TRAINING UPDATE

Lab Location:	SGAH & WAH	**Date Distributed:**	1/1/2014
Department:	Core	**Due Date:**	1/31/2014
		Implementation:	**2/1/2014**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Routine Urinalysis by IQ 200 Series Analyzer® Iris ™

SGAH.U02, WAH.U02 version 5

Description of change(s):

Information added to SOP to make it match current practice

Section	Reason
5.3	Require re-calibration after failures
10.1.2	Add comparison of macro and micro

This revised SOP will be implemented on February 1, 2014

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training all sites (version 5)

Technical SOP

Title	**Routine Urinalysis by IQ 200 Series Analyzer® Iris ™**	
Prepared by	Wendell R. McMillan II	Date: 3/22/2010
Owner	Robert SanLuis	Date: 3/25/2013

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

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TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Urinalysis, Complete	IQ 200 Series IRIS	UAI

Synonyms/Abbreviations
UA

Department
Urinalysis

2. ANALYTICAL PRINCIPLE

The iQ200 Automated Urinalysis System is an in-vitro diagnostic system composed of the ArkRay™ AX-4280 chemistry module, the iQ200 microscopy module, computer and monitor. The system is used to automate the complete routine analysis of urine and body fluid including chemistry, specific gravity by refractometer, color, clarity and the microscopic analysis of the specimen.

IQ WORKSTATION PRINCIPLE

At the workstation monitor, specimen results are reviewed and edited as needed. During the review process, individual images may be displayed. Images may be manually reclassified by the operator. Crystals (UNCX), unclassified casts (UNCC), non-squamous epithelial cells (NSE) and yeast (BYST) may be further subclassified, which is done during the review process. Once the review has been completed and accepted, the results will be sent to the LIS.

AX-4280 ANALYTICAL PRINCIPLE

The AX-4280 instrument performs the urinalysis chemistry panel and determines the specific gravity, color and the clarity of a urine specimen. The chemistry panel is performed using a test strip which tests for the presence of 9 elements – glucose, protein, bilirubin, urobilinogen, pH, blood, ketones, nitrite, and leukocytes. The specific gravity is determined by measuring the refraction angles of light passing through a prism. Color is measured by transmitted light. Clarity is determined by scattered light.

3. Test Methodology of Aution Sticks

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

$$\text{Glucose} \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{1-Napthol-3, 6-disulfonic acid, disulfonic acid} \xrightarrow[\text{Oxidation}]{\text{Peroxidase}} \text{Quinone imine dye*}$$

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

Protein: Protein-error reaction. The protein test pad is particularly sensitive to albumin and less sensitive to Bence-Jones protein, globulins, hemoglobin, and mucoprotein.

$$\text{Protein} + \text{tetrabromophenol blue (pH indicator)} \xrightarrow{\text{Acid}} \text{pH indicator changes to a cyan color}$$

Bilirubin: Azo-coupling reaction. This test is sensitive to Direct-Reacting (conjugated) bilirubin.

$$\text{2-methyl-5-nitroaniline} + \text{Sodium nitrite} \xrightarrow[\text{Diazo reaction}]{\text{Acid}} \text{Diazonium salt}$$

$$\text{Bilirubin} + \text{Diazonium salt} \xrightarrow[\text{Coupling reaction}]{\text{Acid}} \text{Azo dye (reddish brown color)}$$

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Urobilinogen: Azo-coupling reaction. 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.

Urobilinogen + Diazonium salt* $\xrightarrow[\text{Coupling reaction}]{\text{Acid}}$ Azo dye (reddish brown color)

*3, 3'-Dimethoxy-4, 4'-biphenyl bis

PH: pH indicator. PH values of 5.0 – 9.0 may be read within 0.5 units.

H^+ + mixed pH indicator* $\rightarrow\rightarrow\rightarrow$ mixed pH indicator shows urinary pH range of colors**

*Mixed pH indicator: Bromocresol green and Bromoxylenol blue

**color range: yellow ~ cyan

Blood: Activity measurement of pseudoperoxidase in hemoglobin. The blood test pad is more sensitive to free hemoglobin and myoglobin than to intact (non-hemolyzed) erythrocytes. The pad is sensitive to hemoglobin values as low as 0.03 mg/dL. In the total absence of hemolysis, a negative result may occur and not agree with the results of a urine sediment examination.

Cumene hydroperoxide + 3, 3', 5, 5'-Tetramethybenzidine $\xrightarrow[\text{pseudoperoxidase action}]{\text{Hemoglobin}}$ H_2O + Cumene +

Oxidation

Ketones: Reaction as described by Legal. The ketone pad is more sensitive to acetoacetic acid than to acetone. The pad should not react with β-hydroxybuteric acid. Acetone has about 10% reactivity compared to the reaction with acetoacetic acid.

Ketones + Sodium nitroprusside $\xrightarrow{\text{Alkaline}}$ Ketones complex (purple color)

Nitrite: Greiss reaction. The nitrite pad is specific for nitrites and will not react with other constituents normally excreted in urine. There is no correlation to the level of color development and the concentration of bacteria in the urine sample. A negative result during fasting can occur since nitrates will not be excreted into the urine.

Nitrite + Sulfanilamide $\xrightarrow{\text{acid}}$ Diazo-compound + N-1-Naphthylethylenediamine

dihyrochloride $\xrightarrow[\text{coupling reaction}]{\text{acid}}$ Azo dye (pink color)

Leukocytes: Measurement of leukocyte esterase activity. The leukocyte pad reacts with esterase derived from granulocyte white blood cells (WBC) in the urine specimen.

