Iris iRICELL System — Technical Procedure 3341
Beckman Coulter, IRIS Diagnostics Division

Principle

Intended Use

This procedure provides instructions for performing a complete urinalysis using the iRICELL.

The iRICELL™ is an in-vitro diagnostic system composed of the Automated Urine Chemistry Analyzer module (the iChemVELOCITY), the iQ200 Series Automated Urine Microscopy Module, results/analysis processor, computer monitor, mouse and keyboard. The iRICELL provides a fully integrated, automated chemical and microscopic analysis of urine.

The iChemVELOCITY module performs the chemistry panel, determines the specific gravity, color and clarity of a urine specimen. The chemistry panel is performed using a test strip, which detects the presence of 10 constituents by wavelength reflectance. Specific gravity is determined by measuring the refractive index. Color is measured by transmitted light and clarity is measured by scattered light.

The iQ200 module performs the microscopic portion of the urinalysis and provides a quantitative or qualitative count of formed elements such as cells, casts, crystals, and organisms. The iQ200 photographs particles as they are passed in front of a digital camera. The images are classified, counted and stored for verification by the user.

The workcell consists of a computer that is interfaced with an approved chemistry analyzer and the iQ Series modules. At the workcell, results of the chemistry profile are stored for review. The user can verify results including the images of the formed elements. As needed, the user may sub-classify or verify results. After verification the results may be sent to the host computer or printed.

iChemVELOCITY Urine Chemistry Module Principle

The iChemVELOCITY is a urine chemistry analyzer that measures the chemical constituents of the urine using iChemVELOCITY strips, which are read by a dual wavelength reflectance system. The iChemVELOCITY strips consist of a plastic strip containing ten (10) pads impregnated with chemicals specific for the determination of a particular constituent. The ten analytes measured are: glucose, protein, bilirubin, urobilinogen, pH, blood, ketones, nitrite, leukocytes esterase, ascorbic acid and a color compensation pad. A color compensation pad is included on the strip to compensate for the natural color of urine and its effect on the color of the reaction pads. Test strips are placed onto a strip conveyor system by a mechanical extractor. The sample probe mixes the sample, aspirates an aliquot of urine and dispenses it onto each reagent pad. At defined wavelengths, the iChemVELOCITY analyzes the color changes and the intensity of reflected light from the reaction pads. These measurements are used to calculate clinically meaningful results.

Test Methodology of iChemVELOCITY Color, Clarity and Specific Gravity

COLOR: The color of the specimen is measured by transmitted light. The colors obtained are colorless, yellow, orange, brown, red, violet, blue, green, and other.

CLARITY: The clarity or turbidity of the urine specimen is measure by passing a beam of light through the sample and measuring how the light is scattered. The amount of scattered light increases as the specimen becomes more turbid. Clarity is reported as clear, slightly turbid, turbid or opaque.

SPECIFIC GRAVITY: The specific gravity is obtained by measuring the refraction angles of light passing through a triangle prism. An LED emits a beam of light through a slit and a lens. The refractive index changes according to the specific gravity of the sample; the higher the specific gravity, the greater is the angle of measurement. The change in the angle of light is reported as the specific gravity. The result is automatically corrected for elevated protein or glucose concentrations as measured by the test strip.

Test Methodology of iChemVELOCITY Test Strip

BILIRUBIN: This test is based on the coupling of bilirubin and diazonium salt in an acidic medium. A pinkish-tan color proportional to bilirubin concentrate is generated.

UROBILINOGEN: This test is based on the coupling reaction of urobilinogen with a stable diazonium salt in buffer. A pink to red color proportional to the urobilinogen concentration is generated.

KETONES: This test is based on Legal's method in which the test pad contains sodium nitroprusside and glycine in an alkaline medium. A violet color proportional to methylketone is generated.

ASCORBIC ACID: This test is based on Tilman's reaction in which the presence of ascorbic acid leads to the decolorization of the pad from grey-blue to orange. Ascorbic acid results are used to evaluate possible interference with other test strip results only, and are not reported.

GLUCOSE: This two-step enzymatic reaction uses glucose oxidase, peroxidase, and chromogen. Glucose oxidase catalyzes the formation of gluconic acid and H_2O2 via the oxidation of glucose. Peroxidase then catalyzes the reaction of H_2O_2 with a chromogen via the oxidation of chromogen colors ranging between green and grey-blue.

PROTEIN: This test is based on the "protein error of indicators" and the green color developed from the presence of protein. This dye-binding is particularly strong with albumin.

BLOOD: This pseudo-enzymatic test contains organic peroxide and a chromogen. The peroxidase effect of hemoglobin and myoglobin causes a color change to green.

pH: This test contains a mixed indicator which assures a marked change in color between pH 5 and pH 9. Colors range from orange through yellow and green to cyan.

NITRITE: This test is based on modified Griess reaction in which nitrite in the urine reacts with amide to form a diazonium compound. The subsequent coupling reaction yields a pink color in the presence of nitrite. Some Gram positive and non nitrite-forming bacteria are not detected in this test.

LEUKOCYTES: This enzymatic test pad contains an indoxyl ester and a diazonium salt. Granulocyte esterases react with indoxyl ester and diazonium salt to generate a violet color.

