Ketones-Urine, on AUTION 9EB multi-parameter test strip Technical Procedure 3305
ARKRAY USA, Inc., manufacturer

Principle

Intended Use

For the determination of ketones in urine.
AUTION 9EB Strips are manufactured for use only in the AUTION MAX AX-4280 Urine Analyzer.

Clinical Significance

Ketones may appear in the urine due to defective carbohydrate metabolism, high protein and fat diet with low carbohydrate intake. Any of these conditions causes the body to metabolize larger amounts of fatty acids. The intermediary products of fat metabolism are the three ketone bodies: Acetoacetic acid (diacetic acid), acetone, and ß-hydroxybutyric acid. All three appear in ketonuria in relative proportions of 20% acetoacetic acid, 2% acetone, 78% ß-hydroxybutyric acid, although these proportions may change in certain physiologic states.

The most significant cause of ketosis is diabetes mellitus, in which glucose metabolism is sufficiently impaired such that fatty acids are metabolized to meet the body's energy requirements. In untreated or inadequately treated diabetes, excessive amounts of fatty acids are metabolized to meet the body's energy requirements. In untreated or inadequately treated diabetes, excessive amounts of fatty acids are metabolized resulting in ketone excess and ketonuria. Since ketones are excreted in combination with normal basic ions, progressive metabolic acidosis ensues and may lead to a diabetic crisis with coma and death if corrective action is not taken. Ketones are likely to increase in the diabetic as a result of acute infection, surgery, gastrointestinal disturbance or other stress.

In nondiabetic individuals, ketosis occurs whenever increased amounts of fatty acids are metabolized due to diets high in fat or restricted carbohydrate intake. Ketonuria due to restricted intake of carbohydrates may occur in association with fever, anorexia, gastrointestinal disturbances, alcoholism, fasting, starvation, cyclic vomiting, (vomiting) due to pregnancy, cachexia, following anesthesia and due to certain neurologic disorders. Children are more likely to develop ketonuria than adults.

Ketone Bodies in Urine: Metabolism of fatty acids results in the formation of a small amount of acetoacetic acid, which is subsequently metabolized in the peripheral tissues. In conditions in which there is carbohydrate deprivation (starvation) or decrease utilization of carbohydrates (diabetes mellitus), lipids are the main source of energy. This causes an increased production of acetoacetic acid, which may exceed the capacity of the peripheral tissues to metabolize this compound. Thus, the acetoacetic acid accumulates in the blood and is, in part, converted to acetone by spontaneous decarboxylation and in part converted to ß-hydroxybutyric acid.

The relative proportions of ketone bodies in the blood:

ß-hydroxybutyric acid	78%
acetoacetic acid	20%
acetone	2%

Methodology

AUTION Strips 9EB are multi-parameter test strips with ketones intended for use with the AUTION MAX AX-4280 Urine Analyzer to measure certain constituents in urine The AUTION 9EB consist of 9 pads impregnated with chemicals specific for the determination of a particular constituent affixed to a plastic strip.

One of the pads permits the determination of ketones. An additional correction pad is included, to compensate for the natural color of urine and its effect on the color reaction of the ketones pad.

The AUTION MAX AX-4280 Urine Analyzer utilizes dual wavelength reflectance spectroscopy, in combination with AUTION Strips reagent chemistry, to provide semi-quantitative results of ketones. At the defined wavelength for ketones, the AUTION MAX AX-4280 Urine Analyzer analyzes the color and the intensity of reflected light from the ketones pad to calculate clinically meaningful ketones results.

Chemical Reaction Scheme

Legal reaction:

Specimen Collection and Preparation

Acceptable Sample Containers

Sterile collection bottles
BD yellow top urinalysis tubes
BD tiger top urinalysis tubes with preservative.

Gray Top culture tubes are not acceptable.

Sample Collection

A clean freshly voided midstream specimen should be collected in a clean container for routine analysis, and a sterile container for UACII requests. Infant bag collections are acceptable for children ≤ 2 years of age. Other acceptable specimens include catheterized specimens, suprapubic and ostomy collections, as well as kidney or bladder collections from the operating room.

BD tiger top urinalysis preservative tubes must be filled to a level between the marked minimum and maximum lines on the tubes (7-9 mL). Under-filled or over-filled tubes are unacceptable.

For best results, BD yellow top urinalysis tubes without preservative require eight (8) mL for UA or UACII. Urine specimens with a volume < 3 mL will be diluted for microscopic analysis, if possible. Urine specimens with a volume < 1 mL may not have enough volume for microscopic analysis.

Specimens exhibiting gross hematuria cannot be tested on the AX-4280. Gross hematuria may cause incorrect results in subsequent samples.

If analysis cannot be performed within one hour after collection, immediately refrigerate (2° and 8° C) the specimen. Bring the specimen to room temperature prior to analysis. Do not centrifuge the specimen prior to analysis.