3-(N-Toluenesulfonyl-L-alanyloxy)-indole $\xrightarrow[\text{Hydrolysis}]{\text{Esterase from leukocytes}}$ Indoxyl +

2-Methoxy-4-(N-Acid morpholino) benzenediazonium $\xrightarrow{\text{coupling reaction}}$ Azo dye (purple color)

4. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting urine may be used for samples to be analyzed by this method. Transfer contents to Urine Collection Kit to better preserve the sample.
Special Collection Procedures	A first-morning specimen is preferred but random collections are acceptable.
Other	If Urine Collection Kit is not used, submit to Laboratory within 2 hours of collection.

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Urine, freshly voided Random Urine	
Collection Container	Clean or sterile container	
Volume - Optimum **- Minimum**	The specimen volume placed on the iQ200 System must be between 4 and 6 mL. If testing on the AX-4280 module only, the minimum volume is 2 mL. If testing on the iQ200 module only, the minimum volume is 3 mL. See above.	
Transport Container	Urine, random: Urine Collection Kit (preferred) or container at room temperature.	
Stability & Storage Requirements	Room Temp:	2 hours
	Refrigerated (2-8°C):	24 hours
	Frozen:	Unacceptable
Timing Considerations	Test the urine within two hours after voiding, sooner if testing for bilirubin or urobilinogen.	
Unacceptable Specimens & Actions to Take	Specimens with volume ≤ 2 mL are processed using the back up system. Cancel the order and reorder test code UA. Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.	
Compromising Physical Characteristics	If specimen refrigerated, let it return to room temperature before testing.	
Other Considerations	After testing, samples will be held until the next successful QC performance.	

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5. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
iQ™ Lamina™	Iris Diagnostics Division, Ref: 475-0047
Iris Diluent	Iris Diagnostics Division, Ref: 800-3202
Iris System Cleanser	Iris Diagnostics Division, Ref: 800-3203
AX – 4280 Wash Solution Concentrate	Iris Diagnostics Division, Ref: 475-3503
Aution Test Sticks	Iris Diagnostics Division, Ref: 800 – 3510

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.

Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent	**iQ™ Lamina™**
Container	7 Liters
Storage	20 –28 °C
Stability	Stable until the expiration date printed on the bottle.
Preparation	Ready for use.

Reagent	**Iris System Cleanser**
Container	425 ml
Storage	20 –28 °C
Stability	Stable until the expiration date printed on the bottle.
Preparation	Ready for use.

Reagent	**Iris Diluent**
Container	475 ml
Storage	20 –28 °C
Stability	Stable until the expiration date printed on the bottle.
Preparation	Ready for use.

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Reagent	**AX – 4280 Wash Solution Concentrate**
Container	1 Liter
Storage	1 –30° C. Protect from light.
Stability	Wash Solution Concentrate bottle is stable until the expiration date printed on the bottle. The working Wash Solution is stable for 7 days.
Preparation	See Below

Reagent	**AX – 4280 *Working* Wash Solution**
Container	2 Liters
Storage	N/A
Stability	Discard any unused solution after 7 days.
Preparation	a. Place 1800 ml of distilled or deionized water in the wash solution container. b. Add 200 ml of AX-4280 Wash Solution Concentrate c. Protect from light at all time. **Avoid sudsing (no bubbles).**

Reagent	**Aution Sticks 9EB for Urine Chemistry**
Container	Plastic bottle
Storage	1-30°C. Do not freeze. Protect from heat, light and moisture.
Stability	Unopened reagent is stable until the expiration date printed on the bottle. When bottle is opened and sticks are placed on the instrument, sticks will be stable for only 3 days. **Be sure to transfer the absorbent packet with the sticks.**
Preparation	Ready for use.

6. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator Verification Control	Supplier and Catalog Number
iQ® Calibrator, contains 4 x 125 mL calibrator bottles	Iris Diagnostics Division, Calibrator P/N: 800-3103. Bottle reference number : 475-0059

Calibrator	Supplier and Catalog Number
AX–4280 Specific Gravity Calibrator - 1.005	Iris Diagnostics Division, Ref: 475-3501
AX–4280 Specific Gravity Calibrator - 1.040	Iris Diagnostics Division, Ref: 475-3502

5.2 Calibrator Preparation and Storage

NOTE: Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech (6) any special storage instructions; check for visible signs of degradation.

iQ® Calibrator	
Preparation	Ready for use.
Storage/Stability	2 –8 °C. Opened: 24 hrs Unopened: refer to label.

AX – 4280 Specific Gravity Calibrators	
Preparation	Ready for use.
Storage/Stability	20 – 28 °C. Open: 90 days Unopened: date on bottle.

