**Beaumont Laboratory**
**Royal Oak**

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Related Documents: CH.UA.MT.PR.031 –Bile Ictotest; RC.CH.UA.MT.PR.006-Protein in Urine Qualitative Sulfosalicylic Acid (3% SSA); RC.CH.UA.MT.PR.002 –Examination of Urinary Sediment by Phase Microscopy; RC.CH.UA.MT.PR.003—Urinalysis Protocol for Analyzing Bloody Specimens

# Macroscopic and Microscopic Examination of Urine Using the Iris iQ200 Sprint

SOP#RC.CH.UA.IRIS.PR.001

## Principle

The iQ200 Automated Urinalysis System is an in-vitro diagnostic system composed of the iChem Velocity chemistry module, the iQ200 microscopy module, computers and monitor. The system is used to automate the complete routine urinalysis including chemistry, specific gravity by refractometer, color, clarity and the microscopic analysis of the specimen.

The iChem Velocity instrument performs the chemistry panel and determines the specific gravity, the color and the clarity of a urine specimen. The chemistry panel is performed using iChem Velocity test sticks which are read by a dual wavelength reflectance system. The iChem Velocity test stick consists of a plastic strip containing 10 pads impregnated with chemicals specific for the determination of a particular constituent. Ten analytes are measured: glucose, protein, bilirubin, urobilinogen, pH, blood, ketones, ascorbic acid, nitrite, and leukocytes. A color compensation pad is included on the strip, to compensate for the natural color of urine and its effect on the color reactions of the reactive pads. Test strips are dispensed from the strip provider module and placed on the strip conveyor system. The sample probe mixes the sample, then aspirates an aliquot of urine and dispenses it onto each reagent pad. At defined wavelengths the instrument analyzes the color changes and the intensity of reflected light from the reactive pads. These measurements are used to calculate clinically meaningful results. The specific gravity is obtained using an Atago refractometer and a linear photodiode array. A small flowcell is attached to the SG meter and the raw video from the linear photodiode array inside the device extends the measurement range to 1.060. The color and clarity is determined via a flowcell which is illuminated with white light and a photodetector measures the color of the sample. The sample is then illuminated at a 90 degree angle with a second white light to measure the amplitude of light scattered within the flowcell to determine the clarity.

The microscopic portion of the routine urinalysis is performed on the iQ200 module. It auto-identifies and processes specimens by mixing, sampling and analyzing the data obtained from the sample. A portion of the mixed specimen is aspirated and is sandwiched between enveloping layers of a suspending fluid. This fluid or “lamina” is positioned exactly within the depth of focus and field of view of the objective lens of a microscope that is coupled to a video camera. The iQ Lamina is used to position the formed elements in an orthoscopic orientation that presents asymmetric particles with their largest profile facing the direction of view. The camera captures five hundred frames per sample as each field is illuminated by the flash of a strobe lamp. The pictures are digitized and sent to the instrument processor. Individual particle images are classified into one of 12 categories using size, shape, contrast and texture. The

auto-classified categories are RBCs, WBCs, WBC clumps, hyaline casts, unclassified casts (UNCC), squamous epithelial cells, non-squamous epithelial cells (NSE), bacteria, yeast, crystals (UNCX), mucus, and sperm. Images that do not classify as any of these 12 types are placed in the artifact category. Particle concentration is calculated using the number of images and the volume scanned. User defined criteria are checked and results are sent either directly to the host computer or to the workstation monitor for review and/or editing.

The workstation consists of a computer that is interfaced with the iChem Velocity and the iQ200 modules. At the workstation, results of the chemistry profile and the microscopic are collated, compared to user-defined criteria for auto-release, and stored for review. The user can review results including the images of the formed elements. As needed the user may sub-classify or edit results. After review the results may be sent to the host computer for verification.

## Specimen Collection and Handling

1. PATIENT PREPARATION:

No special preparation required for random urine samples. For clean catch midstream urines, patient must follow Vacutainer Urine Collection Kit package directions.

2. TYPE - URINE:

A. Containers:

1. Sterile containers with no preservatives preferably with a screw top.
2. Vacutainer Urine Collection Kit should be used for clean catch midstream urines.
3. BD Vacutainer Plus 16 x 100 plastic conical tubes with preservative.
4. BD Vacutainer Plus 16 x 100 plastic conical tubes without preservative.

B. Volume:

Optimum volume for non Vacutainer specimens is 12 ml or greater. BD Vacutainer specimens when full hold 8mls. A minimum of 2 ml of urine is required for macroscopic examination, and 3 ml for both macroscopic and microscopic examination.

C. Collection:

A first morning clean catch midstream urine is preferred. However, random urine specimens will be accepted.

D. Storage Instructions:

Urine specimens should be examined within 1 - 2 hours after voiding. If this is not possible, refrigerate urine samples at 2 – 8 C. Urine collected in the BD Vacutainer Plus 16 x 100 plastic conical tubes with preservative is stable for 72 hours at room temperature. Specimens without preservatives are stable for 24 hours refrigerated.

Specimens must be brought to room temperature before testing.

E. Causes for rejection:

1. Improperly identified specimen.
2. Insufficient quantity to perform the test.
3. Specimen received in container with preservatives present other than the BD Vacutainer with preservative.
4. Specimens that are greater than 4 hours old and that have not been refrigerated.
5. If a sample is received that has not been refrigerated and the sample would be difficult to re-collect the following statement is attached:

   "Sample greater than 4 hours old may show a loss of casts or other formed elements. Dipstick testing may yield inaccurate results."

