Repaso Urinálisis y Fluídos Corporales

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Board of Certification Exam

- Urinalysis and BF – 5-10% exam
- Urinalysis
  - Physical
    - Color and Clarity
    - Specific Gravity/Osmolality
  - Chemical
    - Reagent Strip
    - Confirmatory Tests
  - Microscopic
    - Cells
    - Casts
    - Crystals
    - Microorganisms
    - Contaminants
    - Artifacts
  - Renal Physiology
  - Disease states

Board of Certification Exam

- Body Fluids – CSF, Amniotic, Synovial, Serous, Semen, Feces
  - Physical
  - Chemical
  - Microscopic
  - Physiology
  - Disease states

Urinalysis Review

Types of Urine Specimens

- Random – any time without patient preparation
  - Convenient
  - Hydration Dependent
- First Morning Void – Void before bed and collects urine specimen immediately upon waking in the morning
  - Useful for metabolite analysis
  - Inconvenient to obtain
- Timed
  - 2 hour post prandial – specimen collected after a meal
  - 24 hour collection – patient voids and then collects all urine for a predetermined amount of time.

Urine Collection Techniques

- Routine void
- Midstream clean-catch – Patient cleans external genital area, passes some urine into the toilet, collects some of the mid-portion of urine in appropriate container, then passes the remaining urine into the toilet.
- Catheterized specimen – collected by inserting a sterile catheter through the urethra into the bladder
- Suprapubic aspiration – collected by puncturing the abdominal wall and the distended bladder using a needle and syringe
- Pediatric collection – collected by securing a plastic bag to the external genital area using hypoallergenic skin adhesive

Collection Guidelines

- Container must be clean, dry, leak-proof, and made of translucent disposable material.
- Must be labeled before or immediately following collection (NOT ON LID)
  - Name, ID#, date/time of collection, preservative used
- Must be transported promptly to the lab or refrigerated
  - Preservative may or may not be added
    - Varies depending on test methodology, how often the test is performed, time delays, and transport conditions
    - Protect from light

Changes in Unpreserved Urine

Physical Change	Results	Cause
Color	Change	Oxidation or reduction of substances (bilirubin to biliverdin, Hgb to Met-Hgb, urobilinogen to urobilin)
Clarity	Decreased	Bacterial proliferation, solute precipitation (crystals and amorphous materials)
Odor	Increased	Bacterial proliferation/bacterial decomposition of urea to ammonia

Changes in Unpreserved Urine

Physical Change	Results	Cause
pH	Increased	Bacterial decomposition of urea to ammonia, loss of CO2
	Decreased	Bacterial or yeast conversion of glucose to form acids
Glucose	Decreased	Cellular or bacterial glycolysis
Ketones	Decreased	Bacterial metabolism of acetoacetate to acetone, volatilization of acetone
Bilirubin	Decreased	Photo-oxidation to biliverdin and hydrolysis to free bilirubin

Changes in Unpreserved Urine

Physical Change	Results	Cause
Nitrite	Increased	Decreased bacterial production following specimen collection.
	Decreased	Conversion to nitrogen
RBC's, WBC's and casts	Decreased	Desintegration of cellular and formed elements, especially in dilute and alkaline urine.
Bacteria	Increased	Bacterial proliferation following specimen collection

Urinalysis

- Laboratory examination of urine consists of 3 components:
  - Physical Examination
    - Color, clarity, odor, concentration (sp.gravity or osmolality) , volume and foam
  - Chemical examination
    - pH, specific gravity, blood, leukocyte esterase, nitrite, glucose, protein, ketones, urobilinogen, bilirubin
  - Microscopic Examination
    - Blood cells, epithelial cells, casts, crystals, microorganisms, contaminants

Color

- Normally different shades of yellow
  - Intensity of color is an indicator of urine concentration and body hydration.
- Color can vary from colorless to amber, orange, red, green, blue, brown, even black.
- Variations can indicate a disease process, metabolic abnormality, ingested food or drug, or excessive physical activity/stress.
- Each laboratory should have an established list of terms for color to assure consistency in reporting

Color cont.

Abnormal Color	Cause	Clinical Correlation
Dark yellow	Concentrated specimen	May be normal following strenuous exercise or in a first morning specimen.
Amber	Large amount of urobilinogen	Does not produce yellow foam when shaken.
Orange	Bilirubiin	Produces yellow foam when shaken and positive strip test for bilirubin
	Pyridium	Drug given for UTI. May have orange foam and pigment that interferes with strip test
	Nitrofurantoin	Antibiotic given for UTIs

Color Cont.

Abnormal Color	Cause	Clinical Correlation
Pink/Red	RBCs	Cloudy urine, positive strip test for blood, RBCs seen microscopically
	Hemoglobin	Clear urine, positive strip test for blood, intravascular hemolysis
	Myoglobin	Clear urine, positive strip test for blood, muscle damage
	Porphyrins	Negative strip test for blood, detect with Watson-Schwartz Test or UV light
	Beets/Blackberries	Certain people are gentically susceptible, pH dependent
	Rifampin	Medication foRBCs, mucous, and clotsr tuberculosis
	Menstrual Contamination	Cloudy, specimen with RBCs, mucous and clots.

Color Cont.

Abnormal Color	Cause	Clinical Correlation
Brown/Black	Methemoglobin	Oxidized hemoglobin, seen in acidic urine after standing, positive strip test for blood
	Homogentisic acid	Seen in alkaline urine after standing
	Melanin or oxidized melanogen	Urine darkens on standing, associated with malignant melanoma
	Metronidazole (Flagyl)	Darkens on standing

Color Cont.

Abnormal Color	Cause	Clinical Correlation
Yellow-green/ Yellow-brown	Blirubin oxidized to biliverdin upon standing or improper storage	Color foam in acidic urine, false negative test strip test for bilirubin
Green/blue-green	Pseudomonas infection	Positive urine culture
	Amitryptiline	Antidepressant
	Methocarbamol (Robaxin)	Muscle relaxant
	Clorets	Breath mint
	Indican	Infection of the small intestine
	Methylene blue	Given for a number of conditions

Foam

- Normal white foam that appears upon agitation and dissipates readily on standing.
- Large amounts of protein-thick, long-lasting, white foam produced when the urine is poured or agitated that does not dissipate.
- Bilirubin – yellow foam upon agitation that dissipates readily on standing
- Not reported in routine Hematology,

Clarity

- Describes the overall visual appearance (transparency) ofa aurine specimen
  - Normal – Clear
    - Cloudiness is caused by suspended particulate mater that scatters light as it passes through the specimen
  - Each laboratory should have an established list of terms for clarity to ensure consistency in reporting.
  - Abnormalities can be caused by :
    - Bacterial growth
    - Precipitation of amorphous solutes
    - Excretion of fat or lymph

Clarity

Non-pathologic causes of turbidity	Pathologic causes of turbidity
Squamous epithelial cells	Red Blood Cells
Mucus	White blood Cells
Semen	Bacteria
Amorphous phosphates,carbonates, urates	Yeast
Fecal contamination	Non-squamous epithelial cells
Radiologic contrast media	Abnormal crystals
Talcum powder	Lymph fluid
Vaginal creams	Lipids

Odor

- Normally the specimen is only faintly aromatic (not reported as part of routine urinalysis)
- Abnormal odors can result from:
  - Allowing specimen to stand at room temperatura/age - smells like ammonia because of bacterial conversión of urea to ammonia
  - Severe UTI – smells pungent or fetid from pus, protein decay and bacteria
  - Ingestion of certain foods/drugs – smell varies, caused by esting food like asparagus or garlic or IV medications containing phenol derivatives
  - Metabolic disorders – diabetes leads to sweet or fruity smelling urine; maple syrup urine disease
  - Cleaning agents – if household containers are used to collect the specimen

Concentration

- A dilute urine has fewer solute particles present per volumen of water, whereas a concentrated urine has more solute particles present per volume of water.
- Color is a crude indicator of urine concentration
- Specific gravity is most often used to rapidly screen urine concentration in the clinical laboratory
- Osmolality is used to obtain more accurate and specific information about urine concentration

Specific Gravity

- An expression of density
  - Ratio of density of an equal voume of pure water (Range 1.002-1.04)
  - Affected by the number of solutes present AND their molecule size
  - Requires solutes to be DISSOLVED in the solution rather tan particles floating in the solution to bring about changes in SG
- Terminology
  - Isothenuric: SG = 1.010
  - Hypothenuric: SG < 1.010
  - Hyperthenuric: SG > 1.010
  - If SG > 1.035, suspect the presence of radiographic contrast media
- Direct measurements- determine true density of urine (all solutes detected and measured)
  - Urinometry or harmonic oscillation densitometric method; only of historical interest
- Indirect measurements
  - Refractometry and reagent strip chemical method; used in current clinical laboratory

Osmolality

- Concentration of a solution expressed in mOsm/kg
- Temperature independent
- Serum normal range= 275-300 mOsm/kg
- Urine normal range= 275-900 mOsm/kg
  - Varies greatly with the patient's diet, health, hydration status and physical activity
- Evaluates the renal concentrating ability of the kidneys
- Depends only on the number of solute particles present
- Measured by:
  - Freezing point depression (most common)
  - Solvent vapor pressure depression
  - Elevation of osmotic pressure
  - Solvent boiling point elevation

Volume

- Normal= 600 – 1800 ml/day with less tan 400 ml excreted at night
  - Nocturia=>500 ml excretion at night
  - Polyuria = any increase in urine excretion
    - Excessive water intake, diuretic therapy, hormonal imblance, renal dysfunction or drug ingestión
  - Oliguria = any decrease in urine excretion
    - Water deprivation, excessive sweating, diarrea, vomiting or renal diseases
  - Anuria = lack of urine excretion
    - Hypotension, hemorrhage, shock, heart failure, urinary tract obstrucion, toxic chemicals, hemolytic transfusión reactions, etc.
  - Affected by individuals diet, health, exercise, fluid intake and ADH secretion

Routine Urinalysis

Reagent strips

- Rapidly screen for pH, protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrite and leukocyte esterase (sometimes SG and ascorbic acid too)
- Plastic strips that contain chemically impregnated test sites on an absorbent pad
- Procedure
  - Dip
  - Remove excess
  - Wait the specified amount of time
  - Compare
  - Can be performed by semi-automated or fully-automated instruments

Confirmation tests

- Protein,sugars, ketones, bilirubin
- Why?
  - Confirm results already obtained by reagent strip
  - Alternative test method for highly pigmented specimens
  - More sensitive for the substsnce of interest than the reagent strip
  - Specificity of the test differs from specificity of the reagent strip

Specific Gravity

- Normal range = 1.002-1.035
- Isosthenuria = SG fixed at 1.010
  - Indicates no concentrating ability
- Hyposthenuric
  - Diabetes insipidus
  - Loss of tubular concentrating ability
- Hypersthenuric
  - Adrenal insufficiency
  - Hepatic disease
  - Congestive heart failure
  - Excess wáter loss (vomiting, diarrea, sweating)

Specific Gravity

- Principle
  - Specific to ionic solutes
    - Polyelectrolyte pH indicator
    - Ions cause reléase of H^+ from pad
  - Indicator – Bromthymol blue
    - pH↓ - goes from blue green - yellow Green
  - Interfering substances
    - False low = High glucose, urea, or pH >6.5
    - False high = Protein of 100-500mg/dl, ketones

pH

- Kidneys play arole in mantaining acid-base balance; correct for respiratory/metabolic acidosis/alkalosis
  - Helps identify defects in renal tubular secretion or reabsorption of acids and bases
  - Use to modify diet/manage disease
- Helps identify crystals or determine if specimen is satisfactory
- pH can range from 4.5-8.0, but not >8.0 or <4.5
  - First morning specimen is usually slightly acidic (5.0-6.0)
  - pH tends to be more alkaline after a meal (alkaline tide)

pH

Acid Urine	Alkaline Urine
Emphysema	Hyperventilation
Diabetes mellitus	Vomiting
Strvation	Renal tubular acidosis
Dehydration	Presence of urease-producing bacteria
Diarrhea	Vegetarian diet
Presence of acid-producing bacteria	Old specimens
High protein diet	
Cranberry juice	
Medication (methionine, mandelic acid, etc.)	

pH

- Principle
  - Double indicator system – Methyl es and bromthymol blue are used to give distinct color changes from orange to blue
- Interfering substances
  - No interferences with test results are known
  - Erroneous results can occur form pH changes caused by
    - Improper storage of specimen with bacterial proliferation
    - Contamination of the specimen container before collection
    - Improper regent strip technique causing the acid buffer from the protein test pad to contaminate the pH test area

Blood

- Blood can enter the urinary tract anywhere form the flomeruli to the urethra or can be a contaminant
  - The presence of any blood is considered abnormal
- Strips pick up the presence of RBCs, hemoglobin, or myoglobin
  - Hematuria
  - Hemoglobinuria
  - Myoglobinuria

Blood

Hematuria	Hemoglobinuria	Myoglobinuria
Renal calculi	Transfusion reactions	Muscle trauma/crush syndrome
Glomerulonephritis	Hemolytic anemias	Prolonged coma
Pyelonephritis	Severe burns	convulsions
Tumors	Strenuous exercise/RBC trauma	Alcoholism/overdose
Exposure to toxic chemicals		Drug abuse
Anticoagulants		Extensive exertion
Strenuous exercise		

Blood

- Principle
  - Pseudoperoxidase activity of the heme portion of ehmoglobin
  - Chromogen reacts with a peroxide presence of hemoglobin or myoglobin to become oxidized; produces color change from yellow to green
  - Interfering substances
    - False positive=Menstrual contamination, microbial perxidase, soaps and detergents
    - False Negative= Ascorbic acid (for some strips), High SG, unmixed specimens, high concentration of nitrite
      - Ascorbic acid reacts with the peroxide impregnated on the blood reagent pad and removes it from the intended reaction (prevents oxidation of the chromogen)