Clinical Significance of iChemVELOCITY Color, Clarity and Specific Gravity

COLOR: Color variation can indicate the presence of a disease process, metabolic abnormality, an ingested food or drug, or may be due to excessive physical activity or stress.

CLARITY: Substances that cause urine turbidity may be pathologic or non-pathologic.

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 the hydration status of the patient.

Clinical Significance of iChemVELOCITY Test Strip Results

BILIRUBIN: The appearance of urinary bilirubin can be a sign of liver disease or extra- or intra-hepatic bilirary obstruction.

UROBILINOGEN: Normal urine has a small amount of urobilinogen (less than or equal to 2.0 mg/dL). The strip is unable to detect a decreased amount, which may appear in infants, patients on antibiotic therapy, or patients with obstructive liver disease. Increased amounts appear in hemolytic anemias and liver dysfunction.

KETONES: Ketonuria appears when there is an increased use of fat instead of carbohydrate as a source of metabolism. Conditions of ketonuria include diabetes mellitus, vomiting, inadequate intake of carbohydrates due to starvation, weight reduction, or pregnancy.

GLUCOSE: The presence of glucose in the urine (called glycosuria) is caused by hyperglycemia or renal condition. Diabetes mellitus is the most common disease resulting in hyperglycemia. Renal conditions causing dysfunction of tubular reabsorption of glucose occur in may conditions, including pregnancy.

PROTEIN: The presence of protein in urine is often the first indicator of renal disease, but its appearance in the urine doesn't always signify renal disease. Although proteinuria may indicate nephritic syndrome, multiple myelomo, 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.

BLOOD: A positive reaction for blood may indicate red blood 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. Very dilute or extremely alkaline urine can also lyse the cells. Myoglobinuria indicates muscular destruction that may appear in hypothermia, convulsions, and extensive exertions.

pH: Along with the lungs, the kidneys are the major regulators 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.

NITRITE: Bacteria, specifically gram negative organisms, are detected by this nitrite reducing reaction. In order for the reaction to take place there must be adequate dietary nitrates, and the urine must be in the bladder at least four hours for the bacteria to react with nitrate for a positive reaction.

LEUKOCYTES: The presence of white blood cells in the urine is an indicator of inflammation. Lysed and intact white blood cells are detected because both may have produced esterase.

iQ Series Instrument Principle

The microscopic portion of a routine urinalysis is performed on the iQ200 module. The iQ200 auto-identifies and processes specimens by mixing, sampling and analyzing the data obtained from the sample. Approximately 1mL 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 the best orientation so that the particles appear with their largest profile facing the direction of view. The camera captures five hundred frames per sample. The flash of a strobe lamp illuminates each field. 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 (WBCC), hyaline casts, unclassified casts (UNCC), squamous epithelial cells, non-squamous epithelial cells (NSE), bacteria, budding yeast, unclassified crystals (UNCX), mucus, and sperm. Any images that do not classify into any one of these 12 categories are placed in the UNCL category. The particle concentration is calculated using the number of images, a normalization factor and the volume scanned. Results are sent to the workstation monitor for verification.

Workstation Principle

At the workstation monitor, specimen results are verified. During the verification process, individual images are displayed. The operator may manually reclassify images. Unclassified crystals (UNCX), unclassified casts (UNCC), and non-squamous epithelial cells (NSE) must be further sub-classified during the verification process. Once the verification has been completed and [Accept] has been chosen, the results will be sent to the LIS.

Specimen Collection

A clean freshly voided midstream specimen should be collected in a clean container for routine analysis, and a sterile container for UACII requests. Do not add disinfectant or detergent to the specimen.

Infant bag collections are acceptable for children ≤ 2 years of age. Other acceptable specimens include catheterized specimens, and suprapubic, ostomy, and surgical kidney or bladder collections.

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

Grey-topped boric acid tubes are unacceptable for Urinalysis.

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

All specimens must be capped tightly. Specimens that leak are unacceptable for analysis.

Specimens exhibiting gross hematuria cannot be tested undiluted on the iQ Series and not at all on the Velocity. Gross hematuria may cause incorrect results in subsequent samples. Grossly bloody samples must be tested on iChem or visually read strips.

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

Sample Stability and Handling

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

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

Urine collected in BD urinalysis preservative tubes will be accepted up to 48 hours after collection.

All specimens should be handled using the principles of Universal Precautions.

Reagents and Supplies

iChemVELOCITY Consumables

CONSUMMABLE	STORAGE	STABILITY
iChemVELOCITY Strips **800-7212** 100 strips/bottle.	Store at room temperature.	Stable unopened until the expiration date on the bottle. Open vial stability is 5 days.
iChem Wash Solution and Wash Filter **800-7704** 4 (7L) bottles/case	Store at room temperature.	Provided ready-to-use. Stable unopened until the expiration date on the bottle. Open vial stability is 3 months.
IRISpec CA/CB/CC **800-7702** 9 bottles (3 bottles of each control) per box	Refrigerate 2 – 8 °C Bring to room temperature prior to use.	Stable unopened until the expiration date on the bottle. Open vial stability is 15 days.
iChemVELOCITY CalChek Kit Strips **800-7703** 2 vials/kit, I vial required quarterly	Store at room temperature.	Stable unopened until the expiration date on the bottle. CalChek strips are single use only.
iChemVELOCITY CalChek Kit: Reagents **800-7703** 2 sets/kit, 1 set required quarterly	Store at room temperature.	Stable unopened until the expiration date on the bottle. Open tube stability = 8 hours.