3. SPECIMEN IDENTIFICATION:

All specimens should be labeled with patient's name, identification number, time and date of collection, and initials of collection personnel.

4. HANDLING CONDITIONS:

**CAUTION:** Gloves, facial protection (full face shield or approved safety glasses with a face mask covering the nose and mouth ), and a lab coat must be worn when handling open specimens. A benchtop safety shield may be used in place of face shields or safety glasses and masks.

## Reagents / Supplies / Equipment

1. REAGENTS

A. iChem Velocity

1. iChem Wash Solution - a ready to use wash solution for the iChem Velocity. It is a 7 L container which is stored at room temperature and is stable until the expiration date on the label. There are 2 filters packaged with each case of 2 wash solution bottles. Change the filter when replacing each bottle of wash solution. Open expiration is 3 months, unopened until date on container.
2. iChem Cal Check
   a. Strips-store at RT, protect from light and moisture, stable until expiration date on package. Single use only.
   b. Reagents-store at RT, protect from light and moisture, use within 8 hours of opening, do not use unopened reagents after expiration date on package.
3. iChem Velocity Test Strips – (100 per vial) - are ready to use strips containing pads that have been impregnated with chemicals used to perform the chemical analysis portion of the urinalysis. They are stored on their side at room temperature and are stable unopened until the expiration date on the vial. Loaded on the instrument, the strips expire in 5 days. 1 – 100 strips may be loaded at one time. Load a quantity of strips appropriate to the testing volume to be performed. Replace the desiccant as indicated on the maintenance log. Store any strips remaining in the bottle on its side.
4. IRISpec™ CA/CB/CC-store at 2-8ºC, protect from light, stable for 15 days at 2-8ºC or until expiration date printed on bottles if unopened. Minimize oxygen exposure. Do not shake. DO NOT PIPETTE out of bottles.
   a. CA-consider analyte concentration reduction if solution turns green, would see negative bilirubin.
   b. CB-may become turbid with time, this does not impact performance.
   c. CC

B. iQ 200

1. iQ Cal-store at 2-8ºC, unopened stability until date on bottle, open stability 24 hours.
2. Iris Diluent- store at RT, unopen stability until expiration date on bottle.
3. Iris Cleanser-store at RT, unopen stability until expiration date on bottle.
4. Lamina-store at RT, change filter with every other new container, stable until expiration date on container.
5. Focus-store at 2-8ºC, unopened stability date on bottle, open stability 30 days.
6. Positive QC- store at 2-8ºC, unopened stability date on bottle, open stability 30 days.
7. Negative QC- store at 2-8ºC, unopened stability date on bottle, open stability 30 days.

8. iQ Lamina - is an isotonic fluid used to stabilize the flow in the focal plane of the microscope objective and to hydrodynamically orient the particles so that their broadest face is toward the microscope during microscopic examination of the urine sample. It is stored at room temperature and is stable until the expiration date on the container. There is a filter in each case of 2 iQ Lamina bottles. Change the filter when replacing the first iQ Lamina bottle from a new case, *i.e.,* every second bottle.

2. SUPPLIES:

Sample Tubes - 16 x 100 mm round bottom glass tubes, BD Urine Preservative tubes or Fisher brand 16 x 100 mm round bottom plastic tubes (preferred).
Gauze pads
Gloves
DiH20

3. EQUIPMENT:

None

## Instrumentation

Iris iQ 200 Sprint System

## Calibration

The iChem Velocity and the iQ200 modules have separate calibration materials. Calibrations are performed as a part of the maintenance schedule. Refer to the Iris Maintenance / Calibration Procedure for details

A. iChem Velocity module Calibrations:

1. Quarterly calibration verification is performed using 5 CalCheck test strips.
2. Quarterly the specific gravity is calibrated using 3 Specific Gravity CalCheck Solutions.
3. Quarterly the clarity is calibrated using 3 Clarity CalCheck Solutions.

B. iQ200 Module Calibration:

The iQ200 module is calibrated monthly as part of the iQ200 monthly maintenance schedule.

## Quality Control

1. Controls Used:

A. IRISpec CA, CB, and CC Controls **-** used to monitor Glucose, Protein, Bilirubin, Urobilinogen, pH, Blood, Ketones, Nitrite, Leukocytes, Specific Gravity and Ascorbic Acid. Each set contains three 100 ml bottles of simulated human urine comprised of appropriate chemicals, biological matter, buffer salts, and preservatives. The controls are stored at 2 – 8° C. They are in a liquid, ready to use form. Unopened, the controls are stable until the expiration date on the package. When properly stored at 2° to 8°C and protected from light, the controls expire 15 days after being opened.

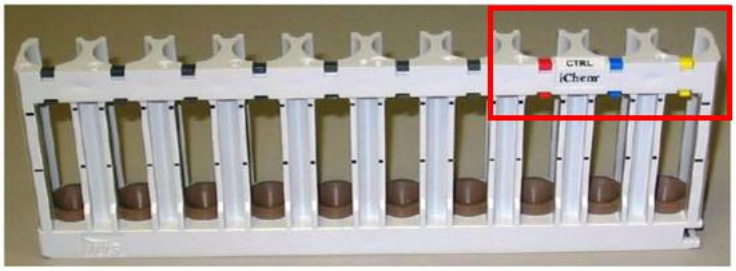
B. iQ Controls / Focus Set – used to monitor particles that are imaged and counted by the instrument. Each 125 ml bottle of iQ Focus and iQ Positive Control is a suspension of fixed human red blood cells in a particulate-free, buffered, isotonically balanced solution. Each 125 ml bottle of iQ Negative Control is a particle-free solution containing no fixed human cells. They are all in a liquid, ready to use form and are kept at 2 - 8° C for long term storage. Unopened, the material is good until the expiration date on the package. Once opened, they can be kept at room temperature. They expire 30 days after being opened.

