APPENDIX A

Urinalysis Automation

Studies have shown that the biggest variable in urinalysis testing is the conscientiousness of the laboratory personnel in their interpretations of the color reactions. This subjectivity associated with visual discrimination among colors has been alleviated by the development of automated reagent strip readers that use a spectrophotometric measurement of light reflection termed reflectance photometry. Reflectance photometry uses the principle that light reflection from the test pads decreases in proportion to the intensity of color produced by the concentration of the test substance. A monochromatic light source is directed toward the reagent pads by placing a filter between the light source and the reflective surface of the pad or by using a light-emitting diode (**LED**) to provide the specific wavelength needed for each test pad color reaction. The light is reflected to a photodetector and an analog/digital converter. The instruments compare the amount of light reflection with that of known concentrations and display or print concentration units or transmit data to a laboratory information system (**LIS**). The ultimate goal of automation is to improve reproducibility and color discrimination while increasing productivity and standardization for reporting urinalysis results.

Several automated instruments are currently available to standardize sample processing, analyze test strips, and report results with consistent quality free of visual discrimination. Additional features include on-line computer capability; bar coding; manual entry of color, clarity, and microscopic results to be included on the printed report; flagging of abnormal results, storing of patient and control results; and minimal calibration, cleaning, and maintenance.

Automated instruments in urinalysis include individual strip readers, semiautomated analyzers, fully automated chemistry analyzers, and the complete urinalysis workstations. Semiautomated instruments are still dependent on an operator for specimen mixing, test strip dipping, and inputting physical and microscopic results. The fully automated chemistry analyzers add urine to the reagent strip, and the workstations have a "walk-away" capability for a complete urinalysis.

The major automated chemistry urine analyzers are the Clinitek 50 and 100 strip readers, semiautomated Clinitek 200/200+, Clinitek 500, and the fully automated Clinitek Atlas (Bayer Diagnostics, Elkhart, IN/Dublin, Ireland) as well as the semiautomated Chemstrip 101, Chemstrip Criterion II, Chemstrip Urine Analyzer, and the fully automated Chemstrip Super Automated Urine Analyzer (Roche-Boehringer Mannheim Diagnostics, Indianapolis, IN), and the semiautomated Rapimat II/T (Behring Diagnostics Inc. Somerville, NJ). A variety of International Remote Imaging Systems workstations are available (Chatsworth, CA). All instruments use reflectance photometry to determine each analyte concentration. The automated urinalysis instruments currently available are listed in Table A–1.

The Bayer Clinitek 50 and 100 strip readers are well suited for small volume laboratories and physician's offices (Figure A–1) and meet the Clinical Laboratory Improvement Amendments (CLIA)-waived standards. Reagent strips are manually dipped and placed on the strip reader, and results are displayed or printed. Patient identification and specimen color and clarity may be manually entered, abnormal results may be flagged, up to 100 test results may be stored in memory, and computer interfacing is available. An additional feature of these strip readers is their ability to provide automated reading of Bayer microalbumin/creatinine and human chorionic gonadotrophin strips.

The Clinitek 200+ is designed for medium-volume to large-volume urine laboratories with a high specimen output of one strip every 10 seconds. Multistix reagent tests strips are used, and the instrument has the ability to report semiquantitative (mg/dL) results or plus (+) and Système International units. All positive results are flagged to indicate a patient sample that requires additional confirmation testing or microscopic evaluation. The operator manually enters urine color and clarity observations and the patient identification number from a keyboard or an optional barcode reader. Bidirectional interface is available to upload and download patient identification information with the host computer. The reflectometer is calibrated daily and

TABLE A–1 **Urinalysis Automation**

Equipment	Manufacturer
Semiautomated Chemistry Instruments	
Clinitek 200/200+	Bayer Diagnostics
Clinitek 500	Bayer Diagnostics
Chemstrip 101	Roche-Boehringer Mannheim Diagnostics
Chemstrip Criterion II	Roche-Boehringer Mannheim Diagnostics
Chemstrip Urine Analyzer	Roche-Boehringer Mannheim Diagnostics
Rapimat II/T	Behring Diagnostics Inc.
Fully Automated Chemistry Instruments	
Clinitek Atlas	Bayer Diagnostics
Chemstrip Super Automated Urine Analyzer	Roche-Boehringer Mannheim Diagnostics
Automated Microscopy	
UF-100 Urine Cell Analyzer	International Remote Imaging Systems
Workstations	
Yellow IRIS	International Remote Imaging Systems
Model 300 Urinalysis Workstation	International Remote Imaging Systems
Model 500 Urinalysis Workstation	International Remote Imaging Systems
Model 939UDx Urine Pathology System	International Remote Imaging Systems

maintenance is required each day for all areas in contact with urine test strips.

