Verification of Automated Urinalysis Instrumentation

Analytical and Clinical Considerations

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Outline

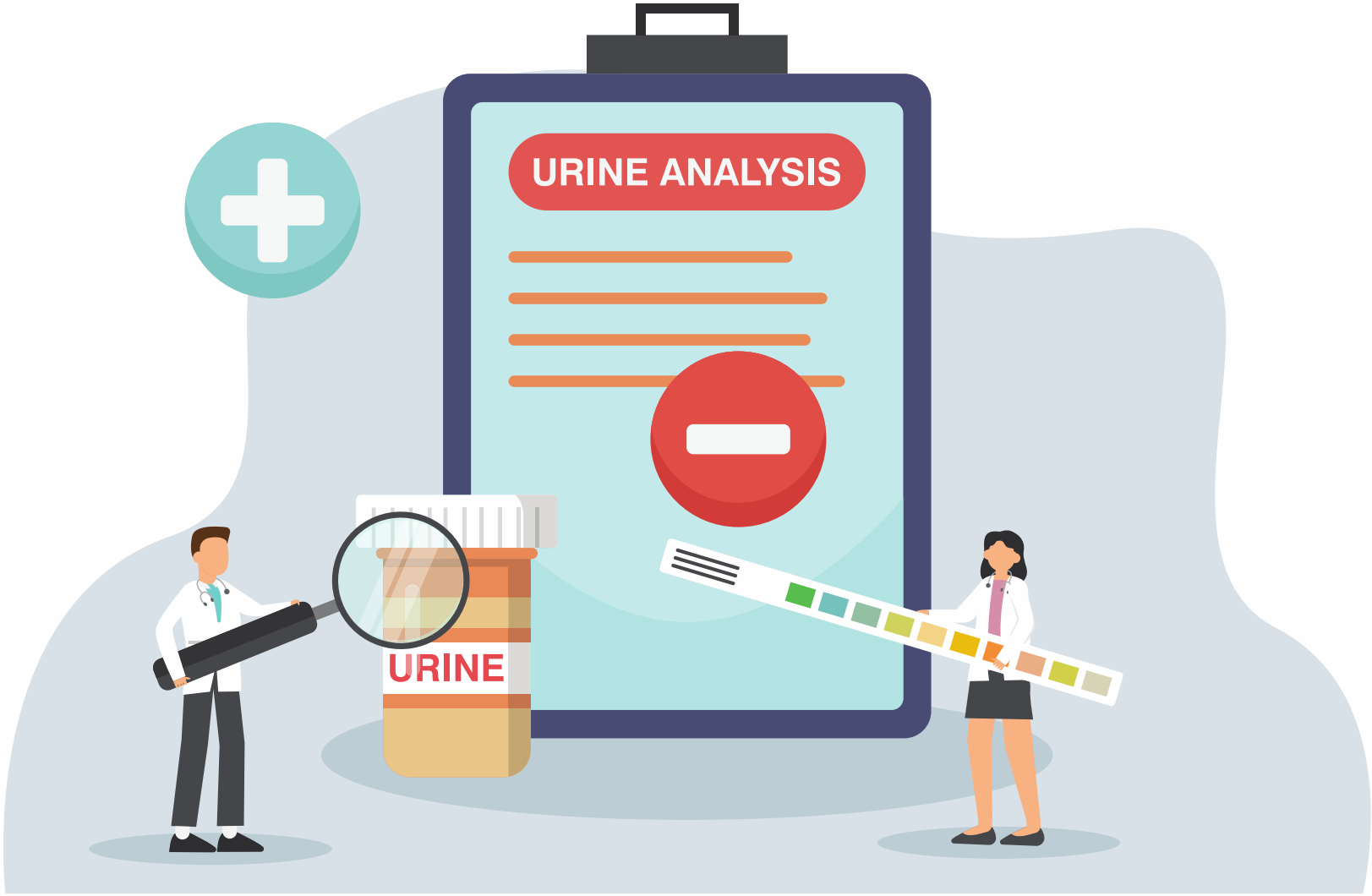
- Components of urinalysis testing
- Clinical utility of urinalysis
- Overview of methods
- Validation/verification essentials for automated methods
- Clinical impacts of automated methods

