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Learning Objectives

- Compare the correlative performances of urinalysis, urine microscopy, and urine culture
- Describe motivations for and clinical outcomes associated with urine reflexive testing
- Contrast various laboratory workflows and operational considerations for implementing reflexive urine testing

Urinalysis – the world's oldest lab test

Physician holding matula into the light for inspection

A chart used to categorize urine

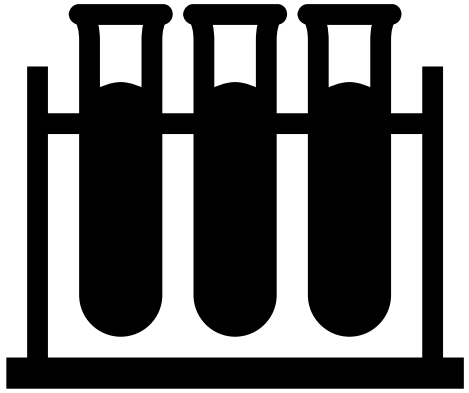
Armstrong JA. Urinalysis in Western culture: a brief history. *Kidney Int*. 2007.

Utility of urinalysis today

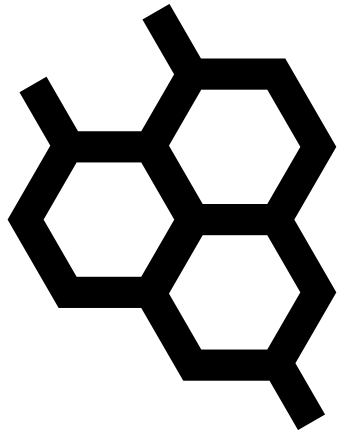
- Kidney disorders
- Diabetes mellitus
- Liver disease
- Hypertension of pregnancy
- Urinary tract infections

Urinalysis may represent 30% of all lab samples received

Modern urinalysis

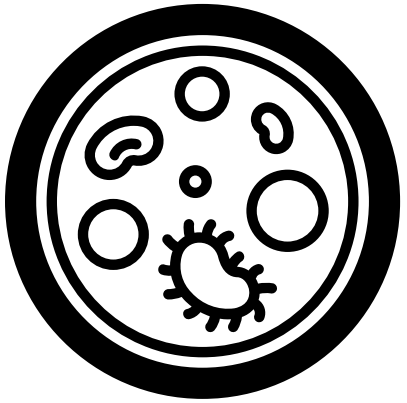


macroscopic and physical



chemical

microscopic



culture

POCT

Micro Lab

Core Lab

Chemical/dipstick urinalysis

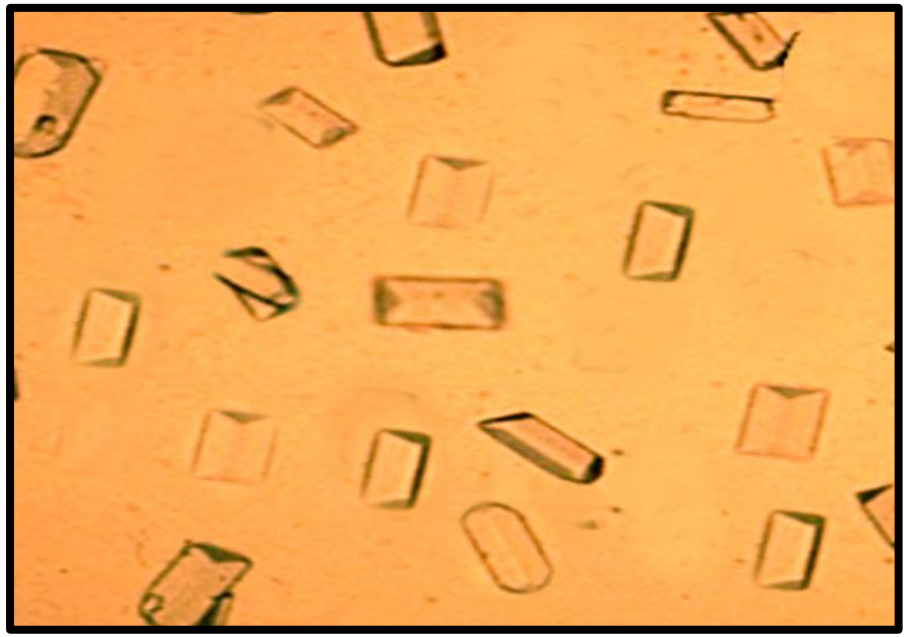
ketones ● protein ● glucose ● leukocyte esterase ● blood ● nitrite ● bilirubin ● pH ● urobilinogen ● specific gravity

Automated analyzers:

- Standardize color interpretation
- Eliminate variance from timing
- Remove operator subjectivity
- High throughput
- May offer sample transfer automation

Microscopic urinalysis

- RBC, WBC, bacteria, yeast, epithelial cells, casts, crystals,…
- Manual
- Particle analyzers
  - Impedance, flow cytometry, digital imaging, light scatter,…
  - Abnormal findings may necessitate manual review
  - Sensitivity limitations in populations with high prevalence of renal disease



Gai M et al. *Clin Chem*. 2003.
https://www.ncbi.nlm.nih.gov/books/NBK537009/

Is microscopy always necessary?