2. When and how to run controls:

The IRISpec CA, CB, and CC and the iQ Controls / Focus Set controls are to be run at the beginning of each shift. As always, controls are processed exactly as patient specimens.

A. IRISpec CA, CB, and CC Controls:

1. Remove one bottle of each control from the refrigerator. Mark the caps of the bottles and note the date opened. Use the same bottles for fifteen days until expired, then open the next three bottles. Do not shake the bottles.
2. **Pour** 3 mL of each control material into a separate sample tube and cover. Return the bottles to the refrigerator **immediately**. Do not pipette the controls.
3. Place the CA tube in position 8 (RED), the CB tube in position 9 (BLUE), and the CC tube in position 10 (YELLOW) of the iChem Velocity QC rack and allow to come to room temperature, out of strong light, before testing.



4. Place the rack on the right side of the iChem Velocity Sampler touching the edge nearest the operator.
5. If the iChem Velocity is in Standby mode (green light), it will automatically detect the rack and will process the samples.
6. When the testing is complete, the results will be reviewed in the QC Review file on the IRIS computer. Compare the control results to the ranges printed on the CA/CB/CC package insert or check the iQ monitor for "Passed Printed" in QC Review.
7. Document QC performance in Biorad Unity or if Unity is not available file the printouts in the respective Iris #1 and #2 control binders.
8. QC failures must be repeated with documenting of resolution in Unity.
   a. The iRISCELL2000 will not allow sample processing when QC failures exist
   b. No patient testing may begin until the QC is in control.
9. New lot implementation
   a. Run current lot and new lot and record results on the CA/CB/CC Quality Control Log.
      i. Record all pertinent information on log sheet
      ii. Results must be within manufacturers suggested ranges.

B. iQ Controls / Focus Set:

1. Before each use, shake the iQ Focus and iQ Positive Control by holding each bottle upside down. Give each bottle five hard, sharp shakes followed by five gentle inversions.
   NOTE: It is very important that you follow the mixing instructions exactly. The cells must be resuspended from where they settle on the bottom of the bottle and the suspension must be mixed to insure uniformity. The shaking cannot be too vigorous (as in vortexing or "cocktail" type shaking) because the cells will become disrupted.
2. Let the bottles sit about 30 seconds until the air bubbles are gone.
3. DO NOT shake or invert the Negative control. This will introduce air bubbles that may be read as particles by the instrument.
4. Place a bar code label with the name "Focus" on the sample tube containing the iQ Focus. Place a bar code label with the name "Control +" on the iQ Positive Control and a bar code label with the name "Control –" on the iQ Negative Control. Use the barcode labels from the current box and be sure to use the correct barcode label for each product. Do not mix barcodes from different lots.
5. If running controls independently of the iQ System Start Up, prepare the color coded control/maintenance rack as follows:

| Position | | Vol | Contents | Function | Barcode |
|---|---|---|---|---|---|
| 1 | | 3 mL | Iris System Cleanser | Cleans lines | No |
| 2 | | 3 mL | Iris Diluent | Rinses Cleanser from lines | No |
| 3 | | 3 mL | Iris Diluent | Rinses Cleanser from lines | No |
| 4 | | | **Empty** | | |
| 5 | | 6 mL | iQ Focus | Focuses camera | Yes |
| 6 | | 3 mL | iQ Positive Control | Primary lot positive control | Yes |
| 7 | | 3 mL | iQ Negative Control | Primary lot negative control | Yes |

6. Place the rack on the STM in front of the iQ Microscopy Unit and press start.
7. When the instrument is finished processing the rack, the results will be printed on the workstation printer (if this option is checked in QC Release Settings). The Instrument Screen will update the date & time of the “Last QC” and “Last Focus” when each result is acceptable.
   Note: If there is an error in barcode label placement or if an iQ Focus or Control fails, the instrument will post a “failed” message, eject the rack and go off line. The tubes in front of the failed tube may be removed for the repeat, unless the failed tube was the iQ Negative Control. In that case, both the positive and negative controls must be run. Remember to go back on line before processing the repeats.
9. File the printout in the respective IRIS #1 and #2 Control binders.
10. New lot implementation
    a. Run current lot and new lot concurrently utilizing the iQ QC rack.
       i. Place new lot of Positive and Negative QC in spots 8 and 9 respectively, identified by the yellow and blue plastic tabs with appropriate bar codes.
    b. Focus is not run
    c. Print and record all pertinent information on iQ New Lot Assay Verification Log for 5 days prior to implementing a new lot.
    d. Results must be within manufacturers suggested ranges

3. Corrective Actions:

If any control value fails to fall in its appropriate range:

A. Repeat the control.
B. If still out of control, repeat the procedure with fresh control.
C. If still out of control, open a new bottle of control and repeat the procedure.
D. If still out of control, notify the supervisor and do **not** run patient samples until the problem is resolved.