The Clinitek 500 is the most recent Bayer benchtop analyzer (Figure A–2). It features an advanced read head design that determines urine color automatically and nonhemolyzed trace blood detection. Specific gravity is measured in 0.005 increments and pH is measured in 0.5 increments. The Clinitek 500 contains a bar-code reader that provides rapid entry of sample identification, color, and clarity values. This instrument includes automatic reagent strip detection, automatic calibration, confirmatory and microscopic sieve functions to flag results for quick review, and a user-friendly interface with a touch-screen display to provide a high-volume throughput of 500 strips per hour. Parameters for each analyte are set at installation to meet laboratory-specific protocol. Memory stores 500 patient results and 200 control results. Results are easily edited and reported by internal storing, transferring to the computer, or by printing. Calibration is automatic and maintenance is minimal.

FIGURE A–1 Clinitek 50 Urine Chemistry Analyzer. (Courtesy of Bayer Diagnostics, Elkhart, IN.)

The fully automated Clinitek Atlas, which is designed for a high-volume urinalysis laboratory, performs 12 tests automatically including urine chemistries using reflectance colorimetry, specific gravity using a fiberoptic refractive index method, color, and clarity (Figure A–3). This instrument offers a "walk-away" capability for urine chemistry testing producing a throughput of 225 samples per hour. Regular urine sample tubes are used and can be placed in a circular 50-position tray or in up to 20 (10-position) linear racks that are compatible with the IRIS/Symex UF-100 Urine Cell Analyzer. Two milliliters of urine are required. STATS may be performed at any time. A reagent pack containing a roll of 490 dry chemistry reagents on a continuous roll minimizes reagent handling. An exact volume of urine sample is pipetted onto the reagent test pad. Reagent pads advance automatically to the reflectance photometer to measure the color change of each reagent pad. Reagent pads then advance automatically to the disposal area. The Atlas uses bar-code sample identification and allows abnormal ranges to be selected for identification and flagging of samples requiring microscopic examination or confirmatory testing. One thousand patient results and 200 control results and calibrations are stored for visual display, printout, or transmission to a laboratory computer system. Standardized controls are run as set by laboratory protocol and a 24-hour within-lot calibration is performed.

Roche-Boehringer Mannheim Diagnostics Chemstrip 101 compact urine analyzer provides simple test strip evaluation. It is designed for small laboratories or physician office laboratories and has a 50-test per hour throughput. Test strips are dipped and placed in a tray, and the start button initiates testing. Incubation timing, analyte mea-

FIGURE A–2 Clinitek 500 Urine Chemistry Analyzer. (Courtesy of Bayer Diagnostics, Dublin, Ireland.)

surement by reflectance photometry, result calculation, and printout are automatic. Software options are available. One hundred patient samples are held in memory, and minimal calibration or maintenance is required. All Roche-Boehringer Mannheim instruments have urine compensation color pads.

The Chemstrip Criterion II, a semiautomated urine test strip analyzer with upgraded software capability, is convenient for mid-sized laboratories. This instrument can measure 100 samples per hour, including urine color, whereas clarity is entered manually. The strip is dipped into the sample and placed on the tray. Test transport, measurement, and disposal are automatic. Individual programming of result ranges, grading, and units is available. Instrument cleaning is minimal and is performed once a day; calibration is required twice a month.

The Chemstrip Urine Analyzer meets the needs of a large urinalysis laboratory by processing 300 strips per hour. It analyzes test strips placed on a transport tray, allows full sample identification, correlates and manages sediment microscopy data, and prints out or transmits results to the laboratory computer system. Bar coding enables microscopic sediment results to be entered and linked to test strip findings and patient data for correlation and patient assessment. Default settings are used to monitor the quality of results. Minimal maintenance is required because the instrument contains a cleaning cycle function and disposable transport and waste trays. Calibration is performed every 2 weeks and printed for a permanent report.

