

به نام خدا



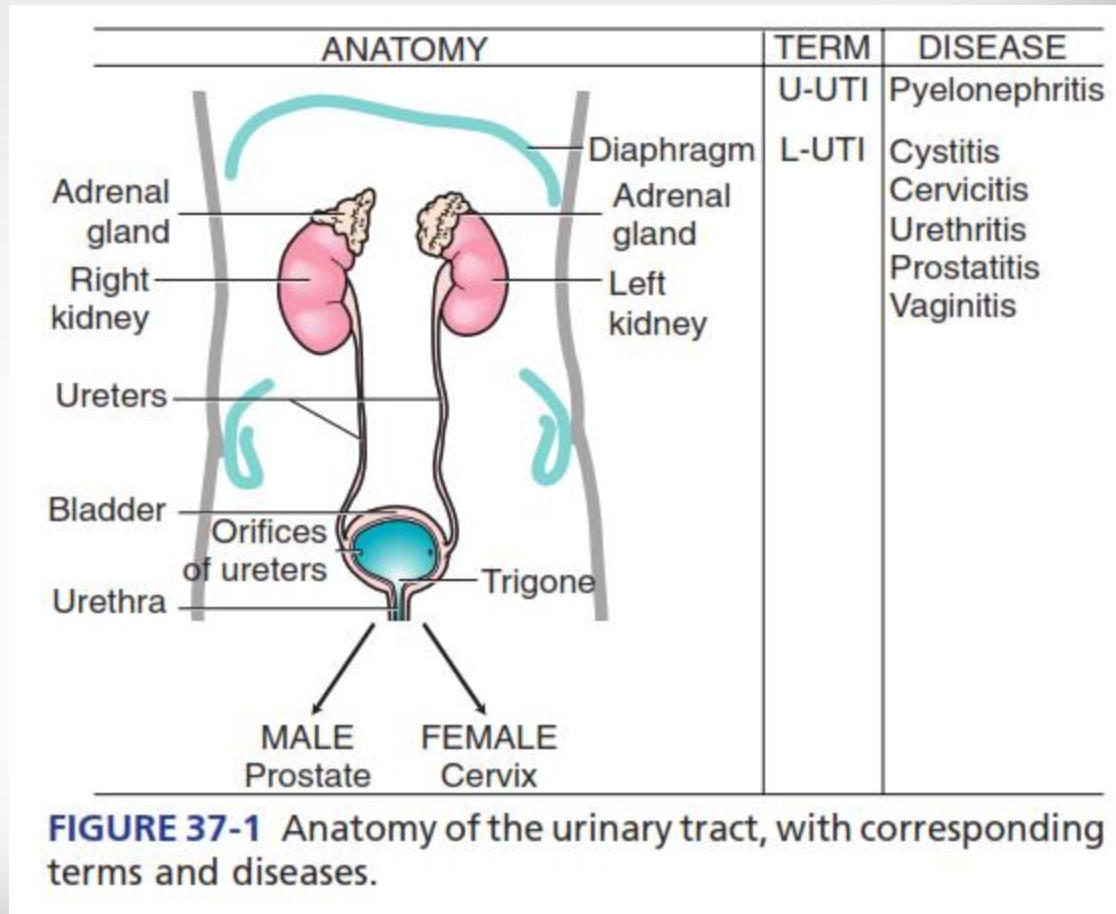
# Bacteriology Lab 2

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# میکروارگانیزم های مجاری ادراری

• کشت ادرار از آزمایش ها رایج در مراکز تشخیص طبی است. ادرار مایعی استریل است اما محیط خوبی برای رشد باکتری هاست. و به آسانی با فلور پوستی ساکن در آنجا آلوده می شود. عفونت های ادراری از شایعترین عفونت های بیمارستانی یا nosocomial infection هستند. عفونت دستگاه ادراری را در urine analysis میتوان تشخیص داد که با وجود باکتری های زیاد . گلبول های سفید در زیر میکروسکوپ نیز انجام می شود. این مساله می تواند دلیل بر عفونت مثانه، عفونت پاراننشیم کلیه، اورتریت ، عفونت حالب یا عفونت مجرا باشد.



## BOX 37-1 Terms and Abbreviations Commonly Used for Urinary Tract Diseases

**Acute urethral syndrome.** Includes dysuria and pyuria. Defined as more than 8 leukocytes/mm<sup>3</sup> of uncentrifuged urine or  $\approx$ 2 to 5 leukocytes/hpf in centrifuged urine sediment.

**Bacteriuria.** The presence of detectable bacteria in the urine. Patients may be symptomatic or asymptomatic (e.g., geriatric or pregnant patients).

**Cervicitis.** Inflammation of the cervix; it may occur as an acute or a chronic presentation. Causative agents include sexually transmitted organisms, such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Symptoms include dysuria, urgency, vaginal discharge, and low back pain.

**Cystitis.** Inflammation of the bladder, presenting as dysuria, urinary frequency, and urgency. It is often caused by gram-negative bacilli, such as *E. coli*, *Proteus*, and *Klebsiella*. It occurs more frequently in women than men. It can also be caused by medication and certain viruses, such as adenovirus.

**Lower urinary tract infection (L-UTI).** A genitourinary (GU) tract infection limited to the urethra (urethritis), bladder (cystitis) and, in males, the prostate (prostatitis). These infections generally appear in adults with dysuria (pain on urination), increased frequency, urgency, and occasionally suprapubic tenderness.

**Prostatitis.** A GU infection in males that involves the prostate; fever is often present.

**Pyelonephritis.** Infection in the kidney. This is often caused by infection in the lower tract ascending to the kidney. Symptoms include fever, chills, nausea, vomiting, and lower back tenderness, as well as dysuria. It can be accompanied by bacteremia.

