

**DEPMEDS LABORATORY PROCEDURES
DEPARTMENT OF CLINICAL SUPPORT SERVICES
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
FORT SAM HOUSTON, TEXAS 78234-6137**

MCCS-HCH

STANDING OPERATING PROCEDURE

1 Mar 95

ALANINE AMINOTRANSFERASE ANALYSIS BY KODAK DT60

I. INTRODUCTION:

A. Alanine aminotransferase (ALT or SGPT) is an enzyme found mainly in the liver. Elevated levels are seen in patients with hepatitis and mononucleosis.

B. The Kodak Ektachem DT Slide (ALT) is a dry, multilayered film in a plastic support. It contains all the reagents necessary to determine alanine aminotransferase activity in 10 uL of serum or plasma. The analysis is based on an enzyme-coupled oxidation of NADH to NAD⁺.

II. PRINCIPLE:

The 10 uL drop of patient sample deposited on the slide is evenly distributed by the slide's spreading layer. The amino group of L-alanine is transferred to α -ketoglutarate in the presence of pyridoxal-5-phosphate (P-5-P) to produce glutamate and pyruvate. The pyruvate formed in the deamination of L-alanine is converted by lactate dehydrogenase (LDH) in the presence of NADH, which is oxidated to NAD⁺. The rate of oxidation of NADH is monitored by reflectance spectrophotometry at 37°C. The rate of change in reflection density measured in a linear region is then converted to enzyme activity in International Units per Liter (U/L).

III. SPECIMEN:

A. Recommended specimen -- 10 uL of serum or plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation.

" Follow universal precautions when performing " " phlebotomy or handling patient specimens, " " calibrators, or other serum based-products. " " Discard contaminated materials with infectious" " waste. "

B. Remove serum promptly from the clot. Hemolyzed specimens should not be used because ALT contamination from red cells will occur. DO NOT freeze the sample; this will cause a loss of ALT activity.

C. If concentration is greater than the analyzer range, dilute with an equal volume of isotonic saline and reanalyze. Multiply the result by two to obtain the original ALT activity.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer -- DTSC Module. See Operator's Manual for additional information.

B. Kodak Ektachem DT Slides for ALT analysis. Store slides refrigerated at temperatures less than 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

- A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 4.
- B. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

- A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are out-of-range, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.
- B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.
- C. See Chemistry Quality Control SOP for control procedures.

VII. PROCEDURE:

- A. Load slide into DTSC Module.
- B. Enter the Patient ID number (usually the SSN).
- C. When the green light is flashing, spot slide with sample or control.
- D. Assay time is approximately 5 minutes.

VIII. CALCULATIONS:

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS:

- A. Expected values: 7-56 U/L.
- B. Panic values: 40% difference from prior value.
- C. Each laboratory should confirm these values.

X. LIMITATIONS:

- A. High total protein samples that are predominately gamma globulins can increase ALT results. The sample should be diluted with an equal volume of isotonic saline and reanalyzed.
- B. EDTA and fluoride oxalate should not be used as anticoagulants.
- C. Analyzer range: 3-950 U/L.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C-50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Alanine Aminotransferase, Publication No. C-336, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

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AMYLASE ANALYSIS BY KODAK DT60

I. INTRODUCTION:

A. High levels of amylase aid in the diagnosis of diseases of the pancreas. Analysis of amylase may be used to differentiate inflammation and hemorrhage of the pancreas and other disorders of the digestive system.

B. The Kodak Ektachem DT Slide (AMYL) is a dry, multi-layered film in a plastic support. It contains all the reagents necessary to determine amylase levels in 10 uL of serum or plasma. The analysis is based on the hydrolysis of a dyed starch by serum amylase. The amylase causes the dyed starch to hydrolyze to smaller dyed saccharides. The intensity of the color is proportional to the amount of amylase in the sample.

II. PRINCIPLE:

The 10 uL drop of patient sample deposited on the slide is evenly distributed by the slide's spreading layer. This layer also contains a dyed starch that reacts with the amylase to hydrolyze to smaller dyed saccharides. The saccharides diffuse to the underlying reagent layer to form a highly colored complex. By measuring the amount of light reflected from the dyed layer after a fixed incubation period, the analyzer can calculate the amount of amylase present in the sample.

III. SPECIMEN:

A. Recommended specimen -- 10 uL of serum or plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation.

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³ Follow universal precautions when performing ³ phlebotomy or handling patient specimens, ³ calibrators, or other serum based-products. ³ Discard contaminated materials with infectious³
³ waste. ³

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B. Remove serum promptly from the clot. Refrigerate specimen at 4°C for up to 24 hours if analysis cannot be performed immediately. Freeze specimen if analysis will not be performed within 24 hours. Allow specimen to reach room temperature prior to analysis.

C. If concentration is greater than the analyzer range, dilute with isotonic saline and reanalyze. A minimal dilution is recommended (less than twofold if possible). Results are considered approximate. For maximum accuracy, dilute with 2% bovine serum albumin in saline (pH 7.4) or a clear patient specimen with low amylase activity. If bovine serum albumin is used,

verify that it is free from contamination by amylase prior to use. Multiply the result by the dilution factor to obtain the original amylase concentration.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer. See Operator's Manual for additional information.

B. Kodak Ektachem DT Slides for Amylase analysis. Store slides refrigerated at temperatures less than 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 3.

B. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are outofrange, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See Chemistry Quality Control SOP for control procedures.

VII. PROCEDURE:

A. Load slide into the DT60 Analyzer.

B. Enter the Patient ID number (usually the SSN).

C. When analyzer reads "Spot Slide," dispense slide onto slide.

D. Assay time is approximately 5 minutes.

VIII. CALCULATIONS:

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS:

A. Expected values: 30-110 U/L.

B. Panic values: 25% difference from prior value and values >150 U/L.

C. Each laboratory should confirm these values.

X. LIMITATIONS:

A. EDTA, oxalate, and citrate should not be used as anticoagulants because they lower results.

B. Analyzer range: 5-900 U/L.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Amylase, Publication No. C311, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

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ASPARTATE AMINOTRANSFERASE ANALYSIS BY KODAK DT60

I. INTRODUCTION:

A. Aspartate aminotransferase (AST or SGOT) is an enzyme found mainly in the heart as well as in other organs. Elevated levels may be associated with myocardial infarctions, various liver diseases, pulmonary emboli, and gangrene.