## Procedure

1. Log On:

    A. At the workstation, access the Logon menu by clicking on “Instrument” which is located at the top right of the computer screen.
    B. Click on “Logon” to access the Logon screen.
    C. In the identifier field, each operator will type in their unique tech code.
    D. In the password field, each operator will type in their unique password.
    E. Click “OK” to logon and close the logon screen.

2. Sample Preparation:

    A. Place the patient’s bar code label on a glass or plastic sample tube. Apply it to the tube so that the start of the barcode(not the label edge) is approximately ½” from the top of the tube. This leaves room for the dilution label should it be required.
    B. Transfer 4.0 – 6.0 mls of a well-mixed urine specimen into the bar coded tube.
    C. Put the sample tube in a sample rack so that the bar code is centered between the uprights and facing toward the instrument when the rack is placed correctly on the system.

3. iChem Velocity and/or iQ200 Instrument Operation:

    A. Load urine chemistry strips into the iChem Velocity strip loader as needed.

        i. Rotate the strip loader to the unlock position and then pull out from analyzer.
        ii. Remove and discard old desiccants and replace with a new one.
        iii. Fully extend the strip loader and tilt at a 45 degree angle.
        iv. Load the urine chemistry strips inside the Test Strip Loader (IRIS logo facing the back of loader).
        v. Retract the strip loader and then shake loader lightly so that the chemistry strips are straight.
        vi. Insert the strip loader inside the analyzer. Rotate the strip loader to the lock position so that the chemistry strips drop inside the strip provider module. The strip provider module will tumble to place the strips in the flat position.
        vii. Look through the strip provider module window to verify strips are flat and in proper direction. If some strips are not flat, rotate strip loader 180 degrees and then pull it out from the analyzer for 5 seconds. Re-insert strip loader and rotate to lock position.

    B. A sample may be run on the iChem Velocity instrument alone, the iQ200 instrument alone or on both instruments. If the sample is to run on both instruments or on the iChem Velocity module alone place the sample rack containing specimens on the right side of the iChem Velocity Sampler.

    C. Ensure that the track on the right side of the Sampler is properly placed in the notch in rack base.

D. Place the rack in the forward right corner of the Velocity side, this will block the sensor and automatically start the instrument.

E. The sample rack will be moved along the sample transport tray to the bar code reader.

F. After the bar code is read, the sample aspirator mixes the sample, aspirates an aliquot, and dispenses the sample onto a test strip.

G. When the sample processing is completed, the sample rack will be automatically transferred, via the bridge, to the iQ200 module.

H. After the rack is transferred via the bridge to the iQ200 module, the sample rack will be moved along the iQ200 Sampler to the bar code reader.

I. The bar code reader reads the specimen bar code.

J. If a microscopic is to be done (as defined by our user criteria), the sample aspirator mixes the sample, aspirates an aliquot and performs the microscopic examination. If a microscopic examination is not to be performed, the tube will be passed. A microscopic will be performed if the specimen dipstick is positive for any parameter except urobilinogen.

K. After sample processing is completed, the sample racks can be unloaded from the left side of the iQ200 sampler.

L. If the specimen needs to be run only on the iQ200 module, place the sample rack on the right side of the iQ200 Sampler and press the start button on the lower left side of the instrument.

M. The iQ200 module will process the specimen. When processing is complete the rack may be removed from the left side of the instrument.

4. Reviewing Instrument Test Results:

A. The iChem Velocity:

1. All sample results that do not have any IRIS error codes will be transmitted to the L.I.S., and autoverified.

2. If the sample is abnormal or meets the criteria requiring a microscopic examination, a microscopic will be reflexed in the L.I.S., the test will be performed on the iQ 200, and the iChem Velocity results can be reviewed at the workstation along with the microscopic findings.

B. The iQ200:

1. If a specimen has an abnormal microscopic result it is not auto-released to the host computer. The results are to be reviewed at the workstation monitor. A microscopic, which is normal by our criteria, will be transmitted to the L.I.S., and autoverified.

2. For a quick review of the specimens needing to be edited/reviewed, click on “Work List”. This brings up the Work List screen, which contains all unreleased specimen results.

3. At this screen, a specimen may be deleted or undeleted.

4. The default list order is 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 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.

5. To review a specimen result, double click on it or highlight it then click on “Specimen” at the top of the screen

6. The Results screen for that specimen will be displayed. On the right side are the chemistry results and on the left are the microscopic.

7. The microscopic screen from left to right lists the particles, the concentration of the particles and a graphic representation of the particle concentration.

8. If the concentration is normal the green bar will display. If the concentration is abnormal the red bar will display.

9. If flags are displayed on the right side of the screen, they must be cleared before any particle type detail can be reviewed.

10. Review Samples in AUPI
    a. To review a specimen double click the specimen row or highlight the row and select Specimens to display the results screen.
        i. Flag conditions must be resolved before accessing the Results screen for that specimen. Select the “Review Flag Specimen” and “Accept.
        ii. On the screen, particles to be reviewed will appear as yellow.
    b. Select Edit to review images for the first yellow particle category displayed in the Results screen.
    c. To display the previous or next yellow category, select ◄ Back or Next ►. If there is no previous or next yellow category to review the Results screen is displayed.
        i. To edit a category that is not yellow click on the category in the Results screen.

11. If everything is acceptable click on the “Accept” button at the bottom right of the screen. The results will be transmitted to the L.I.S..

C. Subclassifying or Reclassifying Particles:

1. In the specimen screen click on the button of the first particle to be reviewed.

2. Images of the particles in that category will be displayed. There may be multiple pages of the same particle type, which will be displayed by best focus and largest to smallest particle size.