The Chemstrip Super Automated Urine Analyzer is a fully automated "walk-away" urine chemistry instrument with the ability to process 300 samples per hour, thereby meeting the needs of a large urinalysis laboratory. Urine specimens are loaded in a 60-position carousel with 55 routine positions and 5 stat positions. Sample volumes are detected and adjusted, and automatically mixed. A sorter mechanism supplies a single test strip from the sorter drum to a sorter position. A gripping mechanism grasps the test strip and dips it into the urine specimen tube. A sensor attached to a mixing rod determines the volume of urine. The dipping mechanism lifts the test strip out of the sample tube while removing excess urine by dragging the strip along the inside of the specimen tube. The dipping mechanism then transfers the test strip to the reflectance photometer position. A transport plate positions the test strip at the reflectance photometer recording head where the specimen is measured at three different wavelengths (555, 620, 660 nm) at 48 seconds and/or 120 seconds after dipping. The result is converted to a concentration value and printed or transferred to a laboratory computer. An optional built-in bar-code reader is available for patient identification. The instrument is calibrated with a special calibration strip once every 2 weeks. This instrument also is incorporated in the IRIS 939UDx workstation.

The Behring Rapimat II/T is a semiautomated urine chemistry instrument that also corrects for urine color using a blanking pad. Its unique feature is selective thermostatic heating of the leukocyte esterase test pad to enhance the enzymatic reaction. The Rapimat II/T includes a measure of ascorbic acid content to check for possible reaction interference. Strips are placed on a conveyer belt to be taken into the instrument. Results are printed with the physical description and microscopic findings. Abnormal results are flagged as determined by the user. It does have bar-code availability and can store 300 sample results. Maintenance requires weekly cleaning; calibration is required every 1000 tests and is printed for a permanent record.

FIGURE A–3 Clinitek Atlas Automated Urine Chemistry Analyzer. (Courtesy of Bayer Diagnostics, Elkhart, IN.)

Automated Microscopy

In a routine urinalysis, a test strip determines the chemical analytes and the formed elements are determined by microscopy. Manual microscopy is not easily standardized because of the high variation among operators even in the same institution. Intensive specimen processing affects accuracy as rare elements such as casts or crystals may be lost during handling. Results are not quantitative because they must be reported in ranges or averages. Overall, manual microscopy is not cost effective because of the poor use of personnel in-batch processing and poor turn-around-time for stats. The development of urinalysis workstations capable of performing microscopic urinalysis has provided a solution to these problems for laboratories with a high volume of urinalysis. The IRIS/Sysmex UF-100 Urine Cell Analyzer is dedicated to microscopic analysis, whereas the Yellow IRIS and its successors, the Model 300 and 500 Workstations and the 939UDx Urine Pathology System (Chatsworth, CA) are designed to perform chemical and microscopic urinalysis.

The IRIS/Sysmex UF-100 Urine Cell Analyzer is designed for large urinalysis laboratories with predominantly normal microscopic results and can process 100 samples per hour. The UF-100 uses laser-based flow cytometry along with impedance detection, light scatter, and fluorescence to identify the individual characteristics and count stained urine sediment particles in a flowing stream. The instrument is easy to operate by placing a 10-position linear rack on the instrument and initiating the autoanalysis with a touch of the screen. Uncentrifuged urine is aspirated into the instrument and the conductivity is measured. The sample is stained with two dyes that radiate an orange and green fluorescence. The DNA within the cells is stained by the orange dye, phenathridine; the nuclear membranes, mitochondria, and negatively charged cell membranes are stained with a green dye, carbocyanine. The stained sample is passed through the flow cell where it is hydrodynamically focused and presented to a laser light beam (488 nm) that produces fluorescence and light scatter. Particles are identified by measuring the change in impedance of the sediment elements as well as the height and width of the fluorescent and light scatter signals, which are presented in scattergrams and histograms. The width of the fluorescent signal measures cellular inclusions and the width of forward light scatter measures the length of cells. Values are presented in a numerical quantitation (cells per microliter) and abnormal results are "flagged" for confirmatory review. An internal quality control system monitors performance. One thousand patient results including scattergrams, histograms, and specimen characteristics are stored. A bidirectional interface is provided to download and report results.