Leukocyte Esterase

- Detects the presence of WBCs
  - Allows to detect WBCs even when they have lysed
- Normal urine=few WBCs may be found
- Significant numbers of leukocytes indicate inflammation anywhere in the kisneys or lower urinary tract
- LE specific for granulocytes, monocytes, and macrophages…NOT LYMPHOCYTES

Leukocyte Esterase

- Principle
  - Action of leulocyte esterase to cleave an ester, impregnated in the reagent pad to form an aromatic compound
  - Followed by diazocoupling with diazonium salt on reagent pad to produce azo dye resulting in a color change form beige to violet
- Interfering substances
  - False positive
    - Vaginal contamination
    - Highly pigmented urine
    - Strong oxidizing agents
  - False negative
    - High SG
    - High glucose or protein levels
    - Ascorbic acid
    - Certain antibiotic drugs

Nitrite

- Detect UTI
  - Bacteria up the urethra into the bladder
  - Usually gram-negative bacilli that are normal bacteria from the intestinal tract
    - E. coli, Proteus, Enterobacter, and Klebsiella (most common)
  - In order for nitrite reduction to occur:
    - Microbe must be a nitrate-reducer; not all bacteria contain the enzyme necessary to reduce dietary nitrates to nitrite
    - Adequate time between voids for bacterial conversion; First morning void or urine that has been in the bladder for at least 4 hours
    - Adequate dietary nitrate consumption

Nitrite

- Principle
  - Diazotization reaction of nitrite with an aromatic amine to form a diazonium salt
  - Followed by azocoupling with aromatic compound on reagent pad; azo dye formed causes a color change from white to pink
- Interfering substances
  - False positive
    - High pigmented or color of urine
    - Not performed on fresh urine
  - False negative
    - Testing urine that has not been in bladder for at least 4 hours
    - Some bacteria do not produce enzyme necessary to reduce nitrate to nitrite
    - Dietary nitrates are absent
    - Ascorbic acid
    - High S.G.
    - Antibiotics

Protein

- Normal adults lose up to 150 mg/24 hr
  - Less than 10 mg/dL
- Presence of protein is an early indicator of renal disease
- Low molecular weight proteins readily pass through the glomerular filtration barrier and are reabsorbed
  - Albumin (most common)
  - Uromodulin/Tamm-Horsfall
  - Microglobulins
  - Proteins from prostatic/vaginal secretions
- High molecular weight proteins are unable to penetrate a healthy glomerular filtration barrier

Proteinuria

- Overflow proteinuria- resulting from increase in LMW plasma proteins passing through the glomerular filtration
  - Caused by severe infections/inflammation, intravascular hemolysis, muscle trauma, or multiple myeloma
- Postrenal – proteins produced by the urinary tract
  - Blood proteins leak into the urinary tract as a result of bacterial/fungal infections, physical injury, menstrual contamination, prostatic fluid contamination

Proteinuria

- Renal proteinuria- defective glomerular filtration barrier or tubular reabsorption defect
  - Glomerular- defective glomerular filtration barrier
    - Causes:
    - Disease states: Amyloidosis, diabetes mellitus, presence of toxic substances, streptococcal glomerulonephritis, collagen disorders, immune complexes seen in dysglobulinemias
    - Functional/Benign sources: strenuous exercise, fever, hypothermia, emotional distress, dehydration, posture (orthostatic proteinuria)
  - Tubular- small amount of protein gets into the glomerular filtrate and isn't reabsorbed
    - Caused by Fanconi's syndrome, exposure to toxic materials, severe viral infections

Protein

- Principle
  - Protein error of indicators- pH held constant by buffer, certain indicator dyes release hydrogen ions as a result of the presence of proteins and cause a color change from yellow to blue-green
- Interfering substances
  - Extremely alkaline or highly buffered urine can overwhelm the buffering capacity of the reagent strip to produce false-positive results

Sulfoslaycilic Acid Confirmation Test

- Performed on clear supernatant; mix with SSA and observe for turbidity
- Sensitive to 5-10 mg/dL of any type of protein
- False positives can result from precipitation of non-protein compounds
  - Look microscopically at the SSA precipitate
  - If crystals form, may be drugs or contrast media
- False negatives can occur with highly alkaline urine

Glucose

- Glucosuria or glycosuria – presence of glucose in the urine
- Normally almost all of the glucose filtered by the glomerulus is reabsorbed by the proximal convoluted tubule
- When blood glucose levels elevate and kidney reaches a reabsorption capacity, threshold level of 160-180 mg/dL, glucose will appear in the urine at detectable amounts
- Can be due to a prerenal or renal condition
  - Diabetes mellitus, gestational diabetes, acromegaly, Cushing's Syndrome, hyperthyroidism, pheochromocytoma, pancreatitis, etc.

Other sugars

- Galactose, fructose, lactose, maltose, and pentoses are not detected by the strip
- Galactose is most clinically significant
  - Represents an "inborn error of metabolism", whereby the enzyme necessary to metabolize galactose to glucose is missing or reduced
    - Failure to thrive, mental retardation, but can be corrected with elimination of galactose in diet

Glucose

- Principle
  - Double sequential enzyme reaction- Glucose oxidase (on pad) catalyzes oxidation of glucose to produce gluconic acid and peroxide.
  - Peroxidase catalyzes the reaction between peroxide and chromogen on pad to form an oxidized colored compound that represents the presence of glucose. (Color change varies with chromogen used)
  - Detects only glucose
- Interfering substances
  - False positive
    - Strong oxidizing agent
    - Contaminating peroxides
  - False negative
    - Ascorbic acid concentration of 50mg/dL or more
    - High S.G.
    - Low temperature
    - Improper storage

Ketones

- Identifies three intermediate products of fat metabolism (acetoacetate, acetone, and β- hydroxybutyrate)
- When carb availability is limited, the liver must oxidize fatty acids as its main metabolic substrate
  - Large amts. of ketones are released in the blood, and when the renal absorption threshold is exceeded, ketones are excreted in the urine
  - Can be due to:
    - Inability to use carbs
    - Inadequate carb intake
    - Loss of body carbs
  - Seen in diabetes mellitus, starvation, malabsorption/pancreatic disorders, strenuous exercise, vomiting

Ketone Principle

- Principle
  - Nitroferricyanide reacts with acetoacetate in an alkaline medium to produce a color change from beige to purple
  - Beta-hydroxybutyrate is not detected, and in some test strips, glycine is added to detect acetone.
- Interfering substances
  - False Positive
    - Some drugs
    - Highly pigmented urines
  - False Negative
    - Improper storage
    - Breakdown of acetoacetic acid by bacteria
    - Acetest can be done as a confirmatory test
    - Serum, urine, plasma or whole blood

Bilirubin

- Breakdown of hemoglobin released from old RBC's
- Indirect bilirubin reversibly binds to albumin and is carried to the liver
- Hepatocytes remove it from the albumin and it is conjugated with glucuronic acid to produce watersoluble, non-toxic bilirubin termed conjugated or direct bilirubin.
- Liver excretes conjugated bilirubin as a constituent of bile and passes into the small intestine
  - Should conjugated bilirubin reenter the circulation, it can be excreted rapidly by the kidneys into the urine
- In the intestinal tract, conjugated bilirubin is converted back to unconjugated form
  - Reduced to urobilinogen where 2-5% is carried to the kidney where it is readily filtered and excreted into the urine

Bilirubin

- Normal levels of bilirubin = non-detected amount (approx. 0.02mg/dL)
- Normal levels of urobilinogen=0.1-1.0 Ehrlich unit/dL
- Increases are seen in:
  - Bile duct obstructions: gallstones, pancreatic cancer
  - Liver damage: hepatitis, cirrhosis, Dubin-Johnson Syndrome, Rotor's Syndrome
- Clinical Significance
  - Prehepatic conditions
    - Increased urine urobilinogen
    - Normal urine bilirubin
  - Hepatic diseases
    - Increased urine bilirubin
    - Increased urine urobilinogen
  - Posthepatic conditions
    - Normal or absent urine urobilinogen
    - Increased urine bilirubin

Bilirubin

- Principle
  - Coupling reaction between bilirubin and a diazonium salt (on pad)
  - Azodye forms producing a color change from light tan-beige or pink depending on manufacturer
- Interfering substances
  - False positives
    - Pigmented materials / drugs in urine
  - False negatives
    - Ascorbic acid
    - Elevated nitrites
    - Old specimens

Ictotest Confirmation Test

- • Less subject to interference than the reagent strip method, and more sensitive (0.05- 0.1 mg/dL)
  - May be positive and dipstick negative
- Procedure
  - 10 drops of urine on special pad
  - Add one tablet
  - 2 drops of water, flows onto pad
  - After 30 seconds, a blue to purple color appears if positive
  - Red or pink- negative

Urobilinogen

- Major excretion is in feces, but small amounts excreted in urine
  - Specimen should be fresh and room temp
  - Choice specimen for urobilinogen is a 2-hour collection following lunch
- Increased in hemolytic conditions or in late liver damage
- Decreased in carcinoma, calculi formation or fibrosis

Urobilinogen

- Principle
  - Coupling reaction between urobilinogen and a diazonium salt (on pad)
  - Azodye forms producing a color change from light pink to dark pink depending on manufacturer
- Interfering substances
  - False positives
    - Other Ehrlich-reactive substances such as porphobilinogen, sulfonamides and p-aminosalicylic acid (test is not specific for urobilinogen)
    - Highly pigmented urine
  - False negatives
    - Formalin
    - High levels of nitrites

Effects of common interfering substances

- Radiographic contrast media- increases S.G. and causes turbidity with SSA test
- Pyridium (Phenazopyridine)- causes highly orange urine; results in false positives for:
  - Protein
  - Bilirubin
  - Urobilinogen
  - Leukocyte Esterase
  - Nitrite
- Ascorbic Acid- reducing agent; results in false negatives for:
  - Blood
  - Glucose
  - Bilirubin
  - Nitrite
  - Leukocyte Esterase

Visualization of urine sediment

Low Power Examination (10X objective)

- **Casts** Each type of cast is graded and enumerated separately, and reported as the average number of casts observed per LPF
- **Squamous epithelial cells.** Reported as few, moderate, or many; or as 1+, 2+, and so forth, per LPF
- **Crystals** Normal crystals are reported as few, moderate, or many per LPF (note some laboratories use high power for this). Abnormal crystals are reported as the average number seen per LPF but should be confirmed before they are reported
- **Mucus.** This can be reported as scant, moderate, or heavy.

Visualization of urine sediment

High Power Examination (40X objective)

- **Red blood cells.** Graded and reported on the basis of the average number seen per HPF. Abnormal forms such as dysmorphic cells are reported.
- **White blood cells.** Graded and reported on the basis of average number seen per HPF. The WBCs are usually neutrophils, but if unusual types such as lymphocytes or eosinophils can be identified, they should be reported
- **Epithelial cells.** Renal tubular epithelial cells (RTE; identified as to type), transitional epithelial cells and oval fat bodies may be reported as few, moderate, or many; or as 1+, 2+, and so forth, per HPF.
- **Miscellaneous.** This category includes structures such as microorganisms, parasites, sperm (reported for men only), and the like. These categories may be graded as few, moderate, or many, or described numerically such as 1+, 2+, 3+, and so forth.

TNTC: too numerous to count

Staining

- Supravital Stains
  - Crystal violet and safranin- gives more detailed image of the internal structure of WBCs, epithelial cells, and casts
- Fat/Lipid Stains
  - Sudan III or Oil Red O- confirms presence of neutral fat or triglyceride (stains red or orange)
- Gram Stain
  - To ID bacteria in the urine and differentiate them as gram positive or negative
- Prussian Blue Stain
  - To visualize hemosiderin (iron stains blue)
- Hansel Stain
  - Used to specifically identify eosinophils
- Acetic Acid
  - Accentuates the nuclear pattern of WBCs and epithelial cells while lysing RBCs

Red Blood Cells

- Appear as smooth, biconcave discs that are moderately refractile
- • Crenated
- • Ghost cells
- • Dysmorphic
- •Normal range- 0-3/HPF
- •Seen in:
- • Glomerular damage
- • Vascular injury within the urinary system
- • Acute infection/inflammation
- • Toxic reactions to drugs
- • Physiologic causes (e.g. strenuous exercise)
- • Other diseases in the GI tract (e.g. acute appendicitis, tumors in the colon)

White blood cells

- Appearance as spherical cells with characteristic cytoplasmic granules
- and nucleus; about double the size of a RBC
- • Glitter cells- in hypotonic urine, WBCs swell and granules exhibit Brownian
- movement
- • Normal- 0-8/HPF
- • Seen in response to inflammatory processes/bacterial infections like
- in pyelonephritis, cystitis, urethritis, prostatitis, yeast infections,
- mycoses, etc.