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


URINE ANALYSIS
URINE

Physical Characteristics

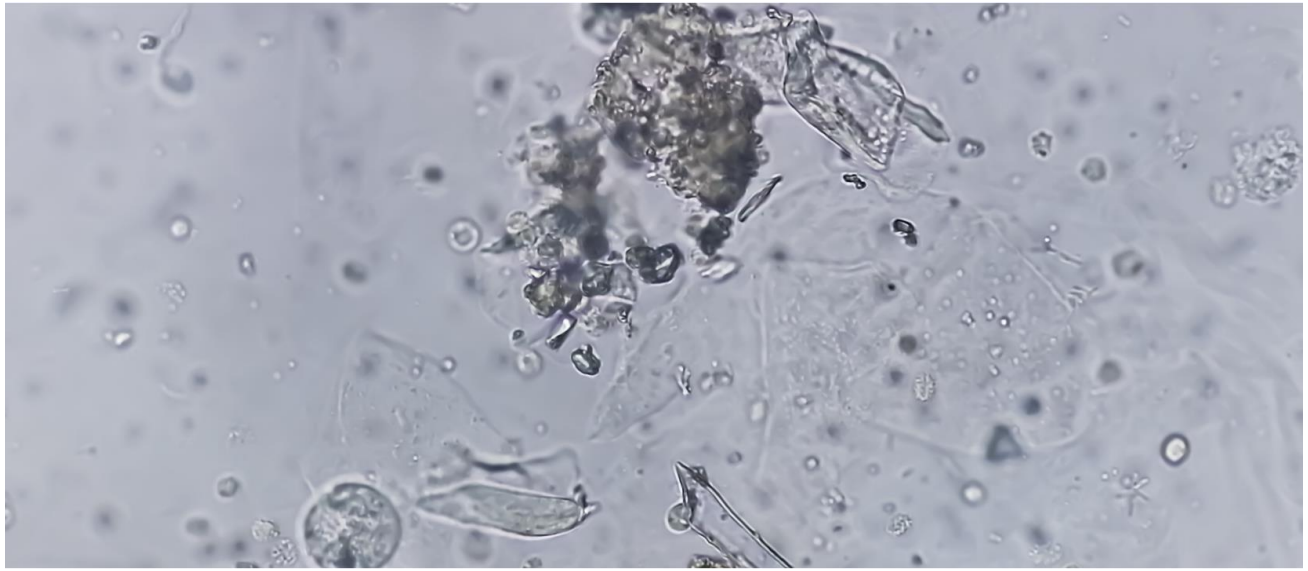
- Color, clarity, odor, foam
- Specific gravity: reagent strip or refractometer