The Models 300 and 500 workstations are self-contained, operator-attended workstations capable of performing specific gravity, routine chemical analysis, and slideless microscopic analysis from an uncentrifuged specimen (Figure A–4). On Models 300 and 500, chemical analysis is performed using Boehringer Mannheim Chemstrip reagent strips and a CHEMSTRIP reflectance photometer. For testing, 6 mL of room temperature urine is required. The patient identification or specimen number is manually entered using a keypad or optional bar-code reader. Color and clarity must be entered or "yellow" and "clear" will automatically be reported. Urine is poured over the strip as it is poured into the instrument for the specific gravity and microscopic, excess urine is blotted by the operator, and the strip is placed on a platform tray and manually transferred to a built-in reflectance reader. Results of the reflectance readings are displayed and reviewed on a video monitor and integrated into the analyzer unit. Urine entering the instrument is divided into two portions for the specific gravity (2 mL) using an IRIS Mass Gravity Meter and to the mixing vessel for the IRIS Slideless Microscope (4 mL). Specific gravity is determined by harmonic oscillation. A standard volume of urine is maintained in a U-shaped tube, and a sound wave of fixed frequency is transmitted into one end of the tube. The change in frequency recorded as the sound wave exits the other end of the tube is directly related to the specific gravity. For microscopic analysis, a stained well-mixed urine specimen is forced in a moving stream through a sheath of envelope fluid into the microscope flow cell at a constant rate (Figure A–5). A process known as hydroplanar positioning forces all particles to flow in a single plane as they pass the optical path of the microscope (Figure A–6). Multiple freeze-frame pictures are taken as a particle passes the microscope and as a high-intensity strobe light flashes; data from these are analyzed by a computer processor as to size and number. Particles are placed into three low-power (100×) and five high-power (400×) groups based on their size, and the low-power and high-power digitized images are presented to the operator on a color monitor. The operator then makes the final identification by touching an appropriate area or category on the monitor screen (Figure A–7). Touch buttons allow any corrections to be made to the computer classification of any particles. When all results are confirmed, the operator touches a button that sends a complete report to the laboratory information system or printer, including patient

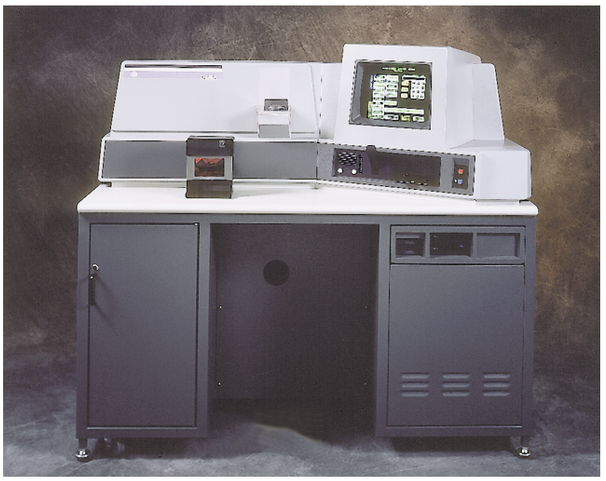

FIGURE A–4 Model 500 Workstation. (Courtesy of International Remote Imaging Systems, Chatsworth, CA.)

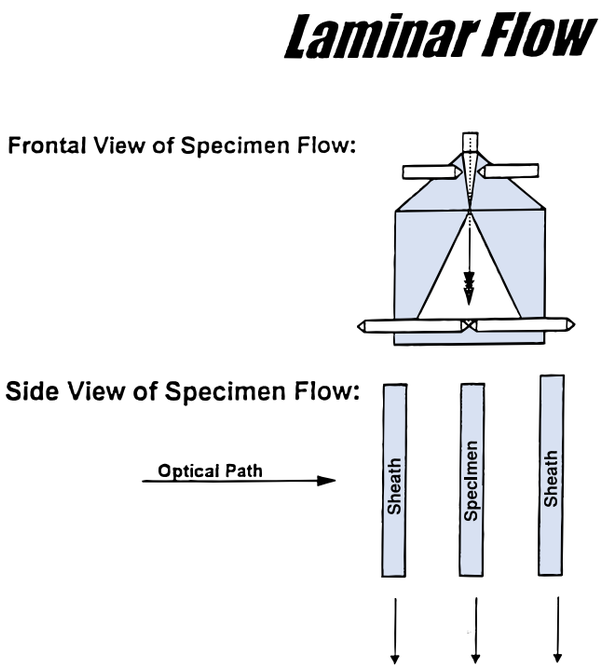

FIGURE A–5 Diagram of laminar flow. (Courtesy of International Remote Imaging Systems, Chatsworth, CA.)