WBCs

- •Correlation
- • Cloudy
- • Foul odor
- • + Leukocyte Esterase
- •Look-alikes
- • Renal Tubular Epithelial Cells
- • RBCs
- •Discrepancies
- • Positive strip leukocyte esterase; no WBCs seen in microscopic exam
- • Cell lysis
- • WBCs in microscopic exam; negative strip leukocyte esterase
- • Misidentification of WBCs
- • WBCs don't contain leukocyte esterase (lymphocytes)

Epithelial cells

- Can result from normal cell turnover or from epithelial damage and
- sloughing caused by inflammatory processes or renal disease
- • Type of cell encountered depends on the location of the disease process that
- is causing the epithelium to be injured
- • Squamous
- • Transitional
- • Renal Tubular

Squamuous epitelial cells

- • Largest and most common epithelial cell found in urine
- • Thin, flagstone-shape with distinct edges; small, condensed, centrally
- located nucleus about the size of a RBC
- • Line the entire female urethra, and the distal portion of the urethra in
- males

Transitional epitelial cells

- • Vary considerably in size; round or pear shaped with a dense
- oval/round nucleus and abundant cytoplasm
- • Few are present in the urine of normal healthy individuals
- • Increased in UTI
- • Line the renal calyces, renal pelvis, ureters, and bladder

Renal tubular epitelial cells

- • Round/oval; small, dense nucleus that is usually eccentric, and
- granular cytoplasm
- • Rarely appear in the urine of normal, healthy individuals
- • More are seen in newborns than in older children and adults
- • Presence of increased amounts of RTEs indicates tubular injury
- • Exposure to heavy metals
- • Drug-induced toxicity
- • Viral infections
- • Pyelonephritis
- • Malignancy

Casts

- •Formed in distal and collecting tubules with a core matrix of uromodulin
- •Factors influencing formation:
- • Acid pH, increased solute concentration, urinary stasis, increased plasma proteins
- •As tubular lumen contents become concentrated, uromodulin forms fibrils
- that attach it to the lumen cells while it traps any substances present in its
- matrix
- •Cast detaches from the tubular epithelial cells and is flushed through the
- nephron and into the urine
- •Appearance varies greatly depending on the diameter and shape of the
- tubule in which they were formed and the length of time spent in the tubule
- • Cylindrical with parallel sides and tapered ends
- ◦ Can be fragile and easily broken with vigorous mixing
- ◦ May disintegrate in hypotonic or alkaline urine

Casts

- • Normal- Few hyaline or finely granular casts
- • Clinical Significance
- • Reflects the status of the renal tubules; number and type of casts reflects the extent
- of tubular involvement and the severity of disease
- • Correlations
- • + Protein
- • Depends on cellular elements present in the cast
- • Classification
- • Based on the composition of the matrix and the substances trapped within them
- • Look-alikes
- • Mucous, fibers, squamous epithelial cells

Hyaline Casts

- Hyaline
- • Most common
- • Composed of homogenous uromodulin matrix; appear colorless with rounded
- ends; shape and size varies
- • Normal- 0-2/LPF
- • Can be seen in strenuous exercise, dehydration, fever, or emotional stress

Waxy casts

- Waxy
- • Edges are well-defined and often have sharp, blunt or uneven ends;
- often broad; have high refractive index
- • Indicate tubular obstruction with prolonged stasis seen in cases like
- renal failure, kidney transplant rejection, and some acute renal
- diseases
- • Correlate with a + dipstick protein

RBC and WBC casts

- • RBC casts
- • Must be able to unmistakably identify RBCs in cast matrix
- • Can degenerate into blood/hemoglobin casts
- • Associated with intrinsic renal disease; glomerular or tubular damage; strenuous exercise
- • Should see + blood and + protein on dipstick
- • WBC casts
- • Leukocytes imbedded in hyaline cast matrix; may not be readily evident because of cellular degeneration
- • Indicates renal inflammation or infection (pyelonephritis, acute interstitial nephritis, etc.)
- • If glomerular damage, then RBC casts will also be present.
- • If tubular disease, RBC casts will be absent.

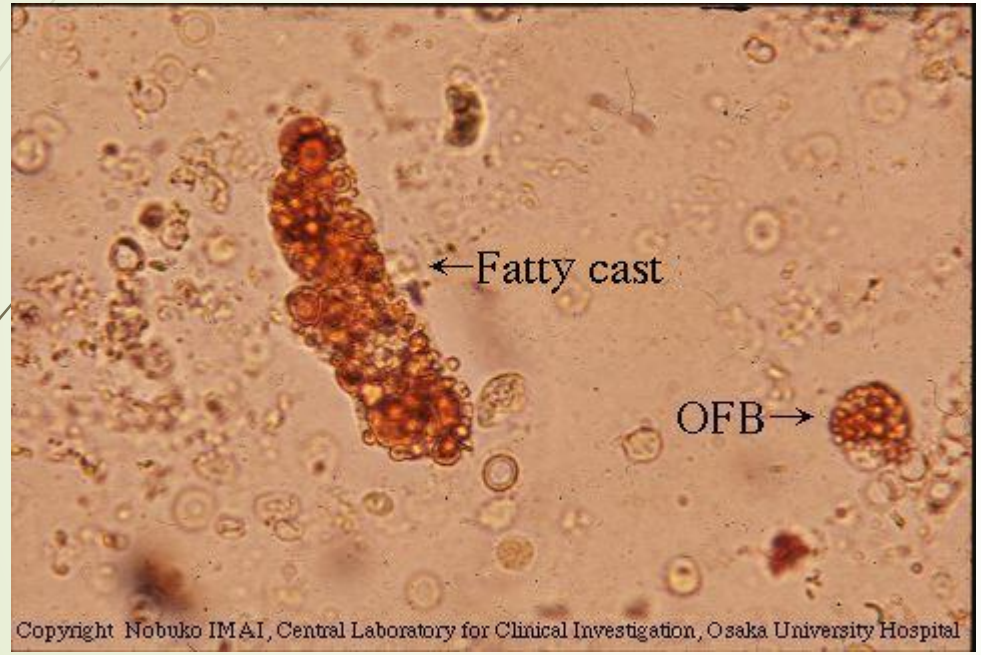
RTE,Mixed Cell and Bacterial Casts

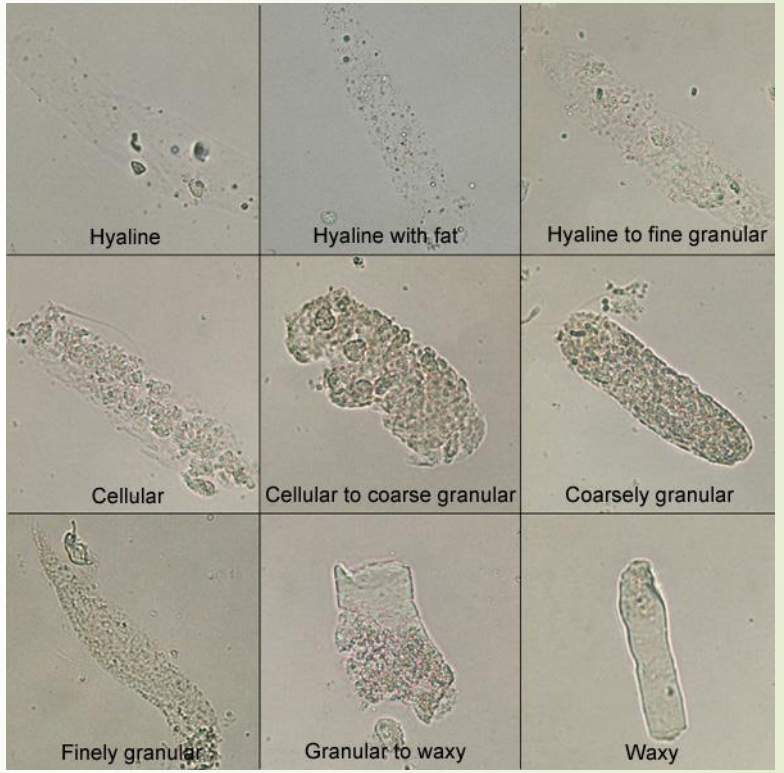
- Renal Tubular cell Casts
  - Non specific markers of tubular injury
  - Seen due to:
    - Heavy metal, chemical or drug-induced toxicity
    - Viral infection
    - Graft rejection
  - Difficult to distinguish from WBC casts
- Mixed cell casts
  - Any combination of cellular elements possible
- Bacterial Casts
  - Usually identified as WBC casts because they are normally present together
  - Diagnostic of pyelonephritis

Other Types of Casts

- Granular casts
  - Granules are a by product of protein metabolism and or cellular breakdown
  - Seen in glomerulonephritis, pyelonephritis, and stress/exercise
- Fatty casts
  - Contain free fat globules, oval fat bodies, or both
  - Indicate renal tubular cell death
  - Stain with Sudan III or Oil Red O and polarize
  - Seen in nephrotic syndrome, severe crush injuries, toxic tubular necrosis, or diabetes mellitus
- Pigmented Casts
  - Hemoglobin, myoglobin or bilirubin
- Brad Casts
  - Indicate cast formation in dilated tubules or the large collecting duct
  - Because several nephrons emty into a single collecting duct, cast formation hereindicates significant urinary stasis because of obstruction or disease

Granular and fatty Casts





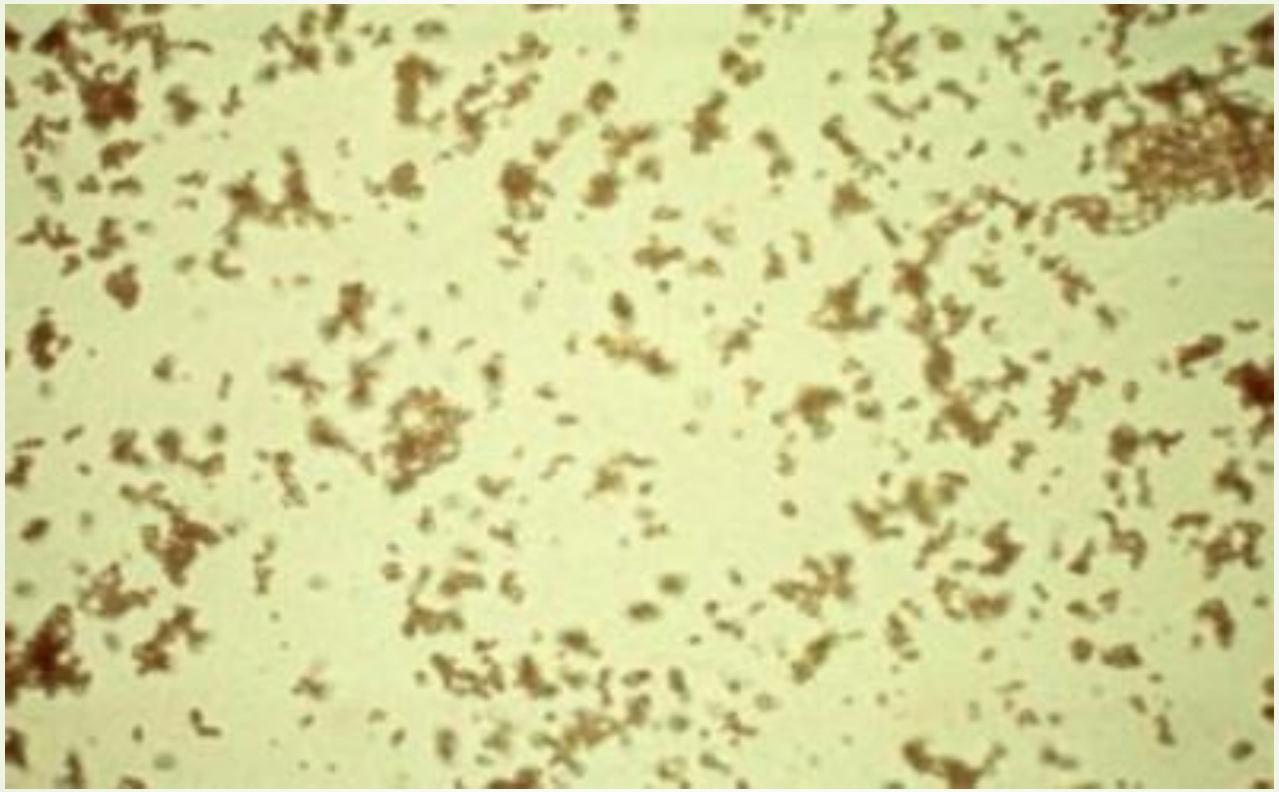
Crystals

- Result from the precipitation of urinary solutes out of solution
- Normally not present in freshly voided urine
- Most crystals are not clinically significant, but some indicate a pathologic process
- Factors contributing to crystal formation:
  - Concentration of the solute in urine
  - Urinary pH
  - Flow of urine through the tubules

Acid Urine Crystals

- Amorphous Urates
  - pH between 5.7-7.0
  - Urate salts precipitate in an amorphous form
  - Appears as small, yellow –brown granules, sand
  - "Brick dust"color from uroerythrin deposition
  - Soluble in alkali and heat
  - No clinical significance
- Acid Urates
  - Neutral to slightly acidic pH
  - Sodium, potassium, and ammonium salts of uric acid that appear as small, yellow-brown balls or spheres
  - Soluble with heat
  - Not clinically significant

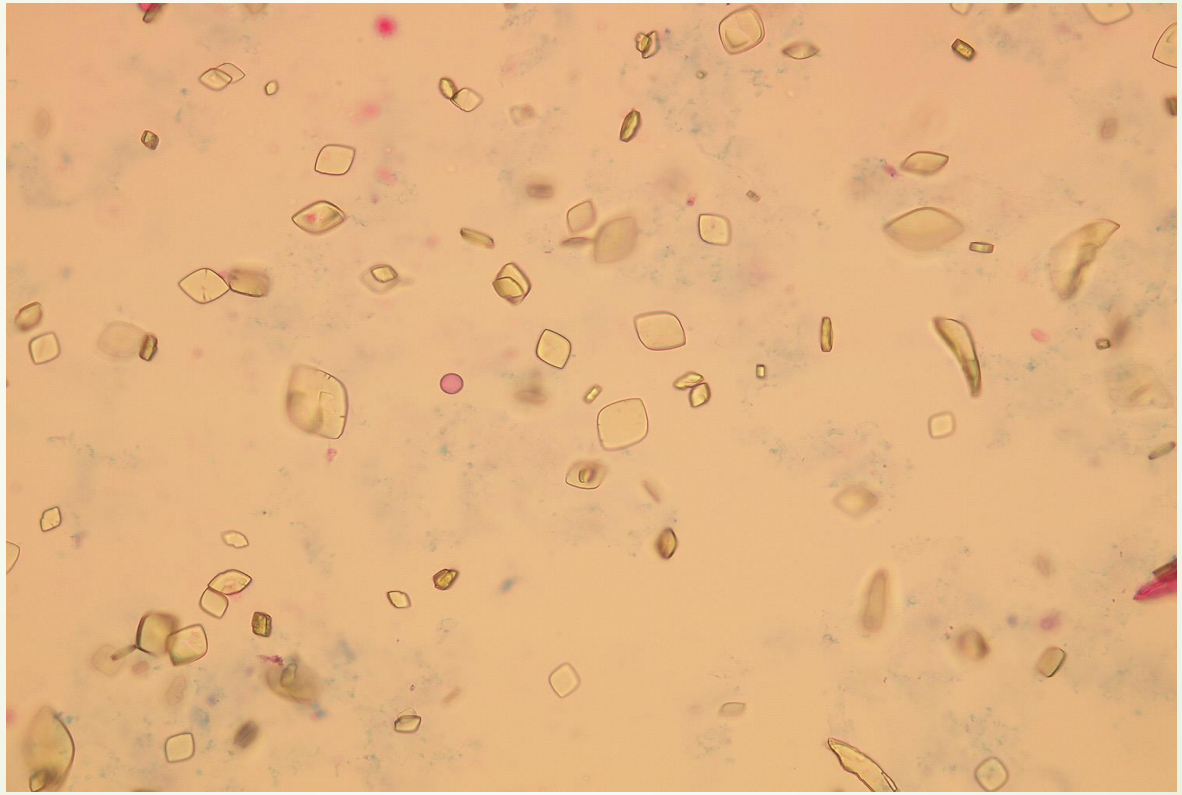
Amorphous Urates



Acid Urine Crystals

- Monosodium Urate
  - Appears as slender, pencil-like prisms (ends are not pointed)
  - Soluble with heat
  - No clinical significance
- Uric Acid
  - Only present in pH less than 5.7
  - Several forms, most common is diamond shape
  - Polarize, soluble in alkali
  - Can appear in the urine of healthy individuals, as a result of administration of cytotoxic drugs, due to gout, or because of increased purine metabolism

