

Piccolo® Renal Function Panel



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

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



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1. Intended Use

The Piccolo® Renal Function Panel, used with the Piccolo® blood chemistry analyzer or the PiccoloXpress® chemistry analyzer is intended to be used for the *in vitro* quantitative determination of albumin, calcium, chloride, creatinine, glucose, phosphorus, potassium, sodium, total carbon dioxide and blood urea nitrogen (BUN) in heparinized whole blood, heparinized plasma, or serum in a clinical laboratory setting or point-of-care location.

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 Renal Function Panel reagent disc and the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer comprise an *in vitro* diagnostic system that aids the physician in diagnosing the following disorders:

Albumin:	Dehydration, renal disease, liver insufficiency with decreased albumin synthesis, severe malnutrition, acute inflammation, chronic inflammation, malignancy, pregnancy and burns.
Calcium:	Parathyroid, bone and chronic renal diseases; tetany.
Chloride:	Dehydration, prolonged diarrhea and vomiting, renal tubular disease, hyperparathyroidism, burns, salt-losing renal diseases, overhydration and thiazide therapy.
Creatinine:	Renal diseases and monitoring of renal dialysis.
Glucose:	Carbohydrate metabolism disorders, including adult and juvenile diabetes mellitus and hypoglycemia.
Phosphorus:	Dehydration, diabetes, parathyroidism, and renal disease.
Potassium:	Renal glomerular or tubular disease, adrenocortical insufficiency, diabetic ketacidosis, excessive intravenous potassium therapy, sepsis, panhypopituitarism, <i>in vitro</i> hemolysis, hyperaldosteronism, malnutrition, hyperinsulinism, metabolic alkalosis and gastrointestinal loss.
Sodium:	Dehydration, diabetes insipidus, loss of hypotonic gastrointestinal fluids, salt poisoning, selective depression of the sense of thirst, skin loss, burns, sweating, hyperaldosteronism, CNS disorders, dilutional, depletion and delusional hyponatremia and ADH secretion syndrome.

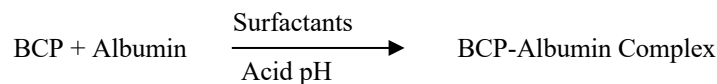
Total Carbon Dioxide:	Primary metabolic alkalosis and acidosis and primary respiratory alkalosis and acidosis.
Blood urea nitrogen (BUN):	Renal and metabolic diseases.

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

3. Principle of Procedure

Albumin (ALB)

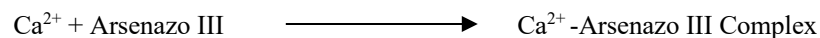
Early methods used to measure albumin include fractionation techniques and tryptophan content of globulins.¹⁻⁵ These methods were unwieldy to perform and did not have a high specificity. Two immunochemical techniques are considered as reference methods, but are expensive and time consuming.⁶ Dye binding techniques are the most frequently used methods for measuring albumin. Bromocresol green (BCG) is the most commonly used of the dye binding methods but may over-estimate albumin concentration, especially at the low end of the normal range.⁷ Bromocresol purple (BCP) is the most specific of the dyes in use.^{8,9}



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.

Calcium (CA)

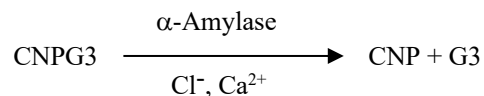
The reference method for calcium is atomic absorption spectroscopy; however, this method is not suited for routine use.¹⁰ Spectrophotometric methods using either *o*-cresolphthalein complexone (CPC) or arsenazo III metallochromic indicators are most commonly used.^{11,12,13} Arsenazo III has a high affinity for calcium and is not temperature dependent as is CPC. Calcium in the patient sample binds with arsenazo III to form a calcium-dye complex.



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

Chloride (CL⁻)

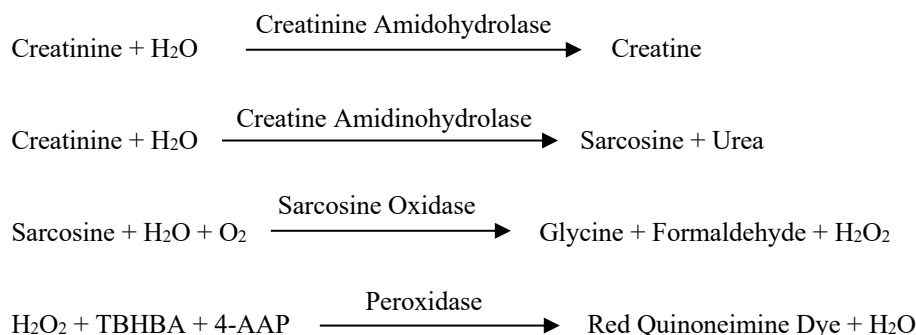
The Abaxis chloride method is based on the determination of chloride-dependent activation of α -amylase activity. Deactivated α -amylase is reactivated by addition of the chloride ion, allowing the calcium to re-associate with the enzyme. The reactivation of α -amylase activity is proportional to the concentration of chloride ions in the sample. The reactivated α -amylase converts the substrate, 2-chloro-*p*-nitrophenyl- α -D-maltotriose (CNPG3) to 2-chloro-*p*-nitrophenyl (CNP) producing color and α -maltotriose (G3). The reaction is measured bichromatically and the increase absorbance is directly proportional to the reactivated α -amylase activity and the concentration of chloride in the sample.¹⁴