The specimen volume placed on the iQ System must be at least 3 mL. If testing on the AX-4280 module only, the minimum volume is 2 mls. If testing on the iQSeries module only, the minimum volume is 2 mL.

Sample Stability and Handling

1. Urine collected without preservative at room temperature must be delivered to the lab within 1 hour of collection.
2. Urine collected without preservative and immediately placed on ice must be delivered within 4 hours of collection.
3. Urine collected in BD urinalysis preservative tubes will be accepted up to 48 hours after collection.

All specimens should be handled using the principles of Universal Precautions, and must be capped tightly.

Specimens that leak are unacceptable for analysis.

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Reagents

Contents: 100 AUTION strips 9EB per container
Reorder # 800-3510

Reactive Ingredients

for ketones pad on AUTION test strip	Concentration
Sodium nitroprusside	12.0 mg/dL

Storage and Handling

Store AUTION strips between 1° and 30°C. DO NOT FREEZE. PROTECT AGAINST HEAT, LIGHT AND MOISTURE (Ambient Humidity). Each vial contains a desiccant to prevent exposure to moisture in the air (humidity). Immediately re-cap vials after removal of desired number of strips.

Reagent Stability

AUTION Strips are stable for two years after the date of manufacture, when stored in their original , unopened container, and maintained at 1° and 30°C.
Once AUTION test strips are placed in the hopper, they are only stable for 72 hours. Allow the hopper to empty at least once every 24 hours.

Acceptable Reagent Performance

Any discoloration of the pad(s) may indicate deterioration. If discoloration is observed or if control/patient results are questionable or in conflict with expected results, check the following:

1. Confirm the AUTION strips are still within the expiration date indicated on the vial.
2. Controls are within the expected range.
3. Results are equivalent when fresh product is tested

If a problem still exist, contact IRIS Customer Service Support at (800) 776-4747

Precautions and Warnings

For In Vitro Diagnostic Use: Utilize standard precautions required for the handling of all laboratory reagents.

Warnings: Toxic. AUTION strips contain one or more of the following chemicals: Diazonium salt and phenol

Gloves: Avoid contact with skin and mucous membranes. Wearing of gloves, when handling blood and body fluids, is included in the Center for Disease Control's recommended universal precautions.

Equipment

This test on the AUTION 9EB strips is used with the AUTION MAX AX-4280 Urine Analyzer. The analyzer is manufactured and supplied by ARKRAY FACTORY, Inc. in Japan and distributed by Iris Diagnostics, A Division of IRIS International, Inc., Chatsworth, California.

For technical assistance, contact IRIS Customer Service Support at (800) 776-4747.

Calibration

Weekly calibration verification is performed using one white and one gray check strip on the AUTION MAX AX-4280 Urine Analyzer. Refer to the Iris Maintenance Procedure, AX-4280 module weekly schedule and check off sheet.

Quality Control

At least two levels of control material should be analyzed each shift. Parallel testing between the old shipment or lot number and the new shipment or lot number will be done to assure acceptable strip performance.

The following controls should be prepared and used in accordance with the package inserts. Allow controls to come to room temperature and mix well for several minutes before testing. Quality Control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Strip lot changes are documented on the IRIS reagent log sheet.

Quality Control Material

Control	Storage
MAS Liquid UA Abnormal Control 1	+2°C to +8°C*
MAS Liquid UA Normal Control 3	+2°C to +8°C*

*Urine controls are received and stored at 2°C to 8°C. Bottles of controls in use are stored at +2°C to +8°C and are good for 30 days

Testing Procedure

Follow the correct testing set-up, testing and control procedures, as outlined in the AUTION MAX AX-4280 Urine Analyzer *Operating Manual.*

Standard Reporting Format

Ketones results, using AUTION Strips, in combination with the AUTION MAX AX-4280 Urine Analyzer, are reported in clinically useful and common units of measure. Results can be printed directly from the urine analyzer and/or transferred to the LIS.

1. Results are reported in concentrations as Negative, Trace, 10 mg/dL, 20 mg/dL, 40 mg/dL, 60 mg/dL, 80 mg/dL, 100 mg/dL, 150 mg/dL, and > 150 mg/dL.

2. Reference interval: Negative.
   Ketones are not normally detected in urine specimens from healthy individuals. However, urine specimens from individuals who are fasting, pregnant, or who undergo regular strenuous exercise, may exhibit significant amounts of ketones. The presence of ketones, in urine specimens from patients with diabetes, may provide a useful marker for metabolic status

3. **Critical Values: Any positive urine ketones result on a child less than one year old is considered critical and will be called according to DOP P&P 110.A**.

Procedural Notes

Limitations

The ketones test pad is more sensitive to acetoacetic acid than to acetone. The pad should not react with ß-hydroxybutyric acid. Acetone has about 10% reactivity compared to the reaction with acetoacetic acid.

Interferences

1. No substances currently can cause false negative results.
2. L-dopa, BSP, PSP, Phenylketone, Cephalosporin and Aldose reductive antienxyme can cause false positive results.

