Verification of Automated Urinalysis Instrumentation

Analytical and Clinical Considerations

Lauren Pearson, DO, MPH
Chief Medical Officer, ARUP Laboratories at University of Utah Health
Associate Professor (Clinical), University of Utah School of Medicine
Outline

Components of urinalysis testing

Clinical utility of urinalysis

Overview of methods

Validation/verification essentials for automated methods

Clinical impacts of automated methods
Objectives

Describe
The chemical and physical principles behind conventional manual and automated methods for urinalysis testing.

List
The studies required to document method performance in CLIA regulations.

Apply
CLIA requirements to document performance characteristics of automated urinalysis methods.
Components of Urinalysis (UA) Testing

Physical, Chemical, Microscopic
COMPONENTS OF UA

Physical Characteristics

- Color, clarity, odor, foam
- Specific gravity: reagent strip or refractometer
COMPONENTS OF UA

Chemical Characteristics

- Qualitative or semiquantitative result
- Glucose, blood, protein, pH, ketones, nitrite, leukocyte esterase, bilirubin, urobilinogen
- Specific gravity, ascorbic acid
COMPONENTS OF UA

Microscopic Examination

• Evaluation of formed elements of the urine
• Casts, crystals, RBCs, WBCs, bacteria
MICROSCOPIC EXAMINATION
Clinical Utility

• Evaluation for infection of the urinary tract
• Hematuria, flank pain, polyuria, etc.
• Monitoring of patients with chronic systemic inflammatory and infectious disorders, transplant patients
• Among others...
Overview of Methods$^{1-4}$

Manual & Automated
Conventional Manual Methods

- Reagent strips for chemical UA
- Bright field microscopy examination of concentrated urine sediment
CONVENTIONAL MANUAL METHODS
CONVENTIONAL MANUAL METHODS
METHODS

Reagent Strips “Dip”

• Simple, low cost
• Add urine to paper impregnated with reagent
• Read manually or by an automated reading device
• If abnormal chemically, microscopic exam may be performed
• May have inadequate sensitivity for some analytes
• Susceptible to interfering substances
REAGENT STRIPS “DIP”
REAGENT STRIPS “DIP”
Automated Methods

• Chemical UA
  » Reagent strip paired with an automated photometric measurements of reagent strip fields
  » Automatically timed and read

• Microscopic
  » Flow cytometry
  » Digital imaging with software
AUTOMATED METHODS
# Automated Microscopic

<table>
<thead>
<tr>
<th>Flow Cytometry</th>
<th>Digital Imaging</th>
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<tbody>
<tr>
<td>• Stains unconcentrated sample</td>
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<tr>
<td>• Interrogated with laser beam</td>
<td>• Passes urine in front of microscope objective to photograph elements</td>
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<tr>
<td>• Light scatter, fluorescence, impedance measured</td>
<td>• Proprietary software used to categorize into user-definable groups</td>
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<tr>
<td>• User-defined flags</td>
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### Why Automated Methods?

<table>
<thead>
<tr>
<th>Laboratory Operations</th>
<th>Performance Characteristics</th>
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<tr>
<td>• Reduced ability to staff clinical labs</td>
<td>• More accurate and precise quantitation of formed elements</td>
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<td>• Efficiency of manual versus automated methods to accommodate increased volumes of testing</td>
<td>• More consistent measurement/semiquantitation of chemical results</td>
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<tr>
<td>• Cost</td>
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Validation/Verification Essentials

Automated UA Methods
VALIDATION ESSENTIALS

Method Validation to CLIA

• Moderate complexity
  » Accuracy
  » Precision
  » Reference interval
  » Reportable range

• High Complexity
  » Add analytical sensitivity and analytical specificity
Essential Steps

Gather your resources
- Review manufacturer’s requirements
- Study what others have done

Write a plan

Execute validation studies

Analyze data and compare to acceptability criteria

Post go-live evaluation
Gather Your Resources

- Package inserts
- Instructions for use
- Literature review
- Understanding components of the automated system and the method behind each
Manufacturer’s Requirements

ACCURACY

PRECISION

REPORTABLE RANGE

CARRYOVER
VALIDATION ESSENTIALS

Write a Plan

• Component studies
• Sample source and type, minimum number
• Proportion of abnormal and normal, patient demographics
• Fresh versus preserved
• Minimum volumes
• Exclusion criteria
• Procedure
  » Storage, processing, mixing, decanting
  » Testing
• Defined acceptability criteria
• Data analysis
Accuracy (Method Comparison)

- Applies to quantitative, semiquantitative, and qualitative results
- Determines bias to an existing method or gold standard reference method
- Analyze samples in duplicate on new method and reference method