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.^{15,16} Enzymatic methods have been developed that are more specific for creatinine than the various modifications of the Jaffe technique.^{17,18,19,20} Methods using the enzyme creatinine amidohydrolase eliminate the problem of ammonium ion interference found in techniques using creatinine iminohydrolase.²¹

In the coupled enzyme reactions, creatinine amidohydrolase hydrolyzes creatinine to creatine. A second enzyme, creatine amidinohydrolase, catalyzes the formation of sarcosine from creatine. Sarcosine oxidase causes the oxidation of sarcosine to glycine, formaldehyde and hydrogen peroxide (H₂O₂). In a Trinder reaction, peroxidase catalyzes the reaction among the hydrogen peroxide, 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) and 4-aminoantipyrine (4-AAP) into a red quinoneimine dye. Potassium ferrocyanide and ascorbate oxidase are added to the reaction mixture to minimize the potential interference of bilirubin and ascorbic acid, respectively.



Two cuvettes are used to determine the concentration of creatinine in the sample. Endogenous creatine is measured in the blank cuvette, which is subtracted from the combined endogenous creatine and the creatine formed from the enzyme reactions in the test cuvette. Once the endogenous creatine is eliminated from the calculations, the concentration of creatinine is proportional to the intensity of the red color produced. The endpoint reaction is measured as the difference in absorbance between 550 nm and 600 nm.

eGFR (calculated)

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

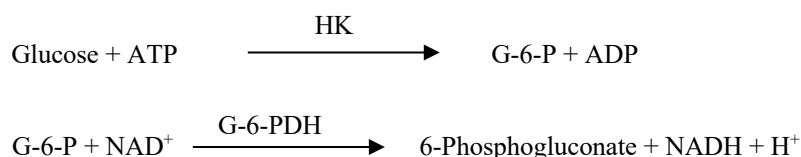
Calculation of the eGFR is performed by the Piccolo using the patient's age, gender and race. The Piccolo method for creatinine is traceable to the IDMS reference method for creatinine so that the following form of the MDRD equation for calculating the eGFR can be used.

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

Glucose (GLU)

Measurements of glucose concentration were first performed using copper-reduction methods (such as Folin-Wu²² and Somogyi-Nelson^{23,24}). 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 Renal Function Panel reagent disc is a modified version of the hexokinase method, which has been proposed as the basis of the glucose reference method.²⁵

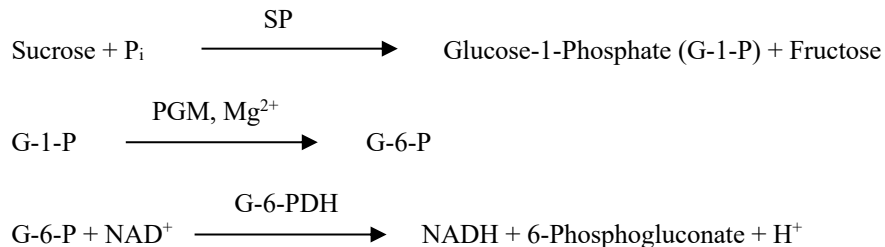
The reaction of glucose with adenosine triphosphate (ATP), catalyzed by hexokinase (HK), produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the reaction of G-6-P into 6-phosphogluconate and the reduction of nicotinamide adenine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



The absorbance is measured bichromatically at 340 nm and 850 nm. The production of NADH is directly proportional to the amount of glucose present in the sample.

Phosphorus (PHOS)

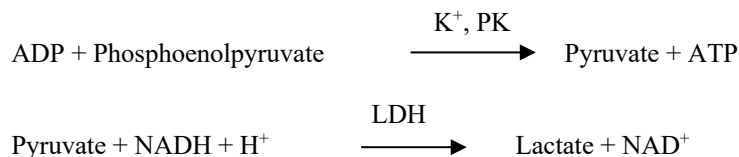
The most applicable enzymatic method for the Abaxis system uses sucrose phosphorylase (SP) coupled through phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6PDH).^{26,27} Using the enzymatic system for each mole of phosphorus present in the sample, one mole of NADH is formed. The amount of NADH formed can be measured as an endpoint at 340 nm.



