Lab 1 - Urinalysis and Urine Culture (UG)

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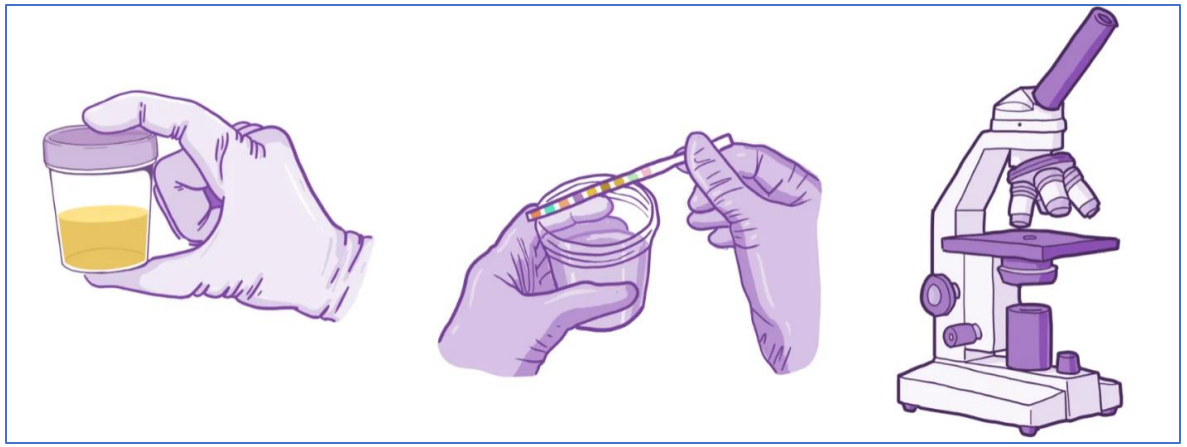
Urine analysis
Definition

- Urinalysis involves the gross examination of urine, chemical evaluation using urine dipstick, and microscopic assessment of urine sediment.
- Further tests include urine culture and urinary electrolyte levels.
- Indications for urinalysis include renal, urinary, and metabolic conditions.

Urinalysis and Urine Culture

1. Methods of urine collection.
2. Characteristics of urine:
   A. Physical
   B. Chemical
   C. Microscopic (cells)
3. Demonstrate the laboratory diagnosis of UTI.



Urine analysis

1- Methods of urine collection

1. Random Specimen
2. First Morning Specimen
3. Midstream Clean Catch Specimen
4. Timed Collection Specimen
5. Catheter Collection Specimen
6. Suprapubic Aspiration Specimen
7. Pediatric Specimen

Urine analysis
1- Methods of urine collection

Random Specimen

- Most commonly
- It is the easiest to obtain and is readily available.
- Could be used for urinalysis and microscopic analysis, although it is not the specimen of choice.

- Sometimes gives an inaccurate view as specimen is too diluted

Urine analysis
1- Methods of urine collection

First Morning Specimen

- Also called an 8-hour specimen
- This is the specimen of choice for urinalysis and microscopic analysis
  - since the urine is generally more concentrated (due to the length of time the urine is allowed to remain in the bladder) and, therefore, contains relatively higher levels of cellular elements and analytes such as protein, if present.

Urine analysis
1- Methods of urine collection

Midstream Clean Catch Specimen

- This is the preferred type of specimen for culture and sensitivity testing
  - reduced incidence of cellular and microbial contamination.
- Patients are required to first cleanse the urethral area with a castile soap towelette
- The patient should then void the first portion of the urine stream into the toilet, urine midstream is then collected into a clean container

Urine analysis
1- Methods of urine collection

Timed Collection Specimen

- Among the most commonly performed tests requiring timed specimens (usually 8 or 24 hours)
  - measuring creatinine, urine urea nitrogen, glucose, sodium, potassium, or analytes such as catecholamines and 17-hydroxy-steroids that are affected by diurnal variations

Urine analysis
1- Methods of urine collection

Catheter Collection Specimen

- This assisted procedure is conducted when a patient is bedridden or cannot urinate independently.
- Specimens may be collected directly from a Foley into an evacuated tube or transferred from a syringe into a tube or cup.

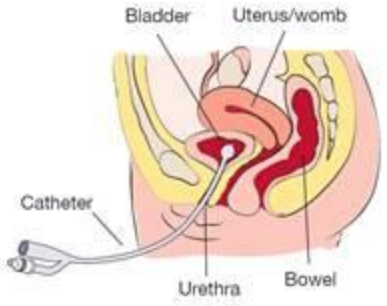


Figure 1 – Female catheter

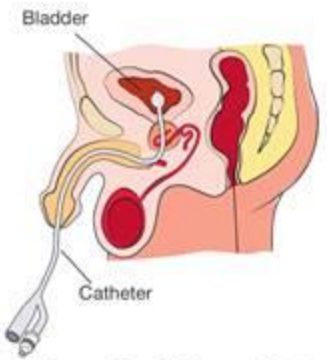
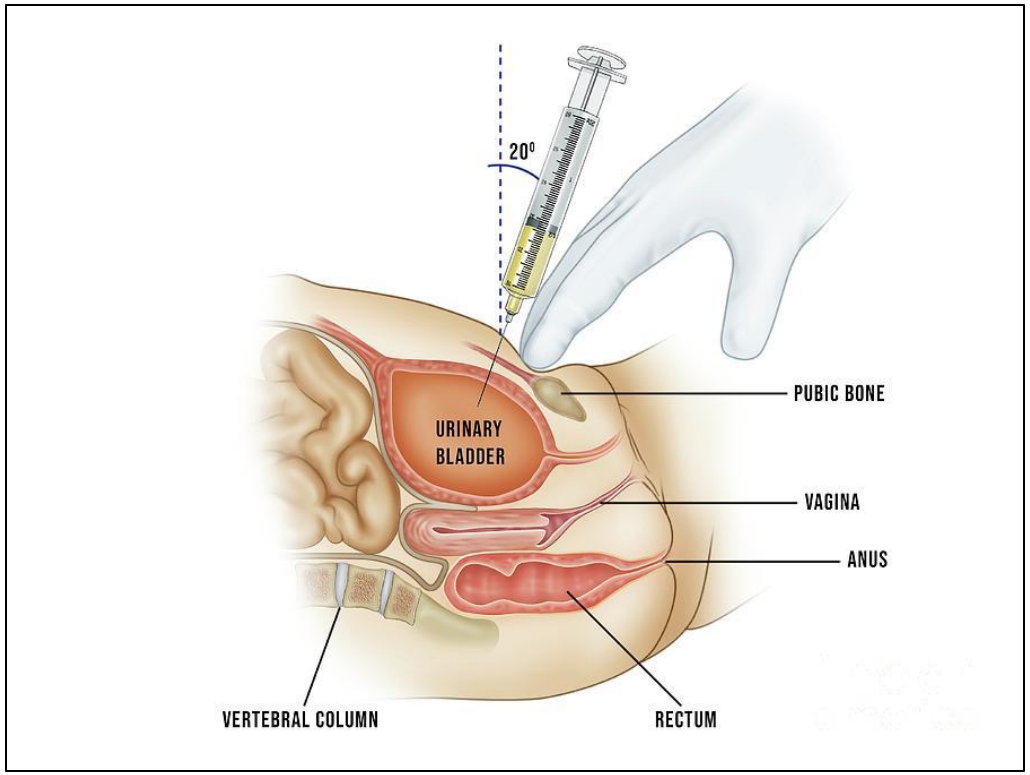


Figure 2 – Male catheter

Urine analysis
1- Methods of urine collection

Suprapubic Aspiration Specimen

- This method is used when a bedridden patient cannot be catheterized or a sterile specimen is required. The urine specimen is collected by needle aspiration through the abdominal wall into the bladder



Urinalysis and Urine Culture

1. Methods of urine collection.
2. **Characteristics of urine:**
   A. Physical
   B. Chemical
   C. Microscopic (cells)
3. Demonstrate the laboratory diagnosis of UTI.

Urine analysis
2.A- Gross urine assessment

- **Urine color**
  - Normal: pale yellow to dark amber → The color of urine depends on the hydration status of the individual.
  - Red urine: Read about “Hematuria.”
  - Black urine: alkaptonuria
  - Green UTI: P.aeruginosa
- **Turbidity** (cloudiness of the urine): cloudy urine suggests infection or chyluria
- **Note:** Certain drugs (e.g., rifampin, phenazopyridine), foods (e.g., beetroot), and types of porphyria cause red discoloration of urine.

Urine analysis

2.A- Gross urine assessment – Urine color

Urine analysis

2.A- Gross urine assessment – Urine color

Clear

Turbid

Red

Urine analysis
2.B- Chemical analysis (Dipstick)

- A diagnostic tool consisting of a urine test strip that allows for quick assessment of potentially pathological changes of various parameters.

Urine analysis

2.B- Chemical analysis (Dipstick) – parameters (1)

- **pH** : Alkaline urine (pH >8) in the setting of UTI is suggestive of a bacterium that produces urease (eg, *Proteus mirabilis*); urease hydrolyzes urea to ammonia (NH3), which is then protonated to ammonium (NH4+), alkalizing the urine. This helps differentiate *P mirabilis* from other nitrate reductase–producing bacteria.
- **Urine specific gravity:** Measures the ratio of urine density over pure water density (normally 1.005–1.030)
  - High urine specific gravity: volume loss, heart failure, presence of large molecules (e.g., glucose, radiocontrast media)
  - Low urine specific gravity: renal failure, diabetes insipidus

Urine analysis

2.B- Chemical analysis (Dipstick) – parameters (2)

- **Heme:** > 90% sensitivity for hematuria (low specificity) → False positive tests for hematuria on dipstick are caused by hemoglobinuria or myoglobinuria.
- **Leukocyte esterase:** Leukocyte esterase is an enzyme released by lysed white blood cells and is a marker of inflammation. Although nonspecific, its presence is supportive of UTI.
- **Protein** (albumin): Read about "Proteinuria."
- **Glucose**: glycosuria is a key finding of diabetes mellitus

A urine dipstick cannot differentiate between hematuria, hemoglobinuria, or myoglobinuria. Therefore, every positive test result for heme must be confirmed with the presence of RBCs on microscopy.