B. The Kodak Ektachem DT Slide (AST) is a dry, multilayered film in a plastic support. It contains all the reagents necessary to determine aspartate aminotransferase activity in 10 uL of serum or plasma. The analysis is based on an enzyme-coupled oxidation of NADH to NAD⁺.

II. PRINCIPLE:

A. The 10 uL drop of patient sample deposited on the slide is evenly distributed by the slide's spreading layer. The slide contains a high concentration of pyridoxal-5-phosphate (P-5-P), to rapidly activate the apoenzyme so that AST is fully active at the end of the lag phase without the need for a long preincubation.

B. In the assay for AST, the amino group of L-aspartate is transferred to α -ketoglutarate in the presence of sodium P-5-P to produce glutamate and oxaloacetate. The oxalo- acetate formed in the deamination of the L-aspartate is converted to malate by malate dehydrogenase (MDH) in the presence of NADH, which is oxidized to NAD⁺. The rate of oxidation of NADH is monitored by reflectance spectrophotometry at 37°C. The rate of change in reflection density measured in a linear region is then converted to enzyme activity in International Units per Liter (U/L).

III. SPECIMEN:

A. Recommended specimen -- 10 uL of serum or plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation.

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³ Follow universal precautions when performing ³ phlebotomy or handling
patient specimens, ³ ³ calibrators, or other serum based-products. ³ ³ Discard
contaminated materials with infectious³
³ waste. ³

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B. Remove serum promptly from the clot. Hemolyzed specimens should not be used because AST contamination from red cells will occur. Refrigerate specimens if analysis is not performed immediately. Freeze specimens if analysis is not performed within 48 hours.

C. If concentration is greater than the analyzer range, dilute with an equal volume of isotonic saline and reanalyze. Multiply the result by two to obtain the original AST activity.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer -- DTSC Module. See Operator's Manual for additional information.

B. Kodak Ektachem DT Slides for AST analysis. Store slides refrigerated at temperatures less than 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 4.

B. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are outofrange, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See Chemistry Quality Control SOP for control procedures.

VII. PROCEDURE:

A. Load slide into DTSC Module.

B. Enter the Patient ID number (usually the SSN).

C. When the green light is flashing, spot slide with sample or control.

D. Assay time is approximately 5 minutes.

VIII. CALCULATIONS:

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS:

A. Expected values: 5-35 U/L.

B. Panic values: 40% difference from prior value.

C. Each laboratory should confirm these values.

X. LIMITATIONS:

A. EDTA and fluoride oxalate should not be used as anticoagulants.

B. High total protein samples that are predominately gamma globulins can increase ALT results. The sample should be diluted with an equal volume of isotonic saline and reanalyzed.

C. Analyzer range: 4-950 U/L.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Aspartate Aminotransferase, Publication No. C338, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

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TOTAL BILIRUBIN ANALYSIS BY KODAK DT60

I. INTRODUCTION:

A. Causes for an increase in total bilirubin may be divided into 3 categories: prehepatic--resulting from various hemolytic states; hepatic--resulting from hepatitis, cirrhosis, and other causes of hepatic necrosis; and posthepatic--resulting from an obstruction of the common bile or hepatic duct.

B. The Kodak Ektachem DT slide (TBIL) is a dry, multi-layered film in a plastic support. It contains all the reagents necessary to determine total bilirubin levels in 10 uL of serum or plasma. The analysis is based on a reaction of bilirubin with a diazonium salt to produce a highly colored dye. The intensity of the color is proportional to the amount of total bilirubin in the sample.

II. PRINCIPLE:

The 10 uL drop of patient sample deposited on the slide is evenly distributed by the slide's spreading layer. The aqueous portion diffuses to the underlying reagent layer where the reaction occurs to produce a colored compound.

By measuring the amount of light reflected from the colored layer after a fixed incubation period, the analyzer can calculate the amount of total bilirubin present in the sample.

III. SPECIMEN:

A. Recommended specimen -- 10 uL of serum or plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation.

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" Follow universal precautions when performing " " phlebotomy or handling patient specimens, " " calibrators, or other serum based-products. " " Discard contaminated materials with infectious" " waste.

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B. Protect specimens from light and analyze as soon as possible after collection. Direct exposure to sunlight is reported to cause as much as 50% loss of bilirubin in one hour, especially when the specimen is kept in a capillary tube. Exposure to normal room light can result in a significant loss of serum bilirubin after 2 to 3 hours.

C. If analysis is not performed immediately, samples can be refrigerated for up to 24 hours.

D. Centrifuge plasma specimens properly to prevent leukocytes and platelets from remaining in suspension. Cellular elements on the slide will cause significant positive errors.

E. Samples that come in contact with alcohol from sterile wipes may become hemolyzed which will increase results.

F. If concentration is greater than the analyzer range, dilute with an equal volume of normal saline and reanalyze. Multiply the result by two to obtain the original total bilirubin concentration. Obtain an approximate result by diluting specimens with an equal volume of isotonic saline or distilled water. The results may show a positive bias of up to 30%.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer. See Operator's Manual for additional information.

B. Kodak Ektachem DT slides for Total Bilirubin analysis. Store slides refrigerated at temperatures lower than 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 3.

B. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are out-of-range, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See Chemistry Quality Control SOP for control procedures.

VII. PROCEDURE:

A. Load slide into the DT60 Analyzer.

B. Enter the Patient ID number (usually the SSN).

C. When analyzer reads "SPOT SLIDE," dispense sample onto slide.

D. Assay time is approximately 5 minutes.

VIII. CALCULATIONS:

The results are reported in mg/dL. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS:

A. Expected values: 0.0-1.4 mg/dL.

B. Panic values: 50% difference from prior value.

C. Each laboratory should confirm these values.

X. LIMITATIONS:

A. Specimens from hemodialysis patients should not be analyzed for total bilirubin. Normal bilirubin levels may be increased by 1.3 mg/dL.