3. If there are images that are incorrectly classified, they may be reclassified **if the reclassification will change the clinical result:**

    a. Example 1: you look at WBC and find 24 images of WBCs with 2 of artifact. Do not take the time to reclassify the artifact as it will not change the clinical result.

    b. Example 2: you click on BYST and see 6 images of amorphous or artifact. Click on ART and leave the screen. This removes the BYST category from the report.

4. On the right side of the screen, all the categories are listed. To reclassify, click on the particle type that an image is to be classified into and then click on the image(s) in question.

5. This transfers the image to the chosen category. Note: if you click on an image in error, re-click on the space to return the image to the screen.

6. Continue to reclassify by clicking on the category and then clicking on all the images that should go into it. Much of the time all the particles will be sub-classified or reclassified into the same category, in this case, leaving the screen moves all images on the screen into the category indicated by the lit button on the right. This saves the time and effort of clicking on the individual images.

7. When all images are not being moved to the same category, move the fewest images individually, then choose the category for the rest of the images and leave the screen.

    Example: You are reviewing UNCC (unclassified casts). A couple of the images are of Cellular casts (CELL) and the rest are Granular (GRAN). Click on the CELL button. Click on the images that are cellular casts. They will disappear from the screen. Then choose GRAN and leave the screen. All the remaining images are now sub-classified as granular casts.

8. If the remaining images are to remain in the original category, click on that category at the upper left corner. This confirms all the images on the screen in their original category. If you do not, all the images on the screen will go to whichever category is highlighted.

9. When everything has been reviewed and edited, return to the specimen screen by clicking on the Results button at the bottom or by continuing through the screens using the right arrow. Review the results. Microscopic results should correlate with results from the iChem Velocity dipstick. The presence of a large number of casts should correlate with a positive protein. A large number of leukocytes should correlate with a positive leukocyte esterase. If a discrepancy exists, repeat the specimen on the iQ 200 System with a new aliquot. Once all classification is acceptable, click on the “Accept” button at the bottom right of the screen.

| If result is positive: | Then consider these findings in micro: |
|---|---|
| Blood | RBC's |
| Leukocytes Esterase | WBC's & Bacteria |
| Protein | Casts |
| Nitrite | Bacteria |
| High Specific Gravity (> 1.035) | Radiographic (X-RAY) crystals |

10. If the specimen does not require further testing (Ictotest, Qualitative Protein or manual microscopic) then the results will autoverify in the LIS.

5. Dilutions:

A. Conditions requiring dilutions:

1. Cloudy and bloody specimens will need to be diluted before performing testing on the iQ200 module. Slightly cloudy specimens and specimens with high amorphous may also need to be diluted.

2. Specimens exceeding the linearity limits of the iQ 200 will need to be diluted. Our linearity is verified at 0-182 / hpf and 0-2912 / lpf. Results from diluted samples still exceeding these thresholds will be reported in the L.I.S. as > 50 / hpf and >50 / lpf.

3. Urine samples that are sent with <3 ml can also be diluted so there is a sufficient amount of sample for iQ 200 testing.

B. To run and result a grossly bloody specimen:

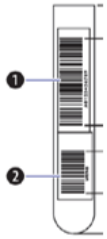
**Grossly bloody specimens cannot be tested using the iChem Velocity module**. Bloody urines may affect the readability of reagent areas on the strips. Color development may be masked or a color reaction may be produced which may be interpreted visually and /or instrumentally as a false positive result.

Please follow procedure RC.CH.UA.MT.PR.003—Urinalysis Protocol for Analyzing Bloody Specimens

C. How to Run Chemistry Testing and a Microscopic Dilution Simultaneously (Results for both portions will appear on the Work List together):

1. If you are following this procedure on a specimen that has already been run on the instrument, delete the accession number from the Work List.

2. Make the appropriate dilution. Place the correct dilution (secondary) barcode below the patient barcode. Do not cover the patient barcode. Make sure there is at least ¼ inch between the last black line of the patient barcode and the first black line of the dilution barcode. The white area of the barcodes may overlap.




3. Set the dilution sample aside.

4. Run the original specimen on the iChem Velocity module. Use the yellow 'Chemistry Only' rack if desired. This will ensure that microscopic is not performed by iQ200.

5. Before the sample is transferred to the iQ200 module, remove it from the iChem Velocity rack. If yellow rack is used, iQ200 will automatically bypass rack for microscopic exam.

6. Place the diluted sample in the rack before the rack reaches the accession area of the iQ200 module. Make sure the barcodes are facing the correct direction.

7. After testing is completed, check above the Chemistry results column. The dilution factor should be displayed under the sample information (Example: 1:2, 1:4. 1:6). If a 1:1 is displayed, the dilution was not processed and the microscopic results were not calculated appropriately on the diluted sample. The displayed results can be multiplied manually and edited in the LIS.

8. Edit the sample as usual and click "Accept" when finished.

D. How to Run a Dilution without Repeating Chemistry Testing:

1. Since the Chemistry portion is not being repeated, you must acknowledge the High Concentration Flag, by clicking on “Accept”.

2. Next, click “Other”, followed by “Separate Micro from Chemistries”, and then click “Accept”.

3. Make the appropriate dilution. Apply the correct dilution barcode to the patient tube below the patient barcode.

4. Place the diluted sample in the rack and place the rack on the iQ200 Sampler. Press the Start button in the top left hand corner on the instrument.