Urine analysis
2.B- Chemical analysis (Dipstick) – parameters (3)

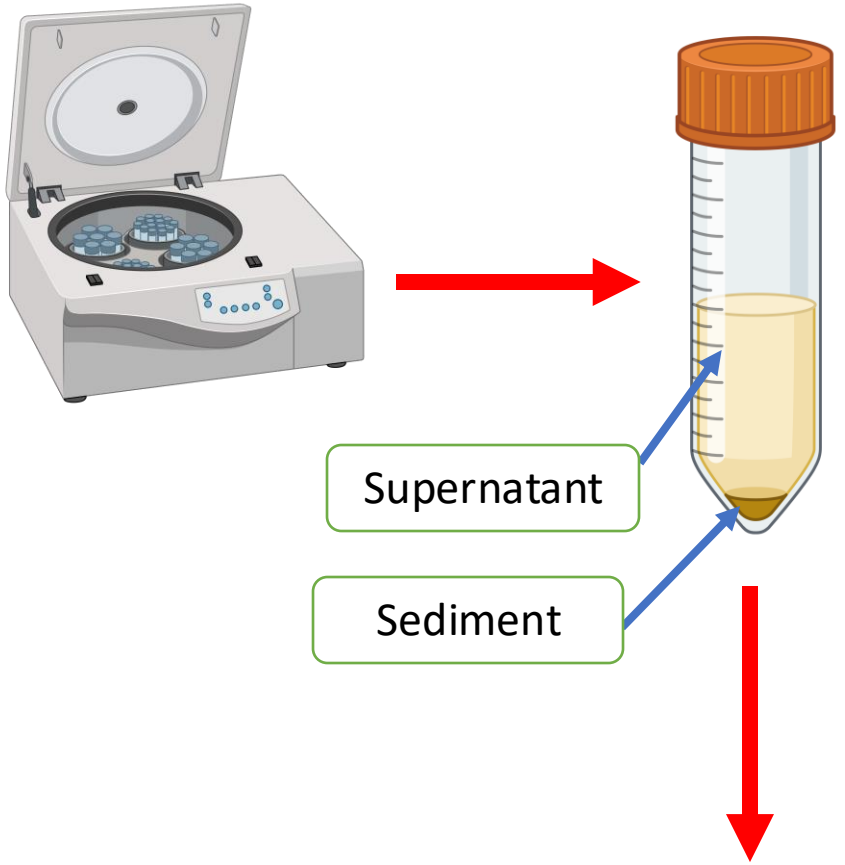
- **Ketones:** ketonuria can help diagnose diabetic ketoacidosis, a complication of type 1 diabetes mellitus
- **Urobilinogen**: Read “Prehepatic jaundice” and “Intrahepatic jaundice.”
- **Nitrite**: Nitrites are a metabolic by-product of bacteria producing nitrate reductase, an enzyme that reduces normal urinary nitrate (NO_3^-) to nitrite (NO_2^-). These bacteria include *Escherichia coli* and *Proteus mirabilis*.

Urine analysis

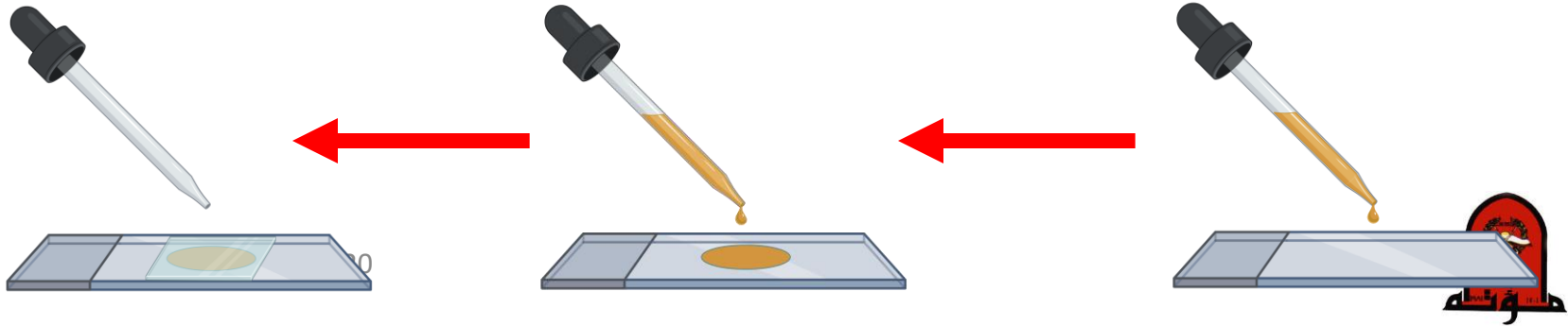
2.B- Chemical analysis (Dipstick) – parameters (4)

Urine dipstick results in urinary tract infection – The most important		
Result	**Pathophysiology**	**Clinical significance**
Leukocyte esterase	• Released by lysed neutrophils/macrophages	• Marker of white blood cells
Nitrites*	• Produced by conversion of urinary nitrate into nitrite by nitrate reductase	• Marker of nitrate reductase–producing bacteria (eg, *Escherichia coli, Klebsiella, Proteus mirabilis*)
pH	• Alkaline urine (pH >8) due to ammonia produced from hydrolysis of urea by urease	• Marker of urease-producing bacteria (eg, *Proteus mirabilis*)
*The absence of nitrites does not exclude these bacteria.		

Urine analysis
2.C- Microscopic examination



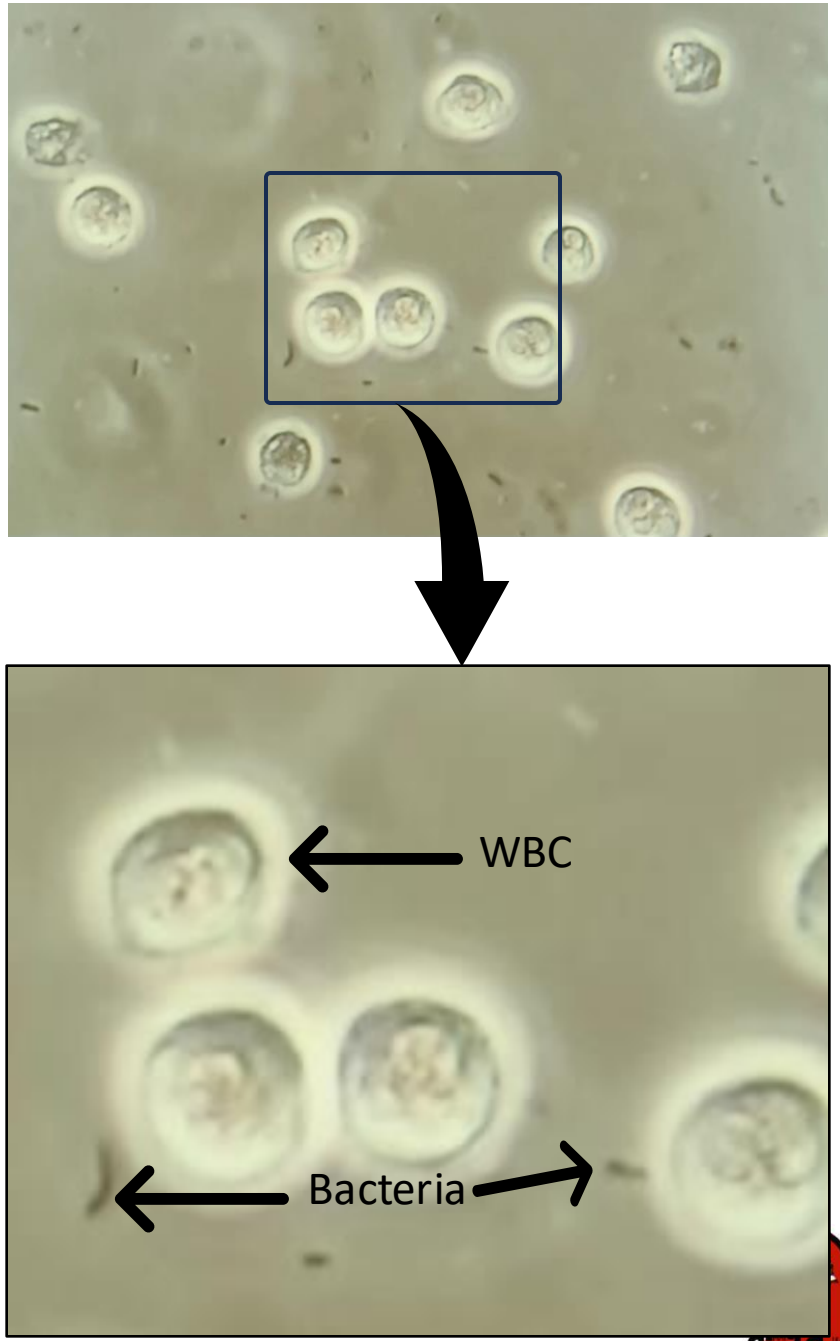
- Urine is centrifuged for 5 mins
- Most of the supernatant is pipetted off, and the remaining pellet is resuspended by gently shaking the tube
- A sample is then applied to a microscopic slide with a cover slip
- **High Power Field (HPF)** → This refers to the area visible when looking through a microscope at high magnification (typically 400x)



Urine analysis
2.C- Microscopic examination

Bacteria

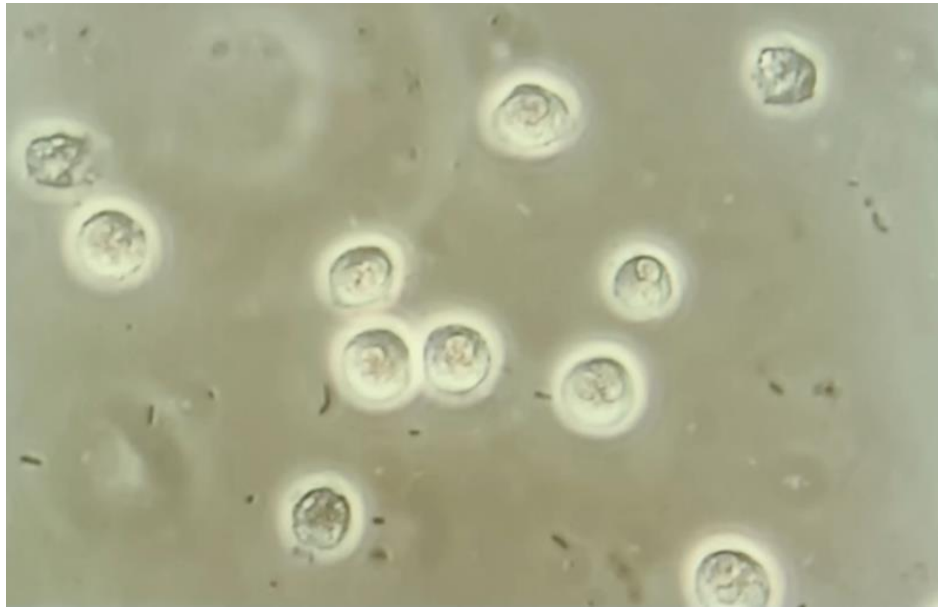
- Bacteria are a common finding on urine microscopy, and consistent with UTI
- However, in the absence of symptoms, particularly if leukocyte esterase and nitrites are negative, it is probably due to poor collection technique