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CLSI GP16-A3:2009 Urinalysis, 3rd Edition

"The decision to perform microscopic examinations should be made by each individual laboratory based on its specific patient population.

- *When requested by the physician*
- *When determined by laboratory protocol*
- *When any abnormal physiochemical result is obtained"*

Screening with urine dipstick

- Debated heavily in literature in 1980s
- Overall sensitivity 95%, specificity 74%
- Most false negatives associated with bacteriuria
- Positive chemical strip test "*can be safely and effectively used as a prerequisite for routine urine microscopic examination*."

Table 1. Association of Biochemical and Microscopic Abnormalities n=1000

Indicator/Condition	Sensitivity	Specificity	Chi-Square
Leuk Ester/pyuria	0.82	0.77	$P < 0.0002$
Nitrite/bacteriuria	0.02	0.99	$P < 0.009$
Leuk Ester/bacteriuria	0.51	0.62	$P < 0.002$
Protein/bacteriuria	0.85	0.33	$P < 0.05$
Hgb/hematuria	0.70	0.92	$P < 0.001$

Wenz and Lampasso, *Am J Clin Pathol*. 1989.

Chemical vs. microscopic urinalysis

Table 1. Comparison of Urine Results Obtained Using Chemstrip® 8 and 9 with Microscopic Examination

n=1000

Part A — Chemstrip 8

Dipstick	Microscopic +	Microscopic −
+	476	182
−	131	211

Sensitivity = 78%
Specificity = 54%

Part B — Chemstrip 9 + leukocyte esterase

Dipstick	Microscopic +	Microscopic −
+	476	245
−	101	178

Sensitivity = 82%
Specificity = 42%

Positive microscopic defined as:

- ≥6 WBC, RBC, or renal tubular cells per hpf
- ≥2+ bacteria
- Presence of casts, pathologic crystals

Morrison and Lum. *Am J Clin Pathol*. 1986.

Urinalysis with reflex to microscopic

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Quest Diagnostics

< BACK

Urinalysis with Reflex to Microscopic

labcorp

Urinalysis, Routine With Microscopic Examination on Positives

Urinalysis, Routine (CHLA Laboratory Guide)

Test Includes

Color, appearance, specific gravity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, leukocyte and nitrite. A microscopic sediment examination will automatically be performed if positive for hemoglobin, protein, nitrite,and/or leucocyte esterase. Request a microscopic sediment examination separately since a microscopic test is not reflexed on normal macroscopic UAs.

Common reflex criteria for microscopy

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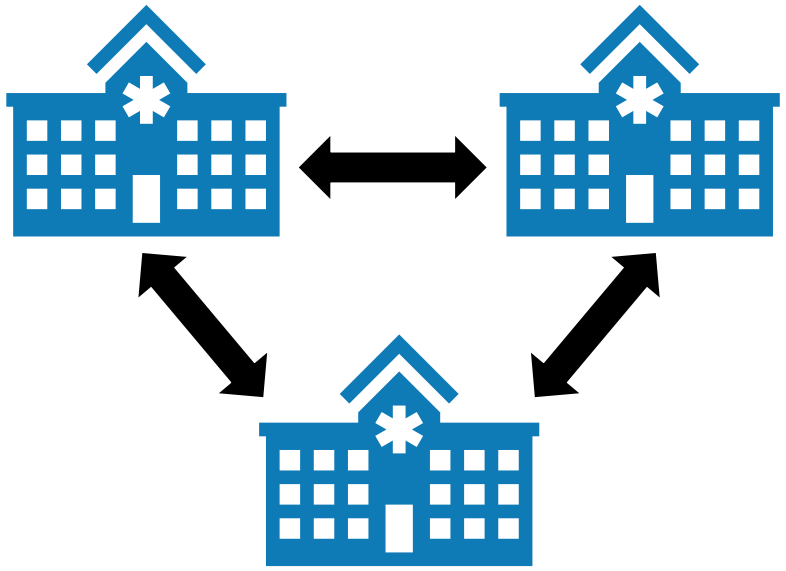
2008 CAP Q-Probes study (n=82 labs):

- Proteins present (99%)
- Leukocyte esterase positive (98%)
- Nitrite present (95%)
- Heme compound present (94%)
- Turbid appearance (80%)
- Bloody appearance (70%)

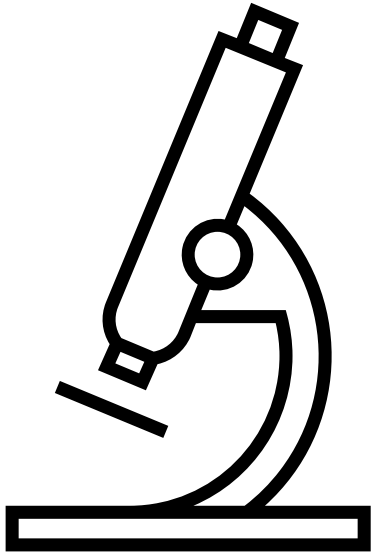
At the time, only 15% of labs reported using an automated microscopic analyzer