Potassium (K⁺)

Spectrophotometric methods have been developed that allow the measurement of potassium concentration on standard clinical chemistry instrumentation. An enzymatic method based on the activation of pyruvate kinase with potassium show excellent linearity and negligible susceptibility to endogenous substances.^{28,29,30} Interference from sodium and ammonium ions are minimized with the addition of Kryptofix and glutamine synthetase, respectively.²⁸

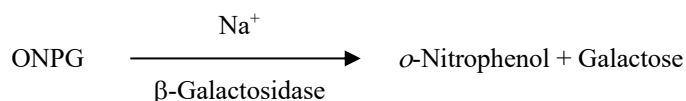
In the coupled-enzyme reaction, pyruvate kinase (PK) dephosphorylates phosphoenolpyruvate to form pyruvate. Lactate dehydrogenase (LDH) catalyzes conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD⁺.



The rate of change in absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD⁺ and is directly proportional to the amount of potassium in the sample.

Sodium (Na⁺)

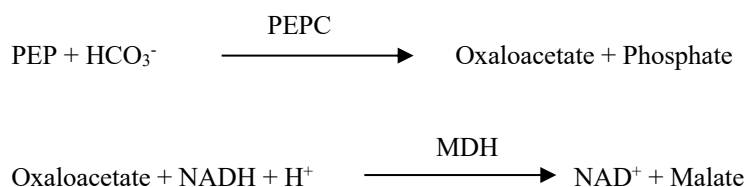
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.^{31,32,33} In the Abaxis enzymatic reaction, β-galactosidase is activated by the sodium in the sample. The activated enzyme catalyses the reaction o-nitrophenyl-β-galactopyranoside (ONPG) to o-nitrophenyl and galactose.



Total Carbon Dioxide (tCO₂)

Total carbon dioxide in serum or plasma exists as dissolved carbon dioxide, carbamino derivatives of proteins, bicarbonate and carbonate ions and carbonic acid. Total carbon dioxide can be measured by pH indicator, CO₂ electrode and spectrophotometric enzymatic methods, which all produce accurate and precise results.^{34,35} The enzymatic method is well suited for use on a routine blood chemistry analyzer without adding complexity.

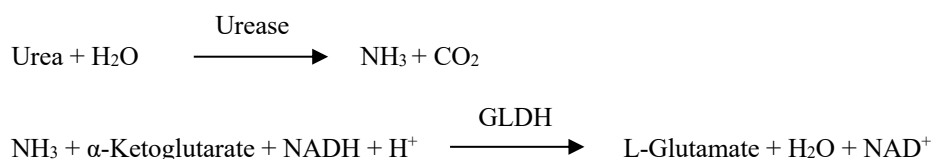
In the enzymatic method, the specimen is first made alkaline to convert all forms of carbon dioxide (CO₂) to bicarbonate (HCO₃⁻). Phosphoenolpyruvate (PEP) and HCO₃⁻ then react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MDH) catalyzes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



Blood Urea Nitrogen (BUN)

Urea can be measured both directly and indirectly. The diacetyl monoxime reaction, the only direct method to measure urea, is commonly used but employs dangerous reagents.³⁶ Indirect methods measure ammonia created from the urea; the use of the enzyme urease has increased the specificity of these tests.³⁷ The ammonia is quantitated by a variety of methods, including nesslerization (acid titration), the Berthelot technique^{38,39} and coupled enzymatic reactions.^{40,41} Catalyzed Berthelot procedures, however, are erratic when measuring ammonia.⁴² 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.⁴³

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



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

4. Principle of Operation

See the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer Operator's Manual, for the Principles and Limitations of the Procedure.

5. Description of Reagents

Reagents

Each Piccolo Renal Panel reagent disc contains dry test-specific reagent beads (described below). A dry sample blank reagent (comprised of buffer, surfactants, excipients, and preservatives) is included in each disc for use in calculating concentrations of albumin (ALB), chloride (CL⁻), calcium (CA), glucose (GLU), phosphorus (PHOS), potassium (K⁺), sodium (NA⁺), total carbon dioxide (tCO₂) and blood urea nitrogen (BUN). A dedicated sample blank is included in the disc to calculate concentrations of creatinine (CRE). Each disc also contains a diluent consisting of surfactants and preservatives.

Table 1: Reagents

Components	Quantity/Disc
N-Acetyl cysteine	60 µg
Adenosine 5'-diphosphate	36 µg
Adenosine 5'-triphosphate	22µg
α -Ketoglutaric acid	19 µg
4-Aminoantipyrine hydrochloride	13 µg
Amylase	0.036 U
Arsenazo III, sodium salt	1.7 µg
Ascorbate oxidase (<i>Cucurbita spp.</i>)	0.3 U
Brij	3 µg
Bromocresol purple, sodium salt	0.2 µg
Calcium acetate	25 µg
Citric acid, trisodium salt	567 µg
2-Chloro-4-nitrophenyl- α -maltotrioxide (CNPG3)	53 µg
Table 1: Reagents (continued)	
Components	Quantity/Disc
Creatine amidinohydrolase (<i>Actinobacillus spp.</i>)	3 U
Creatinine amidohydrolase (<i>Pseudomonas spp.</i>)	1 U
Ethylenediaminetetraacetic acid (EDTA)	182 µg
Ethylenediaminetetraacetic acid (EDTA), disodium salt	15 µg

Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)	4 µg
β-Galactosidase	0.005 U
Glucose-1,6-diphosphate	1 µg
L-Glutamic acid	9.2 µg
Glutamate dehydrogenase	0.1 U
Glutamine synthetase	0.17 U
Hexokinase	0.1 U
Imidazole	29 µg
Lactate dehydrogenase (chicken heart)	0.13 U
Lithium hydroxide, monohydrate	23 µg
Magnesium acetate, tetrahydrate	67 µg
Magnesium sulfate	33 µg
Malate dehydrogenase	0.1 U
Manganese chloride	10 µg
D-Mannitol	675 µg
2-Methyl-4-isothiazolin-3-one hydrochloride (MIT)	4.2 µg
β-Nicotinamide adenine dinucleotide (NAD)	83 µg
β-Nicotinamide adenine dinucleotide, reduced (NADH)	36 µg
<i>o</i> -Nitrophenyl-β-D-galactopyranoside (ONPG)	22 µg
4,7,13,16,21-Pentaoxa-1,10-diazabicyclo[8.8.5]tricosane (Kryptofix 221)	86 µg
Peroxidase (horseradish)	1 U
Phosphoenol pyruvate	57 µg
Phosphoenol pyruvate carboxylase	0.001 U
Phosphoglucomutase	0.035 U
Pluronic F68	1 µg
Polyethylene glycol, 8000	4 µg
Potassium ferrocyanide	0.4 µg
Pyruvate kinase	0.01 U
Sarcosine oxidase (microorganism)	1 U
Sucrose	11 µg
Sucrose phosphorylase	0.07 U
Sodium chloride	57 µg
2,4,6-Tribromo-3-hydroxybenzoic acid	188 µg
Triethanolamine hydrochloride	195 µg
Triton X-100	24 µg
Urease (jack bean)	0.05 U
Buffers, surfactants, excipients and preservatives	

Warnings and Precautions

- For *in vitro* Diagnostic Use
- The diluent container in the reagent disc is automatically opened when the analyzer drawer closes. A disc with an opened diluent container cannot be re-used. Ensure that the sample or control has been placed into the disc before closing the drawer.
- Used reagent discs contain human body fluids. Follow good laboratory safety practices when handling and disposing of used discs.⁴⁴ See the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer Operator's Manual for instructions on cleaning biohazardous spills.
- The reagent discs are plastic and may crack or chip if dropped. Never use a dropped disc as it may spray biohazardous material throughout the interior of the analyzer.
- Reagent beads may contain acids or caustic substances. The operator does not come into contact with the reagent beads when following the recommended procedures. In the event that the beads are handled (e.g., cleaning up after dropping and cracking a reagent disc), avoid ingestion, skin contact, or inhalation of the reagent beads.

Instructions for Reagent Handling

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

Storage

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

Indications of Reagent Disc Instability/Deterioration

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

6. Instrument

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

7. Sample Collection and Preparation

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

- The minimum required sample size is ~100 µL of heparinized whole blood, heparinized plasma, serum, or control material. The reagent disc sample chamber can contain up to 120 µL of sample.
- Whole blood samples obtained by venipuncture must be homogeneous before transferring a sample to the reagent disc. Gently invert the collection tube several times just prior to sample transfer. Do not shake the collection tube; shaking may cause hemolysis.
- Hemolysis may cause erroneously high results in **potassium** assays. This problem may go undetected when analyzing whole blood (release of potassium from as few as 0.5% of the erythrocytes can increase the potassium serum level by 0.5 mmol/L). In addition, even unhemolyzed specimens that are not promptly processed may have increased potassium levels due to intracellular leakage.⁴⁵
- Whole blood venipuncture samples should be run within 60 minutes of collection.⁴⁶ **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.⁴⁷
- Refrigerating whole blood samples can cause significant changes in concentrations of **creatinine** and **glucose**.⁴⁸ 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 or red/black stopper) for serum samples.
- The concentration of **total carbon dioxide** is most accurately determined when the assay is done immediately after opening the tube and as promptly as possible after collection and processing of the blood in the unopened tube. Ambient air contains far less carbon dioxide than does plasma and gaseous dissolved carbon dioxide will escape from the specimen into the air, with a consequent decrease in carbon dioxide value of up to 6 mmol/L in the course of 1 hour.⁴⁹
- Start the test within 10 minutes of transferring the sample into the reagent disc.

8. Procedure

Materials Provided

- One Piccolo Renal Function Panel PN: 400-1027 (a box of discs PN 400-0027)

Materials Required but Not Provided

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

Test Parameters

The Piccolo blood chemistry analyzer or Piccolo Xpress chemistry analyzer operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each Piccolo Renal Function Panel 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 Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer Operator's Manual.