Urine analysis
2.C- Microscopic examination

WBCs

- WBCs are quantified as number of cells /HPF (high power field)
- > 5 WBCs is considered abnormal



↑WBCs

- UTI
- Urinary catheter
- Malignancy
- Chronic interstitial nephritis
- Interstitial cystitis
- Contamination with vaginal secretions

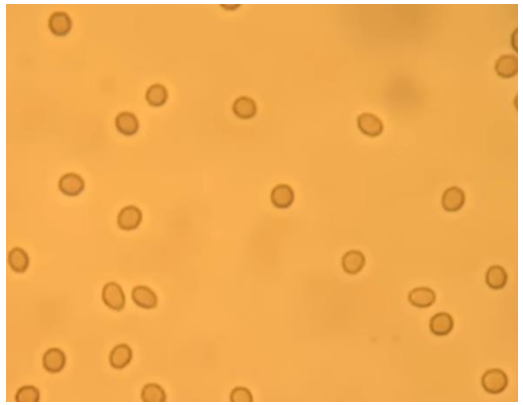
Urine analysis
2.C- Microscopic examination

RBCs

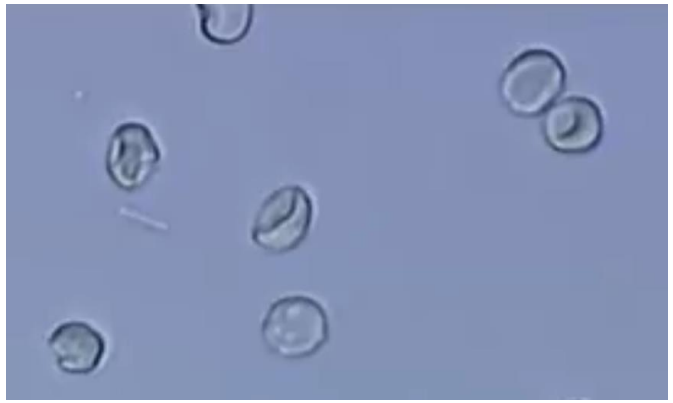
- RBCs are quantified as number of cells /HPF
- Presence 0–3 RBCs is considered normal BUT > 3 is abnormal
- Presence of dysmorphic RBCs suggests glomerular disease

↑RBCs

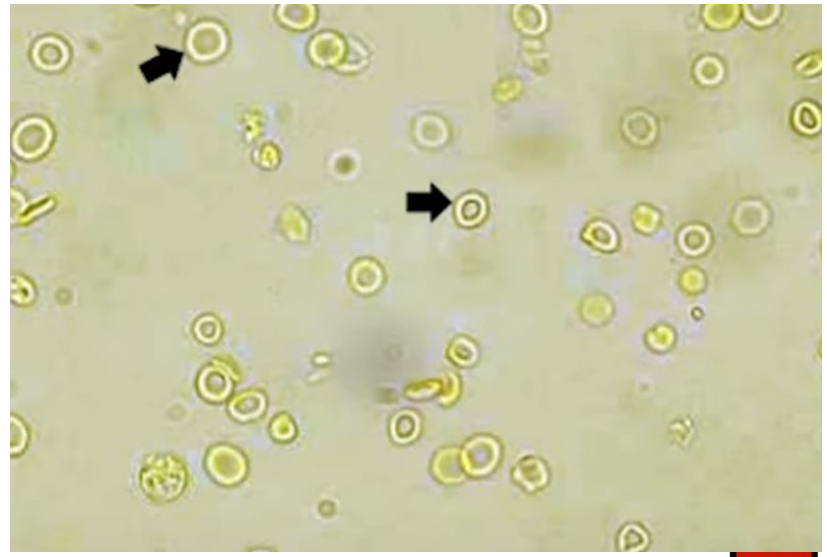
- UTI
- Renal stones
- Malignancy
- Recent instrumentation (ex, Foley catheter)
- Coagulopathy
- Glomerulonephritis
- Sickle cell anemia
- Vigorous exercise
- Contamination with menstrual blood



Lower Power field
→ completely flat



Higher Power field
→ Concaved with little indentation in the middle



Overview of urine changes		
	Definition	**Common diagnoses**
Glycosuria	• Glucose in the urine • Occurs when blood glucose levels exceed 180 mg/dL (renal threshold for reabsorption of glucose)	• Diabetes mellitus
Ketonuria	• Ketones in the urine	• Diabetic ketoacidosis • States of starvation
Proteinuria	• **> 150 mg protein/day** in the urine	• Diabetic kidney disease • Hypertensive nephropathy • Glomerulonephritis (e.g., minimal change disease, focal segmental glomerulosclerosis) • Fever, intense exercise, dehydration • Multiple myeloma
Bacteriuria	• Bacteriuria: presence of bacteria in urine • Significant bacteriuria: **≥ 10^5 colony-forming units/mL** in midstream urinary sample	• Urinary tract infection

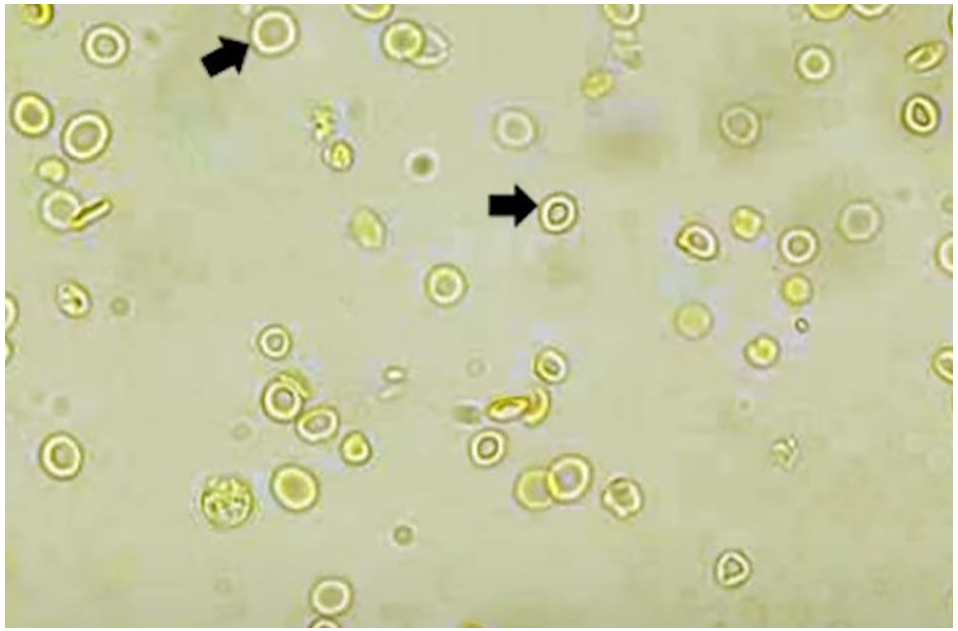
Pyuria	• White blood cells in the urine	• Urinary tract infection
		• Sterile pyuria
		  • Acute tubulointerstitial nephritis
		  • Glomerulonephritis (see nephritic syndrome)
		  • Urogenital tuberculosis

Overview of urine changes		
	Definition	**Common diagnoses**
Hematuria	• Red blood cells in the urine	• Benign prostatic hyperplasia • Urinary tract infection • Urolithiasis • Glomerulonephritis (see nephritic syndrome) • Polycystic kidney disease • Malignancy (e.g., bladder cancer, renal cell carcinoma)

Hemoglobinuria	• Hemoglobin in the urine	• Severe intravascular hemolysis
		  • Microangiopathic hemolytic anemia
		  • Paroxysmal nocturnal hemoglobinuria
		  • G6PD deficiency
		  • Malaria (especially *Plasmodium falciparum*)