**Upper urinary tract infection (U-UTI).** A GU tract infection limited to the renal parenchyma (pyelonephritis) or the ureters (ureteritis). It is often accompanied by L-UTI symptoms in addition to costovertebral flank pain or tenderness and fever. At times, L-UTI precedes the appearance of fever and U-UTI by 24 to 48 hours.

**Urethritis.** Inflammation of the urethra, presenting as dysuria and discharge. Causative agents include *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Ureaplasma urealyticum*. Other causes include trauma, allergic, or chemical factors.

**Urinary tract infection (UTI).** A spectrum of diseases caused by microbial invasion of the genitourinary (GU) tract that extends from the renal cortex of the kidney to the urethral meatus (see Figure 37-1).

## • BOX 72-1 Resident Microbiota of the Urethra

Coagulase-negative staphylococci (excluding *Staphylococcus saprophyticus*)

Viridans and nonhemolytic streptococci

Lactobacilli (adult females)

Diphtheroids (*Corynebacterium* spp.)

Nonpathogenic (saprobic) *Neisseria* spp. (adult women)

Anaerobic cocci

*Propionibacterium* spp. (adult patients)

Commensal *Mycobacterium* spp.

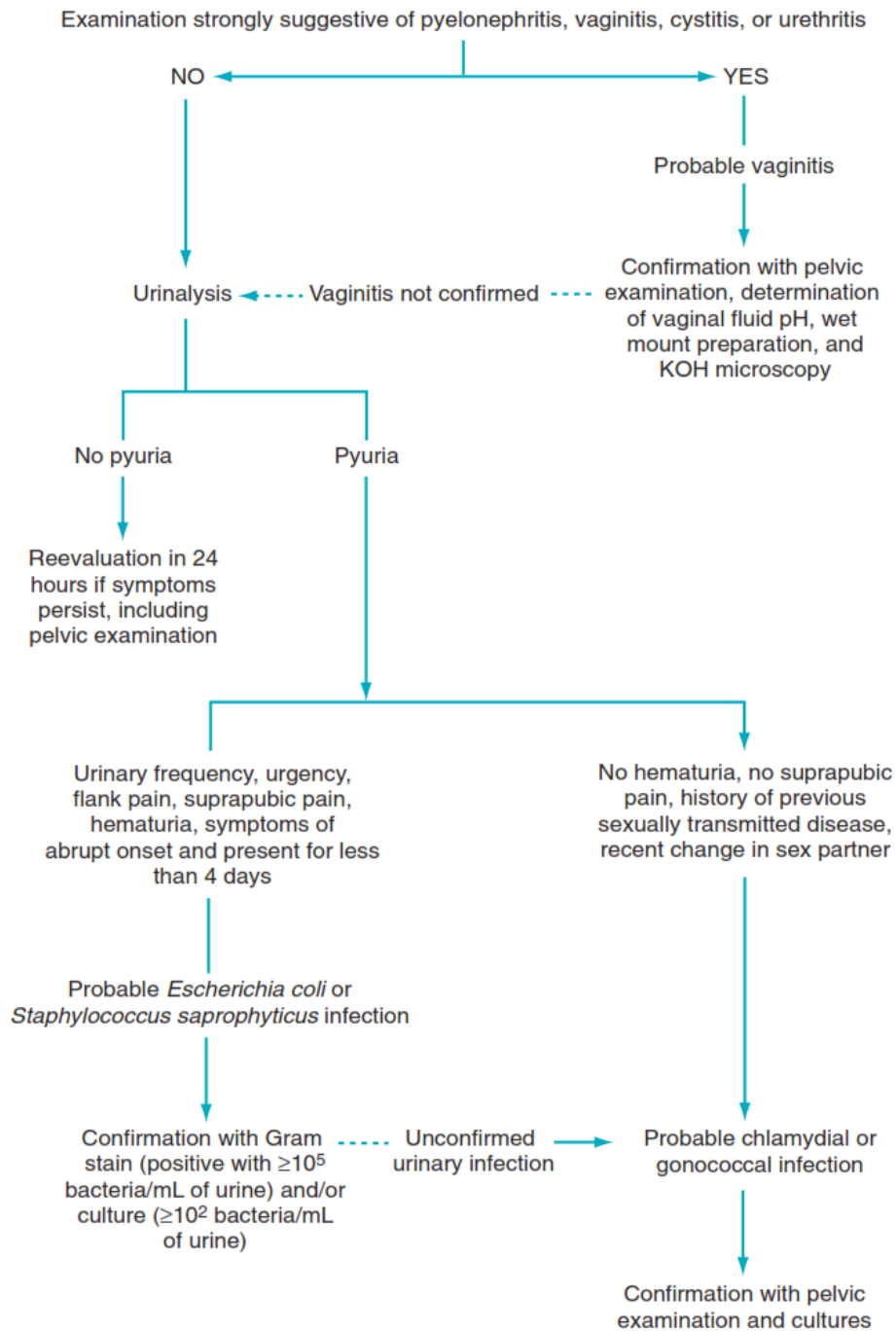
Commensal *Mycoplasma* spp.