Uric Acid



Acid Urine Crystals

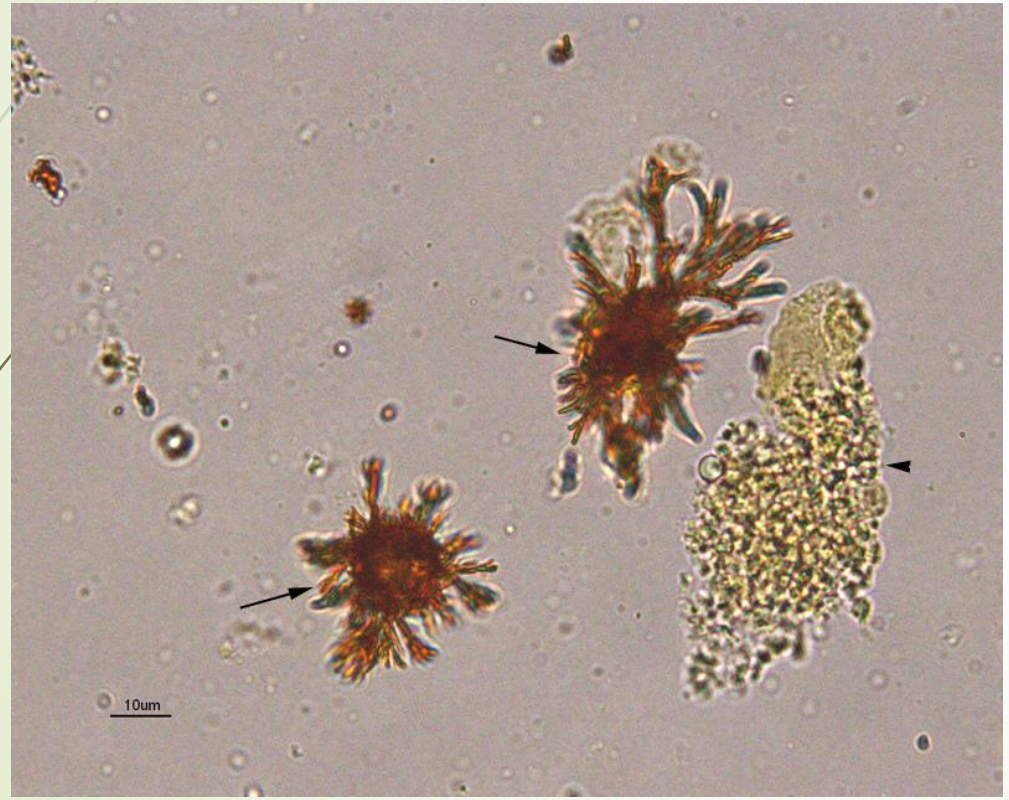
- Calcium Oxalate
  - Most commonly encountered crystal in human urine
  - May vary significantly in color, shape and size
  - Can be found at any pH, polarize
  - Found in the urine of healthy individuals from oxalate consumption, with ethylene glycol consumption, or severe chronic renal disease

Calcium Oxalate

Acid Urine Crystals

- Bilirubin
  - Precipitate when large amounts of bilirubin are present in the urine
  - Appear as small clusters of fine needles, yellow-brown color
  - Dissolve when alkali or strong acids are added
  - Classified as abnormal because they are associated with liver disorders
  - Should be seen with a positive dipstick bilirubin test
- Cystine
  - Appear as colorless, hexagonal plates, clear, refractile
  - Indicate congenital cystinosis or cystinuria, cause renal damage when they deposit in tubules
  - Confirm with cyanide nitroprusside reaction
  - Seen in pH less than 8.0
  - Dissolve in alkali and HCl, do not polarize

Bilirubin and Cystine



Acid Urine Crystals

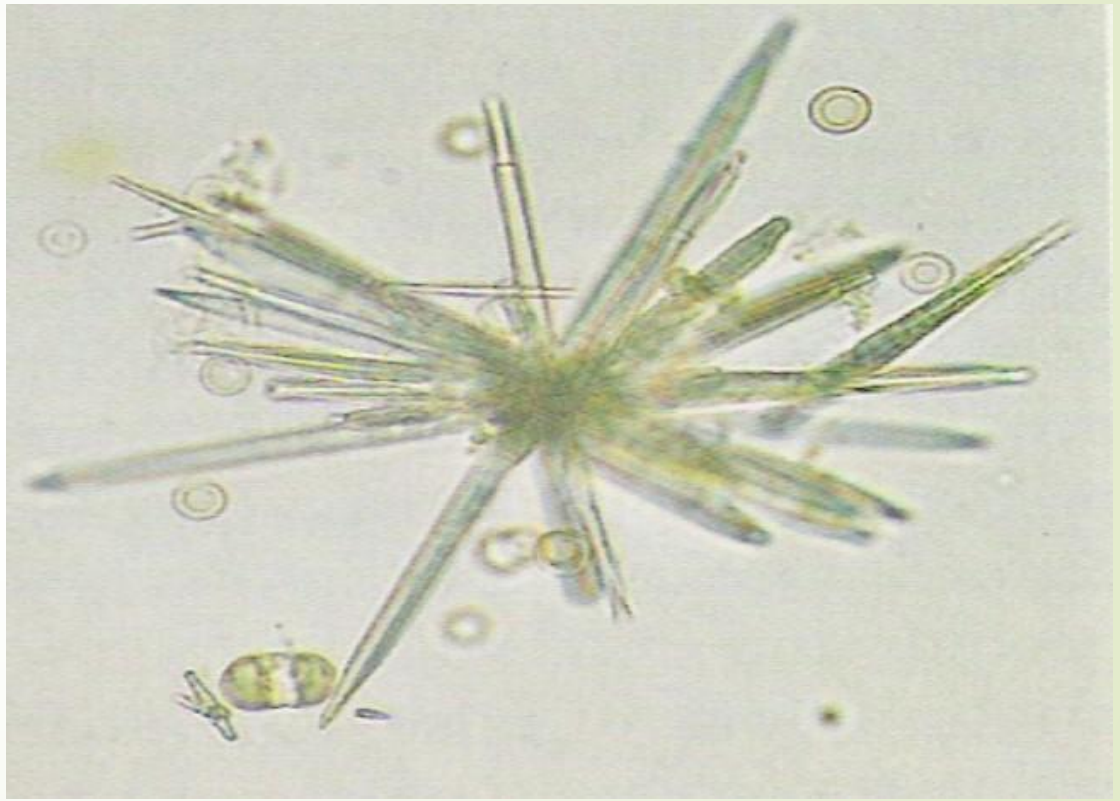
- Tyrosine/Leucine
  - Tyrosine: Fine, delicate needles that arr colorless or yellow, often aggregate to form clusters
  - Leucine: Yellow to brown spheres with concentric circles or radial striations on their surface
  - Both form in acidic urine and dissolve in alkaline solution
  - Abnormal, result of overflow aminoaciduria in patients with inherited metabolic disorders or severe liver disease
  - Should see with a positive dipstick bilirubin
- Cholesterol
  - Appear in clear, flat, rectangular plates with notched corners
  - Form in acid urine and dissolve in chloroform and ether
  - Seen in nephrotic syndrome, lipid disorders, or with ruptured lymphatic vessels into the renal tubules
  - Can be confused with crystals from radiopaque contrast media
  - Often seen with fatty casts, oval fat bodies, and positive dipstick protein

Tyrosine, Leucine and Cholesterol

Acid Urine Crystals

- Medications
  - Medications and metabolites are eliminated from the body via the kidneys; high concentration can cause precipitation out of solution
  - Can form in vivo and cause kidney damage
  - Ampicillin
    - Long, colorless, thin prisms or needles, aggregate into clusters
  - Sulfonamides
    - Yellow to brown bundles of needles that resemble sheaves of wheat, or brown rosettes or shperes with radial striations
    - Polarize
- Radiographic contrast media
  - May appear as colorless, long, pointed needles that occur singly or clustered in sheaves or as flat, elongated, rectangular plates
  - Will present with a high specific gravity

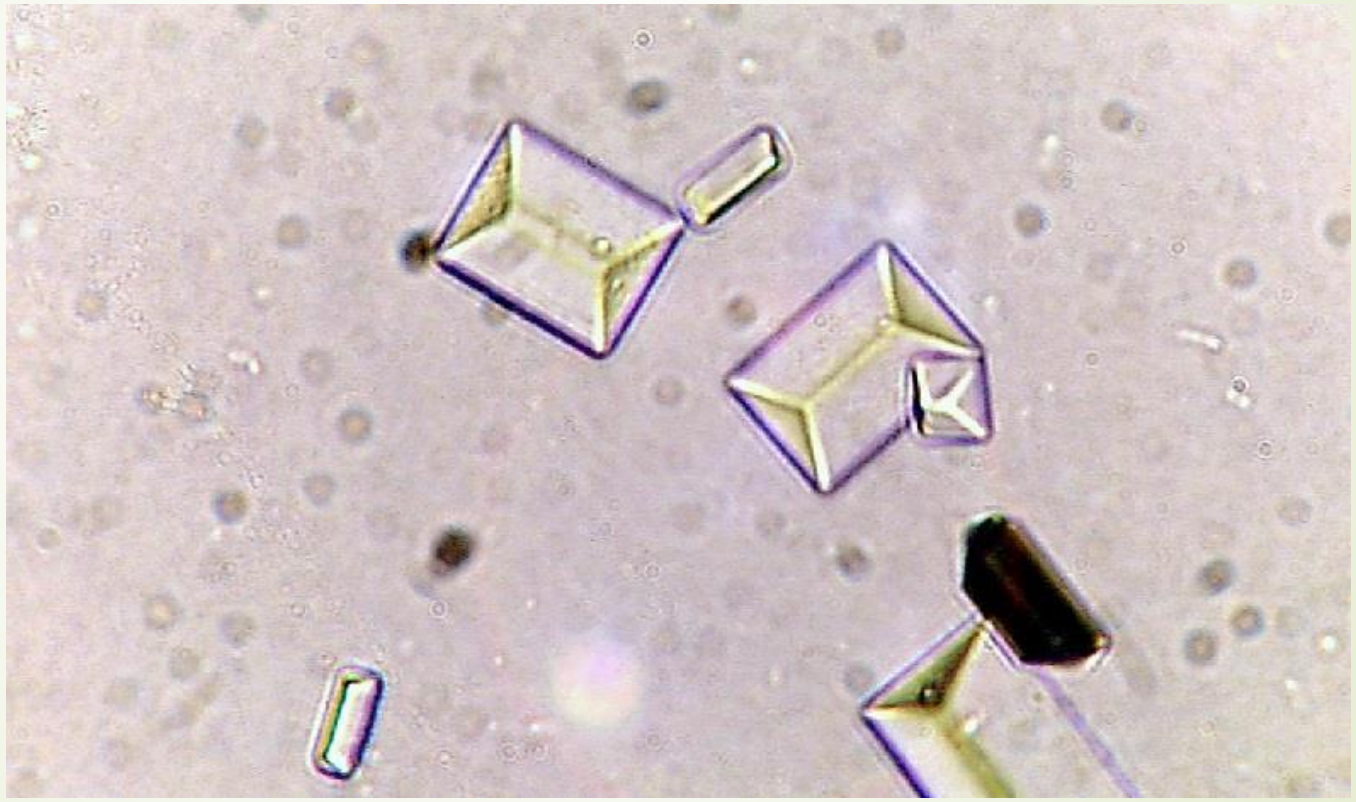
Sulfa and Contrast Media



Alkaline Urine Crystals

- Amorphous phosphate
  - Microscopically indistinguishable from amorphous urates, white precipitate
  - Soluble in acid and do not dissolve with heat
  - No clinical significance
- Triple phosphate
  - Most common crystal in alkaline urine
  - Appears as a 3 to 6-sided prism (coffin lid); size can vary greatly
  - Can be present in urine of healthy individuals; little clinical significance
  - Can be associated with UTIs and renal calculi formation

