VETSCAN® Electrolyte Plus

For Veterinary use only
Customer and Technical Service 1-800-822-2947

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1. Intended Use

The VetScan® Electrolyte Plus reagent rotor used with the VetScan VS2 Chemistry Analyzer utilizes dry and liquid reagents to provide *in vitro* quantitative determination of chloride (CL^-), potassium (K^+), sodium (NA^+) and total carbon dioxide (tCO_2) in heparinized whole blood, heparinized plasma, or serum.

2. Summary and Explanation of Tests

The VetScan Electrolyte Plus reagent rotor and the VetScan VS2 Chemistry Analyzer comprise an *in vitro* diagnostic system that aids the veterinarian in diagnosing the following disorders:

Chloride (CL^-)	Chronic diarrhea, chronic vomiting, renal disease, parathyroid disease, chronic respiratory acidosis or alkalosis, hyperadrenocorticism, hypoadrenocorticism, and thiazide therapy
Potassium (K^+)	Malnutrition and renal disease; this electrolyte is used to diagnose the causes of vomiting, diarrhea and cardiac symptoms
Sodium (NA+)	Dehydration, and diabetes; this electrolyte is used to diagnose the causes of vomiting, diarrhea and cardiac symptoms
Total Carbon Dioxide (tCO_2)	Primary metabolic alkalosis and acidosis and primary respiratory alkalosis and acidosis

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

3. Principles of Procedure

Chloride (Cl^-)
The 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-maltotrioside (CNPG3) to 2-chloro-p-nitrophenol (CNP) producing color and α-maltotriose (G3). The reaction is measured bichromatically and the increase in absorbance is directly proportional to the reactivated α-amylase activity and the concentration of chloride ion in the sample.[1]

$$\text{CNPG3} \xrightarrow[Cl^-,\ Ca^{2+}]{\alpha\text{-Amylase}} \text{CNP} + \text{G3}$$

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 (PK) with potassium and shows excellent linearity and negligible susceptibility to endogenous substances.[2,3,4] Interference from sodium and ammonium ions are minimized with the addition of Kryptofix and glutamine synthetase respectively.

In the coupled-enzyme reaction, PK dephosphorylates phosphoenolpyruvate (PEP) to form pyruvate. Lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD^+. The rate of change in absorbance 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.

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

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

Sodium (NA^+)
Colorimetric and enzymatic methods have been developed that allow the measurement of sodium concentration on standard clinical chemistry instrumentation.[5,6,7] 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. The reaction rate between 405 nm and 500 nm is proportional to sodium concentration.

$$\text{ONPG + } H_2O \xrightarrow[\beta\text{-Galactosidase}]{Na^+} \text{o-Nitrophenol + Galactose}$$

Total Carbon Dioxide (tCO_2)
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, CO_2 electrode and spectrophotometric enzymatic methods, which all produce accurate and precise results.[8,9] 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 (CO_2) toward bicarbonate (HCO_3^-). Phosphoenolpyruvate (PEP) and HCO_3^- 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 tCO_2 in the sample.

$$\text{PEP + } HCO_3^- \xrightarrow{PEPC} \text{Oxaloacetate + Phosphate}$$

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

4. Principle of Operation

See the VetScan VS2 Chemistry Analyzer Operator's Manual for the Principles and Limitations of the Procedure.

5. Description of Reagents

Reagents
Each VetScan Electrolyte Plus reagent rotor contains dry test specific reagent beads. A dry sample blank reagent (comprised of buffer, surfactants, excipients and preservatives) is included in each reagent rotor for use in calculating concentrations of chloride (CL^-), potassium (K^+), sodium (NA^+) and total carbon dioxide (tCO_2). Each reagent rotor also contains a diluent consisting of surfactants and preservatives.

Warnings and Precautions
For *In vitro* Diagnostic Use

- The diluent container in the reagent rotor is automatically opened when the analyzer drawer closes. A rotor with an opened diluent container cannot be re-used. Ensure that the sample or control has been placed into the rotor before closing the drawer.
- The reagent rotors are plastic and may crack or chip if dropped. **Never** use a dropped rotor.

- 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 rotor), avoid ingestion, skin contact, or inhalation of the reagent beads.

- Some reagent beads contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Reagents will not come into contact with lead and copper plumbing when following recommended procedures. However, if the reagents do come into contact with such plumbing, flush with a large volume of water to prevent azide buildup.

Instructions for Reagent Handling
Reagent rotors may be used directly from the refrigerator without warming. Open the sealed foil pouch and remove the rotor being careful not to touch the bar code ring located on the top of the reagent rotor. Use according to the instructions provided in the VetScan VS2 Operator's Manual. A rotor not used within 20 minutes of opening the pouch should be discarded. Rotors in opened pouches cannot be placed back in the refrigerator for use at a later time.