5. Check the iQ200 Results Screen to make sure the dilution was processed. The dilution factor should be displayed under the sample information (1:2, 1:4, 1:6, etc.). If a 1:1 is displayed, the dilution was not processed and the microscopic results were not calculated appropriately on the diluted sample.

6. The microscopic results will “marry” with the Chemistry results in the LIS. This “marriage of results” can only occur one time.

7. After accepting the diluted microscopic results, go the Worklist and delete the original undiluted microscopic results.

E. Heating Urine Samples

In some instances it may be possible to get rid of the cloudiness of the urines by heating the sample

1. Place the urine tube in the 37 C heat bath for 10 minutes.

2. After 10 minutes remove the sample and observe the clarity. If the sample remains turbid you may have to perform a dilution as indicated above.

3. Place the heated sample on the Iris to process as normal.

6. Instrument Error Flags:

A. High Concentration Flag:

Besides visual inspection of a specimen to determine if a dilution is needed, a sample that has this alarm displayed in the Results Screen may need a dilution.

Remedy:

1. Check the Results Screen to find reason such as amorphous for the flag.

2. Clear the flag by selecting “Continue” “Accept”. The microscopic may be evaluated. In the case of amorphous, usually the report can be directly completed by regular evaluation.

3. Visually inspect the specimen and determine the dilution to be performed.

4. Re-run the sample as described in How to Run a Dilution without Repeating Chemistry Testing (See 5C above).

B. Sequential Flags Error:

The iQ200 instrument checks for ongoing problems by monitoring certain flags. If any one of the monitored flags occurs in all three of the last samples run, the alarm is raised and testing is halted. The monitored flags are: FLOW, ILLUMINATION, IMAGE ACQ, LIGHT FLUCTUATION and SHORT SAMPLE. They are all faults that affect the basic analysis process. To avoid wasted specimen and iQ Lamina, the instrument will stop until the problem is solved.

Remedy:

1. Double click the Error Instrument Alarms column and follow the remedy instructions.

2. Check the last three sample results in the Work List to determine the error. If the error is IMAGE ACQ, check the connection on the large cable connecting the Microscopy Module to the Results Processor.

3. In all cases, run a control rack with Iris System Cleanser, Iris Diluent, iQ Focus, iQ Positive and iQ Negative controls.

C. Flow Error

The flow cell may be obstructed. Unless this triggers a Sequential error you may ignore it and simply re-run the specimen.

Remedy:

1. Most samples may simply be re-run but it is good practice to visually inspect the specimen and determine whether a dilution should be performed.

2. Double click the flag to remove it.

3. Re-run the sample either native or diluted as appropriate.

D. Clog Error:

The fluidic system may be obstructed. Unless this triggers a Sequential error you may ignore it and simply re-run the specimen.

Remedy:

1. Most samples may simply be re-run, but it is good practice to visually inspect the specimen and determine whether a dilution should be performed and, if so, what dilution to use.

2. Double click the flag to remove it.

3. Re-run the sample either native or diluted as appropriate.

E. Possible Carryover Error:

A sample previous to the carryover-flagged sample may have interfered with the affected samples results. This flag may occur on the sample following a High Concentration error. The sample that caused the carryover error on the flagged sample; *i.e.,* the specimen that ran before the flagged sample, may need to be diluted. In the following remedy section the High Concentration flagged specimen is referred to as Specimen #1 and the Possible Carryover? flagged specimen is referred to as Specimen #2.

Remedy:

1. Double click the flag to remove it.

2. Open Specimen #2 to the Results screen. Look at the results. If they are all normal, release the specimen as usual. No significant carryover has occurred.

3. If some particle results are abnormal, compare the abnormal results in Specimen #2 to the chemistry results from the previous (High Concentration) specimen, Specimen #1. Example: If Specimen #1 had a 3+ Blood, and the Possible Carryover sample, Specimen #2, shows normal RBCs, there is no carryover. The same logic applies to LEU/WBCs and NIT/Bacteria. If, however, Specimen #2 is high in a particle type that was high in Specimen #1, the *Possible Carryover* specimen, Specimen #2, should be re-run.

4. Particles that may cause a High Concentration with no correlation on the Chemistry result are crystals and yeast. If a Possible Carryover sample shows low abnormal yeast particles, the decision should be delayed until the results are available from Specimen #1 diluted. If the diluted Specimen #1 does not have the yeast that are abnormal in the subsequent sample, the result for Specimen #2 can be released without re-running.

## Calculations

None necessary, unless manual dilutions are performed. If so, please see Section 5 – Dilutions, under procedure.