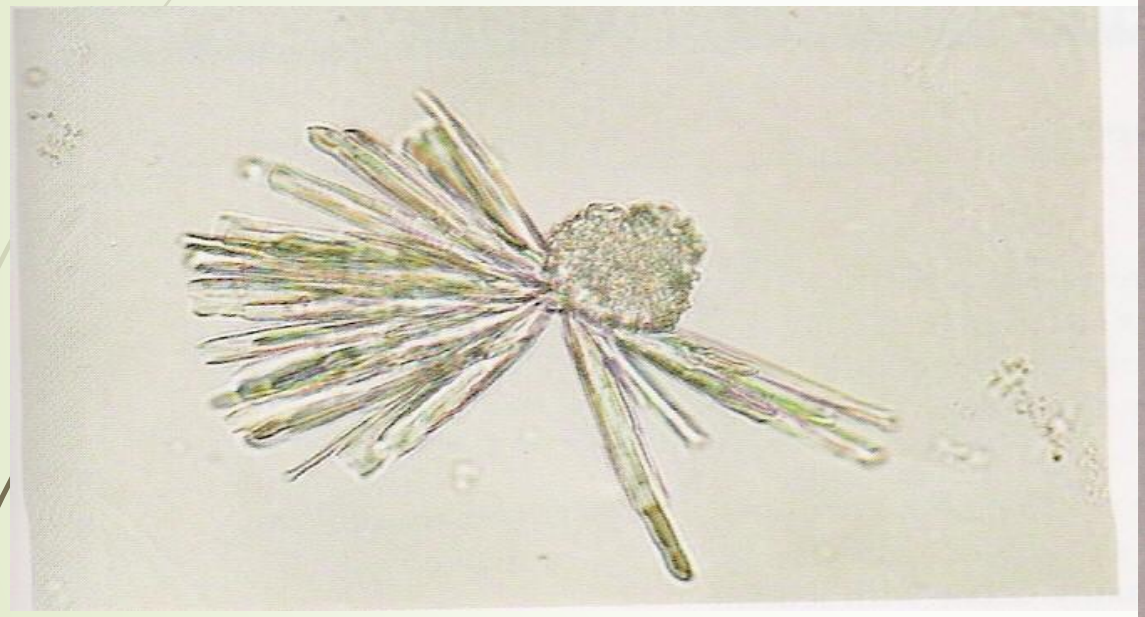
Triple Phosphate



Alkaline Urine Crystals

- Calcium Phosphate
  - Dibasic, colorless, wedge-like prisms arranged in small groupings OR thin, long needles arranged in bundles or sheaves
  - Monobasic-Irregular, granular sheets or flat plates
  - Polarize weakly
  - No clinical significance
- Ammonium Biurate
  - Appear as yellow-brown spheres with striations on the surface; "thorny apple" appearance
  - Appear in alkaline or neutral urine; dissolve with heat
  - Convert to uric acid crystals when acetic acid added
  - Occur most frequently in specimens that have undergone prolonged storage, but can be significant when formed in vivo

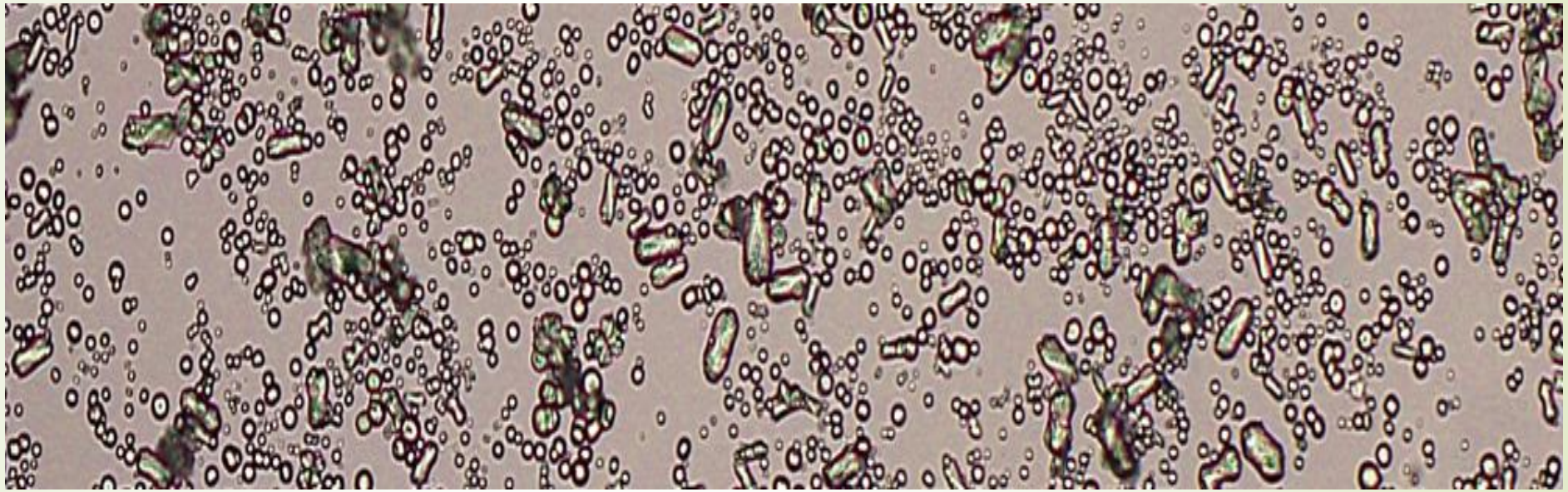
Calcium Phosphate and Ammonium Biurate



Alkaline Urine Crystals

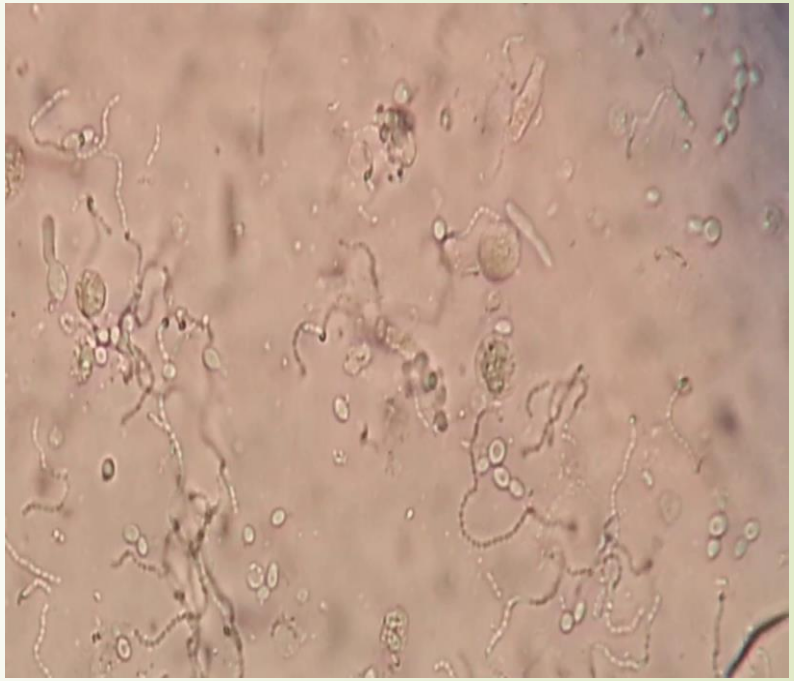
- Calcium Carbonate
  - Appears as small colorless granular crystals (slightly larger than amorphous); usually found in pairs giving them a dumbbell shape
  - Polarize
  - Demonstrate effervescence with addition of acetic acid
  - No clinical significance

Calcium Carbonate



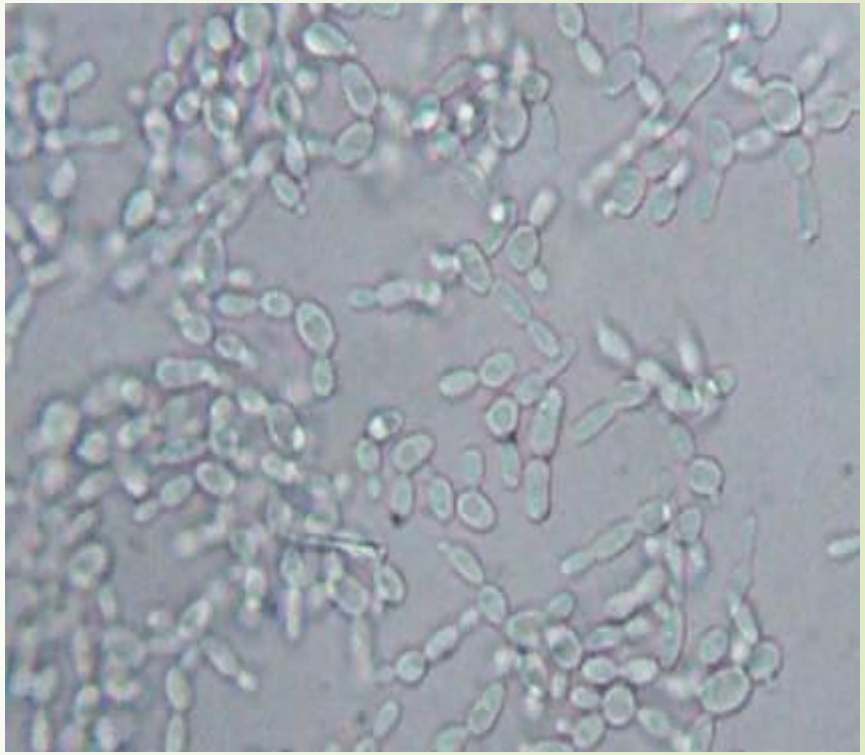
Bacteria

- Observed under high power magnification
- Usually rod-shaped and vary in size from long and thin to short and plump; can be spherical shaped
- Motility often distinguishes bacteria from amorphous substances that may also be present
- Implies the presence of UTI or specimen contamination
  - In UTI bacteria is usually accompanied by WBCs

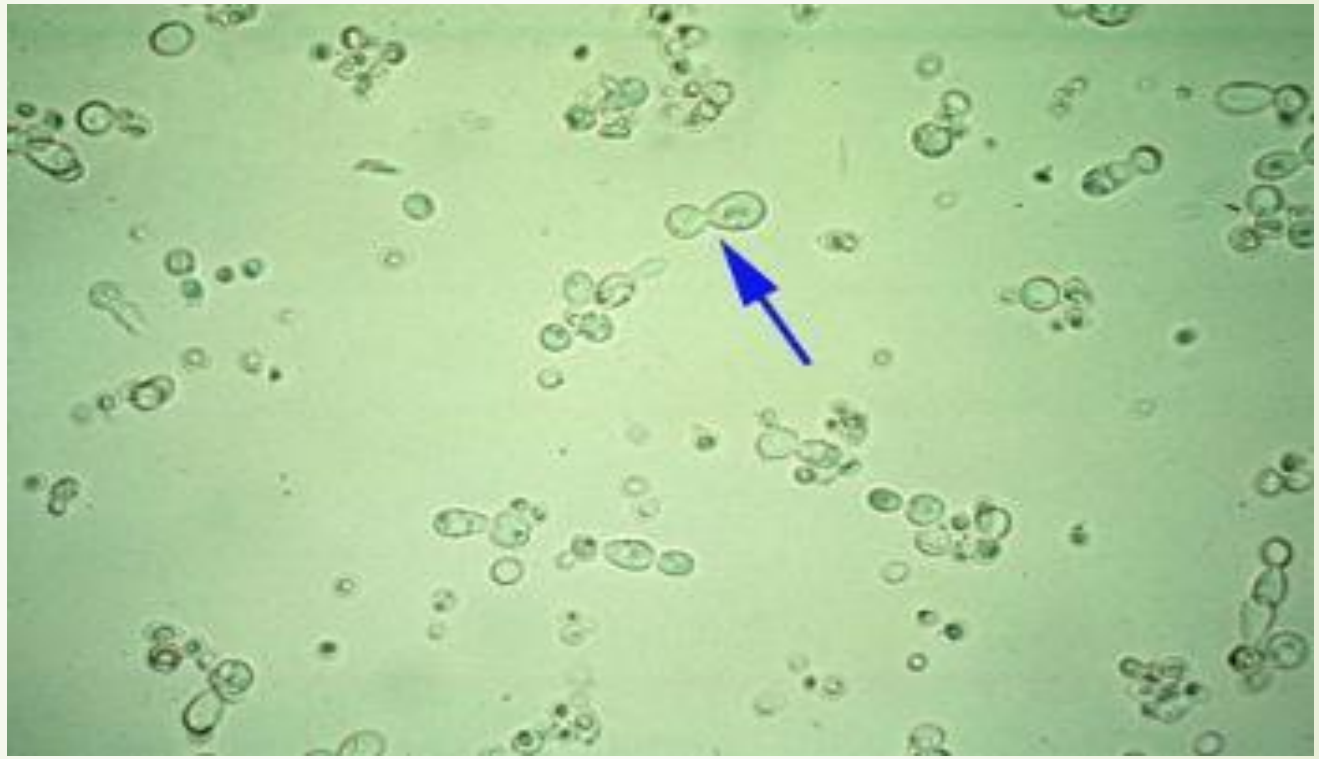


Yeast

- Ovoid cells; resemble RBCs; can show budding or pseudohyphae
- Do not dissolve in acetic acid or stain with supravital stain
- Often represent vaginal infection with subsequent contamination of the urine during collection



Yeast



Oval Fat Bodies

- Oval fat body - Renal tubular epithelial cell absorbs lipid from the glomerular filtrate
  - Can be seen in conditions like nephrotic syndrome, preeclampsia, diabetes mellitus and extreme physical exercise/trauma
  - Lipids most often enter the urine because changes in glomerular filtration barriers; should be accompanied by some degree of proteinuria
- Look-alikes
  - Starch granules and RBCs
  - Contaminants
    - Lubricants, ointments, creams and lotions

Trichomonas Vaginalis

- Protozoan flagellate that causes parasitic gynecological infections in female patients
  - Transmitted sexually
  - Turnip shaped; four anterior flagella, posterior axostyle, and undulating membrane
  - Look-alikes
    - WBCs and renal tubular cells
    - Motility is critical to identification