Calibration

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

Quality Control

For CLIA waived settings, see Quality Control Section pages 9-10 of the Piccolo Xpress Quick Reference Guide. For moderately complex settings, see Section 2.4 of the Piccolo blood chemistry analyzer Operator's Manual or Section 6 (Calibration and Quality Control) of the Piccolo Xpress Operator's Manual. Performance of the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer can be verified by running controls. For a list of approved quality control materials with acceptance ranges, please contact Abaxis Technical Support. Other human serum or plasma-based controls may not be compatible. Quality control materials should be stored as per the package-insert included with the controls.

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

Waived Laboratories: Abaxis recommends control testing as follows:

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

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

9. Results

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

Interpretation of results is detailed in the analyzer 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.

10. Limitations of Procedure

General procedural limitations are discussed in the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer Operator's Manual.

- The only anticoagulant **recommended for use** with the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer is **lithium heparin**. Abaxis has performed studies demonstrating that EDTA, fluoride, oxalate, and any anticoagulant containing ammonium ions will interfere with at least one chemistry contained in the Piccolo Renal Function Panel reagent disc.
- Samples with hematocrits in excess of 62-65% packed red cell volume (a volume fraction of 0.62-0.65) may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down to get plasma. The plasma can then be re-run in a new reagent disc.
- **Any result for a particular test that exceeds the assay range should be analyzed by another approved test method or sent to a referral laboratory. Do not dilute the sample and run it again on the Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer.**

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

Interference

Substances were tested as interferents with the analytes. Human serum pools were prepared. The concentration at which each potential interferent was tested was based on the testing levels in CLSI (formerly NCCLS) EP7-P.⁵⁰

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 blood chemistry analyzer or the Piccolo Xpress chemistry analyzer suppresses any results that are affected by >10% interference from hemolysis, lipemia or icterus. "HEM", "LIP", or "ICT" respectively, is printed on the printout in place of the result.
- Extremely elevated amylase levels (>9,000 U/L) will have a significant effect, >10% increase, on the **chloride** result. The concentration of amylase is not evaluated by the Piccolo system for each specimen.
- The potassium assay in the Piccolo system is a coupled pyruvate kinase (PK) / lactate dehydrogenase (LDH) assay. Therefore, in cases of extreme muscle trauma or highly elevated levels of creatine kinase (CK), the Piccolo may recover a falsely elevated potassium (K⁺) value. In such cases, unexpected high potassium recoveries need to be confirmed utilizing a different methodology.
- For maximum levels of endogenous substances contact Abaxis Technical Support.

Effects of Exogenous and Therapeutic Substances

Thirty-five exogenous and therapeutic substances were selected as potential interferents for Abaxis test methods based on recommendations by Young.⁵¹ Significant interference is defined as greater than $\pm 10\%$ 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
Caffeine	10
Calcium Chloride	20
Cephalothin (Keflin)	400
Chloramphenicol	100
Cimetidine	16
Dopamine	19
Epinephrine	1
Erythromycin	10
Glutathione	30
Hydrochlorothiazide	7.5
Ibuprofen	50
Isoniazide	4
α -Ketoglutarate	5
Ketoprofen	50
L-dopa	5
Lidocaine	1
Lithium Lactate	84
Methicillin	100
Methotrexate	0.5
Metronidazole	5
Nafcillin	1
Nitrofurantoin	20
Oxacillin	1
Oxaloacetate	132
Penicillin G	100
Phenytoin (5,5-Diphenylhydantion)	3
Proline	4
Pyruvate	44
Rifampin	0.5
Salicylic Acid	50
Sulfadiazine	150
Sulfanilamide	50
Theophylline	20

Please see Table 3 for a list of analytes where interference was observed.

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

	Concentration Which Produces > 10% Interference	% Interference ^A Observed
Albumin		
Acetoacetate	102	18% dec
Ampicillin	30	12% dec
Caffeine	10	14% dec
Calcium chloride	20	17% dec
Cephalothin (Keflin)	400	13% inc
Ibuprofen	50	28% inc
α -Ketoglutarate	5	11% dec
Nitrofurantoin	20	13% dec
Proline	4	12% inc
Sulfadiazine	10	14% dec
Sulfanilamide	50	12% dec
Theophylline	20	11% dec
Creatinine		
Ascorbic acid	20	11% dec.
Dopamine	19	80% dec.
L-dopa	5	71% dec.
Epinephrine	1	45% dec.
Glutathione	30	13% dec.
Glucose		
Oxaloacetate	132	11% dec.
Pyruvate	44	13% dec.
Phosphorus		
Nitrofurantoin	20	19% inc.
Oxaloacetate	132	14% dec.
Potassium		
Penicillin G	100	17% inc.
Sulfadiazine	150	12% dec.
Sodium		
Cephalothin	400	12% inc.
Methotrexate	0.5	11% inc.
Penicillin G	100	10% inc.
Total Carbon Dioxide		
Acetaminophen	100	11% inc.
Ascorbic Acid	20	12% dec.
Cephalothin	400	13% inc.
Cimetidine	16	19% dec.
Erythromycin	10	21% dec.
Lidocaine	1	23% inc.
Methotrexate	0.5	80% dec.
Nitrofurantoin	20	13% inc.
Salicylic Acid	50	17% dec.
Sulfadiazine	150	25% dec.