Chemical Characteristics

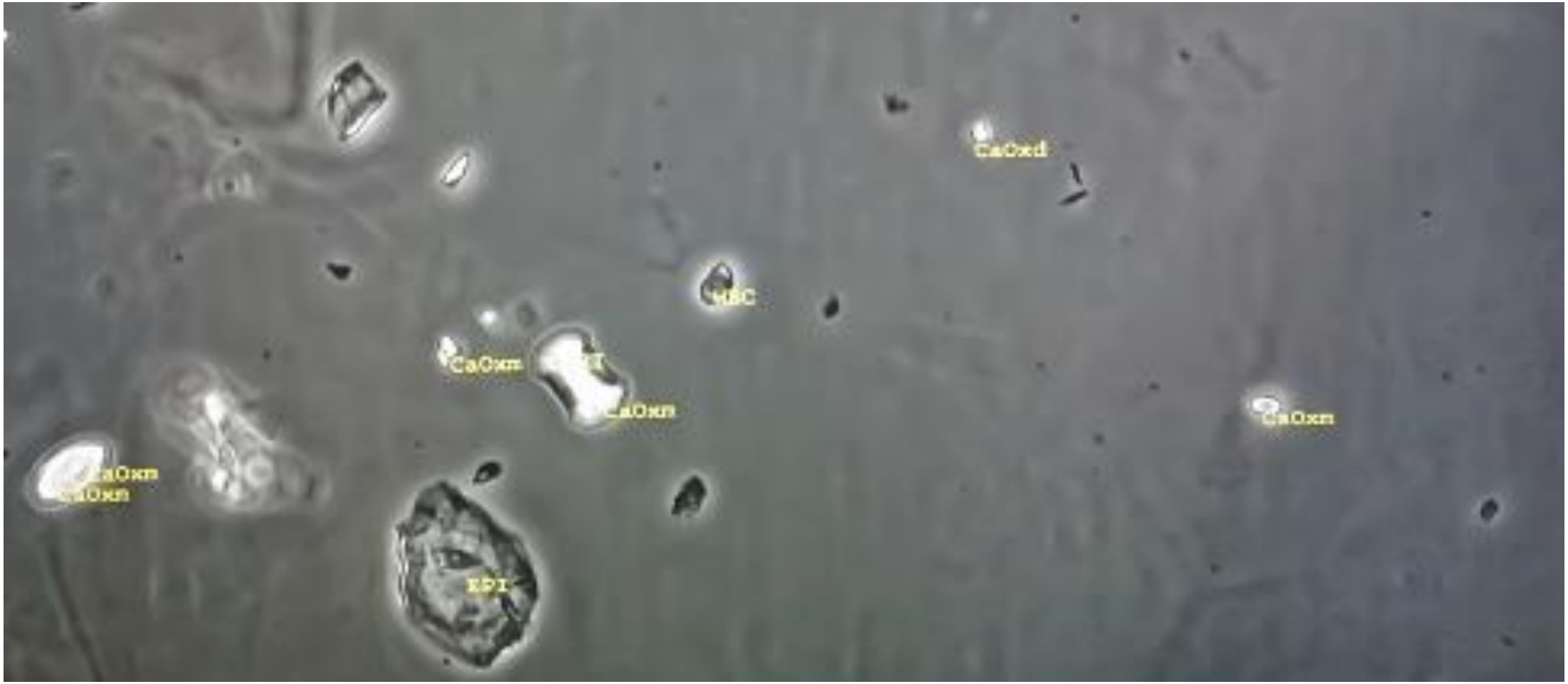
- Qualitative or semiquantitative result
- Glucose, blood, protein, pH, ketones, nitrite, leukocyte esterase, bilirubin, urobilinogen
- Specific gravity, ascorbic acid