Reportable parameters from chemical UA

Quantified formed elements of the urine

Should include flagged parameters
Precision

• Applies to quantitative results
• Within-run and between-run (total)
  » Repeatability
  » Reproducibility
• Analyze materials repeatedly within a run and over several days
Reportable Range

• Required for components that report numerically (not semi-quantitative or qualitative results)
  » e.g., RBC, WBC, BACT

• Verify the lowest and highest results that can be directly reported from the analyzer
  » Linearity experiment

• Must establish reportable limits
Reference Intervals (RI)

Critical when transitioning from manual to automated method

- Verify the current RI, manufacturer’s RI, or other for all reportable parameters

RI verification versus establishing a new RI
Carryover

Should be considered for all components of the automated system

Determines whether samples with high concentration impact subsequent samples with low concentration
Challenges

- Determining what materials to select for each study
- Sample selection, collection, and stability
- Curating abnormal samples and samples from diverse patients
- Differing reporting formats
- Sample processing
- Determining acceptability criteria
- Writing a plan that fulfills requirements for an automated system that has multiple components
Validation Plan

Automated UA Methods
Classified Information

Accuracy

• 40 normal samples, 60 abnormal samples (unpreserved)
• Analyze ASAP and within stability on new and reference method
• Data analysis
  » % agreement, positive and negative agreement for qualitative/semi-quantitative results
    ▪ Within one block or grade
  » Slope, intercept, R value, bias calculated for pH and specific gravity (quantitative results)
**Precision**

- Normal and abnormal control material selected
- Analyze 10+ times consecutively for within-run
- Analyze twice daily for 20 days for total precision
  - Could conduct 5 x 5 study alternatively
- Data analysis
  - Agreement for qualitative and semiquantitative parameters
  - %CV for quantitative
Reportable Range

• Specific gravity
• Diluted 10% saline with deionized water to span the analytical measurement range
• Analyzed samples in triplicate
• Linear regression and percent recovery calculated
Accuracy

• 40 normal and 60 abnormal samples
  » Quantitative and qualitative results compared for agreement
  » Microscopic analysis using conventional microscope on a subset of abnormal samples if comparing two automated methods

• Unit conversion may be necessary to compare results
  » Approach to data analysis and acceptance criteria vary depending on how analytes are measured and reported between systems

• Turbid, abnormally colored, stored specimens more frequently show disagreement

• Effect of sample processing
Precision

- Normal and abnormal materials prepared using reagents and calibrators
  - Bacteria, cast, Epithelial Cells, RBC, WBC
- Two levels of control material analyzed twice daily for 20 days
  - Could conduct 5 x 5 study alternatively
- Compared to manufacturers specification
- Total precision only
Reportable Range

• Serial dilutions spanning the range prepared from stock solutions for WBC, RBC, and bacteria
  » May be provided by the instrument manufacturer
• Each sample in each set analyzed in duplicate
• Linear regression statistics calculated
Accuracy

• Optional, but best practice: determine if the device accurately classifies particles based on images
  » Can use a limited number of abnormal samples
  » Tech confirmation of classification of all images happens in production environment
Precision

- Optional
- Precision of the sensitivity of the nonreportable parameters of the device using commercial QC material specific to the manufacturer
Carryover

• Chemical UA: analyze abnormal followed by normal control material
• Urine particle analyzer: high sample for WBC, RBC, BACT analyzed three times followed by low sample analyzed three times
• Digital imaging device: consecutive analysis of a high solution followed by blank
• Percent carryover calculated using standard equation
Reference Intervals

- Manufacturer may have recommendations for how many samples to include in a verification study
- Analyze on chemical UA and urine particle analyzer
- If a preservative is routinely used, it must be used for samples collected for the study
- Samples must be stored, processed, and tested the same as patient samples
- Exclusion criteria are critical
Take Home Message

• Do what you can with the resources you have!
• Determine how well studied the performance specifications of the new method are
Clinical Validation

- Differences in clinical sensitivity for diverse indications for UA
- Perform a clinical sensitivity study for your unique patient population, method, user-defined parameters
  » Ability to discriminate normal samples differs
- How results are interpreted based on local clinical protocols is important
- Post go-live assessment (optional)
Clinical Impacts of Automated Methods

How do modern methods influence applications of laboratory data in clinical practice?
Microhematuria

• Threshold definition of microhematuria may need to change
• Guidelines are based on gold standard conventional microscopy method
• New methods may result in higher quantities of patients being referred to urology
• Early communication with clinicians about the change in sensitivity of the method for detecting RBCs is essential
Increased Sensitivity

• Possibly increased sensitivity for detecting infection
• May detect smaller quantities of pathologic casts which otherwise could lyse during sample preparation
References


ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.