5.3 Calibration Procedure

5.3.1 Calibration Procedure for the AX-4280 Analyzer

AX-4280 module Calibrations:		
Frequency	AX-4280 module Calibrations: Weekly calibration verification is performed using one white and one gray check strip. Refer to the Iris Maintenance Procedure.	
Tolerance Limits	**IF …**	**THEN …**
	Results fall within the assay specific guidelines and the calibration status displayed is 'acceptable' and QC values are within acceptable range limits:	Proceed with patient analysis.
	Calibration status is displayed as failed, or QC values are outside acceptable limits:	Troubleshoot the assay. Refer to instrument operation manual for specific calibration troubleshooting help. Repeat calibration and control.
Procedure	1. Place the instrument in "STANDBY" by pressing the "STOP" button. 2. Open the test strip feeder and remove the test strips, if there are strips in the hopper. 3. Clean the test strip feeder, test strip tray and waste box following the maintenance procedures as listed above. Line the cleaned waste box with clean paper towels to protect the check strips and return the waste box to the instrument. 4. Remove **one white strip** from the Check Strip tube. **Note: Do not touch the surface of the check strip.** 5. Open the test strip feeder. Press the "CHECK" button on the Operator keypad. **Note: The "SET CHECK STRIPS" screen is displayed.** 6. Place the strip in the provided groove within the test strip feeder. **Note: The printed side must be facing down and the black tab must be positioned towards the back of the instrument.** 7. Press the "START" button. **Note: After the strip is read, the check measurement standby screen is restored and the results will print.** 8. Remove **one gray strip** from the Check Strip tube. **Note: do not**	

	touch the surface of the Check Strip. 9. Repeat steps 5 through 7 using the gray strip. Compare the printed calibration check results with the ranges printed on the label of the Check Strip tube. Remove the check Strips from the waste box, place them in the tube and return the tube to the accessory case. Reload the test strips. Close and lock the test strip feeder. Record that the procedures were completed on the AX-4280 maintenance log.
Dilutions	N/A

AX-4280 S.G. Calibration:		
Frequency	AX-4280 S.G. Calibration: Weekly calibrator is performed using Low Calibrator and High Calibrator. Refer to the Iris Maintenance Procedure	
Tolerance Limits	**IF …**	**THEN …**
	Results fall within the assay specific guidelines and the calibration status displayed is 'acceptable' and QC values are within acceptable range limits:	Proceed with patient analysis.
	Calibration status is displayed as failed, or QC values are outside acceptable limits:	Troubleshoot the assay. Refer to instrument operation manual for specific calibration troubleshooting help. Repeat calibration and control.
Procedure	1. Place the instrument in "STANDBY" by pressing the "STOP" button. 2. Pour at least 2.0ml of SG Low Calibrator into a sample tube. Place the tube in **position "1"** in a routine patient rack. 3. Pour a minimum of 2.0ml of SG High Calibrator into a sample tube. Place the tube in position **"2"** in the same rack. 4. Place the rack on the AX-4280. Press the "SG CAL" button on the keypad. Press "Enter". **Note: The low SG value is displayed.** 5. If the value on the bottle label is different from the value displayed, enter the SG value printed on the label of the SG Low Calibrator bottle. 6. Press "ENTER" **Note: The high SG value is displayed.** 7. Enter the SG value printed on the label of the SG High Calibrator bottle if the value on the bottle is different from the value displayed.	
Dilutions	N/A	

5.3.2 Calibration Procedure for the iQ™200 Module

Criteria	Special Notations	
Frequency	**IQ™200 module Calibrations:** Calibration is performed once a month. Refer to the Iris Maintenance Procedure.	
Tolerance	**IF …**	**THEN …**
	Results fall within the assay specific guidelines and the calibration status displayed is 'Pass' and QC values are	Proceed with patient analysis.

Limits	within acceptable range limits:	
	Calibration status is displayed as failed, or QC values are outside acceptable limits:	Troubleshoot the assay. Refer to instrument operation manual for specific calibration troubleshooting help. Repeat calibration and control.
Procedure	Run a Focus 1. Place provided barcode label on a sample tube. Fill the tube with 6 mL of iQ Focus material and place in position 5 of the Control rack. 2. Iris Diagnostics recommends running Iris System Cleanser in position 1, Iris Diluent in position 2 and 3 before running the Focus. (See section under daily – Perform a Wash Cycle.) 3. Load the Control rack onto the right side of the iQ Series sampler. 4. Press Start. The rack will be processed Run a Calibration 1. Transfer at least 4 mL iQ Calibrator into 10 round-bottom 16 x 100 mm glass test tubes. 2. Place one provided barcode label on the tube that will be placed in the first position, and then load the tubes into the Calibration rack. 3. Load the Calibration rack onto the right side of the iQ Series sampler. 4. Press "START" The rack will be processed and all calculations performed automatically.	
Procedure continued	When the calibration is successful, the date/time and new REF value will be displayed in the Last Calibration field on the Instrument screen. Note: Remember to run Cal Verification after each Calibration with Focus and Positive Control, be sure to mix vigorously 5 times and then gently 5 times. Allow the bubbles to dissipate before pouring.	
Dilutions	N/A	

7. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
iQ™ Focus Set (2 Focus, 1 Negative Control, 1 Positive Control, corresponding labels)	Iris Diagnostics Division, Ref: 800-3104

Controls	Supplier and Catalog Number
IRISpec CA™	Iris Diagnostics Division, Ref:475 – 1227
IRISpec CB™	Iris Diagnostics Division, Ref:475 – 1228

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control	**iQ™ Controls**
Preparation	Ready for use. Mix well
Storage/Stability	Reagent stored at 2 –8 °C Once opened reagent is only stable for 30 days. Unopened reagent is stable until the expiration date printed on the bottle.

Control	**IRISpec CA,™ IRISpec CB™**
Preparation	Must be at room temp before using.
Storage/Stability	Reagent stored at 2 –8 °C Once opened QC is only stable for 15 days. Unopened QC is stable until the expiration date printed on the bottle.