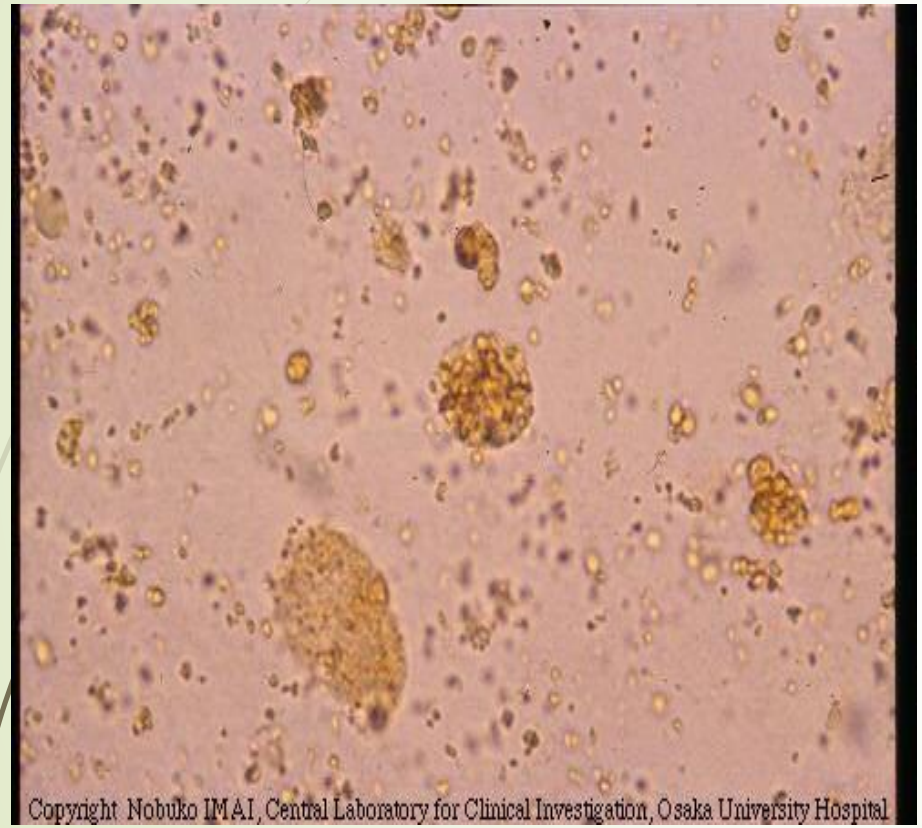
Other Formed Elements in Urine

- Hemosiderin
  - A form of iron from ferritin denaturation
  - Seen in urine sediment 2-3 days after a severe hemolytic episode
  - Identify by staining with Prussian Blue
- Spermatozoa
  - Oval head and thin thread-like tall
  - Indicates recent intercourse or ejaculation
  - Can be found in males and females

Hemosiderin and Spermatozoa

- Hemosiderin
  - Form of iron resulting from ferritin denaturation
  - Seen in urine sediment 2-3 days after a severe hemolytic episode
  - Identify by staining with Prussian Blue
- Spermatozoa
  - Oval head and thin, thread-like tail
  - Indicates recent intercourse or ejaculation
  - Can be found in males and females

Hemosiderin

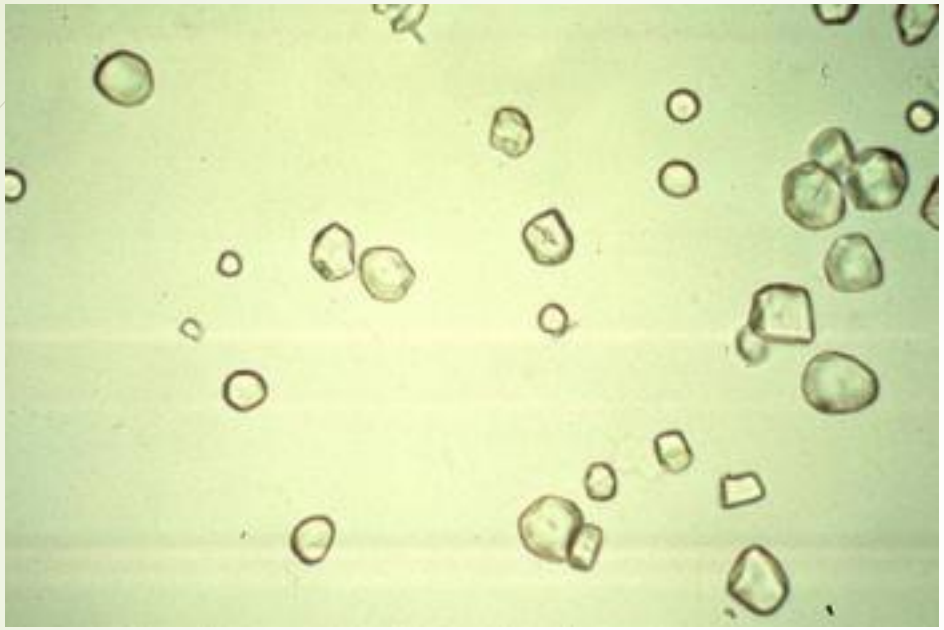


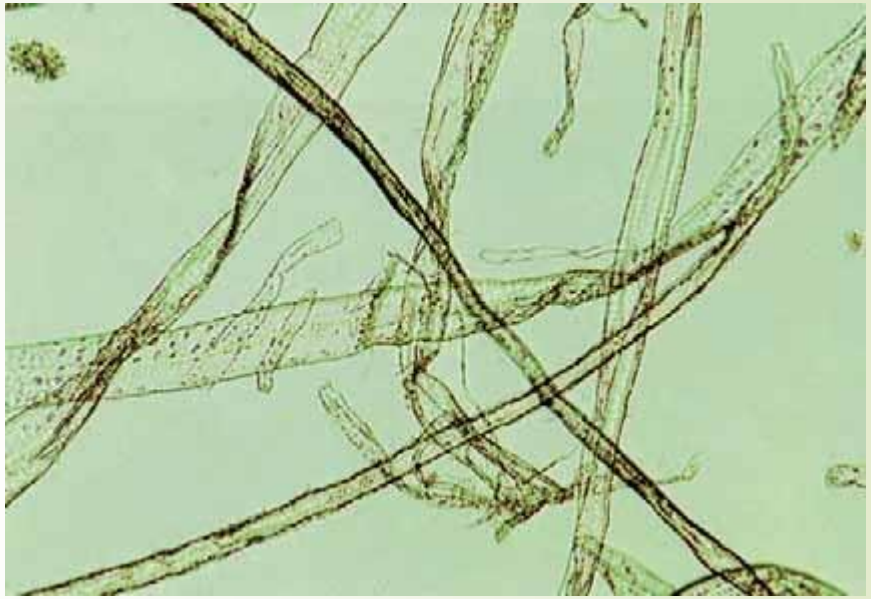
Spermatozoa

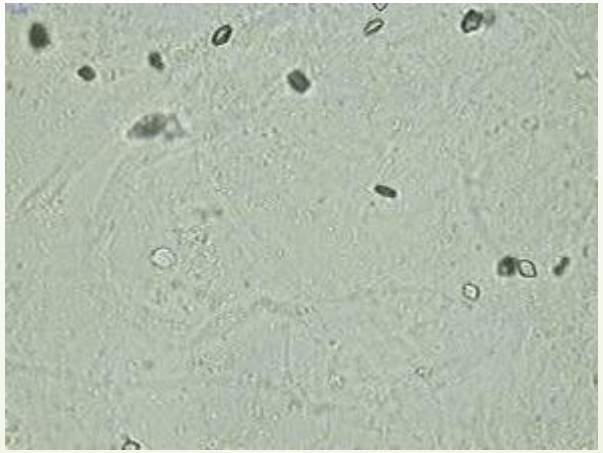
Contaminants

- Fecal Material
  - Result of either improper collection technique through an abnormal connection or fistula between the urinary tract and the bowel.
- Starch
  - Originate form body powders or protective gloves of health care workers
  - Vary in size; central dimple; exhibit a Maltese cross upon polarization.
- Fibers
  - Hair, cotton, other fabric threads
  - Can be large with distinct edges; moderately to highly refractile; polarize
- Mucus threads
  - No clinical significance
  - Appears as delicate, ribbon-like strands with irregular or serrated ends
  - Usually contaminant from vaginal epithelium
  - Commonly misidentified as a hyaline cast

Starch, Fiber , Mucus







Common ID Errors

Element	Identification Errors
RBC	Yeast, oil droplets, air bubbles
WBC	Renal tubular epithelial cells
Oval Fat Body (OFB)	Air bubbles
Squamous Epithelial Cells	Casts
Transitional and Renal Epithelial cells	Resemble each other
Mucus Threads	Hyaline Casts
Bacteria	Amorphous urates/phosphates
Trichomonas	WBCs, renal tubular epithelial cells

Case #1

- Woman, 45 years old, with type 1 diabetes mellitus is admitted to the hospital and has been given a preliminary diagnosis of nephrotic syndrome. She has not been feeling well for the past week and has bilateral pitting edema in her lower limbs. Her admission urinalysis results are:

Physical Exam	Chemical Exam	Confirm Tests
Color: Colorless	SG: 1.010	
Clarity: Clear	pH 5.0	
Large amt. of white foam	Blood: Small	
Physical Exam	Protein: 500 mg/dl	SSE: 4+
	LE: Negative	
	Nitrite: Negative	
	Glucose: 250 mg/dl	
	Ketone: Negative	
	Bilirubin: Negative	
	Urobilinogen: Normal	

Case #1

- Identify any abnormal or discrepant findings
- Which substance most likely accounts for the large amount of white foam observed?
- Explain the most likely reason for the presence of increased RBCs in this patient's urine?
- Is the hemoglobin present contributing to the protein test result?
- Why is glucose present in the urine of this patient?

Case #2

Physical Exam	Chemical Exam	Confirm Tests
Color: colorless	SG: 1.010	Refractometer:1.029
Clarity: Sl. Cloudy	pH: 5.5	
	Blood: Neg	
	Protein: Trace	
	LE: Neg	
	Nitrite: Neg	
	Glucose: >2000	
	Ketones: Small	Clinitest: >5000
	Bilirubin: Neg	
	Urobilinogen:Normal	

Case #2

- Identify abnormal findings
- Diagnosis for glucosuria
- Why is ketone strip test positive
- Explain the two different sp gr results

Case #3

Physical Exam	Chemical Exam	Confirm Tests
Color: Amber	SG: 1.015	
Clarity: Sl. Cloudy	pH: 6.5	
Yellow foam noted	Blood: Neg	
	Protein: Trace	
	LE: Neg	
	Nitrite: Neg	
	Glucose: Neg	
	Ketones: Neg	
	Bilirubin: Neg	Ictotest:Positive
	Urobilinogen:Normal	

Case #3

- Identify abnormal or discrepant findings
- What substance accounts for urine color and foam color ?
- Why is the reagent strip test neg and Icotest pos? How do we report the test?
- What form of bilirubin is present in this urine?
- Why is urobilinogen not increased?

Case #4

Physical Exam	Chemical Exam	Confirm Tests
Color: Yellow	SG: 1.020	Refractometer: >1.035
Clarity: Cloudy	pH: 5.0	
	Blood: Neg	
	Protein: Trace	SSA: 4+
	LE: Neg	
	Nitrite: Neg	
	Glucose: Neg	
	Ketones: Neg	
	Bilirubin: Neg	Ictotest:Positive
	Urobilinogen:Normal	

Case #4

- Microscopic findings:
- RBC: 0-2
- WBC: 0-2
- Casts: Negative
- Epithelial cells Few TE/hpf
- Crystals: Moderate/hpf Type: Unknown

Case #4

- Identify abnormal or discrepant findings
- What is the most likely identity of crystal
- Which sp gr result best indicates patient's ability to concentrate urine?

Case #5

Physical Exam	Chemical Exam	Confirm Tests
Color: Yellow	SG: 1.015	
Clarity: Cloudy	pH: 6.0	
	Blood: Tracw	
	Protein: Trace	
	LE: Neg	
	Nitrite: Neg	
	Glucose: Neg	
	Ketones: Neg	
	Bilirubin: Neg	
	Urobilinogen:Normal	

Case #5

- RBC: 0-2
- WBC: 10-25
- Casts: 2-5 hyaline
- Epithelial cells: Few SE/hpf Moderate TE/hpf
- Bacteria: Moderate/hpf

Case #5

- Identify abnormal or discrepant results
- Most probable diagnosis
- Reasons for negative nitrite test
- Reasons why LE can be negative

BODY FLUIDS

CSF: Cerebrospinal Fluid

- Composition and formation
  - 3rd major BF of the body
  - Adiutl total volume: 140-170 ml
  - Neonate volume: 10-60 ml
- Functions
  - Supply nutrients to nervous tissue
  - Remove wastes
  - Mechanical barrier
- Indications
  - Dx meningitis, Intracraneal hemorrhage, leukemias, malignancies, and CNS disorders

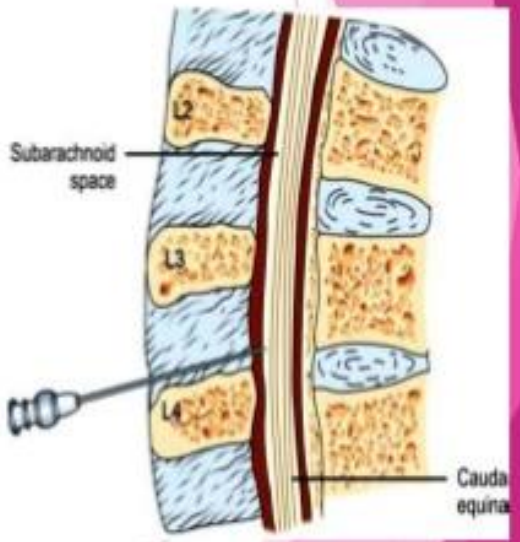
CSF

- Specimen collection
  - Lumbar puncture
  - Three tubes collected
    - Tube 1 – Chem
    - Tube 2 – Micro
    - Tube 3 - Hema