Yeasts (pregnant, adult females)

**TABLE 37-1 Comparative Parameters for Urine in Control Subjects and Patients with Urinary Tract Infections**

Parameter	Ranges		
	Normal	Abnormal	
		Cystitis	Pyelonephritis
Chemistries			
Specific gravity	1.001-1.035		
Volume (average/24 hr)			
Child (1-14 yr)	500-1400		
Adult (younger than 60 yr)	600-1800		
Adult (older than 60 yr)	250-2400		
pH range	4.7-8.0 (6.0 average)		
Protein	Negative to trace		Increased
WBC esterase	Negative		
Nitrite	Negative	Positive	Positive
Microscopic			
WBCs			
Male	0-3/hpf	Variable	Elevated to greatly increased
Female/child	0-5/hpf	Variable	Elevated to greatly increased
RBCs	0-2/hpf	Variable	Variable to greatly increased
Epithelial cells			
Squamous			Variable/hpf
Renal	0-1/hpf		
Transitional	0-2/hpf		
Crystals	Variable	Negative	Negative
Mucus	Variable	Negative	Negative
Casts			
Hyaline	0-2/lpf		
Granular	0-1/lpf		
WBC	Negative	Negative	Positive
Microorganisms			
Bacteria	Less than 1/hpf	Variable	Positive
Yeast	Negative	Variable	Variable
<i>Trichomonas</i> spp.	Negative	Variable	Negative

*hpf*, high-power field; *lpf*, low-power field; *RBCs*, red blood cells; *WBCs*, White blood cells.



**FIGURE 37-3** Evaluation of women with acute dysuria. *KOH*, Potassium hydroxide.



**TABLE 37-3 Flora of Normal Voided Urine\* Defined by Patient's Age and Status**

Patient Age, Status	Usual Flora
Newborn 1-3 days old	Sterile Staphylococci Enterococci Diphtheroids <i>Mycobacterium smegmatis</i>
Prepubertal	Micrococci Streptococci ( $\alpha$ -hemolytic and nonhemolytic) Coliforms Diphtheroids
Adult	<i>L. acidophilus</i> <i>Staphylococcus epidermidis</i> Streptococci ( $\alpha$ -hemolytic and nonhemolytic) <i>Escherichia coli</i> Diphtheroids Yeasts Anaerobic streptococci <i>Listeria</i> spp. <i>Clostridium</i> spp.
Pregnancy	Increase in <i>L. acidophilus</i> Yeasts
Postmenopausal	<i>S. epidermidis</i> Similar to prepubertal flora

\*Usually sterile or <1000 colonies/mL.

## **BOX 37-3** Recognized Microbial Agents of Urinary Tract Infections

### **Common Agents**

Enterococci (including vancomycin-resistant enterococci)  
*Streptococcus agalactiae* (group B streptococci)  
Enterobacteriaceae (especially *Escherichia coli*)  
*Pseudomonas* spp.  
*Streptococcus pyogenes* (group A streptococci)  
*Staphylococcus aureus*  
*Staphylococcus saprophyticus*  
*Candida* spp.

### **Less Common Agents**

*Gardnerella vaginalis*  
*Ureaplasma urealyticum*  
*Mycoplasma hominis*  
*Mobiluncus* spp.  
*Leptospira* spp.  
*Mycobacterium* spp.  
*Chlamydia trachomatis* (males)

### **Agents Often Associated with Multisystem Diseases**

*Salmonella* spp. (with gastroenteritis)  
*Schistosoma haematobium*  
*Cryptococcus neoformans*  
*Trichosporon beigelii*  
*Trichomonas vaginalis*  
*Aspergillus* spp.  
*Penicillium* spp.  
Adenovirus  
Herpes simplex virus

**TABLE 37-4 Urinary Tract Infections Caused by Coagulase-Negative Staphylococci**

Characteristics of Infections	Organism	
	<i>S. epidermidis</i>	<i>S. saprophyticus</i>
Sex and gender of affected patient	Men and women equally	Women 95%, age 16-35 yr
Population at risk	Hospitalized patients with urinary tract complications	Healthy outpatients
Incidence	Common—20% or more of all UTIs for hospitalized patients >50 yr	Uncommon—3.5% or fewer of all UTIs in hospitalized patients
Presentation	90% asymptomatic	90% symptomatic; indistinguishable from <i>Escherichia coli</i> UTIs
Therapy	Often resistant to multiple drugs	Responds readily to traditional urinary tract antimicrobials except nalidixic acid
Outcome	Bacteriuria often persists after therapy.	Relapse rare; occasional reinfection

**TABLE 37-5 Most Common Causative Agents of Urinary Tract Infections Associated with Frequent Clinical Presentation (Disease Syndromes)**