B. Hemoglobin affects bilirubin results. Moderate (2+) hemolysis (150 mg/dL hemoglobin) will:

- lower bilirubin levels less than 10 mg/dL by 7%.
- raise bilirubin levels greater than 10 mg/dL by 7%.

C. Compounds that discolor serum, such as 4-aminosalicylic acid and phenazopyridine, may falsely increase bilirubin results.

D. Results for predominately unconjugated bilirubin (e.g., for neonates) may be up to 10% higher than the reference method.

E. Analyzer range: 0.1-20.0 mg/dL.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C-50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Total Bilirubin, Publication No. C-305, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650, 1988.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer. See Operator's Manual for additional information.

B. Kodak Ektachem DT slides for urea nitrogen analysis. Store slides refrigerated at temperatures lower than 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 3. If not used within 1 hour of reconstitution, refrigerate at 2-8°C.

B. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are outofrange, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.

B. Control fluids should be evaluated for compatibility because they may contain high concentrations of preservatives, stabilizers, or other additives.

C. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

D. See Chemistry Quality Control SOP for control procedures.

VII. PROCEDURE:

A. Load slide into the DT60 Analyzer.

B. Enter the Patient ID number (usually the SSN).

C. When analyzer reads "SPOT SLIDE," dispense sample onto slide.

D. Assay time is approximately 5 minutes.

IX. CALCULATIONS:

The results are reported in mg/dL. No further calculation is necessary unless the specimen has been diluted.

X. RESULTS:

A. Expected values:

Males (18-64 yrs) 9-21 mg/dL.

Females (18-64 yrs) 7-18 mg/dL.

- B. Panic values: <3 mg/dL and >150 mg/dL.
- C. Each laboratory should confirm these values.

XI. LIMITATIONS:

- A. Do not use plasma collected with sodium fluoride.
- B. Specimens that contain hemoglobin increase urea nitrogen. Hemoglobin of 50 mg/dL (slightly hemolyzed) increases BUN levels below 28 mg/dL by 1 mg/dL.
- C. Ammonium ions have shown an increase in urea nitrogen equivalent to their nitrogen content.
- D. Analyzer range: 1-100 mg/dL.

XII. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Urea Nitrogen, Publication No. C301, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

B. Remove serum promptly from the clot. Prolonged contact with clot may lead to lowered calcium values due to dilution by red cell water. Refrigerate specimen at 4°C for up to 24 hours if analysis is not performed immediately. DO NOT freeze serum. Allow specimen to reach room temperature prior to analysis.

C. If concentration is greater than the analyzer range, dilute with an equal volume of sterile distilled water and reanalyze. Multiply the result by two to obtain the original calcium concentration.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer -- DTSC Module. See Operator's Manual for additional information.

B. Kodak Ektachem DT Slides for Calcium analysis. Store slides refrigerated at temperatures less than 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 3.

B. If the laboratory's ambient temperature has changed $\pm 3^{\circ}\text{C}$ or more from the temperature at the time the calcium test was calibrated, the quality control fluids should be checked. If out of control, recalibrate the analyzer for Ca and record the temperature at the time of calibration for future reference.

NOTE: Calibrating at the average laboratory temperature should reduce the necessity for frequent recalibration.

C. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are outofrange, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See Chemistry Quality Control SOP for control procedures.

VII. PROCEDURE:

A. Load slide into DTSC Module.

B. Enter the Patient ID number (usually the SSN).

C. When the green light is flashing, spot slide with sample or control.

D. Assay time is approximately 5 minutes.

VIII. CALCULATIONS:

The results are reported in mg/dL. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS:

- A. Expected values: 8.4-10.2 mg/dL.
- B. Panic values: <6 mg/dL and >13 mg/dL.
- C. Calcium values vary with posture (recumbent patients may have 0.2 to 0.3 mg/dL lower levels). Blood collected with stasis may have 0.2 to 0.3 mg/dL higher levels.
- D. Each laboratory should confirm these values.

X. LIMITATIONS:

A. Keeping sample in an open container at room temperature may result in loss of carbon dioxide and change in pH, causing elevated test results. These changes are minimized by anaerobic handling procedures and prompt analysis, especially for small pediatric samples.

B. EDTA, oxalate, and citrate should not be used as anticoagulants. They are calcium-chelating agents and may cause falsely low values. Sodium fluoride

C. Blood from patients receiving Hypaque® (Wintrop Laboratories) radiographic agents and patient having platelet phoresis should not be used.

D. Results are affected by changes in ambient temperature. If temperature has increased 3°C or more since Ca calibration, values will be lower than expected. If the temperature has decreased 3°C or more since calibration, values will be higher than expected.

E. Analyzer range: 3-15 mg/dL.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Calcium, Publication No. C348, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer -- DTSC Module. See Operator's Manual for additional information.

B. Kodak Ektachem DT Slides for Creatine Kinase analysis. Store slides in a freezer at or below -18°C . Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 4.

B. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. See Chemistry Quality Control SOP for control procedures.

B. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are outofrange, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.

C. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

VII. PROCEDURE:

A. Load slide into DTSC Module.

B. Enter the Patient ID number (usually the SSN).

C. When the green light is flashing, spot slide with sample or control.

D. Assay time is approximately 5 minutes.

VIII. CALCULATIONS:

The results are reported in U/L. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS:

A. Expected values:

Males 55-170 U/L.

Females 30-135 U/L.