^A dec. = decreased concentration of the specified analyte; inc. = increased concentration of the specified analyte

- For the Chloride assay, bromide at toxic levels (≥ 15 mmol/L) can cause a significant effect ($> 10\%$ increase), on the chloride result. Iodide at very high concentrations (30 mmol/L, highest level tested) has no effect. Normal physiological levels of bromide and iodide do not interfere with the Piccolo Chloride Test System.

11. Expected Values

Samples from approximately 90 – 140 adult males and females were analyzed on the Piccolo blood chemistry analyzer to determine the reference intervals for the following assays. These intervals are provided as a guideline only. It is recommended that your office or institution establish normal ranges for your particular patient population.⁵²

Table 4: Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer Reference Intervals

Analyte	Common Units	SI Units
Albumin	3.3-5.5 g/dL	33-55 g/L
Calcium	8.0-10.3 mg/dL	2.0-2.58 mmol/L
Chloride	98-108 mmol/L	98-108 mmol/L
Creatinine	0.6-1.2 mg/dL	53-106 µmol/L
Glucose	73-118 mg/dL	4.1-6.6 mmol/L
Phosphorus (plasma)	2.2-4.1 mg/dL	0.71-1.32 mmol/L
Phosphorus (serum)	2.5-4.4 mg/dL*	0.81-1.42 mmol/L*
Potassium	3.6-5.1 mmol/L	3.6-5.1 mmol/L
Sodium	128-145 mmol/L	128-145 mmol/L
Total Carbon Dioxide	18-33 mmol/L	18-33 mmol/L
Blood Urea Nitrogen (BUN)	7-22 mg/dL	2.5-7.9 mmol urea/L

* There is no observed difference between the concentration of Phosphorus measured in heparinized whole blood and heparinized plasma. However, a small increase (0.3 mg/dL) was observed in serum when compared to heparinized whole blood and heparinized plasma. This increase is consistent with the difference between Phosphorus in serum and plasma as described in the literature.^{53, 54, 55, 56}

12. Performance Characteristics

Linearity

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

Table 5: Piccolo blood chemistry analyzer or the Piccolo Xpress chemistry analyzer Dynamic Ranges

Analyte	Common Units	SI Units
Albumin	1-6.5 g/dL	10-65 g/L
Calcium	4.0-16.0 mg/dL	1.0-4.0 mmol/L
Chloride	80-135 mmol/L	80-135 mmol/L
Creatinine	0.2-20 mg/dL	18-1768 µmol/L
Glucose	10-700 mg/dL	0.6-38.9 mmol/L
Phosphorus	0.2-20 mg/dL	0.06-6.5 mmol/L
Potassium	1.5-8.5 mmol/L	1.5-8.5 mmol/L
Sodium	110-170 mmol/L	110-170 mmol/L
Total Carbon Dioxide	5-40 mmol/L	5-40 mmol/L
Blood Urea Nitrogen (BUN)	2-180 mg/dL	0.7-64.3 mmol urea/L

Sensitivity

The lower limit of the reportable (dynamic) range for each analyte is: albumin 1 g/dL (10 g/L); calcium 4.0 mg/dL (1.0 mmol/L); chloride 80 mmol/L; creatinine 0.2 mg/dL (18 µmol/L); glucose 10 mg/dL (0.56 mmol/L); phosphorus 0.2 mg/dL (0.06 mmol/L); potassium 1.5 mmol/L; sodium 110 mmol/L; total carbon dioxide 5 mmol/L; and blood urea nitrogen 2.0 mg/dL (0.7 mmol urea/L).

Precision

Precision studies were conducted using CLSI (formerly NCCLS) EP5-A guidelines, with modifications based on CLSI (formerly NCCLS) EP18-P for unit-use devices.^{57, 58} Results for within-run and total precision were determined by testing levels of commercially available control materials and in the case of potassium two levels of plasma pools. The studies made use of multiple instruments. Precision for albumin, calcium, creatinine, glucose, sodium and urea nitrogen was performed at one site; total carbon dioxide testing was performed at two sites over 20 days; and chloride and phosphorus testing was conducted at two sites over a period of 5 days. Potassium testing was conducted at a CLIA waived site making use of three analyzers, one lot of reagent discs, and two operators over five days.

Results of the precision studies are shown in Table 6.