Myoglobinuria	• Myoglobin in the urine	• Rhabdomyolysis

Quiz - 1

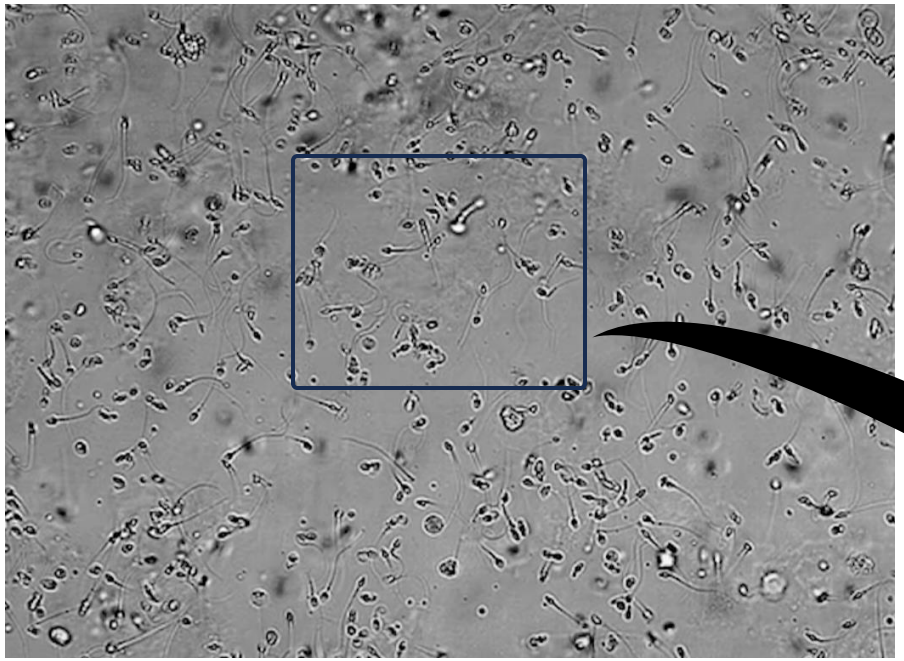


- Answer
  - RBC
  - No nucleus
  - Small size
  - No grainy appearance

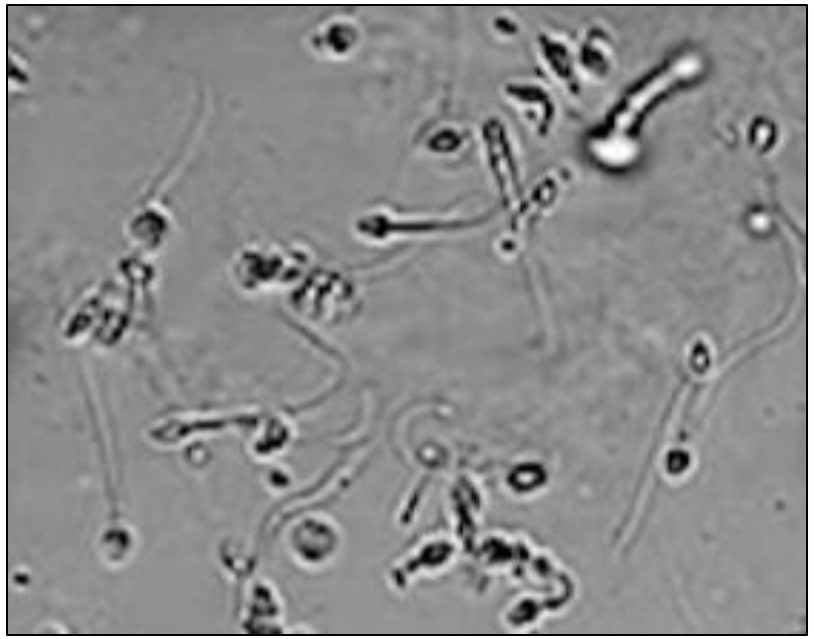
Quiz - 2

- Answer
  - Bacteria
  - Cocci shape, very small cells

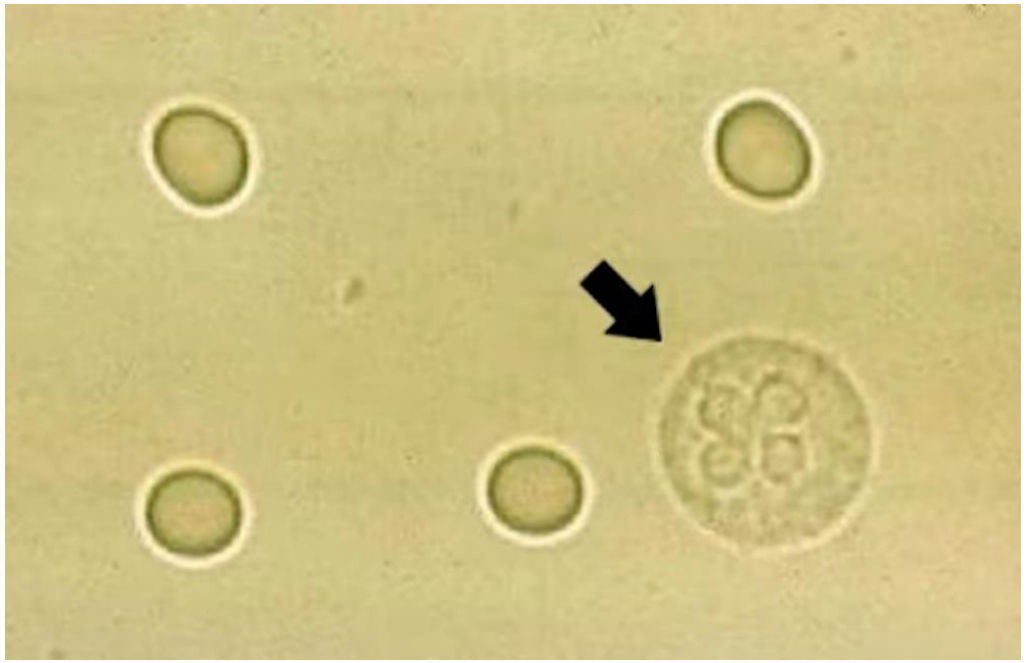
Quiz - 3



- Answer
  - Sperms
  - Head & Tail appearance

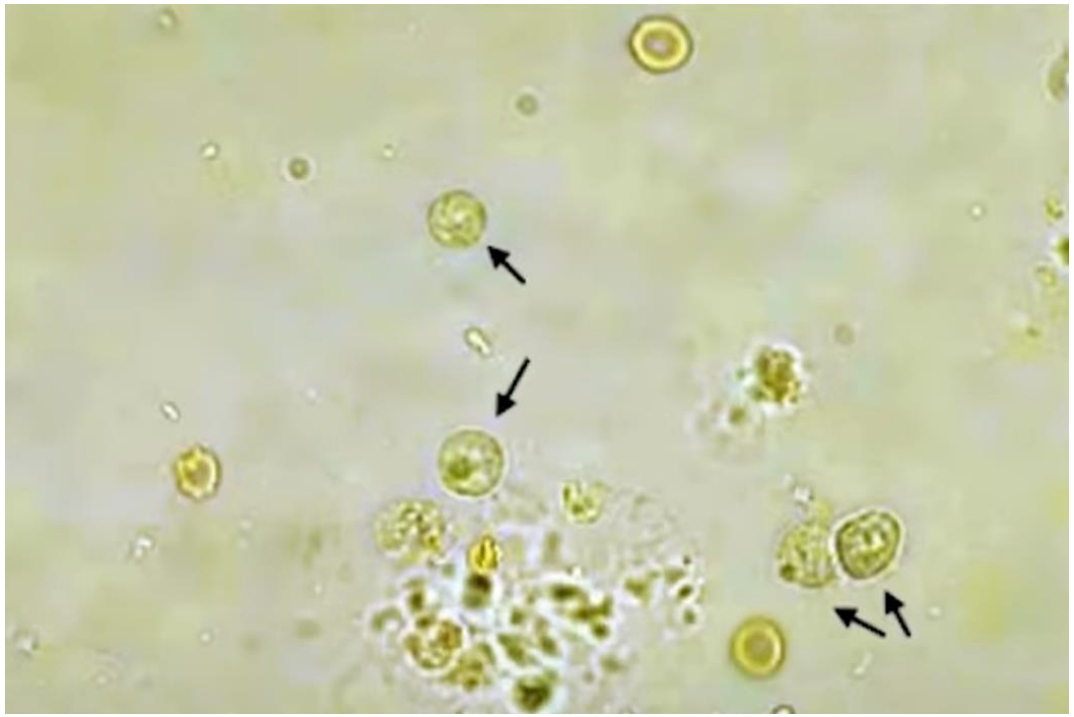


Quiz - 4



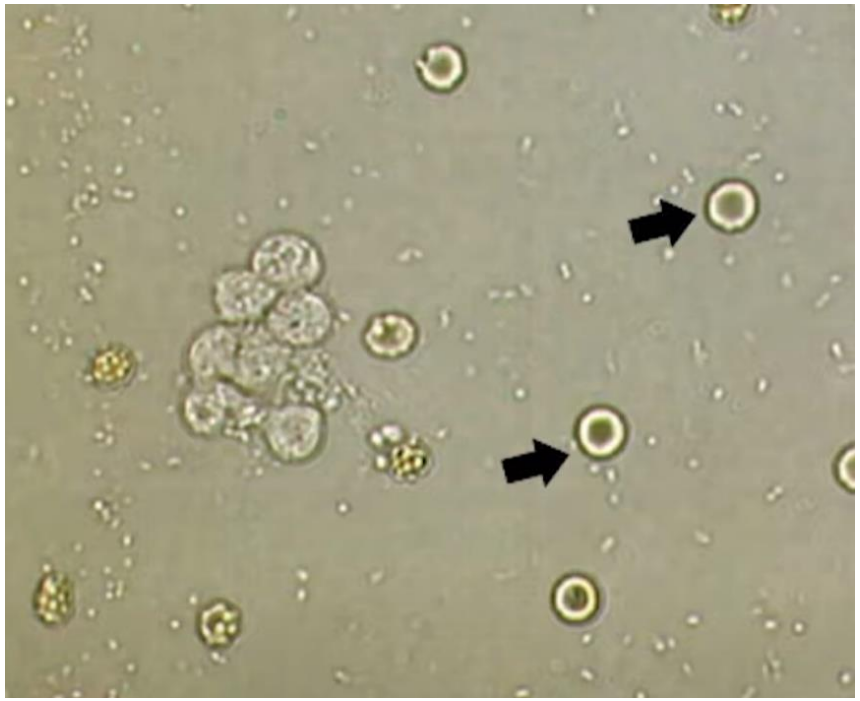
- Answer
  - WBC
  - Large size
  - Round shape
  - Granny appearance

Quiz - 5



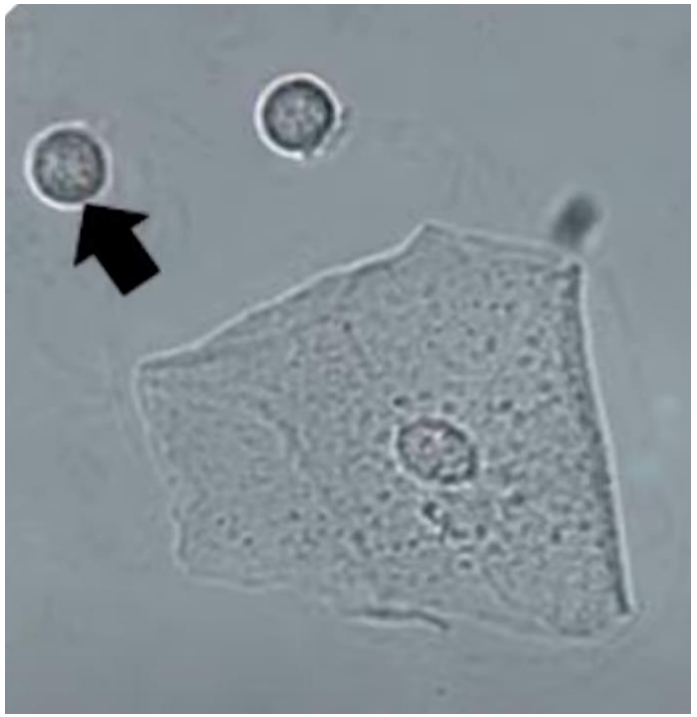
- Answer
  - WBC
  - Large size
  - Round shape
  - Granny appearance