Storage
Store reagent rotors in their sealed pouches at 2-8˚C (36-46˚F). Do not expose opened or unopened rotors to direct sunlight or temperatures above 32˚C (90˚F). Do not allow the rotors sealed in their foil pouches to remain at room temperature longer than 48 hours prior to use. Open the pouch and remove the rotor just prior to use.

Indications of Reagent Rotor Instability or Deterioration
- All reagents contained in the reagent rotor, when stored as described above, are stable until the expiration date printed on the rotor pouch. Do not use a rotor after the expiration date. The expiration date is also encoded in the bar code printed on the bar code ring. An error message will appear on the VetScan VS2 Chemistry Analyzer display if the reagents have expired.

- 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 VetScan VS2 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 VetScan VS2 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 rotor sample chamber can contain up to 120 µL of sample.

- 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.

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

- Whole blood venipuncture samples should be run within 60 minutes of collection; if this is not possible, separate the sample and transfer it into a clean test tube.[10] Run the separated plasma or serum sample within 5 hours of centrifugation. If this is not possible, refrigerate the sample in a stoppered test tube at 2-8°C (36-46°F) for no longer than 48 hours. A plasma or serum sample can be stored at -10°C (14°F) for up to 5 weeks in a freezer that does not have a self-defrost cycle.

- The test must be started within 10 minutes of transferring the sample into the reagent rotor.

- 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.[11]

Known Interfering Substances

- The only anticoagulant recommended for use with the VetScan VS2 Chemistry Analyzer is lithium heparin. Sodium heparin must not be used when collecting blood samples for use with this panel. Abaxis has performed studies demonstrating that EDTA, fluoride, oxalate, and any anticoagulant containing ammonium ions will interfere with at least one chemistry in the VetScan Electrolyte Plus reagent rotor.

- Physical interferents (hemolysis, icterus, and lipemia) may cause changes in the reported concentrations of some analytes. The sample indices are printed on the bottom of each results print-out to inform the operator about the levels of interferents present in each sample. The VetScan Whole Blood Analyzer suppresses any results that are affected by significant interference from hemolysis, lipemia, or icterus.. “HEM”, “LIP”, “ICT” is printed on the results print-out in place of the result.

- 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 particular, even unhemolyzed specimens that are not properly processed may have increased potassium levels due to intracellular potassium leakage.[12]

- The **potassium** assay in the VetScan 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 VetScan may recover a falsely elevated potassium (K^+) value. In such cases, unexpected high potassium recoveries need to be confirmed utilizing a different methodology.

- 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 VetScan system for each specimen.

8. Procedure

Materials Provided

- One VetScan Electrolyte Plus Reagent Rotor PN: 500-1046 (a box of 12 rotors PN: 500-0046-12)

Materials Required but not Provided

- VetScan VS2 Chemistry Analyzer

Test Parameters

The VetScan System operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each VetScan Electrolyte Plus Reagent Rotor is approximately 12 minutes. The analyzer maintains the reagent rotor 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 VetScan VS2 Operator’s Manual.

Calibration

The VetScan VS2 Chemistry Analyzer is calibrated by the manufacturer before shipment. The barcode printed on the barcode ring provides the analyzer with rotor-specific calibration data. Please see the VetScan VS2 Operator’s Manual.

Quality Control

Controls may be run periodically on the VetScan VS2 Chemistry Analyzer to verify the accuracy of the analyzer. Abaxis recommends that a serum-based commercially available control be run. Run controls on the reagent rotor in the same manner as for patient samples. See the VetScan VS2 Operator’s Manual to run controls.

9. Results

The VetScan VS2 Chemistry Analyzer automatically calculates and prints the analyte concentrations in the sample. Details of the endpoint and rate reaction calculations are found in the VetScan VS2 Operator's Manual.

10. Limitations of Procedure

General procedural limitations are discussed in the VetScan VS2 Operator's Manual.

- **If a result for a particular test exceeds the assay range, the sample should be analyzed by another approved test method or sent to a referral laboratory.**

- Samples with hematocrits in excess of 62% packed red cell volume 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 rotor.

Warning: Extensive testing of the VetScan VS2 Chemistry Analyzer has shown that in very rare instances, sample dispensed into the reagent rotor 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 established reference ranges. The sample may be re-run using a new reagent rotor.

11. Performance Characteristics

Linearity
The chemistry for each analyte is linear over the dynamic range listed below when the VetScan System is operated according to the recommended procedure (refer to the VetScan VS2 Operator's Manual). The Dynamic Range table referenced below represents the spectrum that the VetScan System can detect. **The intervals below do not represent normal ranges.**

Table 2: VetScan Dynamic Ranges

Analyte	Common Units	SI Units
Chloride (CL^-)	80 – 135 mmol/L	80 – 135 mmol/L
Potassium (K^+)	1.5 – 8.5 mmol/L	1.5 – 8.5 mmol/L
Sodium (NA^+)	110 – 170 mmol/L	110 – 170 mmol/L
Total Carbon Dioxide (tCO_2)	5 – 40 mmol/L	5 – 40 mmol/L

12. Bibliography

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