Table 6: Precision

Analyte	Sample Size	Within-Run	Total
Albumin (g/dL)	N = 80		
<u>Control 1</u>			
Mean		5.6	5.6
SD		0.09	0.11
%CV		1.7	2.1
<u>Control 2</u>			
Mean		3.7	3.7
SD		0.07	0.11
%CV		2.0	2.9
Calcium (mg/dL)	N = 80		
<u>Control 1</u>			
Mean		8.6	8.6
SD		0.21	0.25
%CV		2.4	2.9
<u>Control 2</u>			
Mean		11.8	11.8
SD		0.39	0.40
%CV		3.3	3.4
Chloride (mmol/L)	N = 160		
<u>Control 1</u>			
Mean		97.8	97.8
SD		1.63	1.74
%CV		1.7	1.7
<u>Control 2</u>			
Mean		113.6	113.6
SD		1.97	2.22
%CV		1.7	2.0
Creatinine (mg/dL)	N = 80		
<u>Control 1</u>			
Mean		1.1	1.1
SD		0.14	0.14
%CV		12.5	13.1
<u>Control 2</u>			
Mean		5.2	5.2
SD		0.23	0.27
%CV		4.4	5.2
Glucose (mg/dL)	N = 80		
<u>Control 1</u>			
Mean		66	66
SD		0.76	1.03
%CV		1.1	1.6
<u>Control 2</u>			
Mean		278	278
SD		2.47	3.84
%CV		0.9	1.4

Table 6: Precision (continued)

Analyte	Sample Size	Within-Run	Total
Phosphorus (mg/dL)	N = 80		
<u>Control 1</u>			
Mean		3.1	3.1
SD		0.12	0.14
%CV		3.7	4.7
<u>Control 2</u>			
Mean		7.3	7.3
SD		0.09	0.15
%CV		1.3	2.0
Potassium (mmol/L)	N = 150		
<u>Control 1</u>			
Mean		3.2	3.2
SD		0.09	0.11
%CV		2.8	3.3
<u>Control 2</u>	N = 149		
Mean		6.2	6.2
SD		0.09	0.11
%CV		2.8	3.3
<u>Plasma Pool 1</u>	N = 150		
Mean		3.2	3.2
SD		0.07	0.09
CV		2.3	2.9
<u>Plasma Pool 2</u>	N = 150		
Mean		5.4	5.4
SD		0.09	0.10
CV		1.6	1.9
Sodium (mmol/L)	N = 80		
<u>Control 1</u>			
Mean		143.5	143.5
SD		2.28	2.28
%CV		1.6	1.6
<u>Control 2</u>			
Mean		120.0	120.0
SD		2.13	2.13
%CV		1.8	1.8
Total Carbon Dioxide (mmol/L)	N = 120		
<u>Control 1</u>			
Mean		21.4	21.4
SD		2.29	2.29
%CV		10.7	10.7
<u>Control 2</u>			
Mean		10.5	10.5
SD		0.90	0.90
%CV		8.6	8.6
Urea Nitrogen (mg/dL)	N = 80		
<u>Control 1</u>			
Mean		19	19
SD		0.35	0.40
%CV		1.9	2.1
<u>Control 2</u>			
Mean		65	65
SD		1.06	1.18
%CV		1.6	1.8

Whole Blood Precision for Potassium

Whole blood precision was tested at a CLIA waived site by two CLIA waiver operators. The study used four Piccolo Xpress Analyzers with 16 replicates per sample for four (4) fresh, lithium heparin whole blood samples.

Table 7: Whole Blood Precision for Potassium

Potassium (mmol/L)	Sample Size	Within-Run	Total
Whole Blood 1	N = 16		
Mean		3.9	3.9
SD		0.06	0.11
CV		1.6	2.8
Whole Blood 2	N = 16		
Mean		4.0	4.0
SD		0.11	0.14
CV		2.9	3.4
Whole Blood 3	N = 16		
Mean		4.0	4.0
SD		0.11	0.15
CV		2.8	3.9
Whole Blood 4	N = 16		
Mean		4.0	4.0
SD		0.11	0.13
CV		2.7	3.4

Correlation

Serum samples were collected and assayed on the Piccolo blood chemistry analyzer and by a comparative method.

Table 7: Correlation of Piccolo® Blood Chemistry Analyzer with Comparative Method (s)

	Correlation Coefficient	Slope	Intercept	SEE	N	Sample Range	Comparative Method
Albumin (g/dL)	0.854	1.001	-0.3	0.22	261	1.1-5.3	Paramax®
	0.896	0.877	-0.1	0.21	100	1.5-5.0	Beckman
Calcium (mg/dL)	0.980	0.98	-0.17	0.31	111	4.6-13.2	Beckman
Chloride (mmol/L)	0.978	0.982	-1.1	1.84	120	71-118	Vitros® 950
Creatinine (mg/dL)	0.993	0.926	0.0	0.15	260	0.4-14.7	Paramax®
Glucose (mg/dL)	0.987	1.009	-2.8	3.89	251	72-422	Paramax®
	0.997	0.943	1.2	4.69	91	56-646	Beckman
Phosphorus (mg/dL)	0.993	1.017	-0.2	0.236	90	0.8 – 11.7	Vitros® 950
Potassium (mmol/L)	0.984	0.99	0.13	0.10	130	1.3-9.5	Siemens
Whole Blood							VISTA
(waived laboratory)							Plasma
Potassium (mmol/L)	0.984	0.98	0.12	0.18	178	1.5-8.6	Siemens
Whole Blood							VISTA
(moderately							Plasma
Complex laboratory)							
Potassium (mmol/L)	0.99	0.98	0.06	0.14	178	1.4-8.5	Siemens
Serum							VISTA
(moderately							Serum
Complex laboratory)							