iQ200 Consumables

CONSUMMABLE	STORAGE	STABILITY
iQ Lamina[a] **800-3102** 2 (7L) bottles/case	Store at room temperature.	Stable unopened until the expiration date on the bottle. Change filter every 2 bottles.
Iris System Cleanser **800-3203** 4 (475 mL) bottles per case	Store at room temperature.	Stable unopened until the expiration date on the bottle.
Iris Diluent[b] **800-3202** 4 (475 mL) bottles per case	Store at room temperature.	Stable unopened until the expiration date on the bottle.
iQ Calibrator **800-3103** 4 (125 mL) bottles/case	Refrigerate 2 – 8 °C	Stable unopened until the expiration date on the bottle. Open bottle stability = 24 hours
iQ Control/Focus Set[c] **800-3104** 1 bottle each Positive and Negative control; 2 bottles of Focus reagent (125 mL/bottle), Lot-specific Barcodes	Refrigerate 2 – 8 °C	Stable unopened until the expiration date on the bottle. Open bottle stability = 30 days
Dilution Code Labels **800-3211**	N/A	N/A

[a] iQLamina 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 largest profile is toward the microscope during image capture. It is stored at room temperature and is stable until the expiration date on the container. There is a filter in each case of two iQ Lamina bottles.

[b] Iris Diluent is an isotonic, particle free fluid used to dilute cloudy specimens and rinse the instrument after Iris System Cleanser.

[c] iQ Positive, iQ Negative Controls and iQ Focus are ready to use bottles of suspended fixed human red blood cells in a particulate-free, buffered, isotonically balanced solution.

Other Items Required

Sample Racks/Routine Racks are racks that hold specimens for processing through the instrument. They are silk- screened with barcodes representing numbers from 1 – 10 and have the corresponding number on the opposite end. They also have lines across the front representing 3 and 6 mL fill volumes.

The iQ Series Calibrator Rack is a rack used for monthly maintenance on the iQ Series module.

The iQ Series maintenance rack is a rack used for daily maintenance and QC.

The ORANGE iQ Series Rack (orange-colored, numbered 23) is used for maintenance of the Color/Clarity module on the iQVELOCITY during monthly maintenance, and for samples run on the iChemVELOCITY that require a dilution on the iQ200 Automated Microscopy Module. Samples run in the ORANGE rack will not run on the Microscopy Module.

Refer to the *IRIS iQ Operator's Manual* for further information about the racks.

Dilution Barcode Labels are secondary barcodes used when dilutions are required on the iQ200 microscopy module. Any specimen that has been diluted must have a dilution barcode properly placed in order for the iQSeries microscopy module to process results accurately.

Calibration

The iChemVELOCITY and the iQ 200 modules have separate calibration materials. Calibrations are performed as a part of the maintenance schedule.

Refer to Technical Procedure *3350 Iris IRICELL Maintenance Procedure* and/or the *IRIS iQ200 Instructions for Use* for further information.

iChemVELOCITY Module Calibrations

A Reflectance Check is performed quarterly using the iChemVELOCITY CalChek Kit Strips.

A Specific Gravity, Color, and Clarity CalCheck is performed quarterly using iChemVELOCITY CalChek Kit Reagents. Ten tubes are used. Tubes 1-3 are for Specific Gravity, tubes 4-7 are for Color, and tubes 8-10 are for Clarity.

iQ Series Module Calibration

The iQ200 module is calibrated monthly as part of the iQ IRICEL monthly maintenance schedule. Refer to Technical Procedure *3350 Iris IRICELL Maintenance Procedure* and/or the *IRIS iQ200 Instructions for Use* for further information.

Quality Control

Quality control is performed as part of the daily maintenance for both the iChemVELOCITY and the iQ200 microscopy module.

iChem VELOCITY

For the iChemVELOCITY, quality control is performed once/day. The condition and accuracy of the system can be checked by performing control measurements using Urine Controls CA/CB/CC. Run the controls in the color-coded control rack for the iChemVELOCITY.

The controls should be prepared and used in accordance with the package inserts. Last run date and time of the iChemVELOCITY controls appear on the main “Instrument” screen. Quality Control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Control results are stored in the instrument and printed monthly for supervisor review. Document out of control results on the back of the maintenance sheets(s).

Quality Control Material

Control	Storage
IRISpec CA/CB/CC controls	+2°C to +8°C

IRISpec controls are stable until the expiration date printed on the bottles.

Protect from light. Minimize oxygen exposure of control; DO NOT SHAKE BOTTLES. Improper storage and handling may reduce analyte concentration (specifically bilirubin), and may prematurely result in a negative reading. A color change from yellow to green for the CA control may indicate that this process has occurred. The CB control may become turbid with time; this does not impact the performance.

Use poured aliquots within one hour of pouring. Discard aliquots after singe use. Do not pour back into bottle.