Microscopic Examination

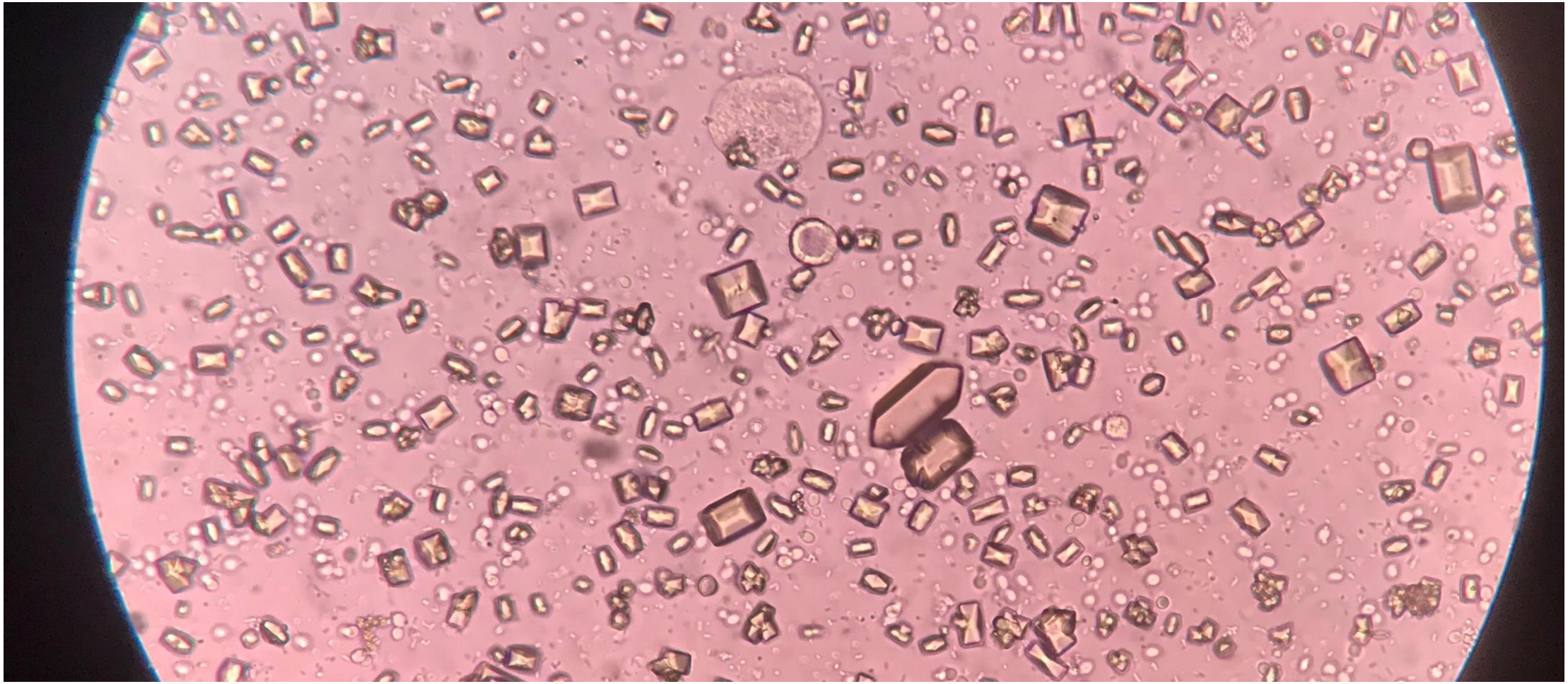
- Evaluation of formed elements of the urine
- Casts, crystals, RBCs, WBCs, bacteria