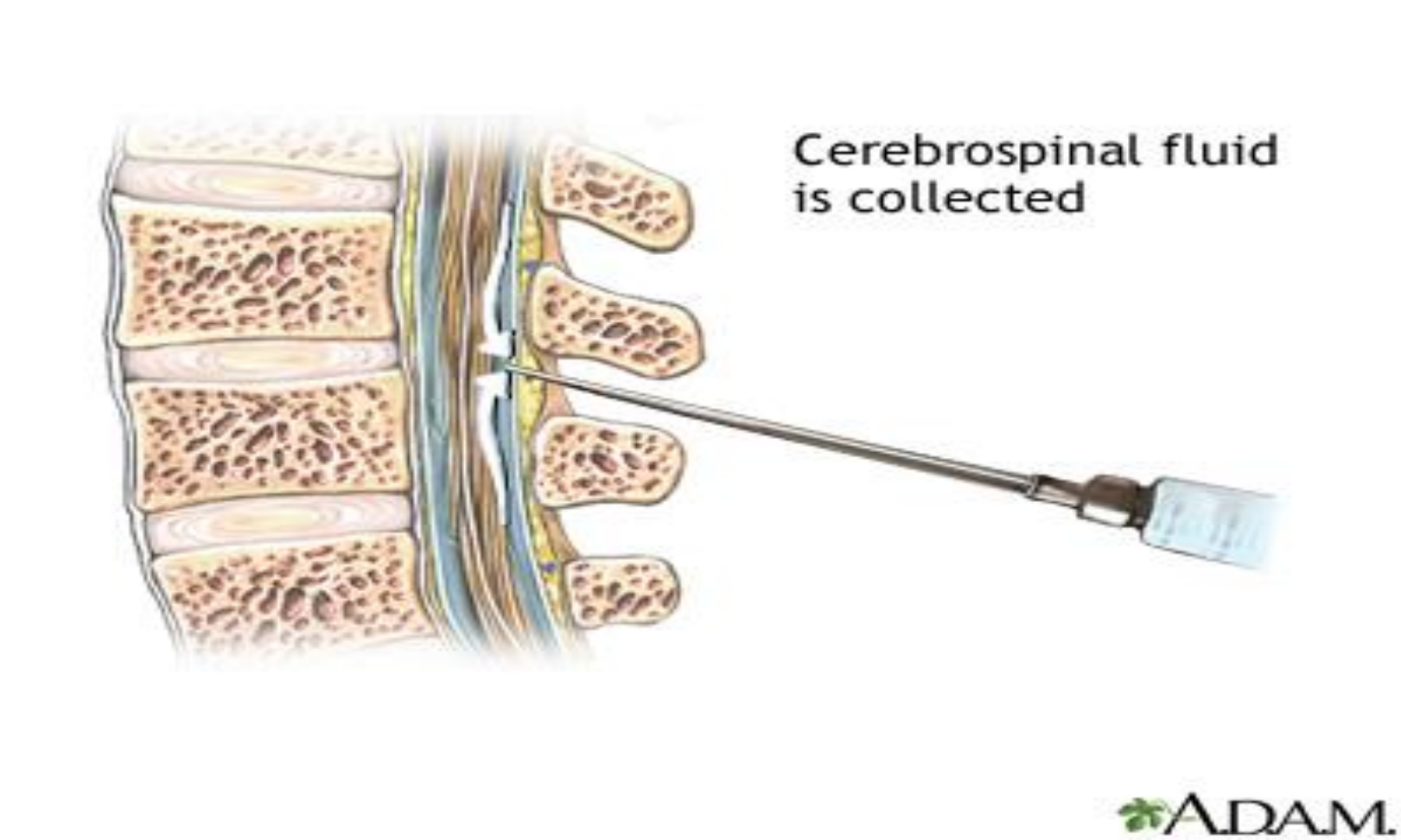
Routine Laboratory CSF Analysis

Collection

- Lumbar puncture
- At interspace of vertebrate Lumber 3rd-4th or 4th-5th
- With complete aseptic techniques
- 3-5 ml of CSF is collected
- It requires certain precautions and careful technique to prevent the introduction of infection or the damaging of neural tissue.

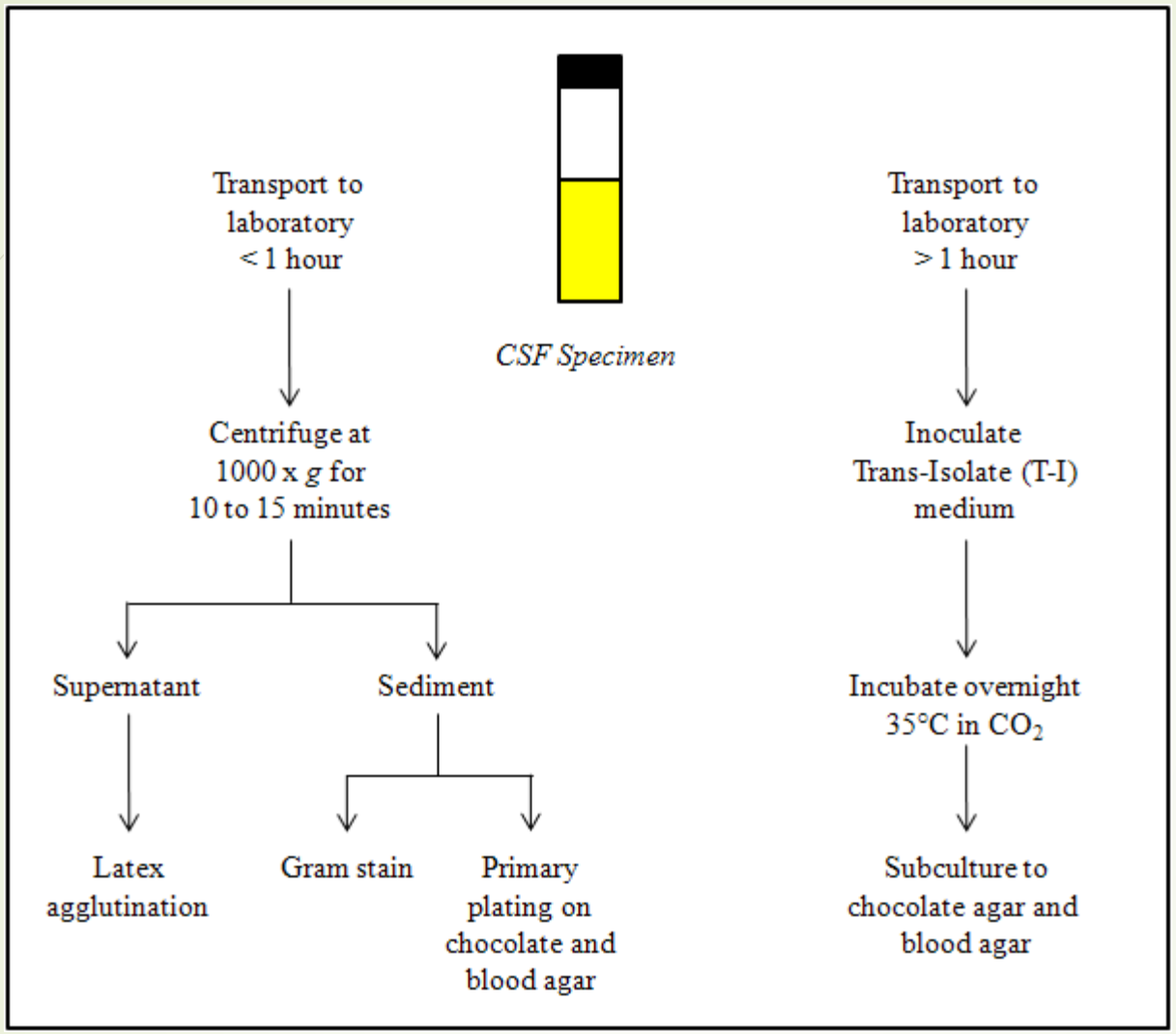


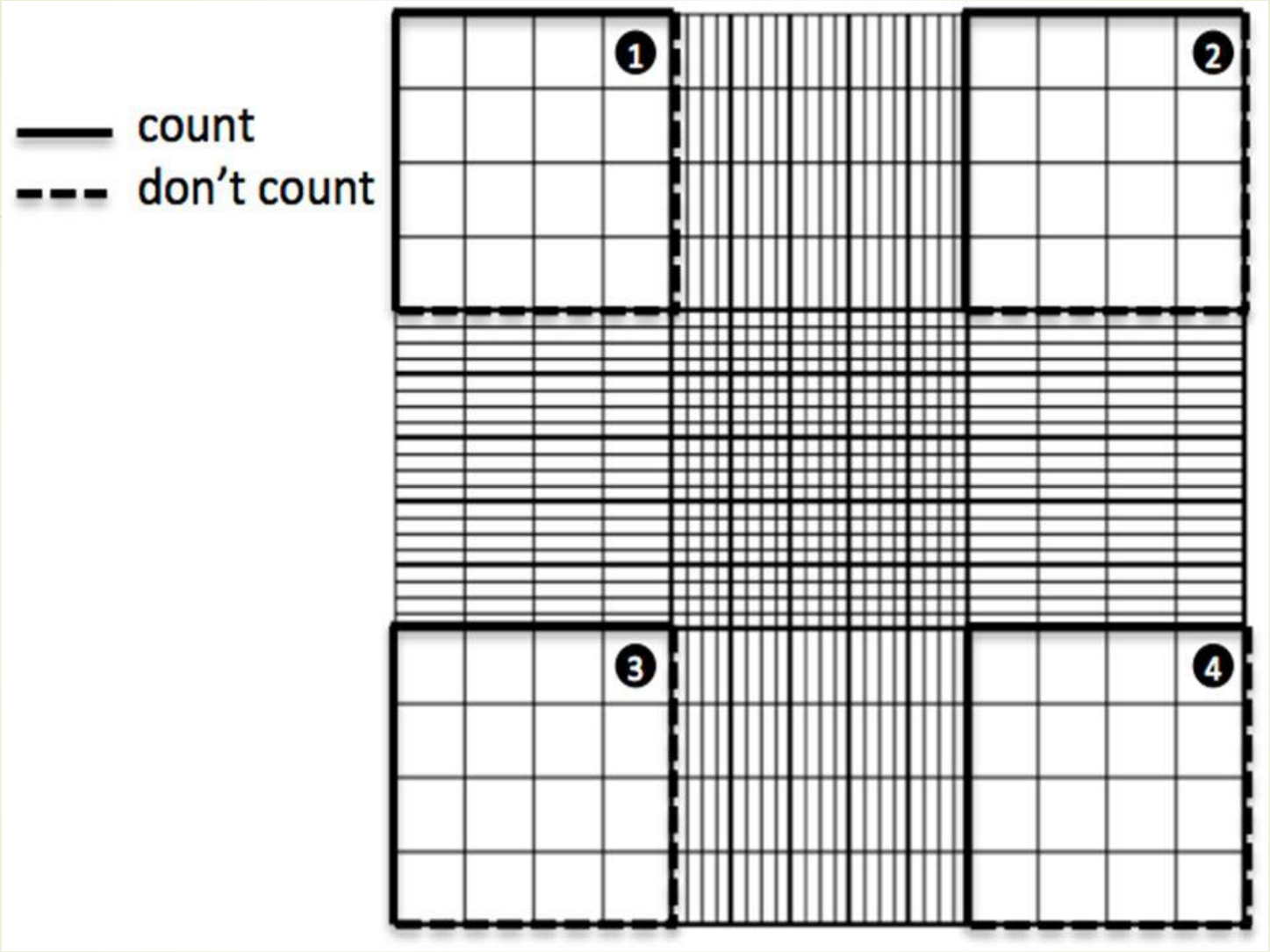
Collection of CSF



CSF

- Physical characteristics/appearance
- Gross examination
- Traumatic tap vs. Intracraneal hemorrhage
- Laboratory procedures
  - CSF count
  - CSF differential
  - Chemistry
  - Microbiology


Transport to laboratory < 1 hour
CSF Specimen
Transport to laboratory > 1 hour
Centrifuge at 1000 x g for 10 to 15 minutes
Inoculate Trans-Isolate (T-I) medium
Supernatant
Sediment
Incubate overnight 35°C in CO_2
Latex agglutination
Gram stain
Primary plating on chocolate and blood agar
Subculture to chocolate agar and blood agar