## Reference Range

| | |
|---|---|
| Specific Gravity | 1.005-1.030 |
| Nitrite | Negative |
| pH | 5.0-8.0 |
| Protein | Negative |
| Glucose | Negative |
| Ketones | Negative |
| Urobilinogen | <2.0 mg/dL |
| Bilirubin | Negative |
| Blood | Negative |
| Leukocyte Esterase | Negative |
| **Microscopic Examination of Sediment** | |
| WBC | 0-2 / hpf |
| RBC | 0-2 / hpf |
| Hyaline Casts | 0-2 / lpf |

## LIS Reporting Results

1. All Results will be reported in the Laboratory Information System.

    i. Template: RUA
    ii. Instrument Menu: XRIQ1 and XRIQ2

2. Report Format:

Macroscopic UA

Color = reported as a color; yellow, dark yellow, red, orange, green, blue, other by the iChem Velocity. The technologist must visually confirm the color.
Clarity = reported quantitatively as Clear, Cloudy, Turbid by the iChem Velocity instrument. Clarity should be confirmed and may be changed to turbid, if necessary.
Specific Gravity = Specific Gravity is reported by refractive index quantitatively with a value to 3 decimal places, in 0.001 increments ranging 1.000 – 1.060.
pH = 5.0, 6.0, 7.0, 8.0, >=9.0
Protein = Negative, 30, 100, >/= 500 mg / dL
Glucose = Negative, 50,150, >/= 500 mg / dL
Ketone = Negative, Trace, 20, >/=80 mg / dL
Bilirubin = Negative, Positive
Blood = Negative, 1+, 2+, 3+
Nitrite = Negative, Positive
Leukocyte Esterase = Negative, 1+, 2+, 3+
Urobilinogen = <2.0, 2.0, >/= 4.0 E.U. / dL

Microscopic UA

RBCs and WBCs are reported as a semi quantitative range listed as follows: 0-2 (Negative), 3-10, 11-24, 25-50, >50 / hpf
Squamous Epithelial Cells are reported as a semi-quantitative range listed as follows: Negative, 1-5, 6-30, 31-50, >50 / lpf.
Hyaline Casts are reported as a semi-quantitative range listed as follows: 0-2 (Negative), 3-5, 6-10, 11-20, >20 / lpf
All pathological casts (granular, cellular, waxy, WBC, RBC) are reported as present
All urine crystals are identified and reported as present.
Non-Squamous Epithelial Cells are classified as Transitional or Renal and reported as a semi-quantitative range as follows: Occasional, 1-5, 6-15, >15 / hpf
Bacteria are reported semi-quantitatively as Negative, 1+, 2+, 3+, 4+.
Budding Yeast and Pseudohyphae are reported as a semi-quantitative range as follows: Rare, Few, Moderate, Many
Sperm is reported as present.
Trichomonas is reported as present. See Confirmatory Testing below.

3. Confirmatory Testing:

Confirmatory testing must be performed under the following conditions:

A. Protein – Perform an SSA on any sample with a positive protein result and a pH > 8. See Procedure RC.CH.UA.MT.PR.006r01 (Protein in Urine Qualitative Sulfosalicylic Acid (3% SSA))

B. Specific Gravity - Confirm specific gravity >1.060 using the Refractometer.

C. Ictotest—Perform an Ictotest on any positive Bilirubin. See Procedure CH.UA.MT.PR.031 (Bile-Ictotest)

D. Manual Microscopic Exam - A manual microscopy may be performed to confirm the identity of any questionable formed element seen when reviewing the iQ screen.

E. Trichomonas must be confirmed manually at the microscope.

F. Previous Sample had Sperm Flag – if sperm is found in a sample where the previous sample had sperm, this flag will be displayed. Ignore the microscopic results and retest the sample.

4. Reflex Testing: A microscopic urinalysis will automatically be ordered for all positive dipstick results EXCEPT urobilinogen.

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**Procedural Notes**

1. In the event the iChem Velocity is not operational, urine chemistry testing may be performed on the Clinitek 500. In the event the iQ 200 is not operational, please refer the procedure Examination of Urinary Sediment by Phase Microscopy (RC.CH.UA.MT.PR.024).

2. While a fresh urine is desired for optimal results, it id especially important for bilirubin and urobilinogen, as these compounds are very unstable when exposed to high temperatures and light.

3. Bloody specimens and substances that cause abnormal urine color, such as drugs containing azo dyes (i.e. Pyridium, Azo Gantrisin, Azo Gantanol) nitrofurantoin (Macrodantin, Furadantin ), and riboflavin, may affect the readability of the reagent areas on urinalysis reagent strips. The color development on the reagent pad may be masked, or a color reaction may be produced on the pad that could be interpreted visually and / or instrumentally as a false positive. Specimens with these problems should have “POSSIBLE COLOR INTERFERENCE” added as a free text comment.

4. Grossly bloody samples –Please follow procedure RC.CH.UA.MT.PR.003 (Urinalysis Protocol for Analyzing Bloody Specimens).

5. Prolonged exposure to room temperature may result in microbial proliferation with the resultant changes in pH.

6. Using a first morning specimen or one that has incubated in the bladder for four hours or more optimizes nitrite tests.

7. Cloudy and turbid specimens must be followed by a diluent tube to help prevent carryover to the next sample. Cloudy or turbid specimens may need to diluted and rerun to get accurate microscopic results. Cloudy or turbid specimens may also be heated.

8. Small particle count data may be helpful in evaluation of bacteria: If you see bacteria (cocci) and do not have a large amount of amorphous present, the All Small Particles (ASP) is a general indicator of small non-amorphous particles, which is primarily bacteria.

9. Care should be taken to correlate the macroscopic and microscopic urinalysis findings. Certain macroscopic results should alert the tech to watch closely for certain microscopic elements. However, it should be noted that the macroscopic urinalysis is a screening test and positive results do not guarantee the finding of a microscopic element, merely the likelihood of finding those cellular elements. The iQ200 will alert the technologist when there is a possible correlation discrepancy. It will be necessary for the technologist performing the microscopic portion of the test to acknowledge the correlation discrepancy prior to accepting the microscopic results in the iQ200.

