Urobilinogen-Urine, on AUTION 9EB multi-parameter test strip Technical Procedure 3311
ARKRAY USA, Inc., manufacturer

# Principle

## Intended Use

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

## Clinical Significance

Urobilinogen is a substance formed by the chemical reduction of bilirubin in the intestine as a result of bacterial activity. The majority of this substance is excreted in the stool, but a very small amount is absorbed from the intestine into the circulating blood. A part of this urobilinogen is then excreted into the urine and a part is re-excreted into the bile. Impairment of liver function may result in decreased biliary excretion of urobilinogen and increased urinary urobilinogen excretion. Excessive hemolysis (e.g., hemolytic jaundice) results in more bilirubin in bile, more urobilinogen formation in the intestine, more reabsorption or urobilinogen into the blood and more urobilinogen in urine.

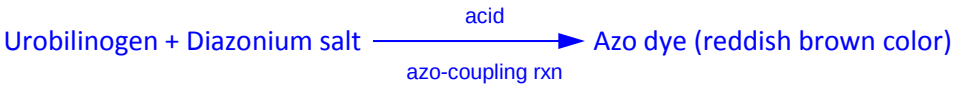
## Methodology

AUTION Sticks 9EB are multi-parameter test strips with urobilinogen 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 urobilinogen. An additional correction pad is included, to compensate for the natural color of urine and its effect on the color reaction of the urobilinogen pad.

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

## Chemical Reaction Scheme

Azo-coupling 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

Urine collected without preservative at room temperature must be delivered to the lab within 1 hour of collection.

Urine collected without preservative and immediately placed on ice must be delivered within 4 hours of collection.

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.

## Reagents

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

### Reactive Ingredients

| for urobilinogen pad on AUTION test strip | Concentration |
|---|---|
| 3,3'-Dimethoxy-4,4'-biphenylbis (diazonium tetrafluoroborate) | 0.16 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 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

Urobilinogen results, using AUTION Sticks, 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, < 2.0 (normal), 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and >12.0 mg/dL.
2. Reference interval: Healthy individuals may excrete a small amount of Urobilinogen and may be increased especially after exercise. Concentrations are generally at their peak in the afternoon.

## Procedural Notes

### Limitations

The urobilinogen test pad is sensitive to Urobilinogen down to approximately 2 mg/dL. The absolute absence of urobilinogen in urine cannot be determined by this method.

### Interferences

1. No substances currently can cause false negative results.
2. Carbapenem can cause false positive results.
3. Urine with high bilirubin causes development of a green color.

### 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 urobilinogen as measured by the AUTION MAX AX-4280.

## Performance Characteristics

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

1. Analytical Sensitivity: 2.0 mg/dL
2. Analytical Measurement Range: 2.0 - 12.0 mg/dL

| **Semiquantitative result** | Normal | 1+ | | 2+ | | 3+ | 4+ | |
|---|---|---|---|---|---|---|---|---|
| **Value (mg/dL)** | <2.0 | 2.0 | 3.0 | 4.0 | 6.0 | 8.0 | 12.0 | >12.0 |

### 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 urobilinogen. 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 urobilinogen, 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 (%) |
|---|---|---|---|---|
| Urobilinogen | 227 | 83.3 | 100.0 | 73.6 |

**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. | |
| Urobilinogen | 21 | 60.45 | 0.84 | 1.39 |

**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

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.
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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 | Atlas 3487.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 | Changed to MAS UA Controls | kdagang | 03/22/2015 | J. Gregg |
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