6.3 Frequency

1.	Quality Control testing is performed once per shift on both AX-4280 and iQ™200 modules.
2.	Controls also need to be tested on the AX-4280 and iQ™200 modules when a new shipment or a new lot number of reagent is received.
3.	Parallel testing between the old shipment or lot number and the new shipment or lot number will be done to assure that it is working properly.

6.4 Tolerance Limits

Step	**Action**
1	Values obtained should fall within the ranges provided by **Iris iQ® Series Automated Urinalysis System Procedure – v5.**
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded, the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. • Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. • Supervisors may override rejection of partial or complete runs only with

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Step	Action
	detailed documentation and criteria for overrides that are approved by the Medical Director. • Consult and follow corrective action guidelines in Laboratory QC Program.

6.5 Review Patient Data

Review patient data for unusual patterns, trends or distributions in patient results, Such as an unusually high percentage of abnormal result.

6.6 Documentation

6.6.1 Document all out of range QC results and resolutions in the "QC Corrective action log".

6.6.2 QC tolerance limits are programmed into the instrument and the LIS. The LIS calculates cumulative mean, SD and CV and stores all information for easy retrieval.

6.6.3 Quality control records are reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee.

6.6.4 Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program

6.7 Quality Assurance Program

6.7.1 Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot, utilize published TEA for acceptability criteria.

6.7.2 Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.

6.7.3 The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.

6.7.4 Monthly QC must be presented to the Medical Director or designee for review and signature.

6.7.5 Consult the Laboratory QC Program for complete details.

8. EQUIPMENT and SUPPLIES

7.1 Assay Platform

The iQ200 Automated Urinalysis System is an in-vitro diagnostic system composed of the AX-4280 chemistry module, the iQ200 microscopy module, computers and monitor.

7.2 Equipment for Manual Method

- Bright field microscope equipped with Low Power (10x) and High Power (40X) Objectives.
- Single plain glass microscope slides 22x22 mm and cover slips for manual method.

- Centrifuge

7.3 Supplies

- Sample Tubes: 16 x 100 mm round bottom plastic (polystyrene) or glass tubes, Kova economy tubes or Urisept tubes. Glass should be used for control material.
- Dilution Barcode Labels: secondary barcodes are available for cloudy and bloody specimen for dilutions on the iQ Series module.
- ArkRay 9EB dipstick: Iris Diagnostics Division, cat # Ref: (800 – 3510)
- Thermal Paper: Iris Diagnostics Division, cat # Ref: (800 – 3519)

9. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

8.1	**Special Handling**
1.	Specimens should be delivered to the laboratory as soon after collection as possible. All specimens should be handled using the principles of Universal Precautions, due to the potential presence of pathogenic material.
2.	Samples that are very dense, very viscous, short in volume (<3ml) or exhibit gross hematuria must be diluted before performing testing on the iQ200 module (see end of section 8)

8.2	**Instrument Set-up Protocol for AX-4280**
1.	Ensure that sufficient supplies and consumables are loaded.
2.	Place the sample rack containing specimens on the right side of the AX-4280 Sampler- if the sample is to run on both instruments or on the AX-4280 module alone
3.	Ensure that the notch of the rack base is placed onto the Sampler track ridge
	Press the **"START"** button located on the upper left side of the AX-4280 module
	Note: if the blue "MEASURE" light is on, place the rack in the forward right corner to activate the sensor. This automatically "starts" the instrument.
	Note: The remainder of the processing is performed automatically on the system
4.	The sample rack will be moved along the sample transport tray to the barcode reader
5.	After the barcode is read, the sample aspirator mixes the sample, aspirates an aliquot, analyzes the SG, color, clarity and dispenses the sample onto a test strip.
6.	When the sample processing is complete, the sample rack will be automatically transferred, via the bridge, to the iQ Series module.

8.3	**Instrument Set-up Protocol for iQ200**
1.	At the workstation, access the Logon menu by clicking on "Instrument" which is located at the top right of the computer screen.

8.3	**Instrument Set-up Protocol for iQ200**
2.	Click on "Logon" to access the Logon screen.
3.	Use the pulldown menu to select your name from the list or type your name in the identifier field. Spelling and case MUST be exact if you choose to type.
4.	Type your password in the password field.
5.	Click "OK" to logon and close the logon screen.

8.4	**Specimen / Reagent Preparation**
1.	Obtain an empty sample tube.
2.	Pour patient sample.
3.	Place the patient's barcode label on a sample tube making sure that the label is approximately ½ inches from the top of the tube. This leaves room for the dilution label should it be required.
4.	Transfer at least 3 mL of well-mixed urine specimen into the barcoded tube. If less than 3 mL, cancel UAI, order UA and run on Clinitek. Refer to Clinitek SOP.
5.	Put the sample tube in position number 1 on the sample rack [Note: The rack's black barcode should be facing to the right]
6.	Position the labeled patient tube so that the patient barcode is centered between the uprights and facing away from the rack logo [toward the instrument when the rack is placed correctly on the system].
7.	Load up to 10 samples in each rack in consecutive positions.