Quiz - 6



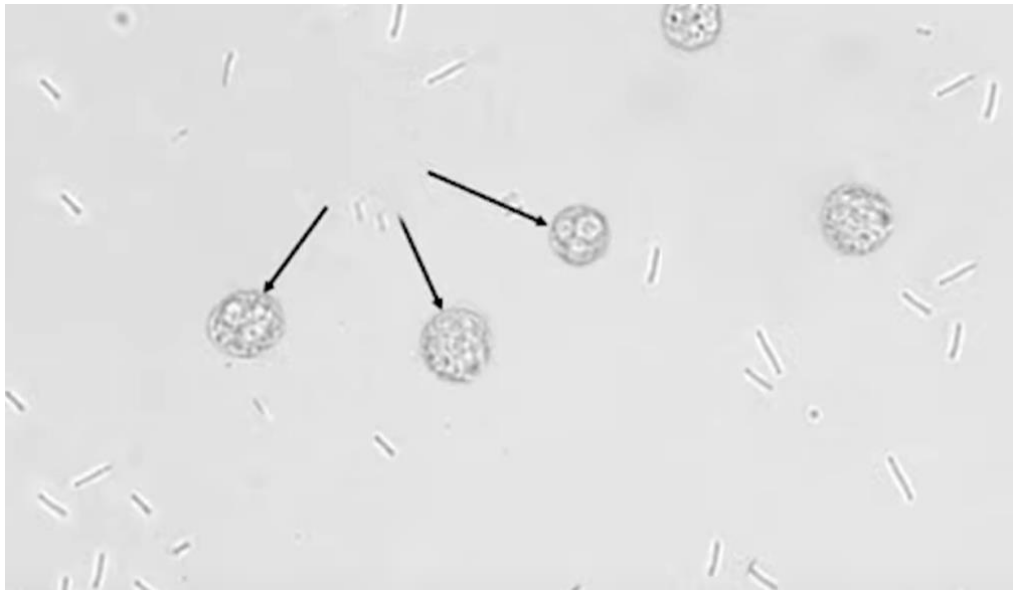
- Answer
  - RBC
  - No nucleus
  - Small size
  - No grainy appearance

Quiz - 7



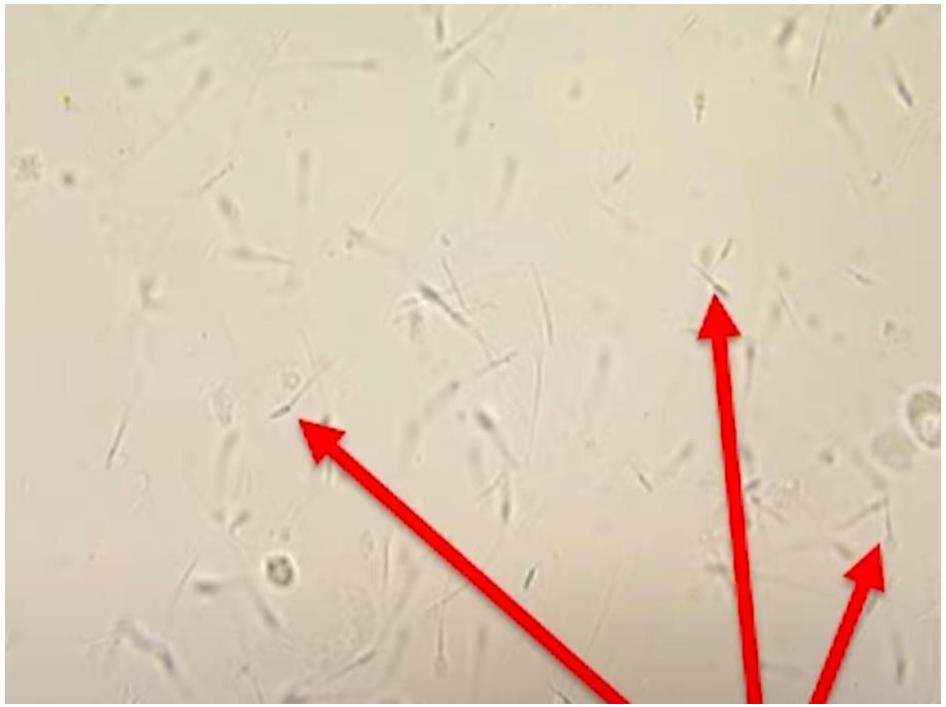
- Answer
  - WBC
  - Large size
  - Round shape
  - Granny appearance

Quiz - 8



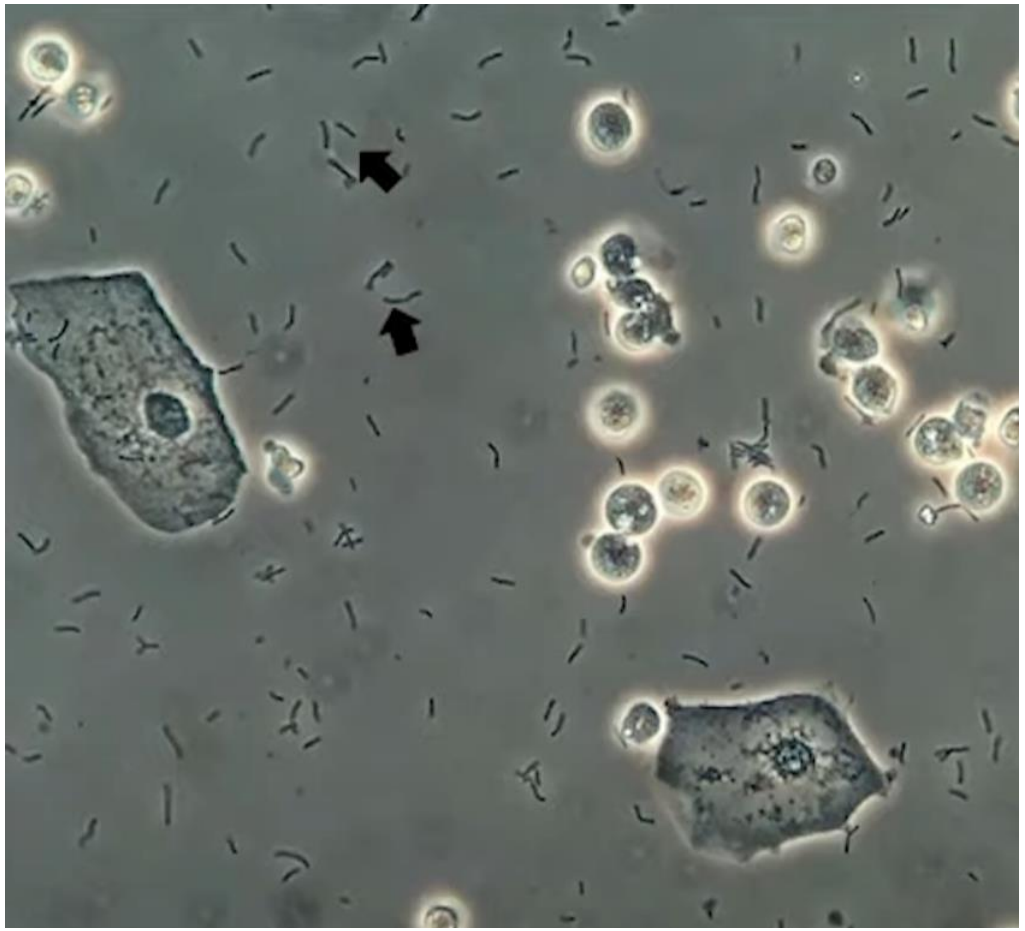
- Answer
  - WBC
  - Large size
  - Round shape
  - Granny appearance

Quiz - 9



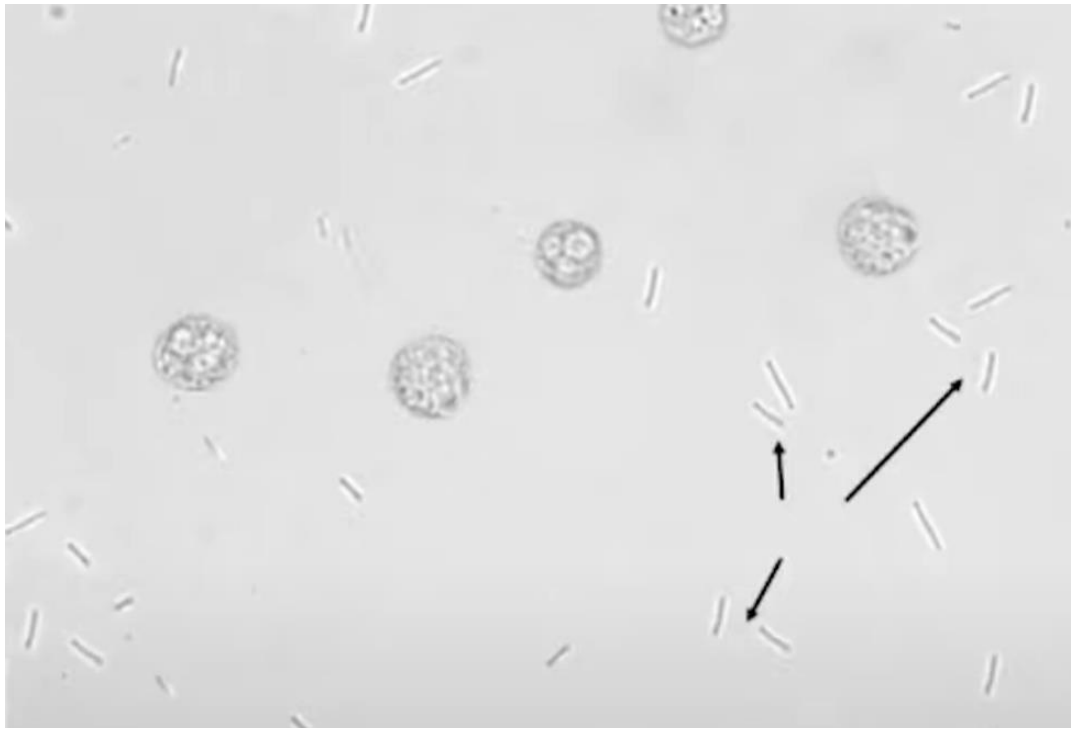
- Answer
  - Sperms
  - Head & Tail appearance