identification, all chemistry results, specific gravity, color, clarity, and microscopic data. Each urine specimen is processed and reported before the next sample may be introduced. Normal samples are complete in 1 minute and abnormal samples with a variety of particles take 2 to 5 minutes. The Model 500 workstation contains a body fluid package to include cerebrospinal, pleural, peritoneal, pericardial, peritoneal lavage, peritoneal dialysate, seminal, and synovial fluids. Calibration is performed daily with calibration strips. Computer capabilities provide the ability to customize reporting units, screen routine samples, report the microscopic examination as negative, perform more extensive microscopic searching on specified patients, store images on floppy disks for later analysis, correct for dilutions, and perform counting and microscopic analysis of other body fluids.

The most sophisticated urinalysis instrument with complete routine urinalysis automation is the 939UDx NNA (neural net automation) Urine Pathology System. Designed for medium to large urinalysis laboratories, the 939UDx is the only automated analyzer that can automatically perform, classify, and report all aspects of a complete urinalysis. The system consists of three components to perform fully automated chemistry test strip results, specific gravity, color and clarity, and microscopic sampling from bar-coded sample tubes on uncentrifuged urine by loading the sample carousel only once. The three components are the Roche-Boehringer Mannheim Chemstrip Super Urin-

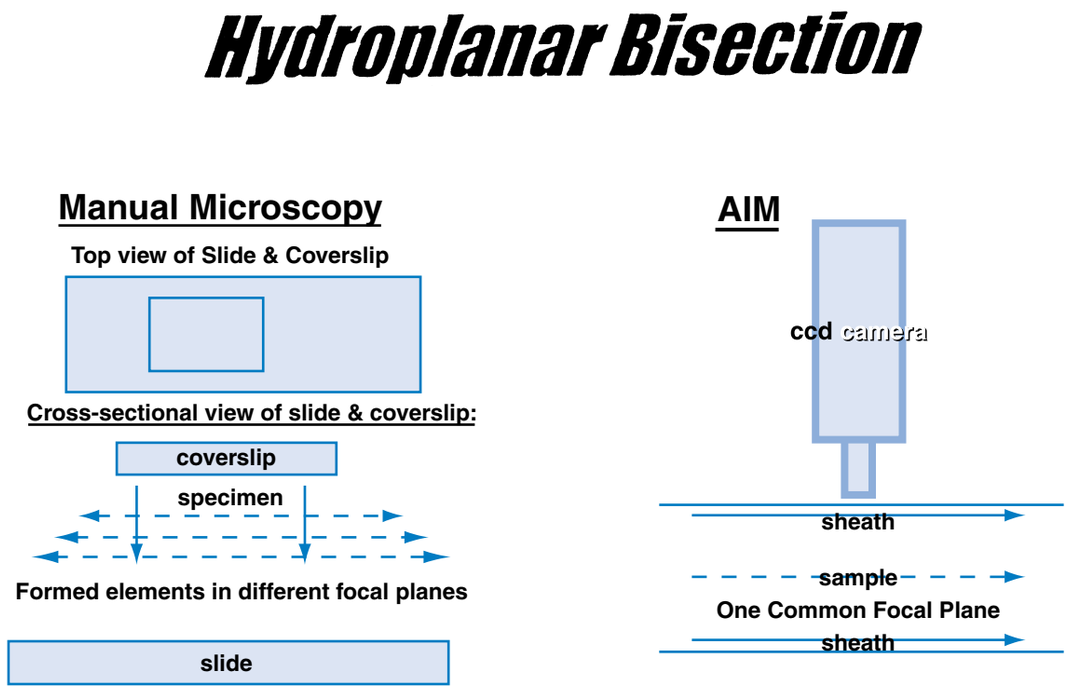

FIGURE A–6 Diagram of hydroplanar positioning. (Courtesy of International Remote Imaging Systems, Chatsworth, CA.)