8.5	**Dilutions**
	Note: Samples that are very dense, very viscous, short in volume (<3ml) or exhibit gross hematuria must be diluted before performing testing on the iQ200 module.
	How to Perform Chemistry Testing and a Microscopic Dilution Using Barcodes. (Results for both portions will appear on the Work List together):
1.	Fix identical patient barcodes onto two separate tubes.
2.	Put the iQ Series **Offline**.
3.	Pour 4 mL of well-mixed urine into one of the tubes.
4.	Place this tube in a specimen rack.
5.	Run on the AX-4280 [if you did not place the iQ series offline, you may manually remove the rack before it crosses the bridge]
6.	Label the second tube with an appropriate secondary dilution barcode below the primary barcode.
7.	Prepare the dilution in this tube using Iris diluent. Refer to addenda for dilution chart.
8.	Replace the original tube that was run on the AX-4280 with the diluted tube.
9.	Put the iQ Series back **Online.**
10.	Place the rack with the diluted sample on the iQ Series sampler.
11.	Press **"START".**
12.	The Chemistry and the Microscopic results will merge automatically.
13.	Verify results as usual.

10. CALCULATIONS

N/A

11. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

10.1.1 Chemistry Panel

Test	Report As
Color	Amber
	Bloody
	Blue
	Brown
	Dark Yellow
	Green
	Orange
	Pale Yellow
	Red
	Straw
	Yellow
Appearance	Clear
	Slightly Cloudy
	Cloudy
	Turbid
Specific Gravity	0.001 – 1.052
pH	≤5.0
	≥9.0
	5.5
	6.0
	6.5
	7.0
	7.5
	8.0
	8.5
Glucose	Negative
	Trace
	1+
	2+
	3+
	4+
Bilirubin	Negative
	1+
	2+
	3+
Urobilinogen	<2.0
	2.0

Test	Report As
	4.0
	8.0
	12.0
	>12.0

Ketone	Negative
	Trace
	1+
	2+
	3+
Blood	Negative
	Trace
	1+
	2+
	3+
Protein	Negative
	Trace
	1+
	2+
	3+
Nitrite	Negative
	Positive
Leukocytes Esterase	Negative
	Trace
	1+
	2+
	3+

10.1.2 Microscopic Analysis

Guidelines for comparing chemistry results with urine microscopic exam

Chemistry Result	Microscopic Findings
Leukocyte esterase – Positive	Look for WBCs
Nitrite – Positive	Look for evidence of infection: bacteria and WBCs
Protein – Positive	Look for large numbers of WBCs, RBCs or bacteria. Casts may be present
Blood and Hemoglobin	Look for RBCs

RBCs and WBCs are **graded per High Power Field (HPF).**

Renal and Transitional epithelial cells are **graded per HPF**

Squamous epithelial cells are **graded per Low Power Field (LPF).**

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Bacteria are **graded per HPF.**

Crystals are **graded per HPF.**

All casts are reported by type, **graded per LPF.**

Yeast is **enumerated per HPF.**

Iris Count vs. Grade Conversion chart (Cells)	
Count	**Grade (value on Iris printout)**
None	Neg
0-2	O0
3-5	O2
6-10	O6
11-20	O11
21-100	O21
>100	TNTC

Iris Count vs. Grade Conversion chart (Casts)	
Count	**Grade (value on Iris printout)**
None	Neg
0-1	O1
2-5	O2
6-10	O6
11-20	O11
>20	OG21

The presence of any questionable constituents should be brought to the supervisor's attention and not released. The specimen should be refrigerated until it can be reviewed by senior laboratory personnel.

We do not report **spermatozoa** on males or adult females.

Cystine, Cholesterol, Leucine, Bilirubin, Tyrosine, Sulfa and Hippuric acid are abnormal crystals. The specimen should be refrigerated until it can be reviewed by senior laboratory personnel or reviewed by a Pathologist. A printout of the crystals in question should also be made in the event that the specimen degrades before it can be reviewed.

10.2 Rounding
N/A

10.3 Units of Measure
Refer to section 10.1.2

10.4 Clinically Reportable Range (CRR)

10.4.1 Chemistry Panel

Color	Yellow (Dark yellow, Pale yellow) Straw
Appearance	Clear
PH	5.0 – 9.0
Specific Gravity	1.001 – 1.030
Glucose	NEG – 4+
Bilirubin	NEG – 4+
Ketone	NEG – 4+
Blood	NEG – 3+
Protein	NEG – 4+
Nitrite	NEG – POS
Leukocytes Esterase	NEG – 3+
Urobilinogen	2.0 - 12.0

10.4.2 Microscopic

WBC	0 - >100/HPF
RBC	0 - >100/HPF
Bacteria	NEG – 4+
Epithelial Cells	NEG – TNTC
Casts	NEG – TNTC
Mucus	NEG – 4+
Crystals	NEG – 3+
Yeast, Oval Fat Body and Trichomonas	NEG – 3+

10.5 Repeat Criteria and Resulting

Chemistry Panel

Test	If the result is…	Then…
Color	Color interference for those findings that are masked.	Report the urine color, appearance and result of the microscopic exam; then release result with the following comment attached "Unable to perform confirmatory test due to color interference"
Ketone	1+, 2+ and 3+	Confirm test if requested by Physician. (see Acetone SOP)
Bilirubin	1+, 2+ and 3+	The comment "Presumptive positive bilirubin. Consider confirmation by serum bilirubin if clinically indicated." will be appended to the result by the LIS.
Specific Gravity	> 1.040	Confirm using a refractometer, if result is > 1.035, report as "> 1.035". Change result in the LIS if appropriate.
pH	≥ 9.0	Perform the 3% Sulfosalicylic Acid test
Protein	Trace	Perform the 3% Sulfosalicylic Acid test