Quiz - 10



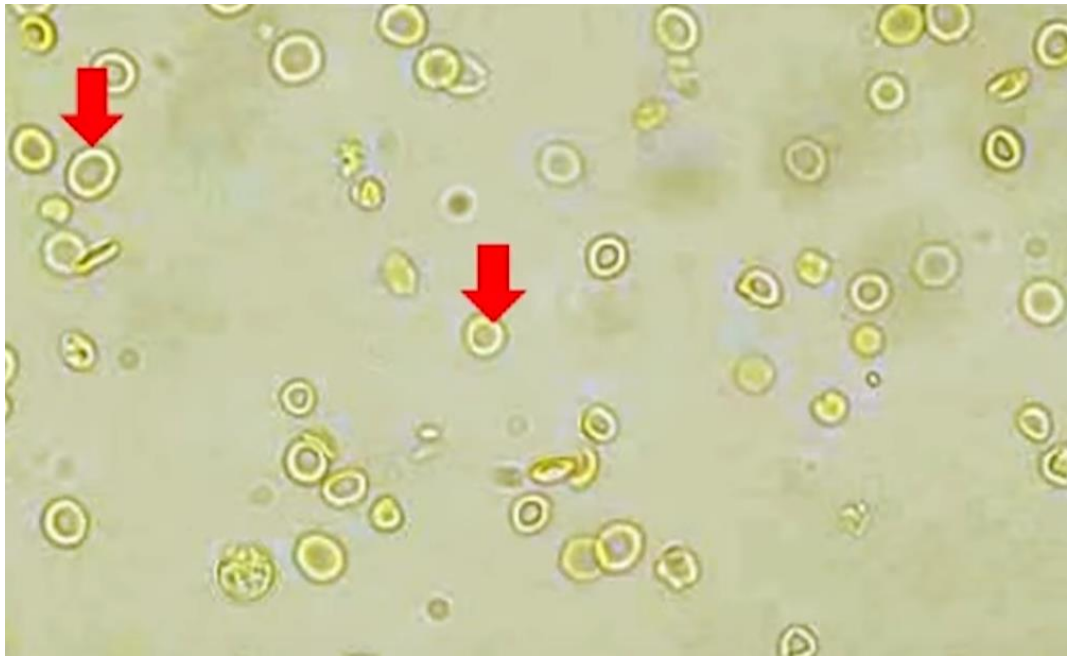
- Answer
  - Bacteria
  - Rod shape, very small cells

Quiz - 11



- Answer
  - Bacteria
  - Rod shape, very small cells

Quiz - 12



- Answer
  - RBC
  - No nucleus
  - Small size
  - No grainy appearance

ن ط ٣/٤

الخدمات الطبية الملكية

مستشفى : .....

اسم المريض الكامل : .....

الرقم الطبي : ..... الفئة : .....

الرقم الوطني : ☐☐☐☐☐☐☐☐☐☐☐☐

الرتبة : ..... ذكر ☐ انثى ☐

العمر : ..... أعزب ☐ متزوج ☐

فحص البول
URINALYSIS

تاريخ الطلب :	القسم / العيادة :	اسم و توقيع الطبيب المشرف :	الطبيب الاختصاصي :

Diagnosis & Relevant Information :

Test	Result	Test	Result
Albumin	NIL	Microscopic	
Sugar	NIL	sediment :	
Other Tests :		R. B. C	0 - 1
		W. B. C	0 - 1
		casts	
		Crystals	
		Other Findings	

Comments :

التسلسل :	التاريخ :	اسم و توقيع المشرف :	اسم و توقيع طبيب المختبر :

الخدمات الطبية الملكية

مستشفى :

فحص البول

URINALYSIS

اسم المريض الكامل :

الرقم العسكري : الفئة :

الرقم الوطني :

الرتبة :

العمر :

ذكر ☐ انثى ☐

أعزب ☐ متزوج ☐

تاريخ الطلب :	القسم / العيادة :	اسم و توقيع الطبيب المشرف :	الطبيب الاستشاري :

Diagnosis & Relevant Information :

Test	Result	Test	Result
Albumin	N.A	Microscopic sediment :	
Sugar	N.A	R. B. C	4 - 6
Other Tests :		W. B. C	8 - 10
		casts	
		Crystals	
		Other Findings	

Comments :

التسلسل :	التاريخ :	اسم و توقيع المشرف	اسم و توقيع فاحص المختبر

ن ط ٣/٤

الخدمات الطبية الملكية

مستشفى : .....

اسم المريض الكامل : .....

الرقم الطبي : ..... الفئة : .....

الرقم الوطني : ☐☐☐☐☐☐☐☐☐☐☐☐

الرتبة : ..... ذكر ☐ انثى ☐

العمر : ..... أعزب ☐ متزوج ☐

فحص البول
URINALYSIS

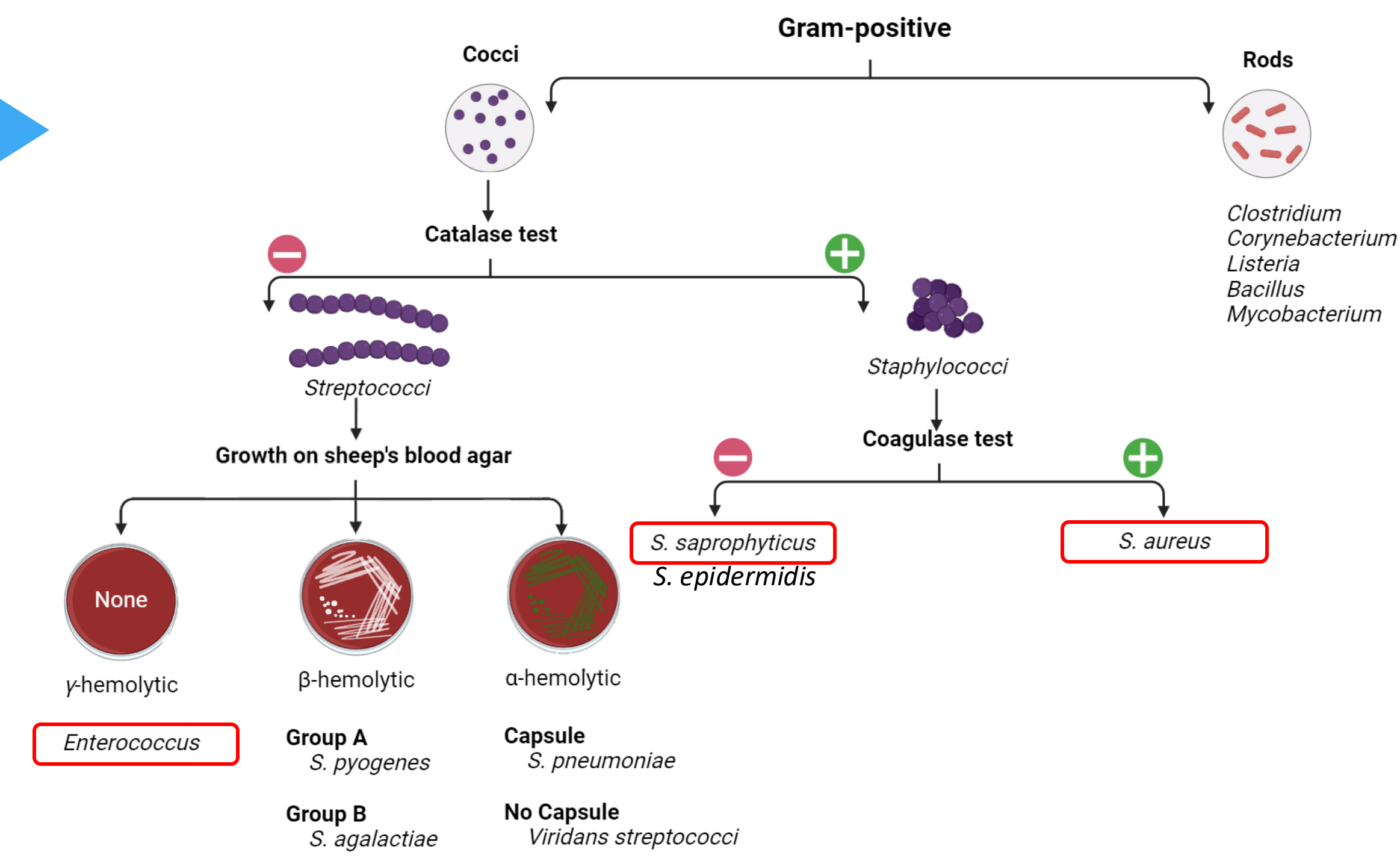
تاريخ الطلب :	القسم / العيادة :	اسم و توقيع الطبيب المشرف :	الطبيب الاختصاصي :

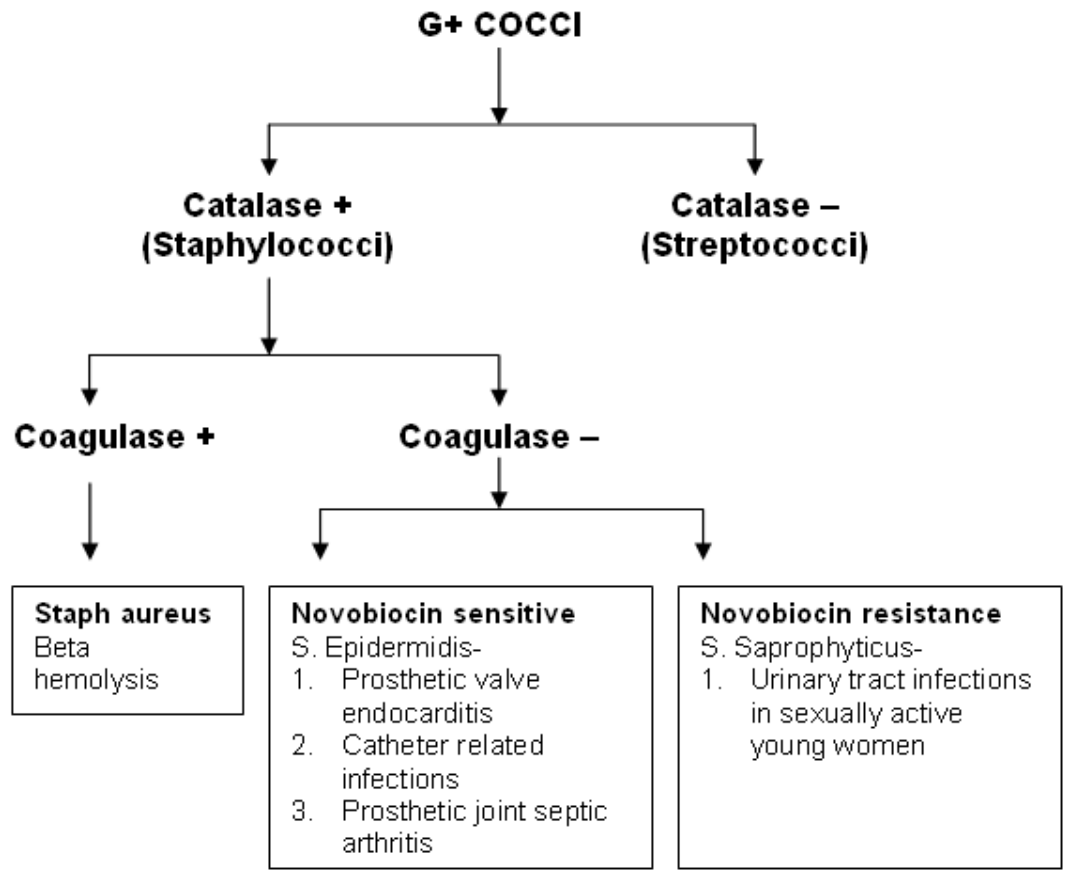
Diagnosis & Relevant Information :