LAC+USC urinalysis practices

- Part of a multi-hospital network with varying UA practices
- Desire to:
  - Evaluate and standardize UA instrumentation
  - Standardize to a UA chemical with reflex to microscopic approach
  - Implement UA with reflex to urine culture as part of a broader effort to decrease CAUTIs



LAC+USC microscopy reflex criteria



Microscopy to be performed if:

- Clarity = Cloudy or turbid
- Glucose ≥ 1000 mg/dL
- Blood or protein positive
- Nitrite or leukocyte esterase positive
- Age <30 days
- Violence Intervention Program locations

Chemical vs. microscopic urinalysis

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An updated assessment

Patient demographics.

Total number of unique patients	**7607**
Age at time of testing	
Mean (range), years	46 (0-105)
Median, years	48
Patient location	
Emergency department	3355 (44.1%)
Outpatient	3293 (43.3%)
Inpatient	959 (12.6%)

Chambliss et al. *J Appl Lab Med*. 2020.

Chemical vs. microscopic urinalysis

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An updated assessment

Contingency table correlating chemical urinalysis results to microscopic urinalysis results.

		Microscopic urinalysis		
Chemical urinalysis		+	−	Total
	+	3892	2128	6020
				64.7% PPV
	−	295	2812	3107
				90.5% NPV
	Total	4187	4940	9127
		93.0% sensitivity	56.9% specificity	

PPV, positive predictive value; NPV, negative predictive value.

Microscopy positive defined as:

- RBC ≥ 4/hpf
- WBC ≥ 4/hpf
- Any bacteria

Chemical vs. microscopic urinalysis

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An updated assessment

Table 3 Microscopic findings in 295 samples negative by chemical urinalysis but positive by microscopic urinalysis.

	RBC (/HPF)	WBC (/HPF)	Bacteria (/HPF)
285 (96.6%)	<4		
10 (3.4%)	≥4		
272 (92.2%)		<4	
23 (7.8%)		≥4	
21 (7.1%)			Negative
198 (67.1%)			Trace
50 (16.9%)			1+
15 (5.1%)			2+
5 (1.7%)			3+
6 (2.0%)			4+

Chambliss et al. *J Appl Lab Med*. 2020.

Can UA help reduce urine cultures?

NEWS RELEASE
March 21, 2019

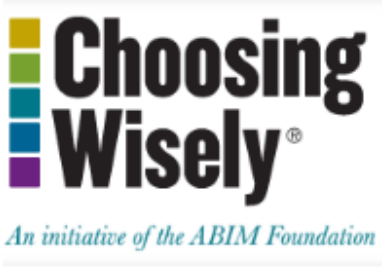
BACTERIA IN URINE DOESN'T ALWAYS INDICATE INFECTION
Testing, Antibiotic Treatment Often Unnecessary, Say IDSA Guidelines

Society for Healthcare Epidemiology of America

View all recommendations from this society

Released October 1, 2015; Revised December 2, 2019

Don't perform cultures (e.g. urine, blood, sputum cultures) or test for *C. difficile* unless patients have signs or symptoms of infection. Tests can be falsely positive leading to over diagnosis and overtreatment.



The American Society for Microbiology

View all recommendations from this society

August 5 2020

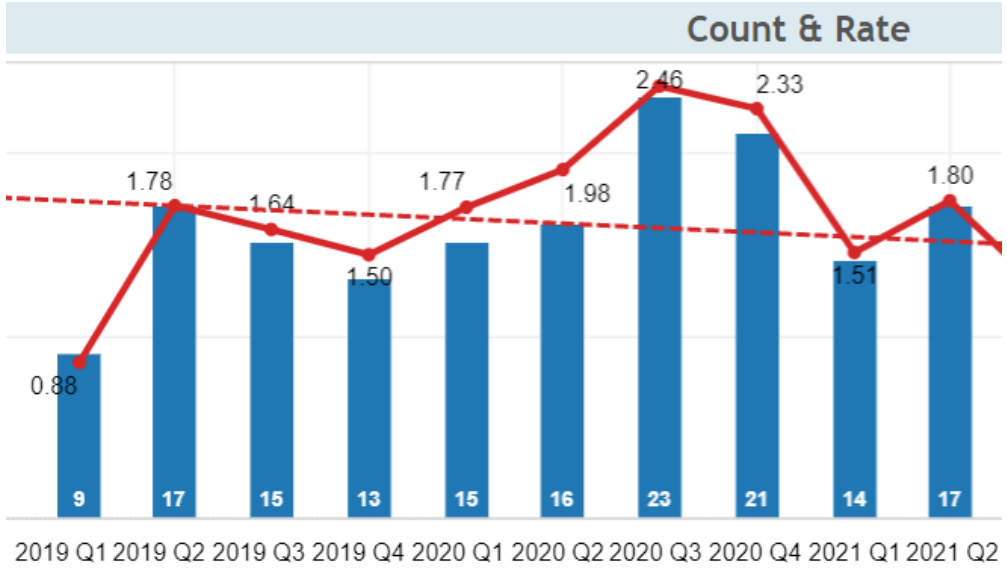
Do not order urine cultures unless patients have symptoms consistent with urinary tract infection (UTI).