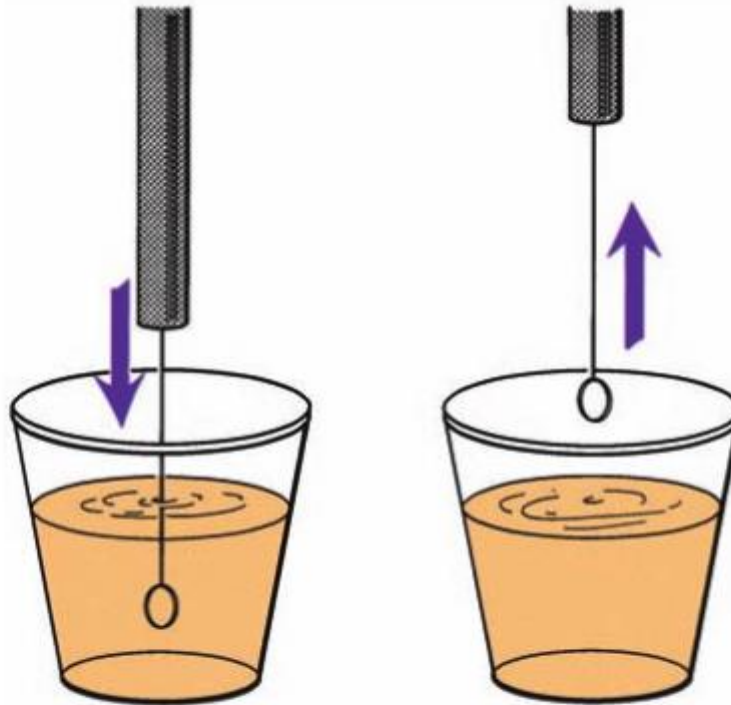
Clinical Presentation	Common Causative Agents
<b>Upper Urinary Tract Infections</b>	
Acute pyelonephritis	Enterobacteriaceae <i>Staphylococcus aureus</i>
Subclinical pyelonephritis	Coagulase-negative staphylococci <i>Candida</i> spp. <i>Mycobacterium</i> spp. <i>Mycoplasma hominis</i>
<b>Lower Urinary Tract Infections</b>	
Acute bacterial cystitis	<i>Escherichia coli</i> <i>Klebsiella</i> spp. Other Enterobacteriaceae Enterococci Coagulase-negative staphylococci
<b>Urethritis</b>	
Acute urethral syndrome	<i>Chlamydia trachomatis</i> <i>Neisseria gonorrhoeae</i> <i>Ureaplasma urealyticum</i>
<b>Other Infections</b>	
Gonococcal urethritis	<i>N. gonorrhoeae</i>
Chlamydial urethritis	<i>C. trachomatis</i>
Vaginitis	
Prostatitis	
Symptomatic bacteriuria	
Catheter-associated (hospital-associated) UTI	<i>E. coli</i> <i>Klebsiella</i> spp. <i>Proteus mirabilis</i> <i>Pseudomonas</i> spp. <i>Candida</i> spp.
Chronic or recurrent (inpatient/outpatient) UTI	Adherent <i>E. coli</i>

UTI, Urinary tract infection.

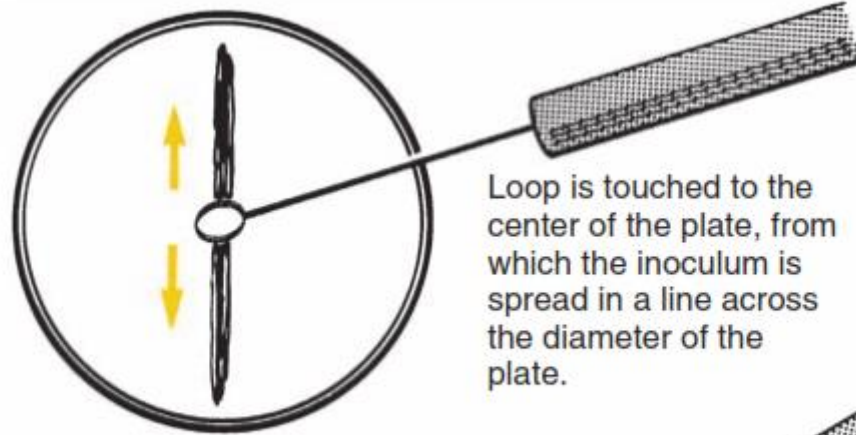
## Pathogens and commensals

Urine specimen	
Common pathogens	commensal flora
<i>Neisseria gonorrhoeae</i>	the urine is sterile except for the urethral mucosa which support the growth of microflora as:
<i>E. coli</i> and other Enterobacteriaceae	
<i>Enterococcus spp</i>	Diphtheroid bacilli
<i>Staphylococcus aureus</i>	<i>Lactobacillus spp</i>
<i>Staph saprophyticus</i>	Coagulase negative <i>Staphylococci</i>
<i>Corynebacterium jeikeium</i>	$\alpha$ Haemolytic <i>Streptococci</i>
<i>Acinetobacter spp</i>	<i>Bacillus spp</i>
<i>Pseudomonas spp</i>	Non pathogenic <i>Neisseria spp.</i>
<i>Gardnerella vaginalis</i>	Anaerobic cocci
$\beta$ -haemolytic streptococci	Commensal <i>Mycobacterium</i>
<i>Salmonella spp</i> (early stage of infection)	Commensal <i>Mycoplasma spp.</i>
<b>Parasites</b>	
<i>Schistosoma haemetobium</i>	
<i>Trichomonas vaginalis</i>	

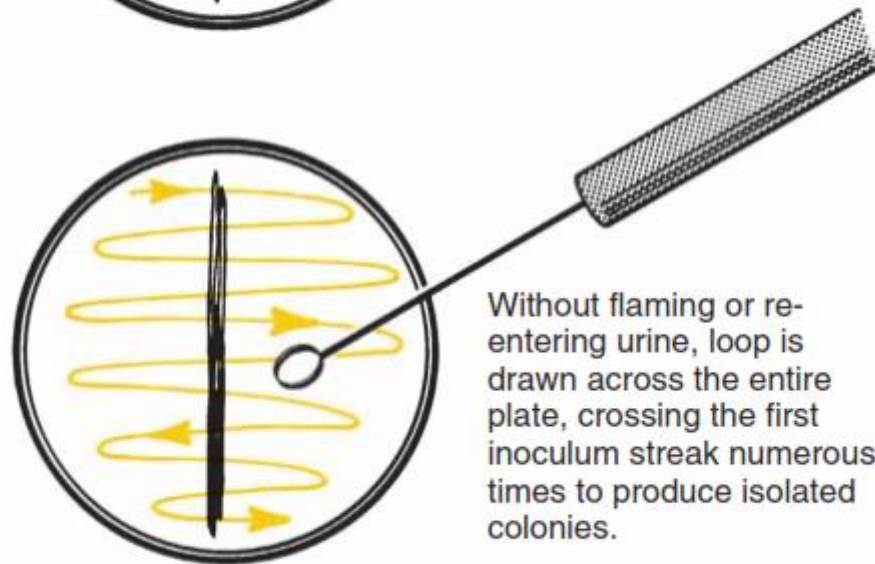
# تشخیص آزمایشگاهی



• **Figure 72-2** Method for inserting a calibrated loop into urine to ensure that the proper amount of specimen adheres to the loop.