FIGURE A–7 Operator editing workstation screen. (Courtesy of International Remote Imaging Systems, Chatsworth, CA.)

alysis Analyzer, the IRIS Flow Microscope Analyzer, and the IRIS ViewStation, all interlinked to provide a complete urinalysis (Figure A–8).

Neural net automation software included in the instrument automatically counts and classifies particles as RBCs, WBCs, bacteria, squamous epithelial cells, nonsquamous epithelial cells, hyaline casts, nonhyaline casts, yeast, crystals, sperm, mucus, WBC clumps, and amorphous. Constituents must meet a predefined minimum concentration to be included in the autoclassification. Laboratories can program the neural net software to block autoreporting of results based on both concentration and types of constituents present.

The instrument can process batches of up to 55 urine samples and process up to 250 specimens without microscopics per hour. The minimum amount of urine required is 7 mL; of this specimen, 3 mL is transported to the Mass Gravity Meter for specific gravity determination and the remaining 4 mL is transported to the sample chamber for staining prior to microscopic imaging. Plastic, glass, or KOVA-style tubes are used. Special pediatric tubes are available for specimens of 3.5 to 6.5 mL. Specimens less than 3.5 mL cannot be run. Uncentrifuged urine specimens are placed in bar-coded tubes and placed into any position of a sample disk carousel with the bar code placed correctly toward the periphery of the carousel. The carousel is placed into the autosampler of the Super UA (Super Urinalysis Analyzer).

With the touch of a button, the Super Urinalysis Analyzer automatically processes test strips and can complete a disk cycle in 12 minutes. As each tube bar code is scanned, a mixing rod mixes the urine and a test strip is dipped into each tube for wetting and placed in the photometer. As each measurement is complete, the result is sent to the ViewStation. After completion of testing for the batch, the carousel is removed from the Super Urinalysis Analyzer and transferred to the Flow Microscope autosampler. After aspiration by the autosampler, the specimen goes to the vacuum chamber where color and clarity are measured; then the sample is delivered into the sample chamber, where it is mixed automatically with stain as the specimen is prepared for microscopy. The stained sample and sheath fluid enter the flow cell at the same rate, then pass into the

FIGURE A–8 Model 939UDx Urine Pathology System. (Courtesy of International Remote Imaging Systems, Chatsworth, CA.)

Imaging Process

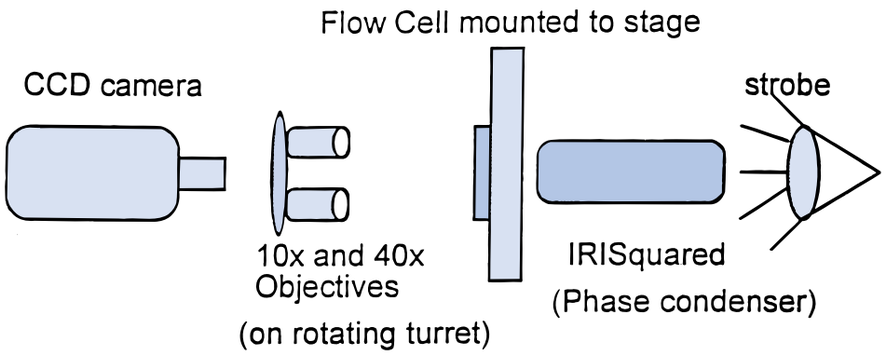

FIGURE A–9 Low-power and high-power imaging process. (Courtesy of International Remote Imaging Systems, Chatsworth, CA.)

IRIS Slideless Microscope, where analytes are oriented and imaged by a video camera. The composition of the sheath fluid and the pressure within the system aids in the hydrodynamic orientation of the sample to obtain maximum exposure of particles to the IRIS Slideless Microscope. As the sheath fluid envelopes the sample, a laminar flow is created that causes the widest cross-section of the particles to face the optical path. As these particles are transported through the flow chamber, the maximum area is bisected by the optical path, creating a planar view on one focal plane. The sample is illuminated with a strobe lamp and viewed with the video camera. As the stream of specimen is illuminated by the strobe lamp, the flashing of the strobe lamp freezes the motion of urine particles. The stop motion pictures are viewed by the 10× and 40× objectives and captured by the video camera as images (Figure A–9). Low-power and high-power examinations are used in the same manner as traditional microscopic procedures.