Refer to Technical Procedure *3350 Iris IRICELL Maintenance Procedure* and/or the *iChemVELOCITY Instructions for Use* for further information.

iQ200

QC on the iQ200 microscopy module is performed once/day using iQ Focus, iQ Positive Control, and iQ Negative Control (packaged together as the iQ Control/Focus Set.

Refer to Technical Procedure *3350 Iris IRICELL Maintenance Procedure* and/or the *IRIS iQ200 Instructions for Use* for further information.

Procedure

Log On

1. At the workstation, access the Logon menu by clicking on “Instrument” which is located at the top right of the computer screen.
2. Click on “Logon” to access the Logon screen.

3. Use the pull-down menu to select your logon from the list.
4. Type your password in the password field. The password is case sensitive.
5. Click "OK" to logon and close the logon screen.
6. Note that the Operator ID appears in the upper corner of the Instrument screen.

Sample Preparation

1. Place the patient's bar code label on a sample tube if required. Apply it to the tube so that the start of the barcode (not the label edge) is approximately ½ inches from the top of the tube. **If the sample is more than slightly turbid, it should be diluted before being run on the iQ200 module.** Refer to the *Dilutions* section, below.
2. At least 3 mL of urine specimen must be in the in the bar coded tube. Mix the sample well by inverting until it is homogenous. Observe sample for mucous or particulate matter; if present, run on the iChem.
3. 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.

iChemVELOCITY and/or iQ200 Series Instrument Operation on the iRICELL

A sample may be run on the iChemVELOCITY instrument alone, the iQ200 module alone, or on both instruments (Iris iRICELL).

1. If the sample is to run on both instruments or on the iChemVELOCITY module alone, place the sample rack containing specimens on the right side of the iChemVELOCITY Sampler. Ensure that the track on the right side of the Sampler is properly placed in the notch in the rack base.

   If the iChemVELOCITY is in Standby (green light lit), place the rack in the forward right corner to block the sensor. This automatically starts the instrument and the rack will move to the sampling position automatically.
2. The sample rack will be moved along the sample transport tray to the bar code reader.
3. After the bar code is read, the sample aspirator mixes the sample, aspirates an aliquot, analyzes the SG, color, clarity and dispenses the sample onto a test strip.
4. When the sample processing is complete, the sample rack will be automatically transferred, via the bridge, to the iQ Series module.

   If the backup iChemVelocity is in use, samples will need to be manually transferred to the iQ200 microscopy module.
5. If the specimen does not need to be run on the iQ200 module, it may be removed from the instrument at this point.
6. After the rack is transferred via the bridge or manually to the iQ Series module, the sample rack will be moved along the iQ200 Series Sampler to the bar code reader.
7. The bar code reader reads the specimen bar code.
8. If a microscopic will be done (as defined by the seive 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. (NOTE: Samples with barcode read errors on the iChemVELOCITY, or those without barcodes will be tested on the iQ Series module regardless of sieve criteria.)
9. After sample processing is complete, the sample racks can be unloaded from the left side of the iQ200 module sampler.
10. If the specimen needs to be run only on the iQ Series module, place the sample rack on the right side of the iQ Series Sampler (on-load station) and press the start button on the upper left side of the instrument.

11. The iQ Series module will process the specimen. When processing is complete the rack may be unloaded from the left side of the instrument.

Reviewing Instrument Test Results

iChemVELOCITY Instrument

1. The results of samples that do not need a microscopic examination will be transmitted to the host computer.
2. The specimen can then be verified at the LIS.
3. If the sample has abnormal or flagged results that meet the criteria requiring a microscopic examination, the results can be reviewed at the workstation along with the microscopic findings.

iQ200 Instrument

1. If a specimen has a microscopic analysis performed, results will not be transmitted to the host computer. The results are to be reviewed at the workstation monitor.

Auto-release will not be turned on. All specimen results must be reviewed.

2. To review specimens, click on “Work List”.
3. This brings up the Work List screen, which contains all specimen results for review.
4. On this screen, a specimen may be deleted or undeleted.
5. 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 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 it 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. Correlate positive chemistries such as protein, blood, leukocyte esterase and nitrite, with microscopic results. Look for the presence of casts, RBCs, WBCs and bacteria in the microscopic images. See *Urinalysis Correlation Chart*. (Attachment A)
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 user-defined abnormal threshold.
10. If flags are displayed on the right side of the screen, they must be acknowledged before any particle type detail can be reviewed.
11. Click on “Review Flagged Specimen” to remove the flag. Then click on “Accept”.
12. In the specimen screen click on the button of the first particle to be verified.
13. Images of the particles in that classification will be displayed. Note: there may be multiple pages of the same particle type.
14. If the classification of particles is acceptable continue to verify by clicking on the right arrow on the right side near the top of the screen. This takes you to the next set of images. Clicking on the left arrow takes you to the previous screen.
15. Continue verifying until you return to the specimen screen.
16. If everything is acceptable after review, click on the “Accept” button at the bottom right of the screen. The results will be transmitted to the host computer.