Loop is touched to the center of the plate, from which the inoculum is spread in a line across the diameter of the plate.



Without flaming or re-entering urine, loop is drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies.

• **Figure 72-3** Method for streaking with calibrated urine loop to produce isolated colonies and countable colony-forming units.



**TABLE 37-6 Manual Screening Methods, Principles of Assay, and Threshold of Detection for Urinary Tract Infections**

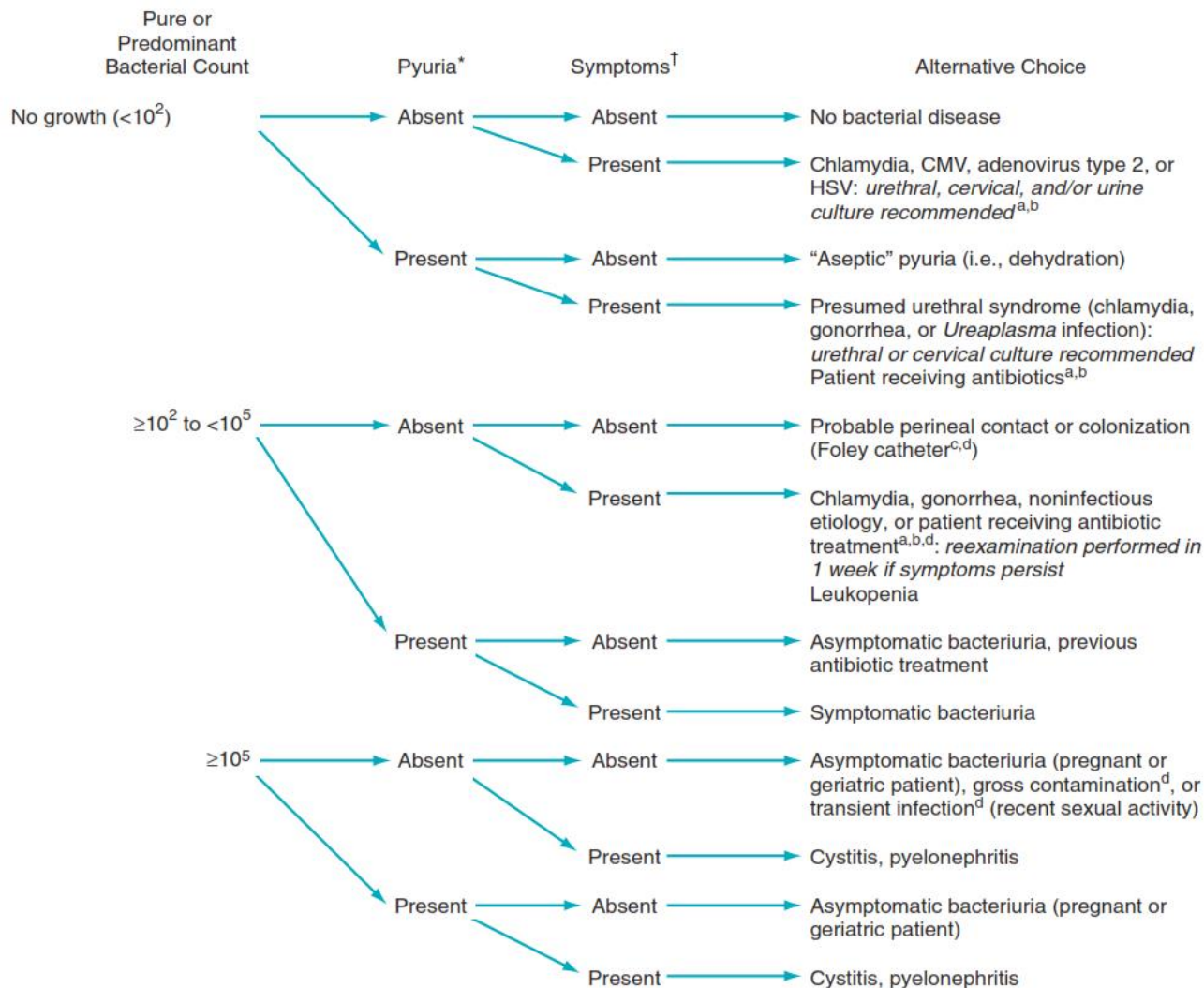
Screens	Principle	Reported Threshold of Detection (CFU/mL)
<b>Manual</b>		
Microscopy Direct, uncentrifuged or centrifuged (cytospin)	Recognition of organism morphotypes and Gram stain	≥One organism/OIF ≥10 <sup>5</sup>
<b>Chemical</b>		
<b>Enzymatic Dipstick</b>		
Nitrate reductase (Griess test)	Gram-negative bacteria reduce nitrates to nitrites	
WBC leukocyte esterase	Measures presence of WBC enzyme	Equivalent to 5 WBC/hpf
Chemstrip LN	Combination testing of nitrate and esterase assays	>10 <sup>4</sup> -10 <sup>5</sup>
<b>Enzyme Tube</b>		
Uriscreen	Measures catalase present in bacteria and somatic cells	<10 <sup>4</sup> -10 <sup>5</sup>
<b>Colorimetric Particle Filtration</b>		
FiltraCheck—UTI	Combination testing of bacteria and WBCs by membrane filtration and detection using safranin O dye	>10 <sup>4</sup>

CFU, Colony-forming unit; hpf, high-power field; OIF, oil immersion field; WBC, white blood cell; UTI, Urinary tract infection.