Urine cultures should only be requested on patients who have clinical signs of UTI. Routine culture of urine in asymptomatic individuals may detect asymptomatic bacteriuria (ASB) which is commonly found in certain populations. Screening for ASB has no clinical benefit and may result in harm (1, 2).

Testing for ASB should only be pursued in specific populations such as pregnant women and individuals who are about to undergo urologic procedures that involve mucosal disruption (2).

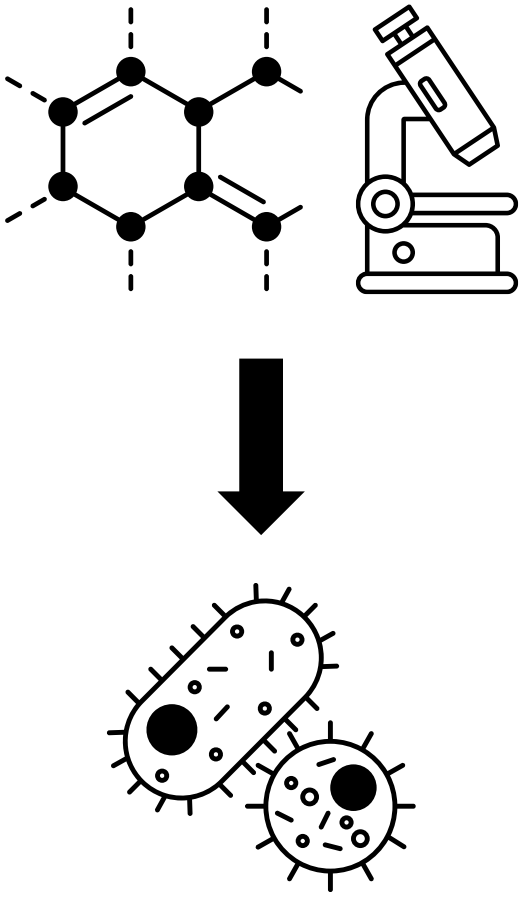
How does over-ordering affect CAUTIs?

- Catheter-associated Urinary Tract Infection
- Reportable data (CDC/NHSN)
- Positive urine culture ≠ UTI
  - Especially with the absence of pyuria
  - Catheterized and non-catheterized patients
  - But still can be defined as CAUTI
- Fewer cultures = fewer CAUTIs?



How can the lab help with compliance?

- Implement urinalysis reflex approaches (?)
- Evaluate and/or define reflex criteria
  - Lack of evidence-based guidance
- Offer various electronic order options
- Clarify which orders are appropriate for which patient populations



Urine culture reflex criteria

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What does the literature tell us?

- Multiple studies show high NPV for pyuria
- WBC > 5 or 10/hpf
- Including studies using automated microscopy
- Positive LE and nitrite can increase sensitivity
- May decrease specificity
- Reflex may eliminate 40-70% of urine cultures
- Performance depends on patient population

Block DR. Automated urinalysis in the clinical lab. MLO 2012.
Humphries RM and Dien Bard J. *J Clin Microbio*. 2016.

Leukocyte esterase reaction

- Enzyme found in WBC
- Catalyzes hydrolysis of esters, releasing alcohols and acids that generate a color reaction
- Bacterial infection is most common cause
- Sensitivity corresponds to approx. 5-15 WBC/HPF
  - Generally considered as clinically significant
- Interferences:
  - False negatives: high glucose, some drugs/antibiotics, ascorbic acid (vitamin C)
  - False positives: oxidizing agents, vaginal discharge contamination

Nitrite reaction

- *Most* bacteria reduce nitrate (plentiful in normal urine) to nitrite
- Nitrites react with amine on pad to form a pink color (Griess reaction)
- Positive corresponds with >10^5/mL bacteria
- Nitrite formation increases as urine is retained in bladder
  - Minimum 4 hr preferred (or first-morning void)
- Nitrite can be produced by contaminant bacteria as voided sample ages (analyze quickly!)
- Interferences/limitations:
  - False negatives: shortened bladder incubation, pathogen that does not reduce nitrate, antibiotics, ascorbic acid, absence of dietary nitrate
  - False positives: contaminant bacteria, old sample

What does our own data tell us?

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Chemical UA vs. urine culture

Contingency table correlating chemical urinalysis results to urine culture results.

		Urine culture		
Chemical urinalysis		+	–	Total
	+	645	1610	2255
				28.6% PPV
	–	55	817	872
				93.7% NPV
	Total	700	2427	3127
		92.1% sensitivity	33.7% specificity	

PPV, positive predictive value; NPV, negative predictive value.

What does our own data tell us?

UCLA Health

Chemical UA vs. urine culture

Association of urine culture results with chemical urinalysis results.