10.6 Reporting Specimens with Abnormal Results: iQ Series Instrument

Step	Action
1	If a specimen has an abnormal microscopic result, it will not be auto-released to the host computer. The results must be reviewed at the workstation monitor.
2	For quick verification, click on "Work List".
3	This brings up the Work List screen, which contains all unreleased specimen results.
4	On this screen, a specimen may be deleted or undeleted.
5	The default list arrangement order is by: time order [oldest first] with any flagged specimens at the top. The list may be sorted for any parameter by choosing Sort Specimen List or by clicking on the heading desired at the top of the row. Clicking a second time will reverse the order; *i.e.,* oldest to newest, newest to oldest or highest specimen number to lowest or vice versa. The small triangle in the header indicates which header is being used to sort at any time.
6	To review a specimen result, double click on the specimen barcode or highlight it, then click on "Specimen" at the top of the screen.
7	The Results screen for that specimen will be displayed. On the right side are the chemistry results and on the left are the microscopic results.
8	The microscopic screen [from left to right] lists the particles, their concentration and a graphic representation of the particle concentration.
9	If the concentration is normal, the green bar will display. If the concentration is abnormal, the red bar will display. The abnormal color is based upon the abnormal threshold.
10	If flags are displayed on the lower right side of the screen, they must be acknowledged before any particle type detail can be reviewed. 1) Click on "Review Flagged Specimen". 2) Then click on "**ACCEPT**". 3) If "Auto-Release" has been enabled and the only parameter preventing auto-release is the flag, the result will be auto-released as soon as the flag is cleared. If the specimen results do not meet the laboratory's criteria for auto-release, the specimen will be moved to the Work List in the time slot it would have occupied, had it not had a flag. 4) It is most efficient to handle all flagged specimens first, and then move on to the regular timed list.
11	In the Specimen screen, click on the button of the first particle to be verified
12	Images of the particles in the selected category will be displayed. Note: there may be multiple pages of the same particle type.
13	If the classification of particles is acceptable, continue to verify by clicking on the arrow in the top right corner of the screen. This takes you to the next set of images. Clicking on the left arrow takes you to the previous screen.
14	Continue verifying until you return to the Specimen screen

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15	If everything is acceptable, click on the "**ACCEPT**" button at the bottom right of the screen. The results will be transmitted to the host computer.
16	Verify the results in the LIS following the Laboratory Results Reporting Procedure.

12. EXPECTED VALUES

11.1 Reference Ranges

11.1.1 Chemistry Panel

Color	Yellow
Appearance	Clear
PH	5.0 – 9.0
Specific Gravity	1.005 – 1.030
Glucose	Negative
Bilirubin	Negative
Ketone	Negative
Blood	Negative
Protein	Negative
Nitrite	Negative
Leukocytes Esterase	Negative
Urobilinogen	<2.0

11.1.2 Microscopic

WBC	0-2/HPF
RBC	0-2/HPF
Bacteria	Negative
Renal Epithelial Cells	0/HPF
Squamous Epithelial Cells	0-2/LPF
Transitional Epithelial Cells	0/HPF
Hyaline Casts	0-1/LPF
All other Casts	0/LPF
Mucus	0/HPF
Crystals	None seen/HPF
Yeast, Oval Fat Body and Trichomonas	Negative/HPF

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

13. CLINICAL SIGNIFICANCE

12.1 Chemistry Panel

Glucose	A small amount of glucose may be detected in normal urine. Generally, the amount of glucose is below the sensitivity level of the method; however, on occasion it may produce a ± (trace) result. Consistently positive glucose results should be clinically investigated.
Protein	Although very small quantities of protein are normally excreted in urine, the amount is generally below the sensitivity level for detection. Positive results may require clinical assessment/additional testing to determine its significance.
Bilirubin	In normal urine, no bilirubin is normally detected. Positive findings should be diagnostically and clinically investigated.
Urobilinogen	Healthy individuals may excrete a small amount of urobilinogen and it may be increased especially after exercise. Concentrations are generally at their peak in the afternoon.
pH	Normal urine pH ranges from 5.0 to 8.0 and is influenced by diet. The typical value for a first morning specimen from healthy individuals is between pH 5.0 – 6.0.
Blood	A blue-green dotted reaction indicates the presence of erythrocytes. Up to 5 erythrocytes/µL may be found in normal urine specimens. Urine from menstruating women may contain blood. A large amount of blood should be clinically investigated.
Ketones	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.
Nitrite	A negative result during fasting can occur since nitrates will not be excreted into the urine. A nitrite concentration as low as 0.08 mg/dL may be detected and produce a positive test.
Leukocytes	Normal urine specimens should not produce a positive reaction. Small amounts of leukocyte esterase, causing a positive reaction, should be repeated using a fresh urine specimen, from the same patient. Positive results require further testing for pyuria.

12.2 Composition of the "Normal" Urinary Sediment

The urinary sediment may be defined as those products derived from the blood and portions of the genitourinary tract which can be identified microscopically as elements contained in the urine. Using this definition, it is obvious that the sediment may be composed of many different elements.

12.2.1 Red blood cells - should be in the range of 0-2/HPF.

12.2.2 White blood cells - may be seen in the range of 0-2/HPF.

12.2.3 Epithelial cells -- are of three types.

A. Renal - derive their origin from the epithelium lining the tubular portions of the nephron. They are polyhedral in shape, measure

approximately 20-30 microns in greatest dimension, and have large central spherical nuclei.

B. Transitional epithelial cells - derive their origin from the transitional epithelium lining the renal pelvis and calices, ureter, urinary bladder, and approximately two-thirds of the urethra.