**TABLE 37-7 Automated Screening Methods, Principle of Assay, and Threshold of Detection for Urinary Tract Infections**

Automated Method	Principle	Threshold of Detection (CFU/mL)
<b>Bioluminescence</b>		
UTI screen	Detects bacterial ATP using enzymatic bioluminescent reaction of ATP with luciferin and luciferase	$>10^4$ - $10^5$
Photometry	If a significant number of organisms are present in the urine specimen, they will grow in the medium to a detectable concentration using photometry.	$>10^4$ - $10^5$
<b>Colorimetric Particle Filtration</b>		
Bac-T-Screen	Automated combination testing for bacteria and WBCs by membrane filtration and detection using safranin O dye	$>10^4$ - $10^5$
Flow cytometry	Detects and quantifies bacteria, WBCs, RBCs with color-coded scatter diagrams	$>10^3$

*ATP*, Adenosine triphosphate; *CFU*, colony-forming unit; *RBC*, red blood cell; *WBC*, white blood cell; *UTI*, Urinary tract infection.



- If patient is receiving antibiotic treatment, the result of the Gram stain, WBC analysis, and culture may not agree.
- Quantitation of organisms and white cells by urinalysis of a centrifuged specimen is of no comparative value for the measurement of leukocyte esterase and bacteria done by microbiologic study, which is performed routinely on a noncentrifuged specimen.
- Interpretation for indwelling catheter has not been established.
- Plates held 72 hours for consultation.

\*Leukocyte esterase (+); equivalent to 5 WBC/hpf.

†Clinical dysuria and frequency.

**FIGURE 37-4** Interpretation of urine culture results using algorithm based on bacterial colony count, pyuria, and symptoms. *CMV*, Cytomegalovirus; *hpf*, high-power field; *HSV*, herpes simplex virus; *WBC*, white blood cell.

**TABLE 37-8 Guidelines for Interpretation of Urine Culture Results and Subsequent Workup**

Colony Count (CFU/mL)*	Symptoms, Clinical Disease, or Patient Population <sup>†</sup>	Urine Source <sup>‡</sup>	No. of Organism Types Isolated	Laboratory Workup Suggested (Inpatient) <sup>§</sup>
<10 <sup>†</sup>		CV, CA	None	None <sup>¶</sup>
≥10 <sup>†</sup>	Pediatric	Suprapubic	≤Two organisms by anaerobic culture	ID and AST
≥ <sup>†</sup>	Symptomatic female, urethritis	CV	Pure culture	ID and AST
≥ <sup>‡</sup>	Symptomatic male, prostatitis	CA	≤Two organisms	ID and AST
		CA	Pure culture	ID and AST
≥10 <sup>‡</sup>		Bladder washout		ID and AST
≥10 <sup>¶</sup>	Cystitis, pyelonephritis	CV	Pure culture	ID and AST
			Two or three organisms	Q and SID
			Three organisms	Q and M or Q and GS

CFU, Colony-forming unit; ID and AST, perform identification and antimicrobial susceptibility testing; Q and GS, quantitate and report Gram stain morphotypes; Q and M, quantitate total amount of bacteria and report as "mixed urethral flora"; Q and SID, quantitate and perform sight identification, identification and sensitivity not indicated, hold plates 72 hours.

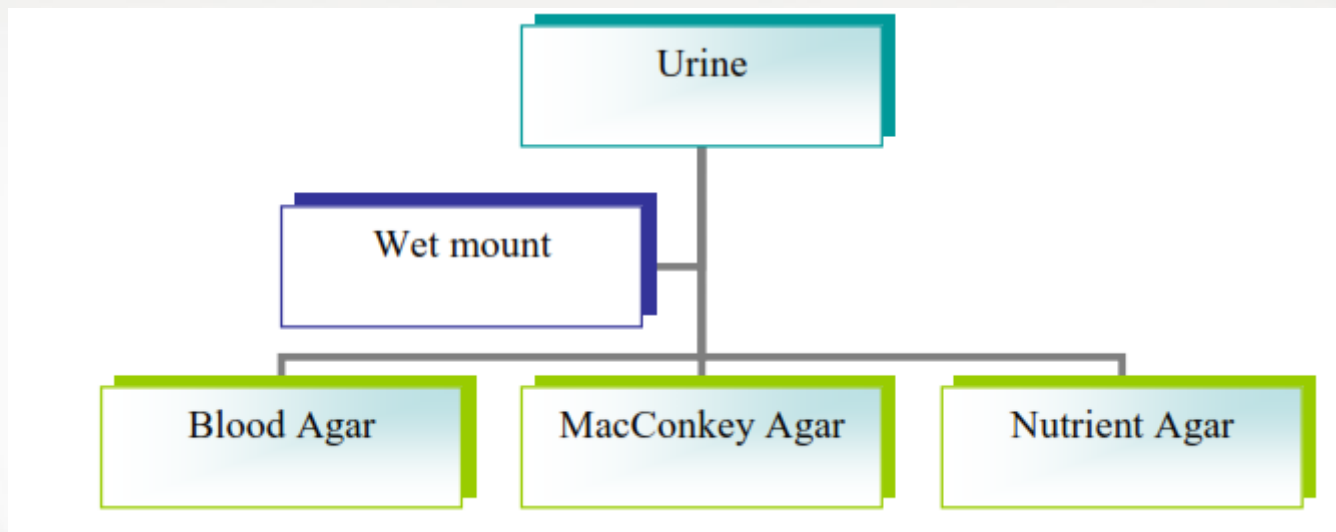
\*Inoculation of 0.01 mL of urine is required to detect 10<sup>2</sup> CFU/mL.