17. Verify the results in the LIS following the Laboratory Results Reporting Procedure.

Subclassifying or Reclassifying Particles

1. In the specimen screen click on the button of the first particle to be verified.
2. Images of the particles in that category will be displayed. Note: there may be multiple pages of the same particle type.
3. If there are images that are incorrectly classified, they may be reclassified if the reclassification will affect the clinical result. If you are reclassifying less than half of the images, it will not affect the clinical result.

   Example 1: you look at WBC and find 24 images of WBCs and 2 of artifact. Do not reclassify the artifact as it will not affect the clinical result.

   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 and is appropriate.
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. 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 before you go to the next screen.
5. Continue to reclassify by clicking on the category and then clicking on all the images that should go into it. If images are grouped together they can be moved at one time by using the click and drag feature. 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.
6. 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 10 images in the UNCC (unclassified casts) category. Two 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.
7. 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 confirm the images, all the images on the screen will go to whichever category on the right is highlighted at that time.
8. When everything has been verified, return to the specimen screen by clicking on the Results button at the bottom or by continuing through the screens using the right arrow.
9. Use the *Microscopic Review Sheet* (Attachment B) to note sediment requiring manual microscopic review before Final Verification in LIS. Once all iQ classification and quantification is acceptable, click on the “Accept” button at the bottom right of the screen.
10. Review samples manually, if required, and manually enter reviewed results. You must confirm the presence of sperm, trichomonas, yeast, fat globules, oval fat bodies and fatty casts, and identify cell types in cellular casts using a microscope and phase or polarizing filters.
11. Verify the results in the LIS following the Laboratory’s Results Reporting Procedure.

Putting a Sample on Hold

The iQ Series software allows the user to start verifying a sample and then save all the changes that have been made without accepting the results. This is accomplished by the use of the “HOLD” button which is part of the verification screen. The “HOLD” button only becomes active after something in the specimen has been changed.

NOTE: **Do not use the Hold button to make changes after reviewing the results manually.** Manually recorded and entered results must be reviewed by another CLS in the LIS for accuracy of manually entered results and clinical correlation in accordance with Technical Procedure 3005, Reporting and Review of Results.

1. In the specimen screen click on the button of the first particle to be verified.
2. Images of the particles in that category will be displayed. Note: there may be multiple pages of the same particle type.
3. Verify the sample as usual.
4. If there is a need to leave this specimen before the verification process is complete, click on the Results button.
5. Click on the "Hold" button. This will save the changes that have been made and return this specimen to the Worklist.
6. When ready to further verify this specimen, highlight it on the Worklist and click on Specimen.
7. All changes previously made will still be present. Continue to verify the specimen and click on Accept when complete.

Manual Orders

The iQ Series allows the user to use Manual Orders in situations where a barcode is not available. The patient identification can be entered and the rack the samples will be placed in selected. From the Specimen Screen, click on Manual Orders.

1. Click on the button that corresponds to the rack the specimens will be placed in.
2. In the first field enter the patient identification information.
3. The second field (Fluid Type) can if left blank if the specimen is a urine.
4. In the third field enter the dilution, if needed. Diluted samples cannot be run on the iChemVELOCITY.
5. The fourth field will automatically change to "**RUN**".
6. Continue to enter manual orders until up to 10 specimens have been entered.
7. Continue to select racks and enter patient information until all have been entered.
8. Click on [**OK**] or [**OK & Print**] to return to the instrument screen.
9. Load specified racks with specimens.
10. Run racks on Iris iRICEL or iChemVELOCITY only as required.
11. Review specimens as above (*Reviewing Instrument Test Results, iQ200 Instrument*).

Dilutions

Diluted samples cannot be run on the iChemVELOCITY.

Cloudy ,bloody, or mucoid specimens will need to be diluted before testing on the iQ Series module. Samples with particle counts above the AMR (>182 particles/HPF) must be diluted to ensure accurate results. Some result flags require dilution. Additionally, low-volume samples (< 3 mL) are diluted to provide sufficient volume for analysis on the iQ200 microscopic module. (Refer to Table #1, below.) Samples containing amorphous sediment can be diluted if the chemistry results do not match the microscopic results, or if there is concern that the amorphous particles are covering other cells.

Refer to Table #2 for the user-defined dilutions available and the volumes of urine and Sample Diluent to be used.

NOTE: Grossly bloody or pyuric specimens cannot be tested using the iChemVELOCITY module. Significant carryover will occur in subsequent specimen(s). Grossly cellular specimens must be tested using the iChem100 instrument. The specimen can then be diluted and run on the iQ Series to obtain the microscopy result. Do not dilute samples more than x101. For grossly bloody or pyuric samples which cannot be accurately evaluated even with a dilution, enter **N** (packed) in the appropriate field, and append the mnemonic **UHW** ("Unable to perform microscopic due to presence of very large numbers of white blood

cells") or **UHR** ("Unable to perform microscopic due to presence of very large numbers of red blood cells").

All dilutions are made with Iris Diluent. Use the mnemonic **GMBD** ("Microscopic performed by dilution") in the Specimen Comment field when samples are diluted.