The particles captured by the video camera are analyzed by the image processor and sorted into groups or ranks, based on particle size. The image data are digitized and sent to the ViewStation computer, where image processing and classifying are finalized and presorted images are displayed for operator review. The image data are logged into the specimen records in the database in hard disk memory. The ViewStation database assembles and consolidates all result

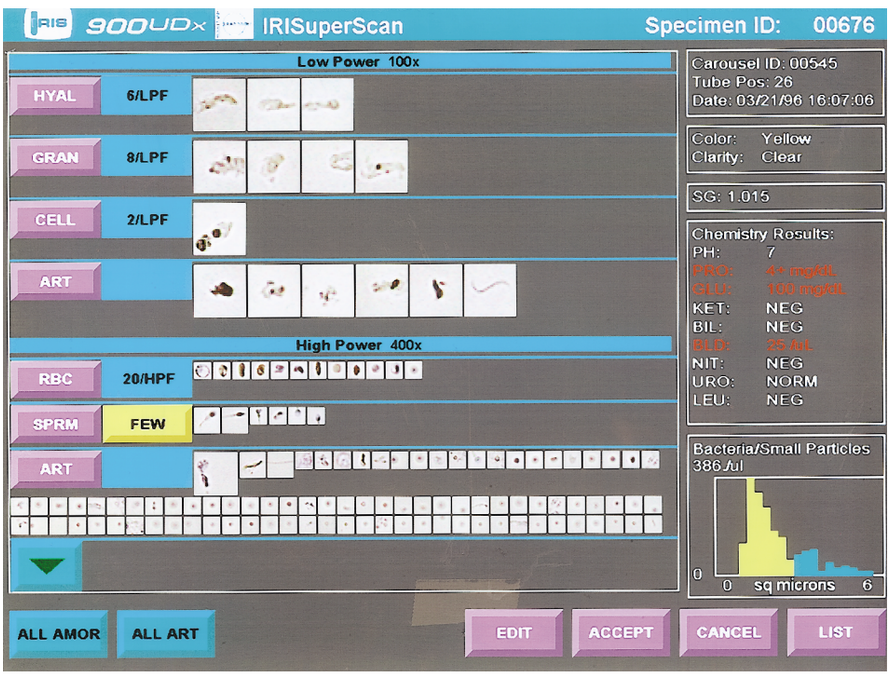

FIGURE A–10 Model 939UDx viewing screen. (Courtesy of International Remote Imaging Systems, Chatsworth, CA.)

information for each specimen from the Super UA and the FlowMicroscope, and displays all the relevant information on the touch screen monitor. Microscopic images, color and clarity, specific gravity chemistry results, and the bacteria histograms are presented to the operator for visual review and editing. Small-particle histograms are a graphic display of size distribution of any small sediment particles (ranging from 1 to 6 μm^2) found during the microscopic examination. The histograms help to decide whether bacteria are present in these small size ranges or if the detected particles are small crystals or amorphous. Results are quantitated as a numerical result or as a graded result with the exception of bacteria. The operator interacts with the monitor to review and confirm analyte images and can edit results at any time (Figure A–10). Abnormal results are flagged according to laboratory specifications. The printer generates a final report. Manual confirmation is needed to identify the types of inclusion casts, nonsquamous epithelial cells and crystals, to verify motility and the flagella of *Trichomonas*, and to confirm fat globules.

The ViewStation software includes specimen tracking, editing, quality control, display configuration, flagging, and archiving/backup features. The Intel Pentium ViewStation computer offers "off-line" result review and release, so that operators in different locations can access each module of the 939UDx.

Calibration on the Super Urinalysis Analyzer is performed every 2 weeks using a calibration strip. The Flow Microscope, specific gravity, color and clarity subsystems are factory calibrated and monitored by the "self test" program. Maintenance is minimal and automatically performed with the start-up self-test procedures.

Additional Information Sources

Bayer Diagnostics: http://www.bayerdiag.com/products.
International Remote Imaging Systems:
http://www.proiris.com/irisbus/products/urinalysis.
Roche-Boehringer Mannheim Diagnostics:
http://www.boehringer-mannheim.com/rapid/urinalysis.