<sup>†</sup>See Table 37-1 for description of clinical diseases, symptoms, and patient population.

<sup>‡</sup>CA, Straight catheterized; CV, clean-catch voided.

<sup>§</sup>Workup required. Any yeast may be quantitated and reported (regardless of number); >100,000 needed to identify to species.

<sup>¶</sup>See figures and text for suggested comments and educational information helpful to physicians.



**TABLE  
72-1**

**Criteria for Classification of Urinary Tract Infections by Clinical Syndrome**

Category	Clinical	Laboratory
Acute, uncomplicated UTI in women	Dysuria, urgency, frequency, suprapubic pain No urinary symptoms in last 4 weeks before current episode No fever or flank pain	$\geq 10$ WBC/mm <sup>3</sup> $\geq 10^3$ CFU/mL uropathogens* in CCMS urine
Acute, uncomplicated pyelonephritis	Fever, chills Flank pain on examination Other diagnoses excluded No history or clinical evidence of urologic abnormalities	$\geq 10$ WBC/mm <sup>3</sup> $\geq 10^4$ CFU/mL uropathogens in CCMS urine
Complicated UTI and UTI in men	Any combination of symptoms listed above One or more factors associated with complicated UTI <sup>†</sup>	$\geq 10$ WBC/mm <sup>3</sup> $\geq 10^5$ CFU/mL uropathogens in CCMS urine
Asymptomatic bacteriuria: female patients	No urinary symptoms	$\pm > 10$ WBC/mm <sup>3</sup> $\geq 10^5$ CFU/mL in two CCMS cultures >24 hours apart
Asymptomatic bacteriuria: male patients	No urinary symptoms	$\pm > 10$ WBC/mm <sup>3</sup> $\geq 10^3$ CFU/mL (suggestive) $\geq 10^5$ CFU/mL (definitive) in one CCMS

\*Uropathogens: Organisms that commonly cause UTIs.

<sup>†</sup>Factors associated with complicated UTI include any UTI in a male patient, indwelling or intermittent urinary catheter, more than 100 mL of postvoid residual urine, obstructive uropathy, urologic abnormalities, azotemia (excess urea in the blood, even without structural abnormalities), and renal transplantation.

CCMS, Clean-catch midstream urine; CFU, colony-forming unit; UTI, urinary tract infection; WBC, white blood cells.

Data from Stamm WE: Criteria for the diagnosis of urinary tract infection and for the assessment of therapeutic effectiveness, *Infection* 20(suppl 3):S151, 1992;

Bennett J, Dolin R, Blaser M: *Principles and practice of infectious diseases*, ed 8, Philadelphia, 2015, Elsevier-Saunders.

**TABLE 72-2**

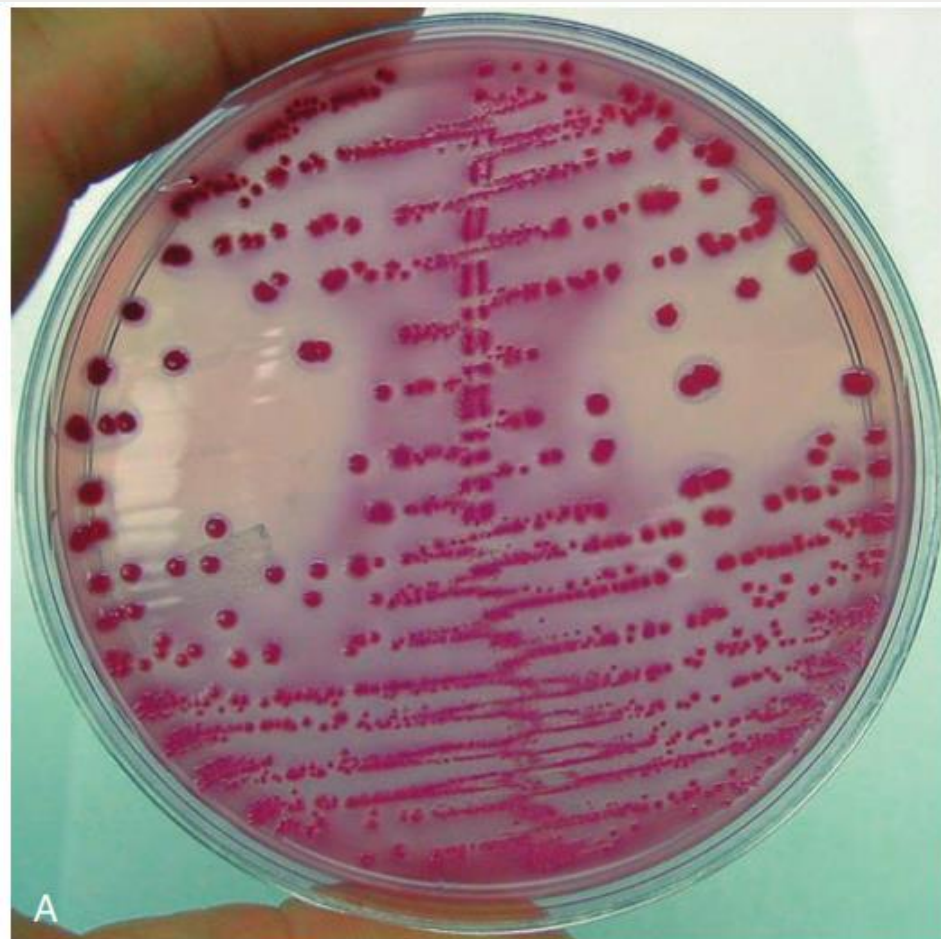
**General Interpretative Guidelines for Urine Cultures**