Table #1: USING DILUTIONS TO TEST LOW-VOLUME CLEAR OR SLIGHTLY TURBID SAMPLES

METHOD	< 1.0 mL	1.0 – 1.9 mL	2.0 – 2.9 mL	≥ 3.0 mL
iChem100	YES	YES	NO	NO
iChemVELOCITY	NO	NO	YES	YES
iQ200, Straight	NO	NO	NO	YES
iQ200, Diluted X2	NO	YES*	YES*	NO
Manual Microscopic	YES	NO	NO	NO

YES=Use this method to perform testing
NO=Do NOT use this method to perform testing.
*Enter mnemonic **GMBD** in Specimen Comment field

Example 1: Two (2) mL of urine is received in the appropriate collection tube. Run the sample undiluted on the iChemVELOCITY, remove the sample and dilute sample x2 before running on the iQ200 automated microscopy module.
Example 2: 0.5 mL of urine is received in the appropriate collection tube. Run the sample with the iChem100 and perform a manual microscopic on the remaining urine.
Example 3: Four (4) mL of urine is received in the appropriate collection tube. Run the sample on the iChemVELOCITY and the iQ200 automated microscopy module.

Table #2: MAKING iQ200 DILUTIONS

LABEL NUMBER	DILUTION	URINE VOLUME	IRIS DILUENT VOLUME
1	x2	1 mL	1 mL
2	x3	1 mL	2 mL
3	x6	250 uL	1.25 mL
4	x11	250 uL	2.5 mL
5	x21	100 uL	2.0 mL
6	x51	100 uL	5.0 mL
7	x101*	50 uL	5.0 mL

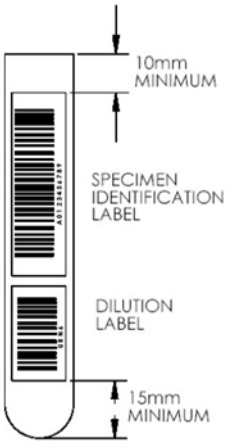
* Do not dilute samples greater than x101.

How to Run Chemistry Testing and a Microscopic Dilution Simultaneously

Chemistry specimens must be run using the ORANGE dilution rack. This rack is specially identified to run on the iChemVELOCITY, but will not processed by the iQ200 Series analyzer. This rack will skip the iQ200 sampling station and be transferred automatically to the unloading station for retrieval.

1. Place identical patient barcode labels onto to two specimen tubes.
2. Pour at least 3 mL of urine into one of the labelled tubes.
3. Place this (undiluted) sample in to the ORANGE dilution rack (number 23).
4. Load the dilution rack onto the chemistry sampler for processing.
5. Make the appropriate dilution using Iris Diluent. (Refer to Table #2, above.) Diluted samples should appear clear or only very slightly turbid. Particle count on diluted samples must not exceed the AMR.

6. 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. (See image at right.)



7. Place the diluted sample into a regular rack (other than the dilution rack number 23). Verify that the barcodes are oriented correctly. Load the dilution tube on the iQ200 Series module, and press Start.
8. After testing is complete, check above the Chemistry results column. The correct dilution factor must be displayed under the sample information (1:2, 1:3, 1:6, etc.). If a 1:1 is displayed, the dilution was not recognized and the microscopic results were not calculated appropriately on the diluted sample. The displayed results can be multiplied manually and edited in the LIS. A comment should be entered into the Edit Comment section documenting the manual change in concentration.
9. Review specimens as above (*Reviewing Instrument Test Results, iQ200 Instrument*).

How to Run a Dilution Without Repeating Chemistry Testing

Chemistry and Microscopic results will be released separately and will not appear on the Work List together.

1. Because the Chemistry portion is not being repeated, you will need to accept the Chemistry results on the original sample. You may do this in either of two ways:

   From the specimen results review screen, Select “Other” on the bottom right. Select “All ART” to move all the particles in the microscopic portion to the Artifact category.

   Or, select “Separate chemistry and microscopic results”. Delete the separated microscopic results.

   Click “Accept” on the iQ Series Results Screen.

   This will allow Chemistry results to be sent to the LIS without the risk of reporting an incorrect microscopic.
2. Make the appropriate dilution. Apply the correct dilution barcode to the patient tube below the patient barcode.
3. Place the diluted sample in the rack and place the rack on the iQ200 Series Sampler. Press the Start button in the top left hand corner on the instrument.
4. The CHEM N/A alarm will be displayed. Check the iQ200 Series Results Screen to ensure the dilution was processed. The dilution factor should be displayed under the sample information (1:2, 1:3, 1:11, etc.).
5. If a 1:1 is displayed, the dilution was not recognized and the microscopic results were not calculated appropriately on the diluted sample. The displayed results can be multiplied manually and edited in the LIS. A comment should be entered into the “Edit Comment” section documenting the manual change in concentration.
6. The microscopic results will consolidate with the Chemistry results in the LIS.

Flags/Alarms Obtained During Analysis

The iQ200 Series instrument monitors certain errors and will give an alarm if any one of the monitored flags occurs in the last three samples run (**SEQUENTIAL FLAGS)**. When an alarm is raised because of a Sequential Flag, testing is halted. The monitored flags are: **FLOW**, **ILLUMINATION**, **IMAGE ACQ**, **LIGHT FLUCTUATION**, and **SHORT SAMPLE**. They are all errors that affect the basic analysis process and may produce erroneous patient results. To avoid wasted specimen and iQ Lamina, the instrument will stop until the problem is solved.

