinical chemistry; proposed guideline. CLSI Document EP7-A. Wayne, PA: CLSI, 2002.
79. Young DS. Effects of drugs on clinical laboratory tests, 3rd ed. Washington, DC: AACC Press, 1990.
80. Clinical and Laboratory Standards Institute. How to define and determine reference intervals in the clinical laboratory, approved guidelines, 2nd ed. CLSI Document C28-A3. Wayne, PA: CLSI, 2008.
81. Clinical and Laboratory Standards Institute. Evaluation of precision performance of clinical chemistry devices; approved guideline. CLSI Document EP5-A. Wayne, PA: CLSI, 1999.
82. Clinical and Laboratory Standards Institute. Quality management for unit-use testing; proposed guideline. CLSI Document EP18-A. Wayne, PA: CLSI, 2002.
83. Clinical and Laboratory Standards Institute. Method comparison and bias estimation using patient samples; approved guideline. CLSI Document EP9-A2. Wayne, PA: CLSI, 2002.