B. Panic values: 40% difference from prior value.

C. Each laboratory should confirm these values.

X. LIMITATIONS:

A. Grossly hemolyzed specimens should not be used. The effect of trace hemolysis is minimized by AK inhibitors.

B. EDTA and fluoride/oxalate anticoagulants will cause low CK results.

C. Analyzer range: 20-1600 U/L.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Creatine Kinase, Publication No. C342, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

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CHLORIDE ANALYSIS BY KODAK DT60

I. INTRODUCTION:

A. Chloride is the major extracellular anion and is significantly involved in maintaining proper water distribution, osmotic pressure, and normal anion-cation balance in the extracellular fluid compartment. Chloride measurements are used primarily in the diagnosis of renal tubular disorders and metabolic acidosis. Abnormally high levels may also be caused by dehydration and decreased renal blood flow.

B. The Kodak Ektachem chemistry DT slide (Cl⁻) uses ion-selective electrodes for potentiometric measurements of ionic chloride. The electrodes in the Ektachem slides are dry film, multilayered, analytical elements coated on a polyester support. Two identical electrodes form a complete concentration cell on a single slide intended to be used for one test and then discarded. The assay is performed on 10 uL of serum or plasma and requires the use of electrolyte reference fluid having a fixed ionic concentration. The slide and reference fluid contain all reagents necessary to determine chloride concentrations.

C. A 10 uL drop each of specimen and reference fluid is deposited simultaneously on the slide by a dual-tip pipet. An electrical potential develops in proportion to the concentration of chloride in the specimen and is measured by a sensitive voltmeter in the DTE module.

II. PRINCIPLE:

A. Electrolyte analysis uses differential potentiometry. The slide contains two identical ion-selective electrodes consisting of a silver and silver chloride layer covered by an ion-selective membrane which makes the electrodes selective for chloride ions. This layer inhibits the effect of normal levels of bromide, uric acid, and other substances that might otherwise interfere with the analysis.

B. A 10 uL drop sample fluid is dispensed on the sample electrode and a 10 uL drop of reference fluid is simultaneously dispensed on the reference electrode.

The two fluids spread toward each other through a paper bridge, forming a liquid junction in the center. Each electrode develops an electrical potential (voltage) in response to the concentration of chloride applied to it. The difference in potential between the two electrodes is proportional to the concentration of chloride in the sample.



III. SPECIMEN:

A. Recommended specimen -- 10 uL of serum or plasma. Collect specimen by standard venipuncture technique. No special patient preparation required and heparin may be used as an anticoagulant for plasma specimen. Handle specimen in stoppered containers to avoid contamination

and evaporation.

Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum based-products. Discard contaminated materials with infectious waste.

B. Remove serum promptly from the clot. Refrigerate specimen at 2-8°C if analysis is not performed within 4 hours; freeze specimen at -18°C if analysis is not performed within 48 hours.

C. Do not dilute specimen for chloride analysis.

D. The specimen should not be drawn from an arm receiving an intravenous transfusion.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT slides for Chloride analysis. Store slides in refrigerator below 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

B. Kodak Ektachem DT60 Analyzer -- DTE Module. See Operators manual for additional information.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1 and 2. If not used within one hour of reconstitution, refrigerate vials at 2-8°C.

B. Calibrate chloride in duplicate by running each bottle twice.

C. See Kodak Ektachem DT60 Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. See Chemistry Quality Control SOP for control procedures.

B. Control fluids should be evaluated for compatibility because they may contain high concentrations of preservatives, stabilizers, or other non-physiological additives. Refer to appropriate package insert for preparation instructions.

C. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are out of range, do not report patient results until problem has been resolved, and the patient samples are repeated with quality controls within acceptable range.

D. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

VII. PROCEDURE:

A. Prepare materials for use. Run controls at beginning of the shift. Warm slides to room temperature before use.

B. Use dual pipet for DTE module. To attach disposable tips press DTE pipet firmly into two of the tips. The tips will click into place (visually inspect for proper seat).

C. Insert and fill dual-sample cup.

1. Gently invert the bottle of Kodak Ektachem DT electrolyte reference fluid, and squeeze at least 4 drops into small well.

2. Using transfer pipet, pipet 4 drops (at least 50 uL) of sample into large well of the cup.

D. Load slide -- manually push the advance lever to move slide into spotting station.

1. Notch on slide in first.

2. Ensure slide barcode is down.

3. An audible tone will tell you when slide is ready to be spotted.

E. Enter patient ID number.

F. Aspirate fluids.

1. Hold pipet in vertical position.

2. Depress button and continue to hold button down as you insert pipet into pipet locator at the aspiration station.

3. Slowly aspirate fluids and wait one second before removing pipet from aspiration station.

4. Wipe outside of the tips in a light quick motion.

5. Visually check fluid levels in both tips for approximately equal fluid levels.

G. Spot slide with fluids.

1. Place pipet into pipet locator dispense station and depress button. Leave pipet in place for one second.

2. Continue to hold button down until completely removed from pipet locator dispense station.

H. Red flashing light indicates slide is incubating; module can not be used until display reads DTE MODULE READY.

I. Assay time is approximately 3 minutes at 25°C.

VIII. CALCULATIONS:

The results are reported in mmol/L. No further calculation is necessary.

IX. RESULTS:

- A. Expected values: 98-107 mmol/L.
- B. Panic values: <85 mmol/L and >115 mmol/L.
- C. Each laboratory should confirm these values.

X. LIMITATIONS:

- A. Therapeutic doses of bromide increase chloride results (3 mmol/L increase for each mmol/L of bromide).
- B. Iodide absorbed from antiseptic preparation increases chloride results (4 mmol/L increase for each mmol/L of iodide).
- C. Abnormal concentrations of triglycerides or total protein may affect the results and give different values than methods that dilute the sample before analysis. Direct ion-selective methods, such as the Kodak Ektachem DT slide, produces appropriate results for clinical use.
- D. Analyzer range: 65-140 mmol/L.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C-50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Chloride, Publication No. C-309, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

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MCCS-HCH

STANDING OPERATING PROCEDURE

1 Mar 95

CARBON DIOXIDE ANALYSIS KODAK DT60

I. INTRODUCTION:

A. Total CO₂ measurements are used together with other clinical and laboratory information (pH, pCO₂) for the evaluation of the acid-base status.