Urine culture result	Number of samples	Positive leukocyte esterase (%)	Positive nitrite (%)	Positive blood (%)	Positive protein (%)	Positive glucose (%)
Negative	1164	205 (17.6)	11 (0.9)	398 (34.2)	353 (30.3)	53 (4.6)
Contaminant	1263	455 (36.0)	22 (1.7)	437 (34.6)	389 (30.8)	62 (4.9)
Positive	700	526 (75.1)	184 (26.3)	419 (59.9)	339 (48.4)	56 (8.0)
Total	3127	1186 (37.9)	217 (6.9)	1254 (40.1)	1081 (34.6)	171 (5.5)

Chambliss et al. *J Appl Lab Med*. 2020.

What does our own data tell us?

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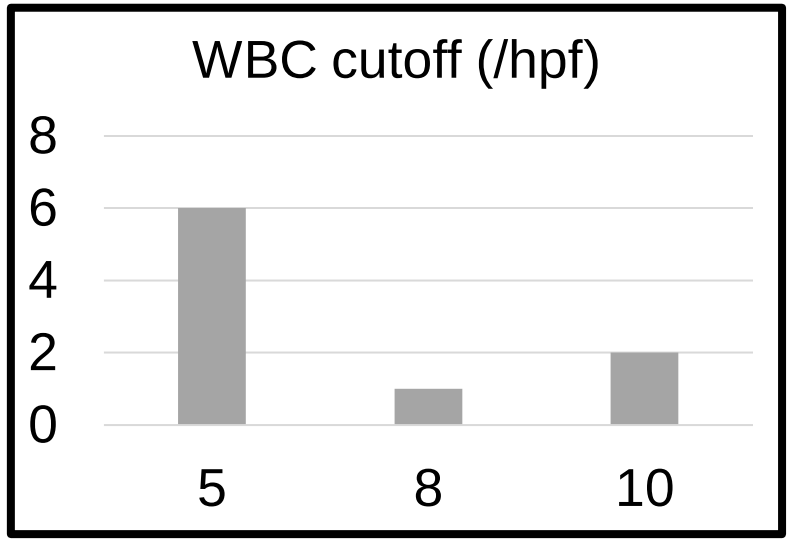
Chemical UA vs. WBC count

Association of WBC count with chemical urinalysis and urine culture results.

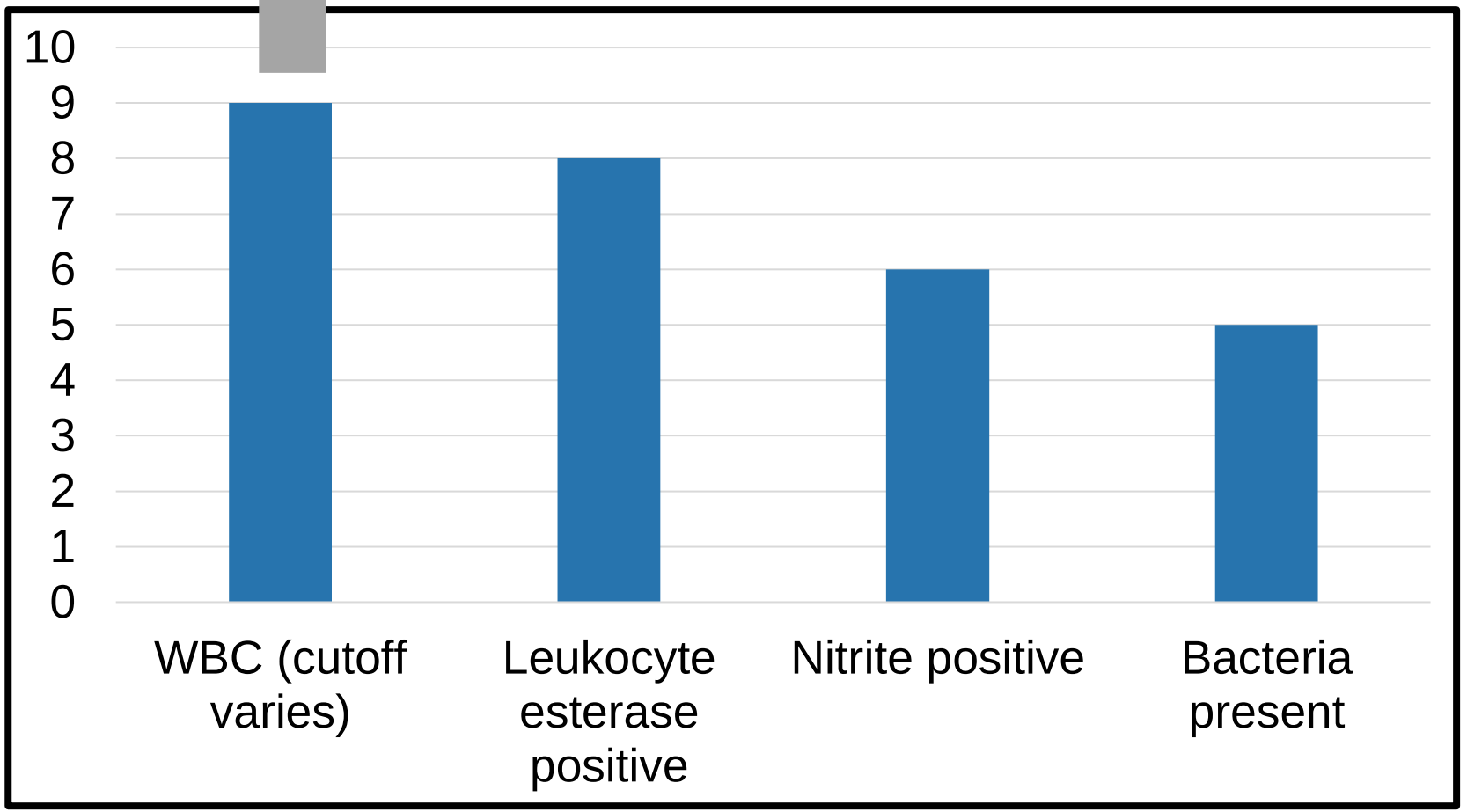
	Chemical UA (n = 9127)						Positive culture (%) (*n* = 700)
WBC/HPF	**Number of samples**	**Positive leukocyte esterase (%)**	**Positive nitrite (%)**	**Positive blood (%)**	**Positive protein (%)**	**Positive glucose (%)**	
0	2819	22 (0.8)	14 (0.5)	274 (9.7)	421 (14.9)	313 (11.1)	43 (6.1)
1-5	4241	637 (15.0)	63 (1.5)	908 (21.4)	1325 (31.2)	499 (11.8)	157 (22.4)
6-10	631	480 (76.1)	39 (6.2)	204 (32.3)	241 (38.2)	78 (12.4)	73 (10.4)
11-20	411	381 (92.7)	28 (6.8)	157 (38.2)	190 (46.2)	50 (12.2)	51 (7.3)
21-50	396	383 (96.7)	61 (15.4)	176 (44.4)	215 (54.3)	57 (14.4)	100 (14.3)
>50	629	618 (98.3)	159 (25.3)	422 (67.1)	461 (73.3)	97 (15.4)	276 (39.4)
Total	9127	2521 (27.6)	364 (4.0)	2141 (23.5)	2853 (31.3)	1094 (12.0)	700 (100.0)