Test	Result	Test	Result
Albumin	+ (1)	Microscopic	
Sugar	+ + (2)	sediment :	
Other Tests :		R. B. C	8-10
		W. B. C	8-20
		casts	
		Crystals	Ca++ oxalate
		Other Findings	

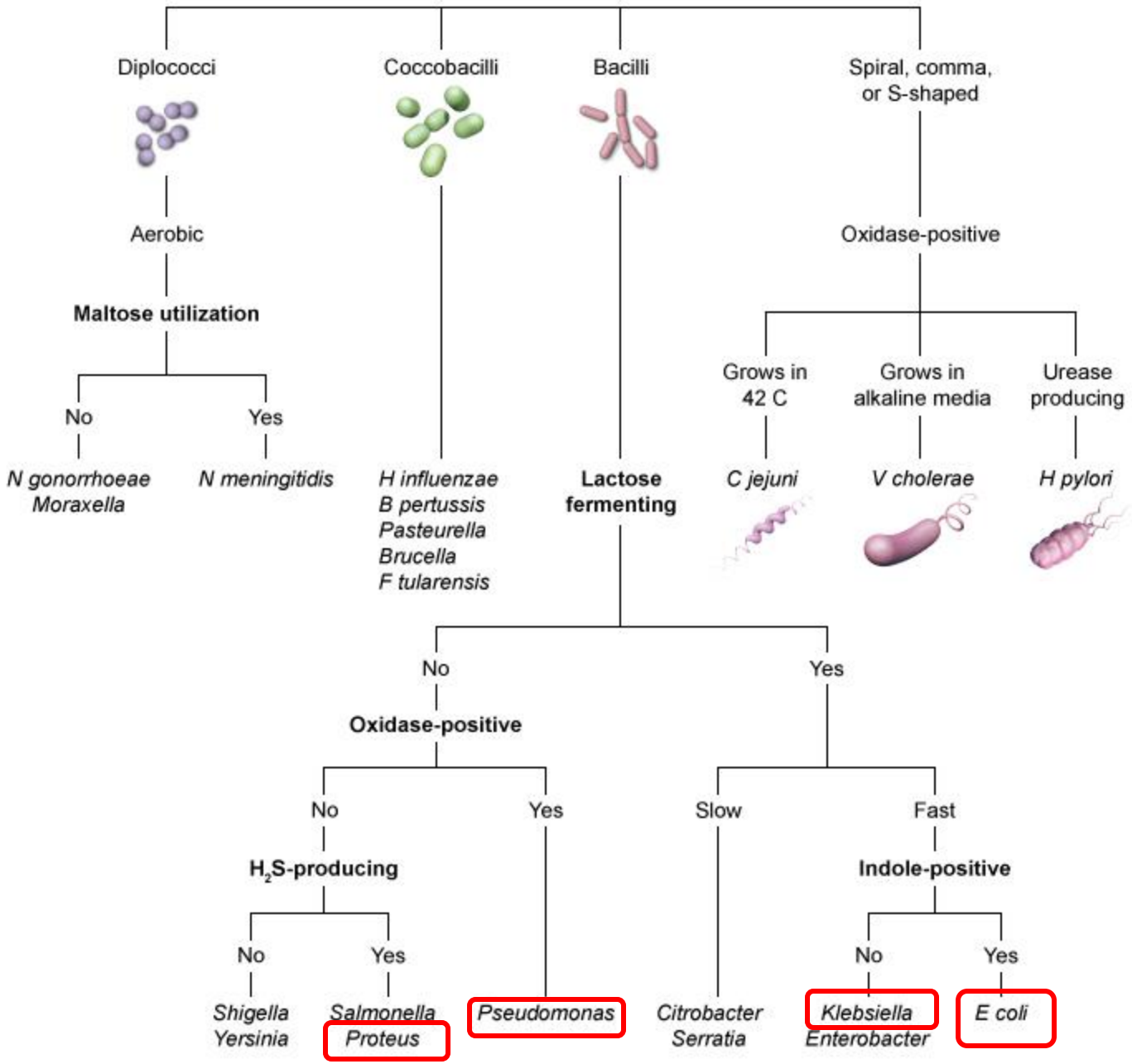
Comments :

التسلسل :	التاريخ :	اسم و توقيع المشرف	اسم و توقيع طبيب المختبر :

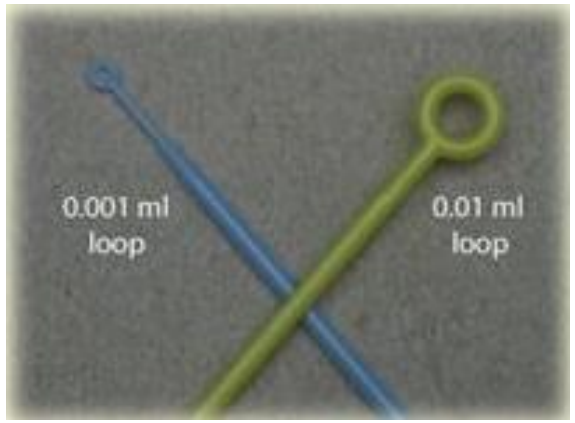

Gram-positive
Cocci
Rods
Clostridium
Corynebacterium
Listeria
Bacillus
Mycobacterium
Catalase test
Streptococci
Staphylococci
Growth on sheep's blood agar
Coagulase test
None
γ-hemolytic
Enterococcus
β-hemolytic
Group A
S. pyogenes
Group B
S. agalactiae
α-hemolytic
Capsule
S. pneumoniae
No Capsule
Viridans streptococci
S. saprophyticus
S. epidermidis
S. aureus

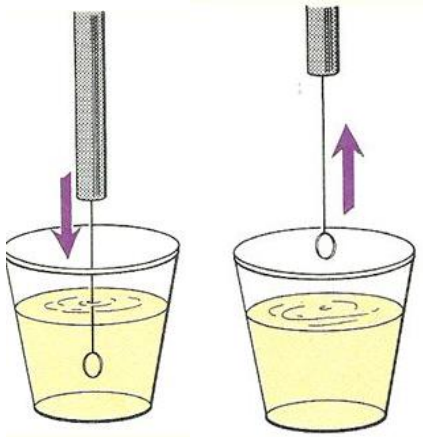

G+ COCCI
Catalase +
(Staphylococci)
Catalase –
(Streptococci)
Coagulase +
Coagulase –
Staph aureus
Beta hemolysis
Novobiocin sensitive
S. Epidermidis-
1. Prosthetic valve endocarditis
2. Catheter related infections
3. Prosthetic joint septic arthritis
Novobiocin resistance
S. Saprophyticus-
1. Urinary tract infections in sexually active young women

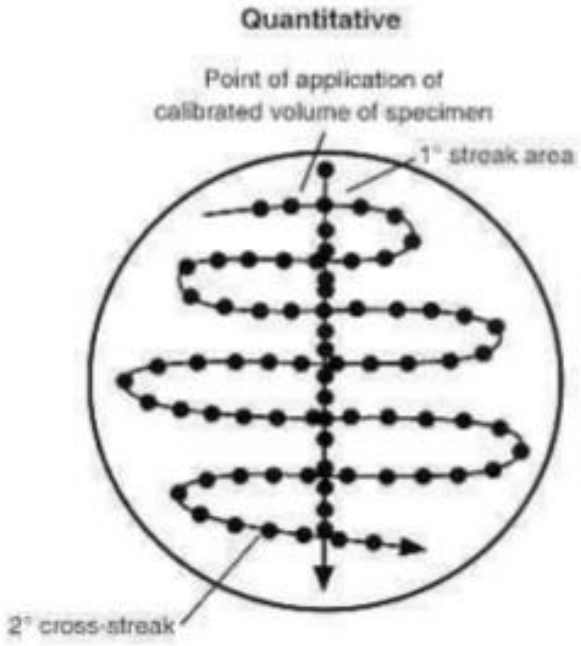
Gram-negative bacteria

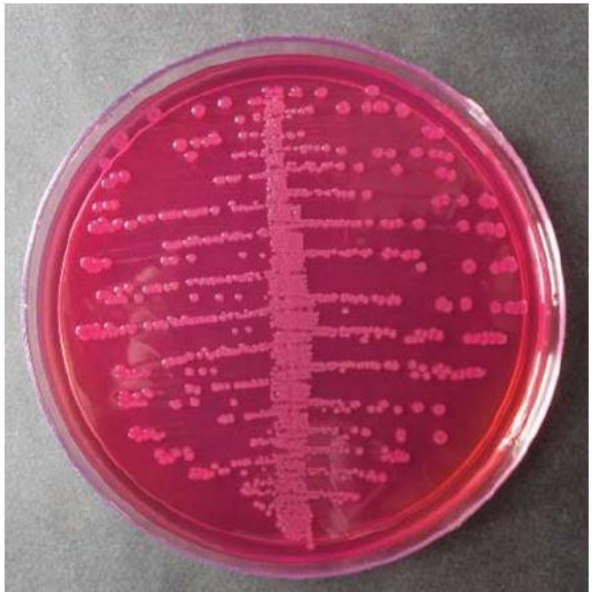


Culture of urine sample









CFU

- **Equation:**
  - dilution factor (DF) X number of colonies (NC)
- **Example 1**
  - Loop used is 0.001 mL (to convert to 1 mL, multiply by 1000)
  - Number of colonies → 5
  - Calculation → CFU = 1000 (DF) X 5 (NC) = 5000 = 5 X 10^3 CFU/mL
- **Example 2**
  - Loop used is 0.001 mL (to convert to 1 mL, multiply by 1000)
  - Number of colonies → 100
  - Calculation → CFU = 1000 (DF) X 100 (NC) = 10000 = 10^5 CFU/mL

CFU (cont.)