B. The Kodak Ektachem chemistry DT slide (CO₂) uses ion-selective electrodes for potentiometric measurements of total serum carbon dioxide. The electrodes in the Ektachem slides are dry film, multilayered, analytical elements coated on a polyester support. Two identical electrodes form a complete concentration cell on a single side intended to be used for one test and then discarded. The assay is performed on 10 uL of serum or plasma and requires the use of electrolyte reference fluid having a fixed ionic concentration. The slide and reference fluid contain all reagents necessary to determine total CO₂ concentrations.

C. A 10 uL drop each of specimen and reference fluid is deposited simultaneously on the slide by a dual-tip pipet. An electrical potential develops in proportion to the concentration of CO₂ in the specimen and is measured by a sensitive voltmeter in the DTE module.

II. PRINCIPLE:

A. Electrolyte analysis uses differential potentiometry. The slide contains two identical ion-selective electrodes. Each consists of a silver and silver chloride layer covered by additional film layers which make the electrodes selective for CO₂ ions. The buffer layer adjusts the sample pH to 8.4 and maintains CO₂, HCO₃, and CO₃ in proper equilibrium or fixed ionic strength condition. The buffer layer has been coated over the ion-selective membrane and is composed of a polymer containing buffer and emulsion (extracts interferents). The ion-selective membrane layer removes substances from the sample that might interfere with the CO₂ analysis.

B. The ion-selective membrane is an ion exchange system consisting of trioctylpropylammonium chloride and membrane solvents in a vinyl resin. The electrical potential at the reference layer/silver chloride layer interface is poised by the chloride ion, while the potential across the membrane is determined by the ratio of carbonate ion activity in the deposited solution to the chloride activity in the reference layer.

C. A 10 uL drop sample fluid is dispensed on the sample electrode and a 10 uL drop of reference fluid is simultaneously dispensed on the reference electrode. The two fluids spread toward each other through a paper bridge, forming a liquid junction in the center. Each electrode develops an electrical potential (voltage) in response to the concentration of CO₂ applied to it. The difference in potential between the two electrodes is proportional to the concentration of CO₂ in the sample.

III. SPECIMEN:

A. Recommended specimen -- 10 uL of serum or plasma. Collect specimen by standard venipuncture technique. No special patient preparation required and heparin may be

used as an anticoagulant for plasma specimen. Handle specimen in stoppered containers to avoid contamination and evaporation.

Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum based-products. Discard contaminated materials with infectious waste.

B. Remove serum promptly from the clot. Refrigerate specimen at 2-8°C if analysis is not performed within 4 hours; freeze specimen at -18°C if analysis is not performed within 48 hours.

C. Do NOT dilute specimen for carbon dioxide analysis.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer -- DTE Module. See Operators manual for additional information.

B. Kodak Ektachem DT slides for Carbon Dioxide analysis. Store slides in refrigerator below 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1 and 2. If not used within one hour of reconstitution, refrigerate vials at 2-8°C.

B. Calibrate carbon dioxide in duplicate by running each bottle twice.

C. See Kodak Ektachem DT60 Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. See Chemistry Quality Control SOP for control procedures.

B. Control fluids should be evaluated for compatibility because they may contain high concentrations of preservatives, stabilizers, or other non-physiological additives. Refer to appropriate package insert for preparation instructions.

C. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are out of range, do not report patient results until problem has been resolved, and the patient samples are repeated with quality controls within acceptable range.

D. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

VII. PROCEDURE:

A. Prepare materials. Run controls at the beginning of the shift. Warm slides to room temperature before use.

B. Use dual pipet for DTE module. To attach disposable tips press DTE pipet firmly into two of the tips. The tips will click into place (visually inspect for proper seat).

C. Insert and fill dual-sample cup.

1. Gently invert the bottle of Kodak Ektachem DT electrolyte reference fluid, and squeeze at least 4 drops into small well.

2. Using transfer pipet, pipet 4 drops (at least 50 uL) of sample into large well of the cup.

D. Load slide -- manually push the advance lever to move slide into spotting station.

1. Notch on slide in first.

2. Ensure slide barcode is down.

3. An audible tone will tell you when slide is ready to be spotted.

E. Enter patient ID number.

F. Aspirate fluids.

1. Hold pipet in vertical position.

2. Depress button and continue to hold button down as you insert pipet into pipet locator at the aspiration station.

3. Slowly aspirate fluids and wait one second before removing pipet from aspiration station.

4. Wipe outside of the tips in a light quick motion.

5. Visually check fluid levels in both tips for approximately equal fluid levels.

G. Spot slide with fluids.

1. Place pipet into pipet locator dispense station and depress button. Leave pipet in place for one second.

2. Continue to hold button down until completely removed from pipet locator dispense station.

H. Red flashing light indicates slide is incubating; module can not be used until display reads DTE MODULE READY.

I. Assay time is approximately 3 minutes at 25°C.

VIII. CALCULATIONS:

The results are reported in mmol/L. No further calculation is necessary.

IX. RESULTS:

- A. Expected values: 22-31 mmol/L.
- B. Panic values: <15 mmol/L and >40 mmol/L.
- C. Each laboratory should confirm these values.

X. LIMITATIONS:

- A. Therapeutic doses of bromide may falsely increase CO₂ results (2 mmol/L increase for each mmol/L of bromide).
- B. Iodide absorbed from antiseptic preparations may falsely increase CO₂ results (12 mmol/L increase for each mmol/L of iodide).
- C. Nitrate may increase CO₂ results (0.6 mmol/L increase for each mmol/L nitrate).
- D. Lactate, hippurate, and other organic acids at significantly elevated concentrations have been reported to increase CO₂ results.
- E. Diatrizoate sodium may increase CO₂ results. Do not use specimens from patients receiving radiographic contrast agents containing this substance.
- G. Abnormal concentrations of total protein and triglycerides may affect the CO₂ results and give different results than methods that dilute the sample before analysis. Direct ion-selective methods, such as used with Kodak Ektachem DT Slides, produce the appropriate results for clinical use.
- H. Analyzer range: 5-50 mmol/L.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C-50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Carbon Dioxide, Publication No. C-308, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

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MCCS-HCH STANDING OPERATING PROCEDURE 1 Mar 95

**CREATININE ANALYSIS BY KODAK DT60
Single-Slide Method**

I. INTRODUCTION:

A. Creatinine measurements are used in the diagnosis and treatment of renal diseases and in monitoring patients on renal dialysis.