Chambliss et al. *J Appl Lab Med*. 2020.

Informal peer survey of culture reflex criteria



n=10 clinical labs



What's common in other major labs?

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Table 2. Urinalysis order options and reflex criteria used by selected major U.S. reference and hospital laboratories[a].

Reference laboratory	UA macroscopic only	UA microscopic only	Complete UA (macroscopic + microscopic)	UA with reflex to microscopic	Criteria for reflex to microscopic	Complete UA with reflex to culture	Criteria for reflex to culture
ARUP Laboratories (University of Utah Health)	X		X		N/A	X	WBC > 5/HPF
LabCorp			X	X	+ protein; + LE; + blood; + nitrite	X	+ nitrite; + LE; WBC > 5/HPF; bacteria ≥ moderate
Quest Diagnostics	X	X	X	X	Not specified	X	+ LE; WBC > 5/HPF; + yeast; + bacteria AND WBC > 5/HPF OR + LE; + nitrite AND WBC > 5/HPF OR + LE
Cleveland Clinic	X		X		N/A		N/A
Johns Hopkins Hospital	X	X	X		N/A	X	Not specified
New York Presbyterian	X	X	X	X	Not specified	X	WBC ≥ 10/HPF
Massachusetts General Hospital				X	+ protein; + LE; + blood		N/A
UCSF Health	X		X		N/A	X	+ protein, LE, or blood; and WBC > 10/HPF

[a]Based on a review of publicly available online test menus accessed 7/16/2021.

A success story at Washington University

- Algorithm tweaked over 7+ years
- Now only reflex culture off of 10 WBC/hpf
  - Initially included blood, protein, LE, nitrite
- Pre-packaged 2-tube collection kit
  - Allows culture stability up to 48 h
- Multiple orders for different patient populations
  - Neutropenic patients – will reflex despite WBC
    - May reflex off of CBC results in the future
  - Strategically place in order sets

Fewer urine cultures — series of changes add up, CAP Today, November 2020.
Urine test ordering—good and going for better, CAP Today, December 2022

Multiple EHR order options

Test name	Definition
UA Reflex to Microscopy WITH Culture *new	If urinalysis is positive for nitrites OR leukocyte esterase, then microscopy and urine culture will automatically be performed
UA Reflex for Neutropenic Patients	If urinalysis is positive for protein (>trace), blood, nitrites, OR leukocyte esterase, then microscopy and urine culture will automatically be performed
UA Reflex to Microscopy WITHOUT Culture	If urinalysis is positive for protein (>trace), blood, nitrites, OR leukocyte esterase, then microscopy will automatically be performed
UA Dip Macroscopic	Macroscopic Dipstick Urinalysis only
UA Microscopy	Urine Sediment Examination only