count
don't count
1
2
3
4

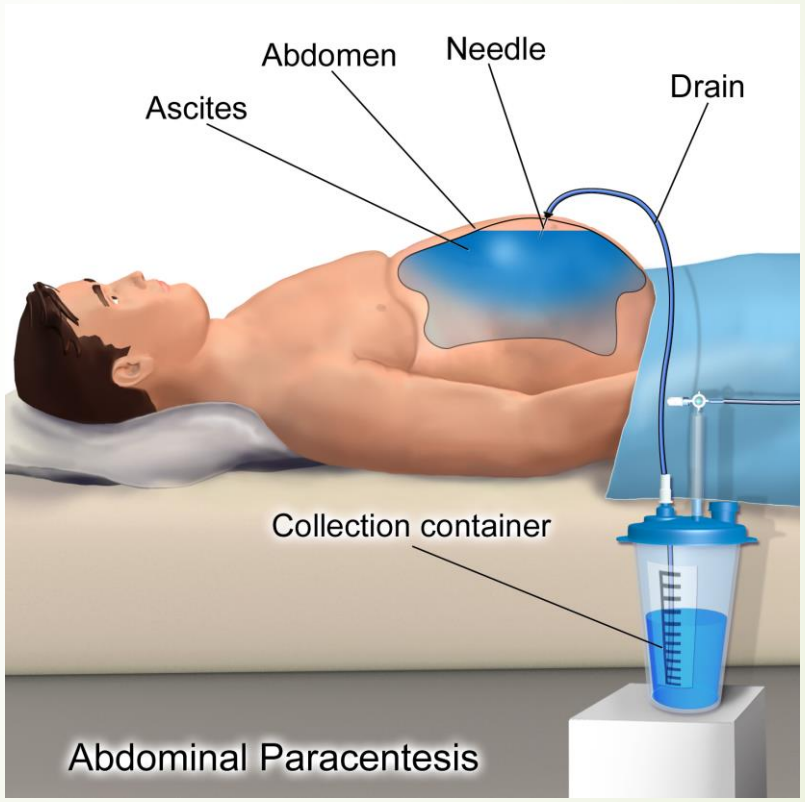
Serous Fluids

- Composition and formation
  - Ultrafiltrate of plasma

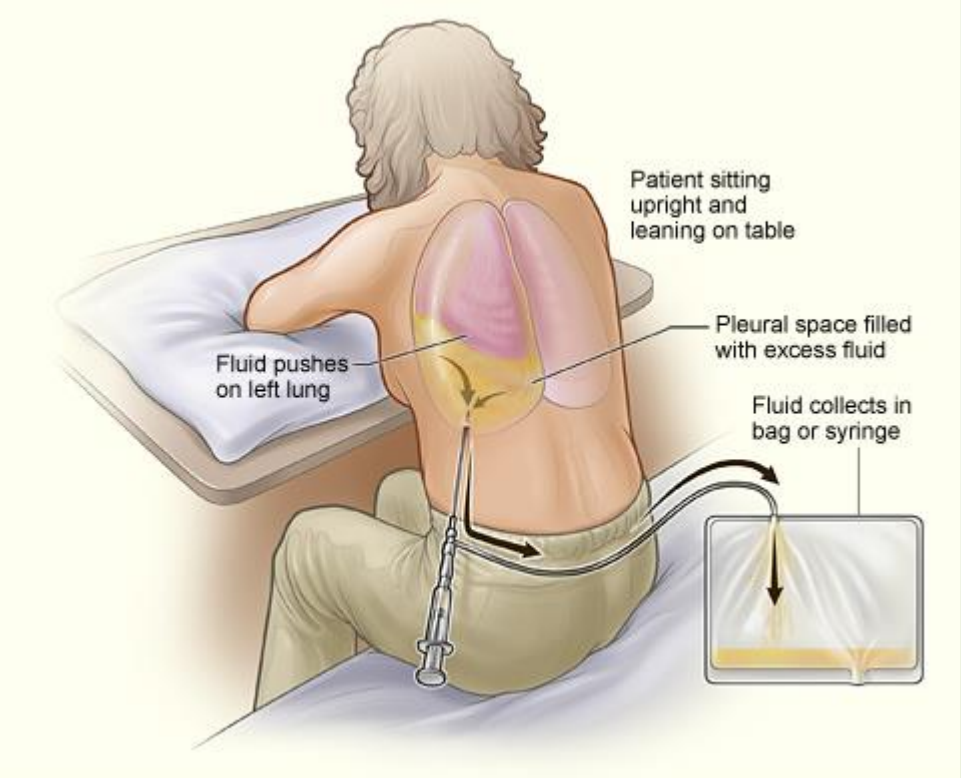
Serous Fluids

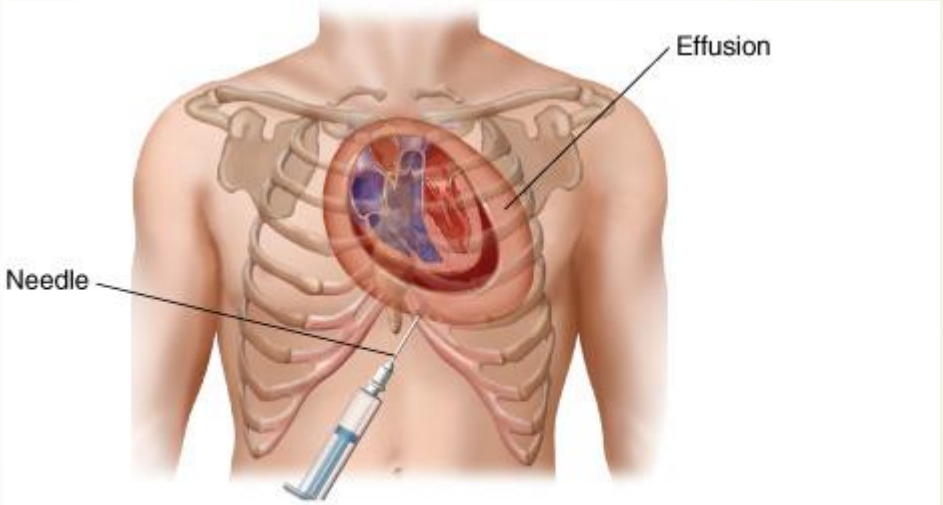
- Peritoneal - paracentesis
- Pleural - thoracentesis
- Pericardial - pericardiocnetesis

Peritoneal Fluid



Pleural Fluid - Thoracentesis





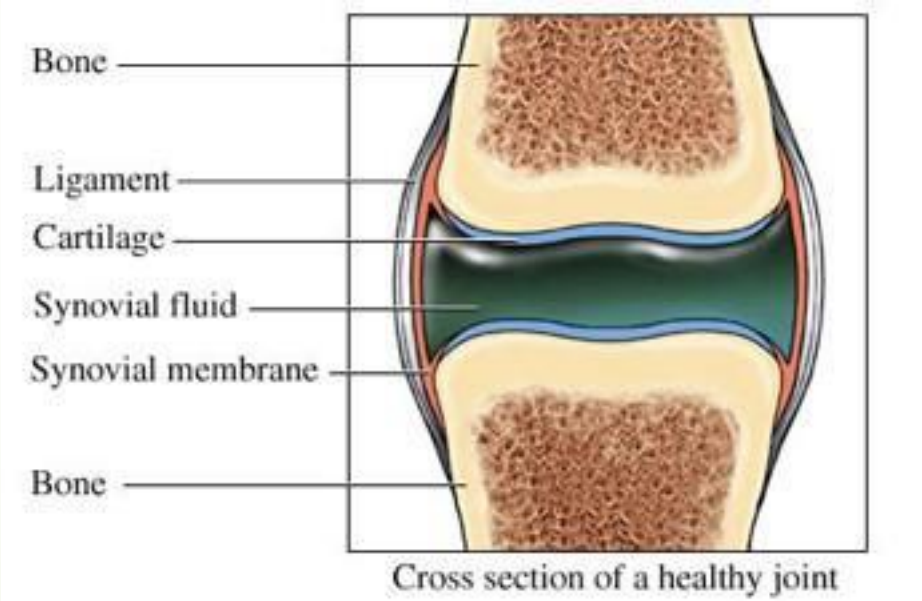
Source: Hanson CW III: *Procedures in Critical Care*: http://www.accessmedicine.com

Transudate vs Exudate

- Non-inflammatory
- Trans means movement of fluid due to changes in pressure gradients
- What do you remember about oncotic pressure and serum albumin levels???
- What is hydrostatic pressure?

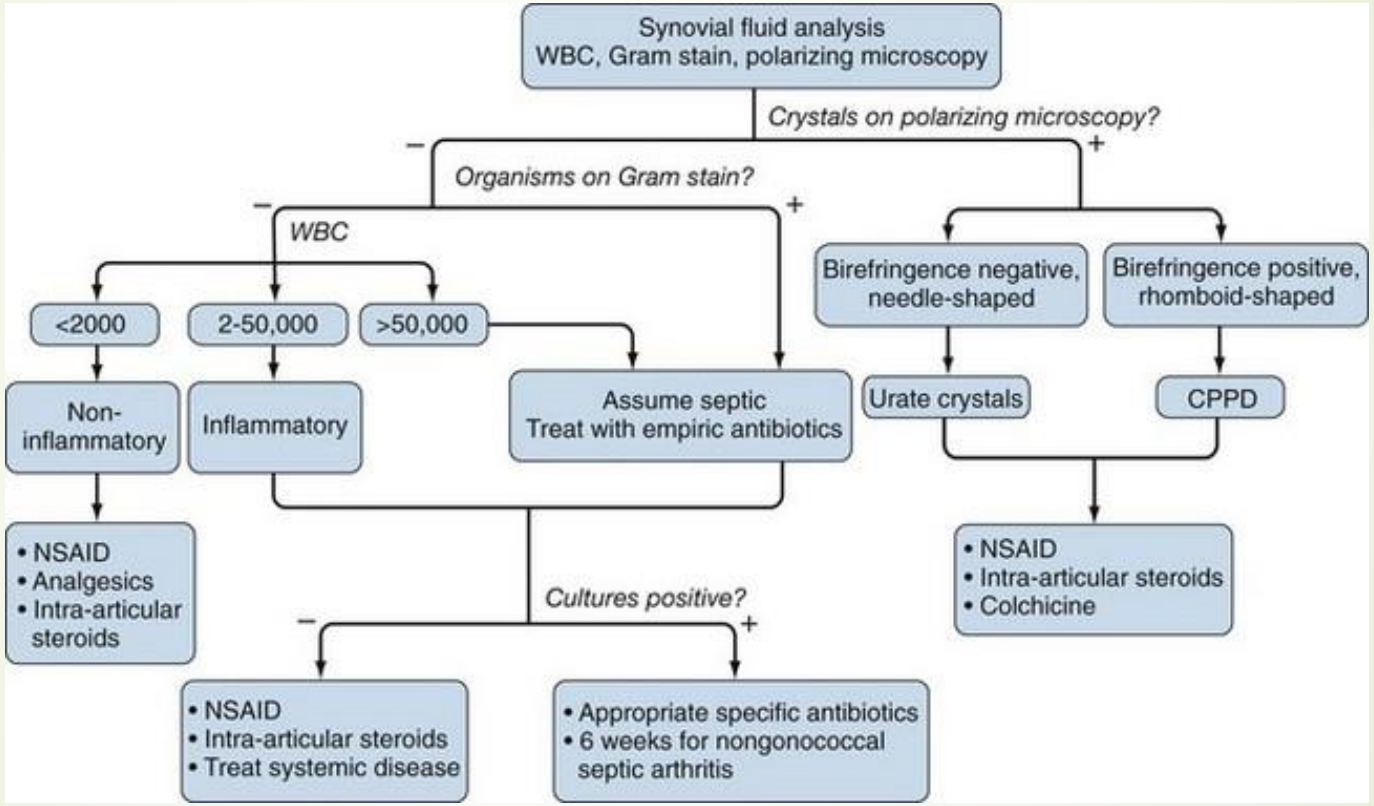
- Inflammatory in nature
- Exudate means there is a release of fluid.
- Exudative pleural effusion are due to changes in capillary permeability.
- The capillaries are inflammed and are not as selective and allow fluid to leak into the pleural space.

Synovial Fluid



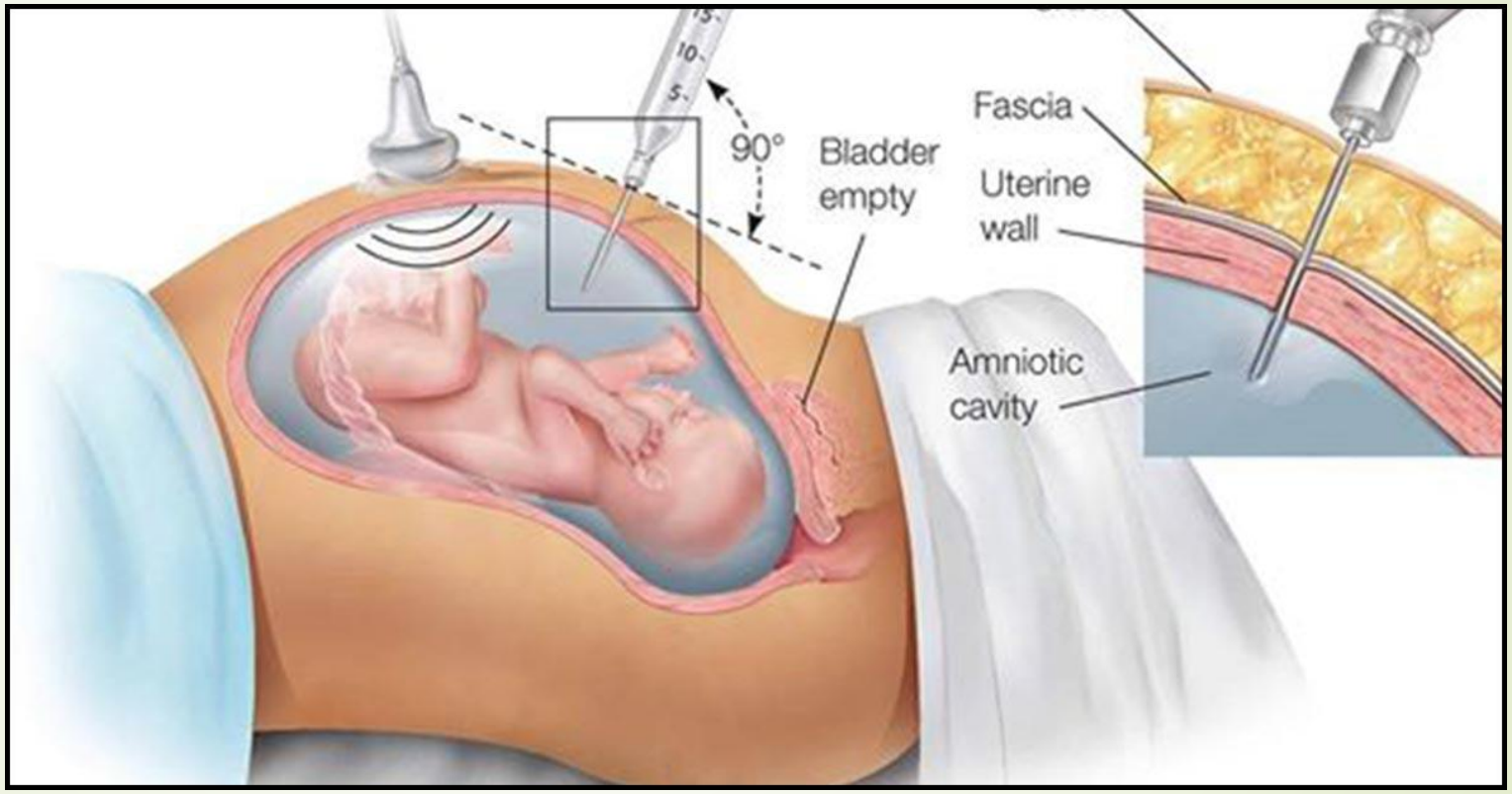
Cross section of a healthy joint

Crystals in synovial fluid



Seminal Fluid

Ammniotic Fluid



Sweat