Sodium (mmol/L)	0.937	0.782	27.7	3.79	113	116 - 154	Radiometer KNA® 2
Total Carbon Dioxide (mmol/L)	0.947	0.903	2.0	0.84	60	6 – 39	Cobas® Fara
Blood Urea Nitrogen (mg/dL)	0.983	0.946	0.0	0.66	92	6 – 38	Beckman

It should be noted that serum will typically give higher results for K⁺ compared to whole blood or plasma for physiological reasons. The variation can range from approximately 0.2 to 0.9mmol/L and is dependent on a number of factors. The primary effect is dependent upon the number of blood cells present in the patient sample.⁸²

Results of Untrained User Study

An “untrained user” study was conducted in which participants were given only the test instructions and asked to perform testing of 3 discs with blinded randomized samples. The samples consisted of serum pools prepared at three levels for each of the ten analytes, albumin, calcium, chloride, creatinine, glucose, phosphorus, potassium, sodium, total carbon dioxide, and blood urea nitrogen (BUN). The participants were not given any training on the use of the test or instrument. A total of 62 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

Albumin

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	3.1	3.5	4.2
Mean value By Piccolo(g/dL)	3.0	3.5	4.2
SD	0.08	0.09	0.07
%CV	2.7%	2.5%	1.8%
Observed Range	2.9 – 3.2	3.3 – 3.7	4.0 – 4.4

Calcium (CA)

	Level 1	Level 2	Level 3
Number	62	62	62
Target concentration	8.1	10.5	13.2
Mean value By Piccolo (mg/dL)	8.03	10.52	13.1
SD	0.14	0.15	0.18
%CV	1.7%	1.4%	1.4%
Observed Range	7.7 – 8.4	10.1 – 11.0	12.6 – 13.4

Chloride (CL⁻)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	93	105	115
Mean value By Piccolo (mmol/L)	94.6	106	115.5
SD	1.66	1.5	1.74
%CV	1.8	1.4	1.5
Observed Range	90 – 100	102 - 108	110 - 119

Creatinine (CRE)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	0.9	2.1	6.9
Mean value By Piccolo (mg/dL)	0.89	2.07	6.89
SD	0.10	0.10	0.11
%CV	11.2%	4.8%	1.6%
Observed Range	0.7 – 1.2	1.8 – 2.3	6.5 – 7.2

Glucose

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	96	131	363
Mean value By Piccolo (mg/dL)	95.2	130.3	365.8
SD	1.08	1.33	2.85
%CV	1.1%	1.0%	0.8%
Observed Range	93 – 98	125 – 133	351 – 373

Phosphorus (PHOS)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	2.2	4.2	7.3
Mean value By Piccolo (mg/dL)	2.2	4.2	7.3
SD	0.10	0.11	0.09
%CV	4.5	2.6	1.2
Observed Range	2.0 – 2.5	4.0 – 4.5	7.1 – 7.5

Potassium (K⁺)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	3.4	5.6	7.2
Mean value By Piccolo (mmol/L)	3.42	5.66	7.19
SD	0.11	0.14	0.14
%CV	3.3	2.5	1.9
Observed Range	3.2 – 3.7	5.2 – 5.9	6.7 – 7.5

Sodium (NA⁺)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	122	141	158
Mean value By Piccolo (mmol/L)	122.1	140.8	157.5
SD	1.25	1.15	1.63
%CV	1.0	0.8	1.0
Observed Range	118 - 127	138 - 143	154 - 162

Total Carbon Dioxide (tCO₂)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	21	28	33
Mean value By Piccolo (mmol/L)	20.3	27.6	34.4
SD	1.03	1.26	1.27
%CV	5.1	4.6	3.7
Observed Range	18 – 23	23 - 30	32 - 38

Blood Urea Nitrogen (BUN)

	Level 1	Level 2	Level 3
Number	62	62	62
Target Concentration	15	42	72
Mean value By Piccolo (mg/dL)	15.1	41.0	72.2
SD	0.35	1.0	1.3
%CV	2.3%	2.5%	1.8%
Observed Range	14 – 16	37 – 43	68 – 75

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



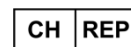
Serial Number



Caution



Temperature
Limitation



Authorized
Representative in
Switzerland

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).

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