Washington University study

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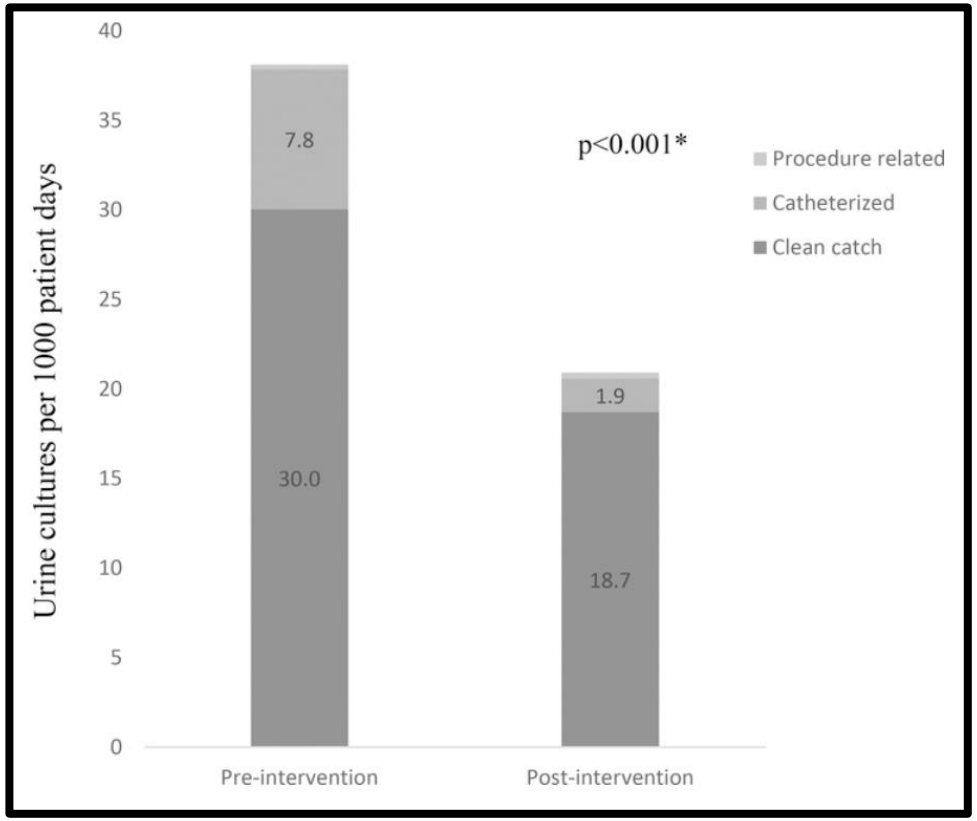
Intervention of new reflex orders decreased urine cultures and increased culture positivity rate



	Pre-	Post-
Urine cultures per 1000 patient days	38.1	20.9
Percent positive cultures	25.5%	29.7%
CAUTIs per 1000 patient days	0.30	0.30

Munigala S et al. *Infect Control Hosp Epidemiol*. 2019.

Other outcomes reported in the literature

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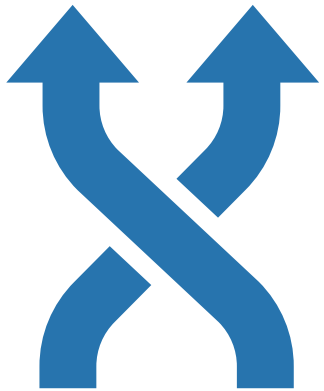
Table 4. Summary of outcomes studies of urinalysis reflexive urine culture practices.

Study setting	Reflex intervention criteria	Summary of outcomes	References
Emergency department of a suburban, tertiary care academic medical center	>10 WBC/HPF, positive nitrites, or any bacteria	Predicted to eliminate 604/1546 (39%) urine cultures	[51]
Outpatient urology clinic	>5 WBC/HPF	Avoid 69% of culture orders, with 7% missed positive urine cultures Predicted cost savings of $46,800 in 2006 ($77.63/culture)	[52]
400-bed acute care hospital	evaluated multiple criteria; ≥5 WBC/HPF and positive nitrite yielded the highest PPV	Pre-intervention: 45% ($n = 590$) were inappropriately started on antibiotics. Post-intervention: 9% ($n = 81$) inappropriate initiation of antibiotics	[54]
3 urban emergency departments	Positive LE or positive nitrite or ≥ 6 WBC/HPF or ≥ few yeasts or ≥ moderate bacteria	Urine culture orders/100 ED visits decreased: 15.2 vs. 9.3 Number of normal or negative urine cultures decrease by 2.42 cultures/100 ED visits	[58]
685-bed adult and 292-bed pediatric tertiary academic medical center	Positive nitrites or small or greater LE or ≥ 5 WBC/HPF	Urine culture rate: decreased by 6.9 cultures/1000 patient days CAUTI rate: increased by 0.2/1000 catheter days	[59]
727-bed acute care and long-term care health care system	>10 WBC/HPF	Acute care: 3.58 (pre) vs. 1.82 (post) cultures performed/100 days	[60]

Variability in observed clinical outcomes

Variability in:

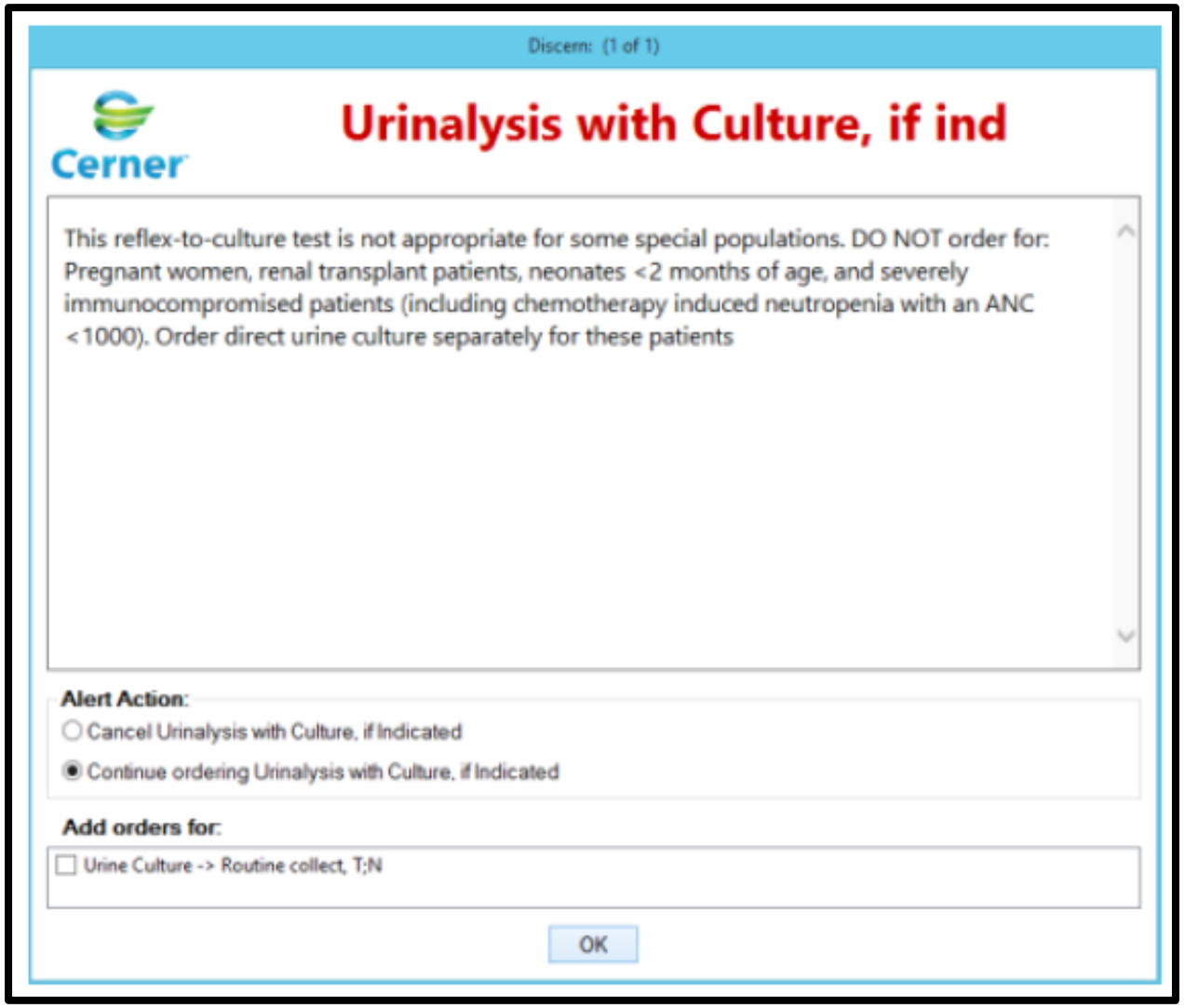
- UA and urine culture practices
- Reflexive algorithm design
- Patient populations
- Adherence to algorithms
  - e.g., ability to order culture despite UA results



Laboratory workflow considerations

- LIS order
- Specimen containers x 2
- Label printing
- What triggers Microbiology to start culture?
- Where are tubes stored/held?
- Provider education/clinical decision support

Clinical decision support (example)



Final considerations for reflex algorithms

- Multiple stakeholders
  - In lab: Core, Micro, possibly POCT
  - Outside of lab: ID, Primary Care, Nephrology, Urology, OBGYN, Pediatrics…
- Consider your patient population(s)
- Design and position EHR orders intelligently
  - Most effective way to influence physician ordering
- Likely to reduce cultures, but long-term clinical/reportable outcomes remain uncertain

Emerging technologies for UTI diagnosis

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Method	Advantages	Challenges
Urinalysis	• fast • cheap(er)	• not specific to UTI • no treatment information
Urine culture	• allows for susceptibility testing • cheap(ish)	• slow • limited by type of bacteria that will grow • must differentiate between contamination and true infection
Rapid molecular detection	• fastest • potentially higher detection rates	• may be limited by genetic targets • must differentiate between contamination and true infection • most expensive

Acknowledgements

- **LAC+USC and Harbor-UCLA CLS's**
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- **Taryn Fox (Cerner)**

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