MICROSCOPIC EXAMINATION



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

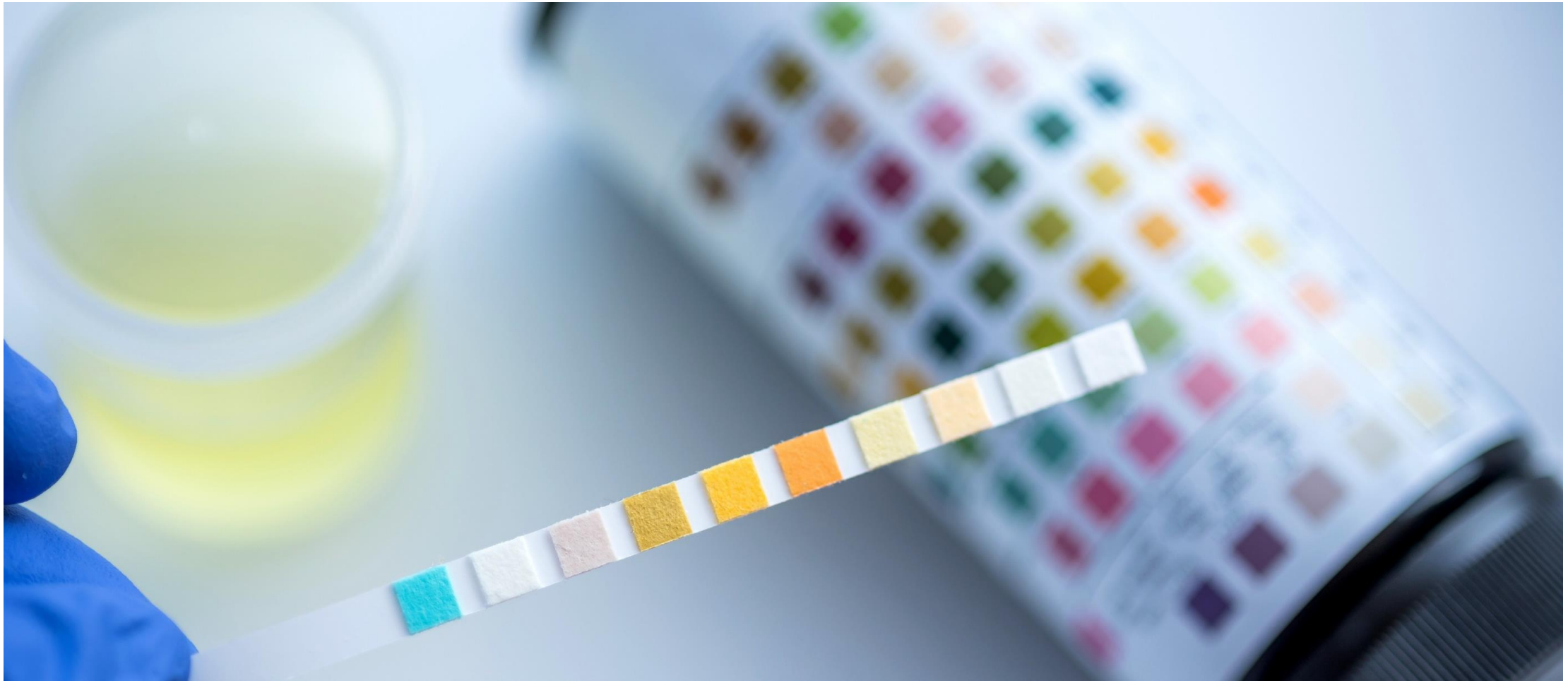
CONVENTIONAL MANUAL METHODS

CONVENTIONAL MANUAL METHODS

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

Flow Cytometry

- Stains unconcentrated sample
- Interrogated with laser beam
- Light scatter, fluorescence, impedance measured
- User-defined flags

Digital Imaging

- Stains unconcentrated sample
- Passes urine in front of microscope objective to photograph elements
- Proprietary software used to categorize into user-definable groups

Why Automated Methods?

Laboratory Operations

- Reduced ability to staff clinical labs
- Efficiency of manual versus automated methods to accommodate increased volumes of testing
- Cost

Performance Characteristics

- More accurate and precise quantitation of formed elements
- More consistent measurement/semiquantitation of chemical results