- **Example 3**
  - Loop used is 0.01 mL (to convert to 1 mL, multiply by 100)
  - Number of colonies → 5
  - Calculation → CFU = 100 (DF) X 5 (NC) = 500 = 5 X 10^2 CFU/mL
- **Example 4**
  - Loop used is 0.01 mL (to convert to 1 mL, multiply by 100)
  - Number of colonies → 100
  - Calculation → CFU = 100 (DF) X 100 (NC) = 10000 = 10^4 CFU/mL

General Criteria to Diagnose UTI

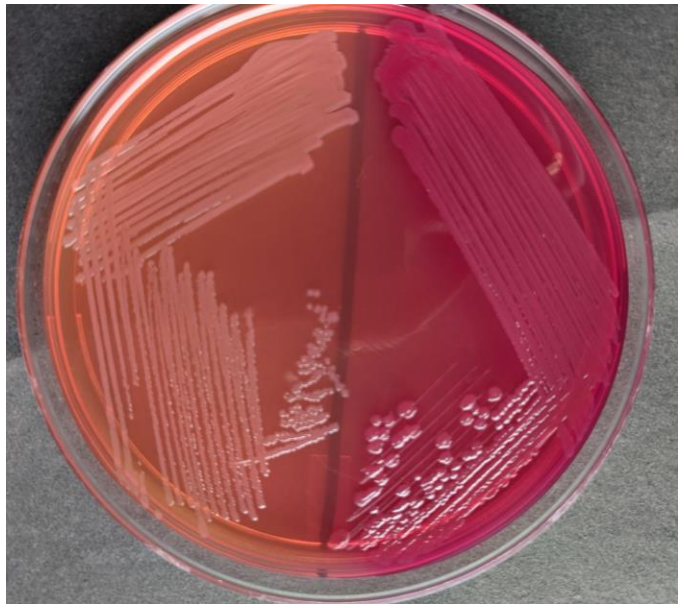
- **Suprapubic Aspiration:**
  - Any growth.

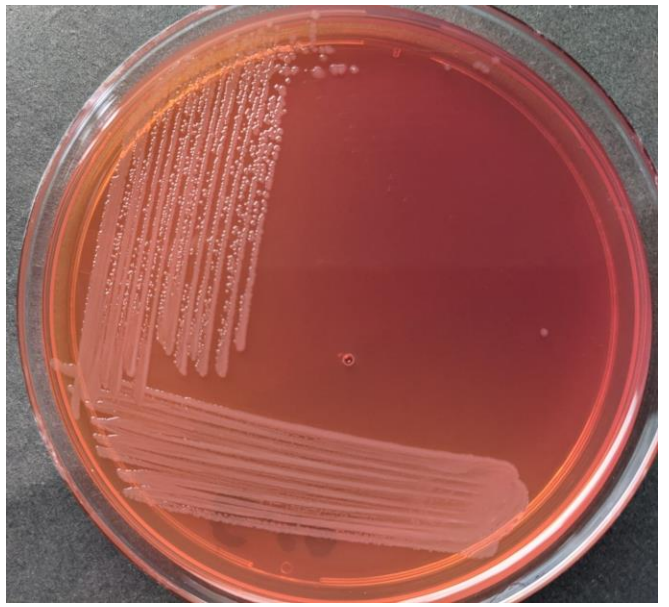
- **Catheterization:**
  - Greater than 100 colony forming units/ml.

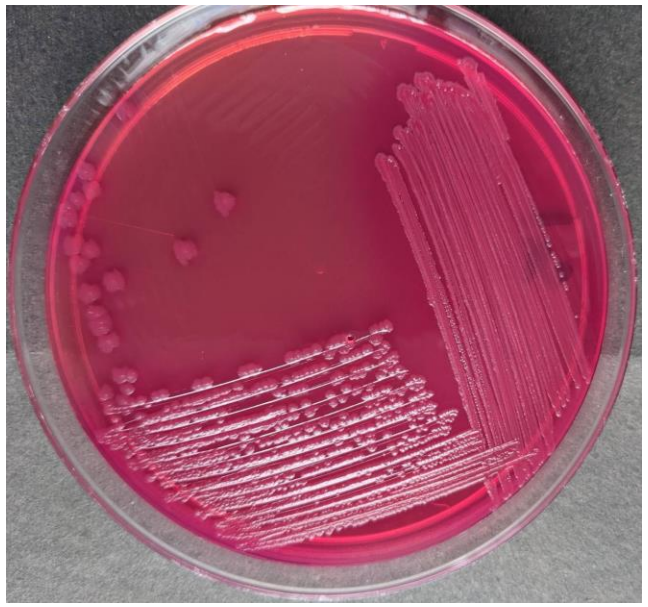
- **Midstream Clean Catch:**
  - Greater than 100,000 colony forming units/ml.

Urine Culture

- **MacConkey Agar:** Used as a selective and differential medium for:
  - Distinguishing lactose fermenters from non-fermenters
    - Lactose fermenters appear as pink to red colonies
  - Inhibiting gram-positive bacteria

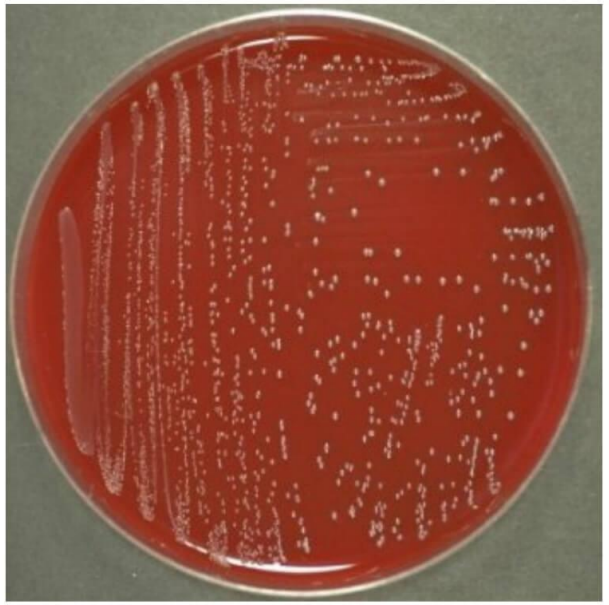






Urine Culture

- **Blood Agar:** Often used for:
  - Detection of fastidious organisms
  - Hemolysis patterns that help with organism identification
  - Growth of a wide range of bacteria



Enterococcus faecalis **on Blood Agar.**

الخدمات الطبية الملكية

مستشفى : .....

فحص الأحياء الدقيقة

MICROBIOLOGY

اسم المريض الكامل : .....

الرقم الطبي : ..... الفئة : .....

الرقم الوطني : ☐☐☐☐☐☐☐☐☐☐

الرتبة : ..... ☐ ذكر ☐ انثى

العمر : ..... ☐ أعزب ☐ متزوج

تاريخ الطلب:	القسم/العيادة :	اسم وتوقيع الطبيب المشرف :	الطبيب الاختصاصي :

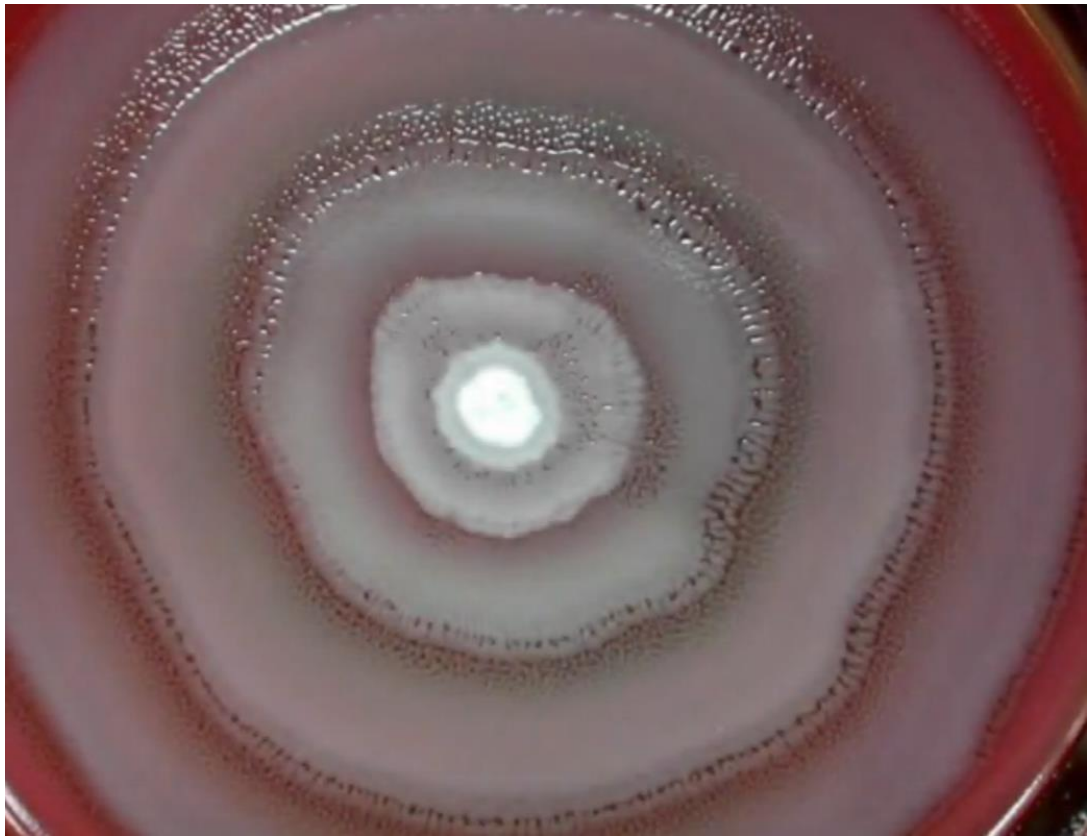
Diagnosis :	Type & Source of Specimen:	Test Requested:

Sensitivity Test		For Lab. Use
Sensitive	Resistant	Result : E. coli $>10^5$
Aug	Am	
TaU_2	CN	
AK	NA	
CIP	SXT	
oFx		

التسلسل:	التاريخ:	اسم وتوقيع المشرف :	اسم وتوقيع طبيب المختبر :

Remember

- Stones → Proteus mirabilis
- Swarming on blood agar → Proteus mirabilis



Thank You