B. The Kodak Ektachem DT Slide (CRSC) is a dry, multi-layered film in a plastic support. It contains all the reagents necessary to determine creatinine activity in 10 uL of serum or plasma. The analysis is based on an enzymatic method that produces a colored end product.

II. PRINCIPLE:

A. The 10 uL drop of patient sample deposited on the slide is evenly distributed by the slide's spreading layer. The creatinine diffuses to the gel layers where it is hydrolyzed to creatine, initiating the series of reactions shown in the reaction sequence below.

B. Creatine present in the sample is reacted within the first 4 minutes of the 5.5 minute incubation at 37°C. The rate of change between 4 and 5.5 minutes is due to creatinine hydrolysis and is proportional to creatinine concentration.

III. SPECIMEN:

A. Recommended specimen -- 10 uL of serum or plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation.

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³ Follow universal precautions when performing ³ phlebotomy or handling patient specimens, ³ calibrators, or other serum based-products. ³ Discard contaminated materials with infectious³
³ waste. ³

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B. Remove serum promptly from the clot. Refrigerate specimens at 2-8°C if analysis is not performed within 4 hours; freeze specimens at -18°C if analysis is not performed within 48 hours.

C. If concentration is greater than the analyzer range, dilute with an equal volume of isotonic saline and reanalyze. Multiply the result by two to obtain the creatinine concentration of the original specimen.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer -- DTSC Module. See Operator's Manual for additional information.

B. Kodak Ektachem DT Slides for Creatinine analysis. Store slides in a freezer at or below -18°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 4. If not used within 1 hour of reconstitution, refrigerate vials at 2-8°C.

B. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. See Chemistry Quality Control SOP for control procedures.

B. Do not use controls prepared with a diluent containing Tris buffer, which causes a decrease in results. Liquid serum controls often contain high creatine levels and may give L-11 error codes.

C. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are outofrange, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.

D. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

VII. PROCEDURE:

A. Load slide into DTSC Module.

B. Enter the Patient ID number (usually the SSN).

C. When the green light is flashing, spot slide with sample or control.

D. Assay time is approximately 5.5 minutes.

VIII. CALCULATIONS:

The results are reported in mg/dL. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS:

A. Expected values:

Males 0.8-1.5 mg/dL.

Females 0.7-1.2 mg/dL.

- B. Panic values: <0.3 mg/dL and >7.5 mg/dL.
- C. Each laboratory should confirm these values.

X. LIMITATIONS:

- A. EDTA and fluoride/oxalate anticoagulants will cause low creatinine results.
- B. Lidocain: Patients on long-term lidocain therapy may show an increase of up to 1.0 mg/dL. Ninety percent of patients receiving intravenous lidocaine will show less than a 0.3 mg/dL increase.
- C. Proline: Patients receiving hyperalimentation fluid that contains proline may show an increase of up to 2.0 mg/dL.
- D. Tris buffer (control fluid) causes an approximate 50% decrease in results.
- E. Creatine: Results that are affected by creatine interference are suppressed and an error code is reported. The true creatinine concentration can be determined after the sample is diluted and reanalyzed.

The criteria for suppressing results depends on both the creatinine and creatine concentrations. At creatinine concentrations up to 10 mg/dL, creatine concentrations of approximately 6 mg/dL or greater will generate an L-11 error code. Samples with creatinine concentrations of 10 mg/dL or greater and creatine values approximately 4.5 mg/dL or greater will generate the message "results above the analyzer range." Dilute samples and reanalyze.

The residual bias due to creatine will be less than 0.15 mg/dL for low creatinine samples and less than 2% for high creatinine samples.

- F. Dipyrone (Metamizol®) at 40 mg/dL shows a -.6 mg/dL bias at a creatinine concentration of 1.0 mg/dL.
- G. *N*-acetylcysteine: Patients receiving *N*-acetylcysteine (*Fluimucil*, *Mucomyst*) intravenously have been reported to show a large negative bias.
- H. Analyzer range: 0.05-16.5 mg/dL.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Creatinine, Publication No. C353, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

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MCCS-HCH STANDING OPERATING PROCEDURE 1 Mar 95

GLUCOSE ANALYSIS BY KODAK DT60

I. INTRODUCTION:

A. Glucose measurements are used primarily in the diagnosis and treatment of endocrine disorders that affect the regulation of blood glucose levels.

B. The Kodak Ektachem DT slide (GLU) is a dry, multilayered film in a plastic support. It contains all the reagents necessary to determine glucose concentrations in 10 uL of serum or plasma. The analysis is based on the enzyme-catalyzed reaction of glucose with molecular oxygen, followed by a second reaction that produces a highly colored complex.

II. PRINCIPLE:

The 10 uL drop of patient sample deposited on the slide is evenly distributed by the slide's spreading layer. Glucose from the sample reacts with molecular oxygen in the presence of the enzyme, glucose oxidase. The second reaction leads to formation of a highly colored complex. By measuring the amount of light reflected from the colored layer after a fixed incubation period, the analyzer can calculate the amount of glucose present in the sample.

III. SPECIMEN:

A. Recommended specimen -- 10 uL of serum or plasma. Collect specimens by standard venipuncture technique. Heparin may be used as an anticoagulant for plasma specimens. Handle specimens in stoppered containers to avoid contamination and evaporation.

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³Follow universal precautions when performing ³ phlebotomy or handling patient specimens, ³ calibrators, or other serum based-products. ³ Discard contaminated materials with infectious³
³ waste. ³

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B. Remove serum promptly from the clot to avoid metabolism of glucose by the cells. Refrigerate specimens at 2-8°C if analysis is not performed within 4 hours; freeze specimens at -18°C if analysis is not performed within 48 hours.