1. Select “Delete Flagged Specimen” to remove the results from the Work List.
2. Check the last three sample results in the Work List to determine if there is a Sequential error.

3. If the errors are sequential, resolve the problem prior to rerunning the sample.

   Example 1: If the observed error is **IMAGE ACQ**, check the connection on the large cable connecting the iQ Series Camera to the Results Processor.

   Example 2: If the observed error is **FLOW**, the sample may be mucoid, and/or the flowcell may require cleaning or is obstructed. Run the iQ Control rack with Iris System Cleanser, Iris Diluent, iQ Focus, iQ Positive and iQ Negative controls. (Consult the Iris iQ User Manual on the S-drive for maintenance and troubleshooting guidelines.)
   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, the appropriate dilution to make.

Limitations and Interferences

iChemVELOCITY

ANALYTE	CAUSES OF FALSE NEGATIVE RESULTS	CAUSES OF FALSE POSITIVE RESULTS
BILIRUBIN	Elevated concentrations of nitrite may inhibit the reaction. Bilirubin is light sensitive and prolonged exposure of urine specimens to light may result in diminished or false negative values.	Some urine specimens may contain impurities such as food dyes and therapeutic pigments that produce a yellowish or reddish discoloration of the test pad that may lead to the interference. Elevated Urobilinogen concentrations may slightly enhance the response to this test pad.
UROBILINOGEN	This test is inhibited by elevated concentrations of formaldehyde and nitrite ≥10 mg/dl. Prolonged exposure to light may lead to diminished or false negative values.	Food dyes and medications that have an intrinsic red color in acidic medium such as red beets, azo dyes, phenazopyridine and *p*-aminobenzoic acid may produce false positive results.
KETONES	Elevated concentrations of phenylpyruvic acid may interfere with the test pad and produce a variety of colors. Phthaleins and anthraquinone derivates exhibit a red color in alkaline medium and this may mask the response. Large amounts of levodopa and medications containing sulfhydryl groups may produce atypical color reactions.	N/A
ASCORBIC ACID	No interferences reported.	No interferences reported.
GLUCOSE	Ascorbic acid concentrations of up to 50 mg/dL did not interfere with glucose assay test results (no false negative results). Acetoacetic Acid concentrations of up to 200 mg/dL did not interfere with glucose assay test results (no false negative results). High specific gravity, acidic pH values and gentisic acid may inhibit color formation.	Cleaning agents such as hypochlorite and peroxide may lead to false positive results.
PROTEIN	Food dyes such as red beets and therapeutic pigments such as methylene blue and pyridium may mask the coloration of the test pad. Interference may occur with high specific gravity. Interference may also occur with disinfectants, wetting agents and blood substitutes (quaternary ammonium compounds, polyvinylpyrolidone, chlorohexidine).	Highly buffered alkaline urine may produce false positive results.

ANALYTE	CAUSES OF FALSE NEGATIVE RESULTS	CAUSES OF FALSE POSITIVE RESULTS
BLOOD	Reducing agents such as ascorbic acid, uric acid, glutathione and gentisic acid may cause false negative results. Samples with a pH of 5 may interfere with this test. High concentrations of nitrite can delay the reaction. Ascorbic acid of ≥10 mg/dl can cause a negative blood reaction.	Preservatives (formalin) and cleaning agents such as hypochlorite may result in false positives.
pH	No interferences reported.	No interferences reported.
NITRITE	A negative response in the presence of bacteriuria may be caused by the following: non-nitrite producing microorganisms, low nitrate diet, antibiotic therapy, strong diuresis, or insufficient urinary retention time in the bladder.	Food dyes and therapeutic pigments such as red beets and pyridium may cause false positive responses.
LEUKOCYTES	High concentrations of protein, glucose, cephalexin and gentamicin may diminish the color response. The test can be negative in the presence of visible leukocytes if they have not lysed and/or are not granulocytes.	False positive results may occur in the presence of preservatives such as formaldehyde and formalin. Test results may be positive in the absence of observable cells if the granulocytes have lysed.
SPECIFIC GRAVITY	N/A-measured by Refractometer	N/A-measured by Refractometer
COLOR	N/A –measured by scattered light	N/A –measured by scattered light

Standard Reporting Format - Chemistry

VOLUME	Report number of milliliters if < 8 mL urine specimen submitted AND manual microscopic analysis is indicated.
COLOR	None, Yellow, Orange/Amber, Brown, Red, Green, and Other
CLARITY	Clear, Slightly Turbid, Turbid and Opaque
SPECIFIC GRAVITY	Refractive index reported quantitatively with a value to 3 decimal places, ranging from 1.000 to1.050 in 0.001 increments
pH	Quantitative pH units
GLUCOSE, PROTEIN, KETONES, UROBILINOGEN	Semi-Quantitatively in mg/dL
BILIRUBIN, BLOOD LEUKOCYTES	Semi-Quantitatively as Negative, Trace, Small, Moderate or Large
NITRATE	Semi-Quantitatively as Negative or Positive

Standard Reporting Format – Automated and Manual Microscopic

A microscopic analysis is only performed when the following sieve criteria are met.