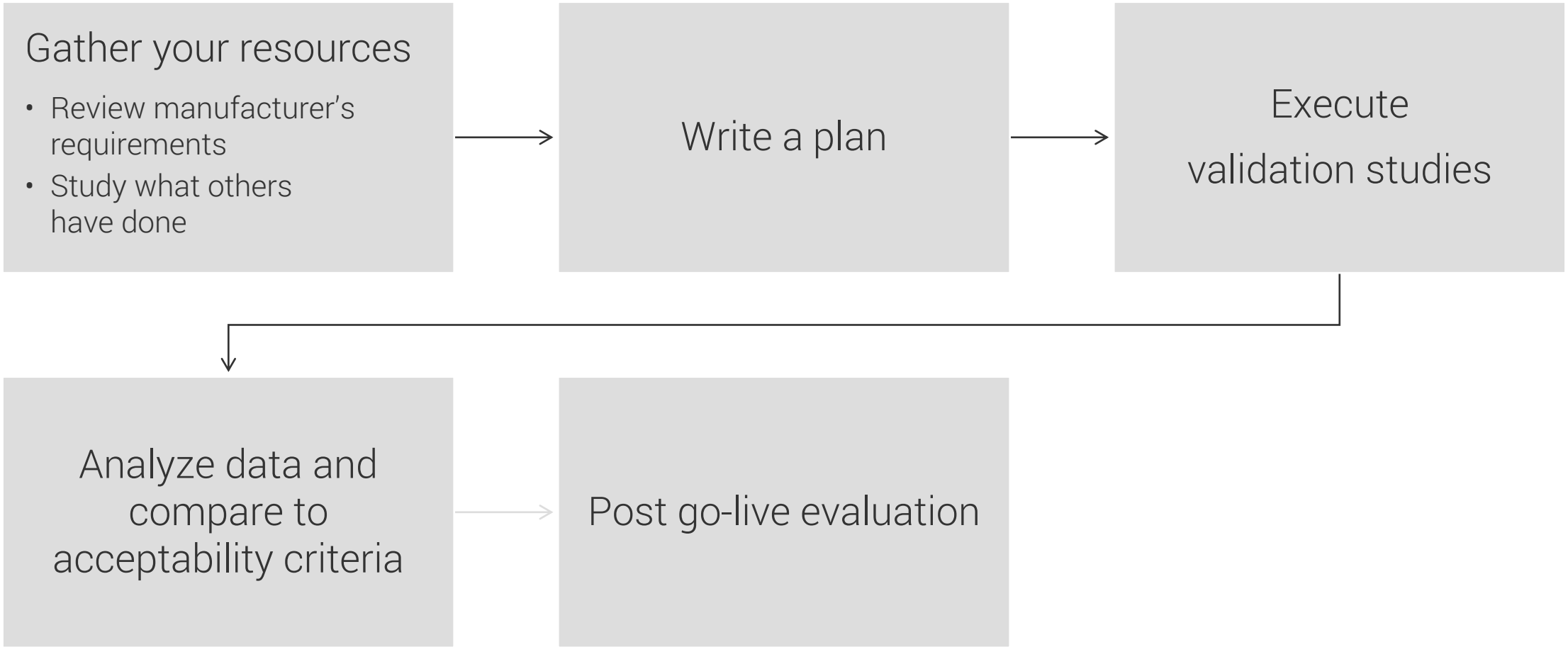
Validation/Verification Essentials

Automated UA Methods

Method Validation to CLIA[5]

- Moderate complexity
  - » Accuracy
  - » Precision
  - » Reference interval
  - » Reportable range
- High Complexity
  - » Add analytical sensitivity and analytical specificity

Essential Steps



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

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
  - Reportable parameters from chemical UA
  - Quantified formed elements of the urine
    - Should included flagged parameters
- Determines bias to an existing method or gold standard reference method
- Analyze samples in duplicate on new method and reference method

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

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

Review > J Clin Pathol. 2017 Feb;70(2):94-101. doi: 10.1136/jclinpath-2016-203958.
Epub 2016 Oct 31.

Validation and verification of automated urine particle analysers

Giuseppe Enrico Bignardi

PMID: 27802413 DOI: 10.1136/jclinpath-2016-203958

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

1. Haber, M, et al, eds. *Color Atlas of Urinary Sediment*. 2010. Second ed. College of American Pathologists. 2019:38–63.
2. McPherson RA, Pincus MR, eds. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. Expert Consult. Online and print. Elsevier Health Science; 2011:445–479.
3. Bignardi GE. *J Clin Pathol*. 2017;70:94–101.
4. Oyaert M. *Ann Lab Med* 2019;39:15–22.
5. Code of Federal Regulations. www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493. [Amended Apr 2023; accessed May 2023]
6. Clinical and Laboratory Standards Institute. Evaluation Protocols. Various publication dates. Wayne, PA

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