C. Gross lipemia may interfere with elevated glucose results. Dilute lipemic specimens before analysis.

D. If concentration is greater than the analyzer range, dilute with an equal volume of isotonic saline or distilled water and reanalyze. Multiply the result by two to obtain the original glucose concentration. Diluted glucose samples have shown values up to 5% lower than the true concentration.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer. See Operator's Manual for additional information.

B. Kodak Ektachem DT slides for Glucose analysis. Store slides refrigerated at temperatures lower than 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 3.

B. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are outofrange, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See Chemistry Quality Control SOP for control procedures.

VII. PROCEDURE:

A. Load slide into the DT60 Analyzer.

B. Enter the Patient ID number (usually the SSN).

C. When analyzer reads "SPOT SLIDE," dispense sample onto slide.

D. Assay time is approximately 5 minutes.

VIII. CALCULATIONS:

The results are reported in mg/dL. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS:

A. Expected values:

Males 75-110 mg/dL.

Females 65-105 mg/dL.

B. Panic values: <40 mg/dL and >400 mg/dL.

C. Each laboratory should confirm these values.

X. LIMITATIONS:

A. Ascorbic acid causes lower glucose results. In patients who ingest 500 mg of vitamin C four times a day, the glucose result may be as much as 3 mg/dL low.

B. Low total protein (less than 5 g/dL) causes slightly lower glucose results (as much as 5% low). High total protein (greater than 10 g/dL) causes higher glucose results (greater than 5% high).

C. Hemolysis causes a decrease in glucose results of up to 10% in the presence of 3+ hemolysis, equivalent to 250 mg/dL hemoglobin.

D. Analyzer range: 20-450 mg/dL.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Glucose, Publication No. C300, Clinical Products Division, Eastman Kodak Co., Rochester,
NY 14650.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer. See Operator's Manual for additional information.

B. Kodak Ektachem DT slides for Total Protein analysis. Store slides refrigerated at temperatures lower than 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1, 2, and 3.

B. See Kodak Ektachem DT60 Analyzer Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are outofrange, do not report patient results until the problem is resolved, and the patient samples are repeated with quality control samples within acceptable range.

B. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

C. See Chemistry Quality Control SOP for control procedures.

VII. PROCEDURE:

A. Load slide into the DT60 Analyzer.

B. Enter the Patient ID number (usually the SSN).

C. When analyzer reads "SPOT SLIDE," dispense sample onto slide.

D. Assay time is approximately 5 minutes.

VIII. CALCULATIONS:

The results are reported in g/dL. No further calculation is necessary unless the specimen has been diluted.

IX. RESULTS:

A. Expected values: 6.3-8.2 g/dL (63/82 g/L).

B. Panic values: <4 g/dL and >9 g/dL.

C. Each laboratory should confirm these values.

X. LIMITATIONS:

A. Hemolysis causes an increase in measured values. An average increase of 4.5% interference for each 200 mg/dL hemoglobin has been observed.

B. Dextran causes an increase in measured values. An increase of up to 1.0 g/dL (10 g/L) has been noted at a dextran concentration of 1.0 g/dL (10 g/L). The degree of bias may be dependent on the particular lot of dextran used.

C. Analyzer range: 2.0-11.0 g/dL (20-110 g/L).

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Total Protein, Publication No. C310, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

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STANDING OPERATING PROCEDURE

1 Mar 95

SODIUM ANALYSIS BY KODAK EKTACHEM

I. INTRODUCTION:

A. Sodium is the major cation of extracellular fluids. The kidneys regulate sodium content of the body. Low sodium levels may be caused by excessive urine loss, diarrhea, Addison's disease and tubular disease. High sodium levels may occur in severe dehydration, some types of brain injury, diabetic coma, and excessive intake of sodium salts.

B. The Kodak Ektachem chemistry DT slides (Na) use ion-selective electrodes for potentiometric measurements of ionic sodium. The electrodes in the Ektachem Clinical Chemistry slides are dry film, multilayered, analytical elements coated on a polyester support. Two identical electrodes form a complete concentration cell on a single slide which is intended to be used for one test and then discarded. The assay is performed on 10 uL of serum, or plasma specimens and requires the use of electrolyte reference fluid having a fixed ionic concentration. The slide, along with the reference fluid, contains all the reagents necessary to determine sodium concentrations.

C. A 10 uL drop each of specimen and reference fluid are deposited simultaneously on the slide by a dual-tip pipet. An electrical potential develops in proportion to the concentration of sodium in the specimen and is measured by a sensitive voltmeter in the DTE module.

II. PRINCIPLE:

A. Electrolyte analysis uses a procedure called differential potentiometry. The slide contains two identical ion-selective electrodes consisting of a silver and silver chloride layer over which an ion-selective membrane has been added to make the electrodes selective for sodium ions.

B. A 10 uL drop of sample fluid is dispensed on the sample electrode and a 10 uL drop of reference fluid is simultaneously dispensed on the reference electrode. The two fluids spread toward each other through a paper bridge, forming a liquid junction in the center. Each electrode develops an electrical potential (voltage) in response to the concentration of sodium applied to it. The difference in potential between the two electrodes is proportional to the concentration of sodium in the sample.

III. SPECIMEN:

A. Recommended specimen -- 10 uL of serum or plasma. Collect specimen by standard venipuncture technique. No special patient preparation required and heparin may be used as an anticoagulant for plasma specimen. Handle specimen in stoppered containers to avoid contamination and evaporation.

Follow universal precautions when performing phlebotomy or handling patient specimens, calibrators, or other serum based-products. Discard contaminated materials with infectious waste.

B. Remove serum promptly from the clot. Refrigerate specimen at 2-8°C if analysis is not performed within 4 hours; freeze specimen at -18°C if analysis is not performed within 48 hours.

