Glucose-Urine, on AUTION 9EB multi-parameter test strip Technical Procedure 3304
ARKRAY USA, Inc., manufacturer

Principle

Intended Use

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

Clinical Significance

Glucose appears in the urine when the blood level exceeds the renal threshold (approximately190 mg/dL). Renal glucosuria is a benign condition which occurs when the tubular reabsorption rate is below normal permitting glucose to spill into the urine in the presence of an acceptable glucose level. This condition may occur after a heavy meal or due to emotional stress, pregnancy and many other conditions. Diabetes mellitus is the major cause of glucosuria with levels reaching as high as 10%. Diabetic urine is usually light in color with an increased specific gravity by refractometer due to the high glucose.
Glucosuria with hyperglycemia is seen in the following conditions:

1. Endocrine disorders involving the pituitary, pancreas, or adrenal glands such as acromegaly, Cushing's syndrome, hyperadrenocorticism.
2. Pancreatic tumors, hyperthyroidism, pheochromocytoma, hemochromatosis, carcinoma, pancreatitis and cystic fibrosis.
3. Central nervous system disorders such as brain tumor, hemorrhage, hypothalamic disease or asphyxia.
4. Metabolic disturbances associated with burns, infection, fractures, myocardial infarction, and uremia.
5. Liver disease and glycogen storage diseases.
6. Feeding after starvation.
7. Drugs: Thiazides, corticosteroids and ACTH, birth control pills.
8. As a normal occurrence in late pregnancy.

Glycosuria without hyperglycemia is usually due to renal tubular dysfunction. This inability to reabsorb glucose may be the result of drugs, poisons or endogenous toxins as in Fanconi's syndrome, galactosuria or amino acid disorders.

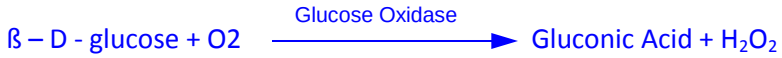
Methodology

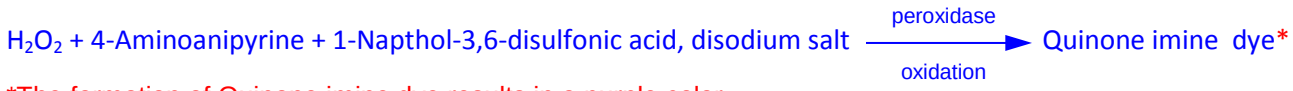
AUTION Strips 9EB are multi-parameter test strips with glucose intended for use with the AUTION MAX AX-4280 Urine Analyzer to measure certain constituents in urine. The AUTION Strips 9EB consist of a plastic strip containing 9 pads impregnated with chemicals specific for the determination of a particular constituent. One of the pads permits the determination of glucose. An additional correction pad is included, to compensate for the natural color of urine and its effect on the color reaction of the glucose 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 glucose. At the defined wavelength for glucose, the AUTION MAX AX-4280 Urine Analyzer analyzes the color and the intensity of reflected light from the glucose pad to calculate clinically meaningful glucose results.

Chemical Reaction Scheme

Glucose Oxidation reaction. The glucose test pad reacts specifically with ß-D-glucose and should not be affected by other reducing sugars such as sucrose, lactose, and fructose.





*The formation of Quinone imine dye results in a purple color.

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 Glucose pad on AUTION test strip	Concentration
Glucose oxidase	700 I.U.
Peroxidase	175 P.U.
4-Aminoantipyrene (4-AAP)	14.0 mg
1-Napthol-3,6-disulfonic acid, disodium salt	14.0 mg

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 exists, 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

Glucose 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, 50 mg/dL, 70 mg/dL, 100 mg/dL, 150 mg/dL, 200 mg/dL, 300 mg/dL, 500 mg/dL, 1,000 mg/dL and > 1000 mg/dL.
2. Reference interval: Negative.
   A small amount of glucose may be detected in normal urine. Generally, the amount of glucose is below the sensitivity level of this method; however, on occasion may produce ± (trace) results. Consistently positive glucose results should be clinically investigated.
3. **Critical Values: Any positive glucose 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 glucose test pad reacts specifically with ß - D - glucose and should not be affected by other reducing sugars such as sucrose, lactose and fructose

Interferences

1. Large amounts of ascorbic acid can cause false negative results.
2. Oxidizing substances such as hypochlorite and chlorine can cause false positive results.
3. Urines with a pH of < 4.0 can also 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 glucose as measured by the AUTION MAX AX-4280.

Performance Characteristics

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

1. Analytical Sensitivity: 30 mg/dL
2. Analytical Measurement Range: 30 - 1000 mg/dL

Semiquantitative result	Trace		1+		2+		3+		4+	
Value (mg/dL)	30(Trace)	50	70	100	150	200	300	500	1000	>1000

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 glucose. 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 glucose, 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 (%)**
Glucose	227	97.4	98.5	96.9

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.	
Glucose	21	43.28	1.28	2.95

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 glucose 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

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9. *AUTION Strips 9EB For Urine Chemistry*, package insert, issued: Dec. 2001, Rev.: Dec. 2011; supplied by ARKRAY, Inc., Kyoto, Japan.

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Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	07/09/2008	3475.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
03/21/2015	Change to MAS UA Controls	kdagang	03/22/2015	J. Gregg