C. Squamous epithelial cells - are the easiest of the epithelial cells to recognize. They are the largest, measuring 30-50 microns in diameter and contain a small central nucleus which can be compared to the size of a red blood cell. Squamous cells line the terminal one-third of the urethra in men and women, and also the vagina in females.

12.2.4 Mucus in excessive amounts can be indicative of inflammation or infection. It is important not to confuse mucus with hyaline casts, since both have a low refractive index.

12.2.5 Casts - may be defined as cylindrically shaped coagulums of protein formed in the tubular portion of the nephron and excreted in the urine. They may be acellular or may contain blood cells or epithelial cells and are named according to their morphologic characteristics. Hyaline and granular casts are present in the normal urine. They are usually seen in small numbers, and are not indicative of primary intrinsic renal disease.

12.2.6 Cylindroids - are nothing but casts, usually hyaline with tails. They have no significance clinically other than they are true casts and should be designated as such.

12.2.7 Crystals - may be present in the normal urine sediment and vary greatly as to the type and number. They are dependent on the osmolality and pH of the urine, the state of hydration of the patient, and a host of other factors for their formation.

12.2.8 Bacteria - not normally seen in urine, but reported as a few: (<5 field), moderate (>5 but up to 10 field), and (many ≥10 field) under 400X (high. power).

12.2.9 PH of Urine and Morphologic Characteristics

pH of Urine	**Morphologic Characteristics**
Acid Amorphous Urates	Granules, pinkish-brown
Uric Acid	Multiple forms (rhombic plates, needles, iosettes) colorless to brown.
Alkaline Ammonium biurate	Yellow-brown "thorn apples"
Calcium carbonate	Colorless, minute "dumbbell" shaped or granules.
Acid or Neutral pH Calcium oxalate	Octahedral or “dumbbell” shaped, colorless
Alkaline or Neutral pH Amorphous phosphates	Granules, colorless or white
Triple phosphates	Prisms (coffin-lids) or feathery, colorless
Calcium phosphates	Plates or wedge shaped prisms, colorless

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12.3 The Abnormal Urine Sediment

12.3.1 **WBC's and RBC's** - blood cells are present in abnormal numbers in the urine sediment in various diseases of the urinary tract. Increased numbers of either white or red blood cells is pathologic. If these cells are present in excessive numbers (Hematuria and pyuria respectively), the patient should be carefully investigated for the presence of disease involving the genitourinary tract.

12.3.2 **Epithelial Cells** Renal, transitional and squamous may all be present in normal or abnormal urine sediment. Large numbers of transitional or squamous epithelial cells in the sediment are not considered pathologic in themselves. However, the presence of increased numbers of these cells having abnormal cytological characteristics, such as nuclear hyperchromatismand irregularities of size and shape is seen in dysplastic and neoplastic states involving the genitourinary tract. Renal epithelial cells may be present in abnormal numbers in diseases which primarily involves the tubular portions of the nephron or in diseases which affect the tubules "secondarily.

12.3.3 **Casts - Hyaline casts** are seen in the normal as well as abnormal urine. In normal individuals, especially after severe strenuous exercise, they may be seen in large numbers. Hyaline casts are present in the urine of patients having intrinsic renal disease or in many disease states that involve the kidneys secondarily. Their presence in disease states is ordinarily accompanied by
proteinuria which may be slight or massive. They may occur in small or large numbers.

12.3.4 **Granular casts** are also present in normal urine sediment, although not in as great a number as hyaline casts. They are seen in large numbers in patients with intrinsic renal disease, especially when accompanied by cellular casts.

12.3.5 **White blood cell casts** when seen in the sediment indicates renal pathology and further clinical investigation of the patient is mandatory.

12.3.6 **Red blood cell casts** are invariably indicative of intrinsic renal disease, and ordinarily pinpoint the glomerulus as the site of injury. If the red cells within the cast degenerate and lyse, the resulting formation is called a blood cast, which is easily recognized by the golden-brown color.

12.3.7 **Epithelial casts** derive their origin from renal epithelial cells that line the nephron. Their presence in the urine implies intrinsic renal disease involving the renal tubules.

12.3.8 **Fatty casts** are casts in which either free fat or oval fat bodies have become incorporated into the cast matrix. They are easily recognized by the "maltese-cross" appearance under polarized light. The presence of fat bodies in the urine sediment usually indicates damage to the tubular portion of the nephron with the concomitant sloughing of the cells into the lumen and their appearance in the urine.

12.3.9 **Waxy casts** are thought to evolve, at least partially, from preexisting granular and cellular casts. Having the highest refractive index of all the casts, they are easily recognized by their irregular margins, smooth surface

and blunt or broken off ends. The presence of many of these casts in the urine portends a poor patient prognosis since it indicates a long renal transit time and therefore depressed renal function.

12.4 Abnormal Crystals found in Urine

There are only a relatively small number of abnormal crystals commonly present in the urine. These crystals are almost always associated with urine's of acid of neutral pH, and are indicative of a patient with a metabolic disease. **Cystine, cholesterol**,
leucine, bilirubin, tyrosine, sulfa, and **hippuric acid** are abnormal crystals.