C. Do NOT dilute specimen for sodium analysis.

IV. REAGENTS AND MATERIALS:

A. Kodak Ektachem DT60 Analyzer -- DTE Module. See Operator's Manual for additional information.

B. Kodak Ektachem DT slides for Sodium analysis. Store slides in refrigerator below 8°C. Warm slides to room temperature for at least 15 minutes before using. Discard slides that are not used within 15 minutes after they are unwrapped.

V. CALIBRATION:

A. Use Kodak Ektachem DT-Plus Calibrator and Diluent Set. Instrument calibration requires Kodak Ektachem DT-Plus Calibrators, bottles 1 and 2. If not used within one hour of reconstitution, refrigerate vials at 2-8°C.

B. Calibrate sodium in duplicate by running each bottle twice.

C. See Kodak Ektachem DT60 Calibration SOP for calibration procedures.

VI. QUALITY CONTROL:

A. See Chemistry Quality Control SOP for control procedures.

B. Control fluids should be evaluated for compatibility because they may contain high concentrations of preservatives, stabilizers, or other non-physiological additives. Refer to appropriate package insert for preparation instructions.

C. Run quality control materials in normal and abnormal ranges (low and high controls) at the beginning of each shift. Always run a set of controls after calibrating the analyzer. If the controls are out of range, do not report patient results until problem has been resolved, and the patient samples are repeated with quality controls within acceptable range.

D. Variability is expressed as standard deviation (SD) and coefficient of variation (CV) and plotted on a Levy-Jennings graph.

VII. PROCEDURE:

A. Prepare materials for use. Run controls if at the beginning of the shift. Remember to warm slides to room temperature before use.

B. Use dual pipet for DTE module. To attach disposable tips press DTE pipet firmly into two of the tips. The tips will click into place (Visually inspect for proper seat).

C. Insert and fill dual-sample cup.

1. Gently invert the bottle of Kodak Ektachem DT electrolyte reference fluid, and squeeze at least 4 drops into small well.

2. Using transfer pipet, pipet 4 drops (at least 50 uL) of sample into large well of the cup.

D. Load slide--manually push the advance lever to move slide into spotting station.

1. Notch on slide in first.

2. Ensure slide barcode is down.

3. An audible tone will tell you when slide is ready to be spotted.

E. Enter patient ID number.

F. Aspirate fluids.

1. Hold pipet in vertical position.

2. Depress button and continue to hold button down as you insert pipet into pipet locator at the aspiration station.

3. Slowly aspirate fluids and wait one second before removing pipet from aspiration station.

4. Wipe off the outside of the tips in a light quick motion.

5. Visually check fluid levels in both tips for approximately equal fluid levels.

G. Spot slide with fluids.

1. Place pipet into pipet locator dispense station and depress button. Leave pipet in place for one second.

2. Continue to hold button down until completely removed from pipet locator dispense station.

H. Red flashing light indicates slide is incubating; module can not be used until display reads DTE MODULE READY.

I. Assay time is approximately 3 minutes at 25°C.

VIII. CALCULATIONS:

The results are reported in mmol/L. No further calculation is necessary.

IX. RESULTS:

- A. Expected values: 137-145 mmol/L.
- B. Panic values: <120 mmol/L and >160 mmol/L.
- C. Each laboratory should confirm these values.

X. LIMITATIONS:

A. Cationic surfactants may cause an increase in results. A benzalkonium chloride concentration of 10 mg/L may increase sodium results by 50 mmol/L. Do NOT use heparinized catheters containing benzalkonium chloride.

B. Ethanol levels of approximately 150 mg/dL (33 mmol/L) may increase sodium results by 2 mmol/L.

C. Rare specimens with abnormal ion composition will cause a junction potential that will normally decrease, and occasionally increase, sodium results.

D. Analyzer range: 95-215 mmol/L.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C-50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Sodium, Publications No. C-307, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

A. Prepare materials. Run controls at the beginning of the shift. Warm slides to room temperature before use.

B. Use dual pipet for DTE module. To attach disposable tips press DTE pipet firmly into two of the tips. The tips will click into place (visually inspect for proper seat).

C. Insert and fill dual-sample cup.

1. Gently invert the bottle of Kodak Ektachem DT electrolyte reference fluid, and squeeze at least 4 drops into small well.

2. Using transfer pipet, pipet 4 drops (at least 50 uL) of sample into large well of the cup.

D. Load slide -- manually push the advance lever to move slide into spotting station.

1. Notch on slide in first.

2. Ensure slide barcode is down.

3. An audible tone will tell you when slide is ready to be spotted.

E. Enter patient ID number.

F. Aspirate fluids.

1. Hold pipet in vertical position.

2. Depress button and continue to hold button down as you insert pipet into pipet locator at the aspiration station.

3. Slowly aspirate fluids and wait one second before removing pipet from aspiration station.

4. Wipe outside of the tips in a light quick motion.

5. Visually check fluid levels in both tips for approximately equal fluid levels.

G. Spot slide with fluids.

1. Place pipet into pipet locator dispense station and depress button. Leave pipet in place for one second.

2. Continue to hold button down until completely removed from pipet locator dispense station.

H. Red flashing light indicates slide is incubating; module can not be used until display reads DTE MODULE READY.

I. Assay time is approximately 3 minutes at 25°C.

VIII. CALCULATIONS:

The results are reported in mmol/L. No further calculation is necessary.

IX. RESULTS:

- A. Expected values: 3.6-5.0 mmol/L.
- B. Panic values: <2.5 mmol/L and >6.0 mmol/L.
- C. Each laboratory should confirm these values.

X. LIMITATIONS:

- A. Testing has not identified any substances that interfere with test for potassium.
- B. Analyzer range: 1.0-11.0 mmol/L.

XI. REFERENCES:

Bishop, M., et al., Clinical Chemistry Principles, Procedures, and Correlations. 2d ed., Philadelphia: J.B. Lippincott, 1992.

Kodak Ektachem DT60 Analyzer Operator's Manual, Publication No. C-50, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.

Kodak Ektachem DT Slides, Sodium, Publication No. C-306, Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650.