10. Analytical Measurement Range: pH is measured from 5.0 to 9.0 in 1.0 increments. Specific Gravity is measured from 1.000 to 1.060. Microscopic particles are measured from 0-1000 / uL or 0-180 / hpf. IQ200 linearity limits are 0-182 particles / hpf and 0-2912 particles / lpf.

11. Clinical Significance of Tests Performed on the iChem Velocity:

A. Specific Gravity: - Specific gravity is a measure of the dissolved substances present in the urine. Specific gravity is one measure of the concentrating and diluting ability of the kidneys and hydration status of the patient.

B. pH: - Along with the lungs, the kidneys are the major regulator of acid-base balance. Freshly voided urine has a pH of 5.0 – 6.0. The pH of urine can be controlled by dietary regulation and medication.

C. Protein: - The presence of protein in urine is mostly indicative of renal disease, but its appearance in the urine doesn't always signify renal disease. Although proteinuria may indicate nephrotic syndrome, multiple myeloma, glomerulonephritis, and pre-eclampsia, a transient mild proteinuria can be present after exposure to cold, strenuous exercise, high fever, dehydration, or an acute phase of a severe illness. The strip is primarily sensitive to albumin.

D. Glucose: - Glucosuria is usually indicative of hyperglycemia due to diabetes but can also be seen in patients with other causes for hyperglycemia, in patients with renal tubular dysfunction and in pregnancy due to increased glomerular filtration.

E. Ketones: - Ketonuria appears when there is an increased use of fat instead of carbohydrate for metabolism. Conditions of ketonuria include diabetes mellitus, vomiting, and inadequate intake of carbohydrates due to starvation or weight reduction, or pregnancy.

F. Bilirubin: - The appearance of urinary bilirubin can be a sign of liver disease or extra- or intra-hepatic biliary obstruction.

G. Blood: - A positive reaction for blood may indicate red cells, hemoglobin, or myoglobin present in the urine. Hematuria can be seen due to bleeding as a result of trauma or irritation (renal calculi, glomerulonephritis, tumors, toxic or chemical exposure). Hemoglobinuria occurs when there is lysis of red cells in the urinary tract, intravascular hemolysis or transfusion reactions. A very dilute or extremely alkaline urine can also lyse the cells. Myoglobinuria indicates muscular destruction that may appear in hypothermia, convulsions, and extensive exertions.

12. Limitations and Interferences:

| ICHEM VELOCITY ANALYTE | CAUSES OF FALSE NEGATIVE RESULTS | CAUSES OF FALSE POSITIVE RESULTS |
|---|---|---|
| Glucose | Increased amounts of ascorbic acid.<br>High specific gravity, acidic pH, and gentisic acid. | Presence of oxidizing substances such as chlorine or hypochlorite, pH <4.0. |
| Protein | Urine with pH <3.0.<br>Interference may occur with high specific gravity. | Urine with large amount of Hgb, pH >8.0, contrast medium, disinfectants including quaternary ammonium compounds. |
| Bilirubin | Ascorbic acid, uric acid and nitrites.<br>Prolonged exposure to light. | Presence of urobilinogen, Etodiac. |
| Urobilinogen | Elevated concentrations of formaldehyde and nitrite >10mg/dL.<br>Prolonged exposure to light. | Presence of Carbapenem.<br>Food dyes drugs with red color. |
| Blood | Urine with elevated specific gravity, protein, or ascorbic acid.<br>pH = 5.0 | Presence of oxidizing substances such as chlorine or hypochlorite. |
| Ketones | Drugs levodopa and those containing sulfhydryl groups.<br>Increased concentrations of phenylpyruvic acid | N/A |
| Nitrite | Non-nitrite producing bacteria, low nitrate diet, antibiotic therapy, diuresis. | Food dyes and pyridium. |
| Leukocytes | Urine with glucose >500, protein >300, cephalexin, gentamicin | Formaldehyde. |
| pH | N/A | N/A |
| Ascorbic Acid | N/A | N/A |

13. Origin and Clinical Significance of Casts:

| CAST TYPE | ORIGIN | CLINICAL SIGNIFICANCE |
|---|---|---|
| Hyaline | Tubular secretion of Tamm-Horsfall protein that aggregates into fibrils | Glomerulonephritis, Pyelonephritis Chronic Renal Disease, Congestive Heart Failure, Stress and exercise |
| RBC | RBCs enmeshed in or attached to Tamm-Horsfall protein matrix | Glomerulonephritis, Strenuous exercise |
| WBC | WBCs enmeshed in or attached to Tamm-Horsfall protein matrix | Pyelonephritis |
| EPITHELIAL | Tubular cells remain attached to Tamm-Horsfall protein fibrils | Renal Tubular Disease |
| GRANULAR | Disintegration of WBC casts, Bacteria, Urates, Tubular cell lysosomes, Protein aggregates | Stasis of urine flow, Urinary tract infection, stress and exercise |
| WAXY | Hyaline casts | Stasis of urine flow |
| FATTY | Renal tubular cells, Oval fat bodies | Nephrotic syndrome |
| BROAD | Formation in collecting ducts | Extreme stasis of urine flow |

**References**

1. Iris iChem Velocity Operators Manual, 301-7146 English Rev B 11/2/2011.

2. Iris iQ200 Operators Manual, Rev B 11/2003.

3. Urinalysis and Body Fluids. S.K. Strasinger, 2nd Edition, 1989.

4. A Handbook of Routine Urinalysis, Sister Laurine Graff, J.B. Lippincott Company, 1983.

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