Result	Specific Specimen Type/Associated Clinical Condition, if Known	Workup
$\geq 10^4$ CFU/mL of a single potential pathogen or for each of two potential pathogens	CCMS urine/pyelonephritis, acute cystitis, asymptomatic bacteriuria, or catheterized urines	Complete*
$\geq 10^3$ CFU/mL of a single potential pathogen	CCMS urine/symptomatic male patients or catheterized urine or acute urethral syndrome	Complete
$\geq$ Three organism types with no predominating organism	CCMS urine or catheterized urine	None; because of possible contamination, ask for another specimen
Either two or three organism types with predominant growth of one organism type and $< 10^4$ CFU/mL of the other organism type(s)	CCMS urine	Complete workup for the predominating <sup>†</sup> organism(s); description of the organism(s)
$\geq 10^2$ CFU/mL of any number of organism types (set up with a 0.001- and 0.01-mL calibrated loop)	Suprapubic aspirates, any other surgically obtained urine (including ileal conduits, cystoscopy specimens)	Complete

\*A complete workup includes identification of the organism and appropriate susceptibility testing.

<sup>†</sup>Predominant growth is  $10^4$  to  $10^5$  CFU/mL or more.

CCMS, Clean-catch midstream urine; CFU, colony-forming unit.



• **Figure 72-4** Culture results illustrating some of the various interpretative guidelines. **A**, Growth of  $10^5$  CFU/mL or more of a lactose-fermenting gram-negative rod in a clean-catch midstream (CCMS) urine sample from a patient with pyelonephritis; complete workup would be done. **B**, Growth of  $10^5$  CFU/mL or more of a lactose-fermenting gram-negative rod (*arrow A*) and less than  $10^4$  CFU/mL of another organism type (*arrow B*) from a CCMS urine; only the organism with a colony count of at least  $10^4$  to  $10^5$  CFU/mL would be worked up completely. *CFU*, Colony-forming units.



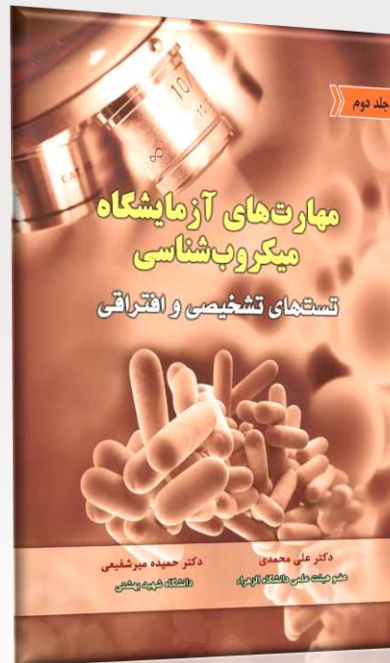
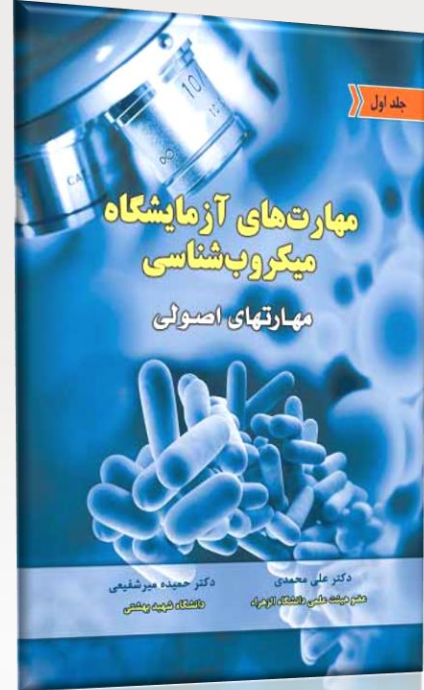
# مواد و روش ها

۱	نمونه مجهول	۷	تست اکسیداز
۲	سواب	۸	محیط های IMViC
۳	لوپ	۹	محیط های TSI, LIA و اوره
۴	محیط های کشت NA. MAC, EMB, BA	۱۰	محیط بایل اسکولین و نمک ۰.۵٪
۵	تست کاتالاز (آب اکسیژنه)	۱۱	محیط مولر هینتون آگار و دیسک نوویوسین
۶	کیت رنگامیزی گرم	۱۲	میکروسکوپ و روغن امرسیون

# کار عملی

## ادامه.

- (۱) ابتدا از نمونه ی مجهول رنگامیزی گرم تهیه نمایید.
- (۲) بر حسب نتیجه ی رنگامیزی باکتری را در محیط های زیر کشت دهید:
- (۳) EMB, MAC, BA ، NA
- (۴) پس از مشخص شدن نتایج ادامه کار را با تست ها و محیط های اختصاصی افتراقی زیرانجام دهید.
- (۵) تست های اکسیداز و کاتالاز
- (۶) تست IMViC ، TSI, LIA، مولر هیتتون ، بایل اسکولین و نمک ۶.۵ درصد و اوره



## منابع:

- **مهارت‌های آزمایشگاه میکروپشناسی** ، جلد ۱-۳
- دکتر علی محمدی-عضو هیئت علمی دانشگاه الزهرا (س).
- دکتر حمیده میرشفیعی - دانشگاه شهید بهشتی

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