14. PROCEDURE NOTES

- **FDA Status:** Approved/Cleared
- **Validated Test Modifications:** None

15. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

PH is measured from 5.0 to 9.0 in 0.5 increments.
Specific Gravity is measured from 1.005 to 1.040 in 0.001 increments
*Refer to 9EB package insert for dipstick ranges.
Microscopic particles are measured from 0-1000/uL, 0-182/HPF, or 0–2857/LPF

14.2 Precision

N/A

14.3 Interfering Substances

ANALYTE	CAUSES OF FALSE NEGATIVE RESULTS	CAUSES OF FALSE POSITIVE RESULTS
Glucose	Increased amounts of ascorbic acid.	Presence of oxidizing substances Such as chlorine or hypochlorite, pH <4.0.
Protein	Urine with pH <3.0.	Urine with large amount of Hgb, PH >8.0, contrast medium, disinfectants including quaternary ammonium compounds.
Bilirubin* *Unstable at room temperature and in light	Ascorbic acid, uric acid and nitrites.	Presence of urobilinogen, Etodiac.
Urobilinogen* *Urine with high bilirubin causes development of green color	N/A	Presence of Carbapenem.
Blood	Urine with elevated specific gravity, protein or ascorbic acid	Presence of oxidizing substances such as chlorine or hypochlorite
Ketones	N/A	Drugs such as L-Dopa, BSP, PSP,

ANALYTE	CAUSES OF FALSE NEGATIVE RESULTS	CAUSES OF FALSE POSITIVE RESULTS
		Phenylketone, Cephalosporin, Aldose Reductive antienzyme
Nitrite	Urine with elevated specific gravity, or ascorbic acid.	N/A
Leukocytes	Urine with glucose >500mg/dL, protein >300 mg/dL; pH 5.0 or less; elevated specific gravity	Formaldehyde
pH	N/A	pH may increase in urine older than 72 hours

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

16. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

17. RELATED DOCUMENTS

1. Iris Quality Control Procedure
2. Iris iQ Series Automated Urinalysis System Procedure
3. Iris iQ Series Automated Urinalysis System Maintenance Procedure
4. Iris iQ Series Operator's Manual
5. Current Iris Diagnostics, Aution Sticks 9EB for Urine Chemistry Insert
6. Laboratory QC Program
7. Acetone, Chemistry procedure
8. 3% Sulfosalicylic Acid, Urinalysis procedure

9. Specific Gravity using the Refractometer, Urinalysis procedure
10. Iris Maintenance Logs (AG.F46)
11. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls

18. REFERENCES

1. Iris AX-4280 Operator's Manual, 700-3093 Rev D
2. Iris iQ200 Operator's Manual, 300-4426 Rev B 08/2006
3. Iris Diagnostics, Aution Sticks 9EB for Urine Chemistry Insert, rev Feb 2005
4. Fundamentals of Urine and Body Fluid Analysis, Nancy A. Brunzel, 2nd edition, 2004
5. QDHE 704 v4.0 Routine Urinalysis by Clinitek(R) Atlas(TM) /Sysmex(R) UF100, corporate issue 1/11/2010.
6. CLSI. *Urinalysis; Approved Guidelines - Third Edition*. CLSI document GP16-A3, Wayne, PA: Clinical Laboratory Standards Institute; 2009

19. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
000	9/01/2011		Update owner	L Barrett	C Reidenauer
000	9/01/2011	3.2	Edited "Storage and stability"	A. Chini	C Reidenauer
000	9/01/2011	4	Add reagent information for sticks	A. Chini	C Reidenauer
000	9/01/2011	6.3	Change QC frequency to once per shift	A. Chini	C Reidenauer
000	9/01/2011	6.7	Remove "run Cal. as unknown" statement	A. Chini	C Reidenauer
000	9/01/2011	10.4.2	Edit Yeast, Oval & Trich high end val.	A. Chini	C Reidenauer
000	8/26/2011	10.5	Revise criteria for specific gravity and pH	A. Chini	C Reidenauer
000	9/01/2011	11.1.1	Change color and appearance	A. Chini	C Reidenauer
000	9/01/2011	14.1	Revise AMR for Specific Gravity	A. Chini	C Reidenauer
000	9/01/2011	15	Update to standard content	L Barrett	C Reidenauer
000	9/01/2011	16	Add QC Program, confirmatory test SOPs current Tea information & source	L Barrett A. Chini	C Reidenauer
000	9/01/2011	17	Add items 5-6	L Barrett	C Reidenauer
001	2/24/2012	3.2	Add random urine as acceptable	A. Chini	R SanLuis
001	2/24/2012	10.5	Delete criteria for glucose, add criteria for protein, revised criteria for pH and specific gravity	A. Chini	R SanLuis
002	3/25/2013		Update owner	L. Barrett	R. SanLuis
002	3/25/2013	3.1	Add urine collection kit	L. Barrett	R. SanLuis
002	3/25/2013	10.5	Add process if reagent unavailable	A. Chini	R. SanLuis
003	6/18/2013	10.5	Remove confirmatory test for bilirubin and process if reagent unavailable, add message for positive result	L. Barrett	R. SanLuis

003	6/18/2013	16	Remove Ictotest SOP, add maintenance logs	L. Barrett	R. SanLuis
004	12/2/2013	5.3	Require re-calibration after failures	L. Barrett	R. SanLuis
004	12/2/2013	10.1.2	Add comparison of macro and micro	L. Barrett	R. SanLuis
004	12/2/2013	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L. Barrett	R. SanLuis

20. ADDENDA

Iris Dilution Conversion Chart

Iris Dilution Conversion Chart	
Dilution Barcode Label Number	**Dilution Factor**
1	1:1
2	1:2
3	1:3
4	1:4
5	1:5
6	1:6
7	1:10
8	1:10
9	1:20