Color - other than Yellow or none

Clarity – other than clear

Leukocyte Esterase ≥ trace

Nitrite - positive

Blood ≥ trace

Protein ≥ trace

WBC/HPF	AUTOMATED: 0-182 negative, 0-5, 6-25, 26-100, >100, packed append canned text "**UHW**" ("Unable to perform microscopic due to presence of very large numbers of white blood cells.") **NOTE:** Report clumping
RBC/HPF	AUTOMATED: 0-182 negative, 0-5, 6-25, 26-100, >100, packed append canned text "**UHR**" ("Unable to perform microscopic due to presence of very large numbers of red blood cells.") **NOTE:** Report clumping and dysmorphic RBCs
BACTERIA/HPF	few = approximately 1-10/HPF moderate = approximately 11-100/HPF many = approximately greater than 100/HPF **NOTE:** Each chain or cluster should be counted as one.
CRYSTALS/HPF	few, moderate, many; LEUCINE, CYSTINE, TYROSINE: positive **NOTE:** Identify using references and biochemical tests as necessary. If unable to identify, crystals should be reported as CRYSTALS, OTHER, and a description that includes shape, solubility, birefringence, other identifying characteristics included as a comment.
EPITHELIAL CELLS/HPF	AUTOMATED: 0-182 negative, 0-5, 6-25, 26-100, >100, packed Identify as squamous, transitional, or renal tubular cells.
MUCUS/HPF	few, moderate, many
CASTS/LPF	AUTOMATED: 0-2900 negative, 0-5, 6-25, 26-100, >100, packed Identify casts by type seen. **NOTE:** Suspected cellular casts must be classified by cell type. If unable to do so, report number of CELLULAR CASTS/LPF and add the LIS mnemonic "**NODIST**" – "Unable to distinguish cell type." as a comment. Mixed cell casts must have cell types noted in a result comment.
SPERM, TRICHOMONAS, AMORPHOUS SEDIMENT	Report as PRESENT if seen.
YEAST	Report as PRESENT if seen. Report as BUDDING and/or HYPHAE yeast.
OTHER	Comment on the presence of any other elements found.

For manual microscopic result entry see Attachment C.

Confirmatory Test

Sulfosalicylic Acid Test is performed on urines that have both a positive protein result and a pH ≥ 8.0. See Technical Procedure 3356, *Sulfosalicylic Acid – Urine Protein*.

Analytical Measurement Range

pH is measured from 5.0 to 9.0 in 0.5 increments.

Specific Gravity is measured from 1.000 to 1.050 in 0.001 increments, or > 1.050.

Microscopic particles are measured by the IRIS iQ200 from **0-182**/HPF, or **0–2857**/LPF

Reference Intervals

Chemistry Results

Color	None - Yellow
Clarity	Clear – Slightly Turbid
Specific Gravity	1.002 – 1.030
pH	4.8 – 7.8
Glucose	Negative
Ketones	Negative
Bilirubin	Negative
Urobilinogen	Negative – 2.0 mg/dL
Protein, Qualitative	Negative – Trace
Blood	Negative
Leukocyte Esterase	Negative
Nitrite	Negative

Microscopic Results

WBC	0-5/HPF	CRYSTALS	
WBC Clumps	Absent	Amorphous	Absent/Present
RBC	0-5/HPF	Calcium Oxalate	Negative-Few/HPF
RBC Clumps	Absent	Uric Acid	Negative-Few/HPF
Dysmorphic RBC	Absent	Triple Phosphate	Negative-Few/HPF
Bacteria	Negative-Few/HPF	Calcium carbonate	Negative-Few/HPF
Squamous Epithelial Cells	0-5/HPF	Calcium Phosphate	Negative-Few/HPF
Transitional Epithelial Cells	0-5/HPF	Leucine	Negative/HPF
Renal Epithelial Cells	0-5/HPF	Cystine	Negative/HPF
Mucus	Negative-Few/LPF	Tyrosine	Negative/HPF
CASTS			
Hyaline Casts	0-5/LPF	Budding Yeast	Absent
Granular Casts	Negative/LPF	Hyphae Yeast	Absent
WBC Casts	Negative/LPF	Oval Fat Bodies	Negative/HPF
RBC Cast	Negative/LPF	*Trichomonas sp.*	Absent
Epithelial Cell Casts	Negative/LPF	Sperm	Absent
Cellular Casts (ID if possible)	Negative/LPF		
Mixed Cell Casts (ID cells)	Negative/LPF		
Waxy Casts	Negative/LPF		
Fatty Casts	Negative/LPF		

References

1. iChemVELOCITY Operators Manual
2. Iris iQ200 Operators Manual
3. iChemVELOCITY urine chemistry strip insert
4. Fundamentals of Urine and Body Fluid Analysis, Nancy A. Brunzel, 2nd edition, 2004.
5. Urinalysis and Body Fluids, Susan King Strasinger, 5th edition, 2008.
6. GP16-A2: CLSI Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline-Second Edition

Prepared By	Date Adopted	Supersedes Procedure #
Kathy Dagang	04/01/2018	3340

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
04/01/2018	New			