Carryover

Studies were performed to assess the amount of analyte carried over by the AUTION MAX AX-4280 Urine Analyzer from one specimen reaction into subsequent specimen reactions. The studies consisted of the measurement of four identical high control samples immediately followed by four identical low control samples. Results of the testing demonstrated zero carryover for bilirubin as measured by the AUTION MAX AX-4280.

Gross hematuria may cause incorrect results in subsequent samples. Do not test specimens exhibiting gross hematuria. If carryover is suspected, sample a few tubes of saline and rerun samples that followed a sample that may have caused carryover.

Performance Characteristics

The performance characteristics of the ketones pad test on the AUTION Strips have been determined in clinical and analytical studies. In clinical studies, the sensitivity of the ketones pad test depends on several inherent factors, including pH, Specific Gravity and interfering factors (see Limitations). Generally, the ketones reagent pad test on the AUTION strips have been developed to be specific for ketones being tested.

Sensitivity and Range

Sensitivity and linearity studies were performed using a series of contrived control materials which covered a wide range of known analyte concentrations. The results of the studies using the AUTION MAX AX-4280 Urine Analyzer demonstrated analytical sensitivity and linearity over the measurement range as shown for ketones.

1. Analytical Sensitivity: Acetoacetic acid 5 mg/dL
2. Analytical Measurement Range: 5 - 150 mg/dL

Semiquantitative result	Trace	1+	2+	3+	4+
Value (mg/dL)	<10	10 20	40 60	80 100	150 >150

Method Comparison

The performance of the AUTION MAX AX-4280 Urine Analyzer was evaluated in comparison with a commercially available automated urinalysis system. Urine samples were obtained from a hospital laboratory and included both normal and abnormal levels of urine ketones. Some of the native urine samples were spiked to elevated levels in order to achieve the desired range of abnormal values. Both urinalysis systems provide semi-quantitative results. Therefore, for purposes of data analysis, for ketones, results for the individual urine samples were referred to the cut-off value for each system to discriminate between negative (normal) and positive (abnormal) findings. Overall agreement, sensitivity (positive agreement), and specificity (negative agreement) between the AUTION MAX AX-4280 Urine Analyzer and the comparative system are shown in the following table.

Analyte	**No. of Samples**	**Overall Agreement (%)**	**Sensitivity (%)**	**Specificity (%)**
Ketones	227	96.5	100.0	95.2

Validation Studies performed at UCDHS

See attached validation method comparison sheet.

Precision

Reproducibility of the AUTION MAX AX-4280 Urine Analyzer was evaluated by performing replicate measurements of a control material within a single run. Measurement results were reported by the instrument in the reflectance values format instead of concentration values in order to allow for a continuous measurement output.

Within Run Precision Data

Analyte	Number of Replicates	Reflectance Value		CV (%)
		Mean	S.D.	
Ketones	21	52.43	3.04	5.79

Precision Studies performed at UCDHS

See attached AUTION MAX AX-4280 Precision Reflectance Report sheet.

Additional Information

For more detailed information on the AUTION Strips 9EB multi-parameter test strips with ketones and the AUTION MAX AX-4280 Urine Analyzer, refer to the *AUTION Strips 9EB for Urine Chemistry* package insert and the AUTION MAX AX-4280 Urine Analyzer *Operating Manual*.

References

1. Free, A.H.,et. al. *Clincal Chemistry*, 1957; 3:716.
2. Free, H.N.,et. al. *Clincal Chemistry*, 1960; 6:352
3. Graff, Sr. L., *A Handbookof Routine Urinalysis*, J.B. Lippincott Co., Philadelphia; 1983; 7-67.
4. Hayashi, Y., *Modern Medical Technology, Basic Clinical Technology and Analytical Toxicology*, Igaku-shoin, 1973.
5. Hayashi, A., et. al., *Journal of Japan Diabetes Society*, 1992; 35:819.
6. Henry, J.B., et. al., *Clinical Diagnosis and Management by Laboratory Methods*, 17th ed. Saunders, Philadelphia; 1984; 394:1441.
7. Ringsrud, K.M, and Linné, J.J. *Urinalysis and Body Fluids, A Color Text and Atlas*, Mosby-Year Book, St. Louis; 1995; 34-73.
8. Schersten, B. and Fritz, H. Subnormal Levels of Glucose in Urine. JAMA 1967. 201; 129-132.
9. *AUTION Strips 9EB For Urine Chemistry*, package insert, issued: Dec. 2001, Rev.: Dec. 2011; supplied by ARKRAY, Inc., Kyoto, Japan.

Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	07/09/2008	3472.T

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
			07/09/2008	G. Kost
			09/15/2009	G. Kost
			10/12/2010	G. Kost
			11/16/2011	G. Kost
09/25/2012	Added acceptable sample containers	M.Inn	11/20/2013	G. Kost
03/20/2015	Change to MAS UA Controls	kdagang	03/22/2015	J. Gregg