# Body Fluids II

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# Today's Discussion

- Semen Analysis
- Amniotic Fluid Analysis
- Fecal Analysis
- Bronchial Lavage Analysis

# Semen Analysis

# Collection

- Collected after 2 days of sexual abstinence but not more than 7 days
  - Prolonged abstinence- higher volume and decreased motility
- WHO- recommends 2 or 3 specimens collected not less than 7 days or more than 3 weeks apart
- If collected outside of the lab, must be delivered at room temperature within 1 hour
- Majority of sperm contained in 1st portion of ejaculation
  - 1st part missing= count decreased, pH falsely high, won't liquefy
  - Last part missing= semen volume decreased, count falsely high, pH falsely low and the specimen will not clot
- Specimens awaiting analysis kept at 37 °C and delivered to the lab within 1 hr. of collection

# Gross Examination

- Volume
- pH
- Appearance
  - Color
  - Liquefaction
  - Viscosity


https://www.invitra.com/en/seminogram/macroscopic-analysis/

# Gross Examination

## Volume

- Normal= 2-5 mL
- Measured in graduated cylinder having 0.1 mL increments
- Increased Volume:
  - Period of extended abstinence
- Decreased Volume:
  - Infertility
    - Indicate improper functioning of one of the semen –producing organ(s)
    - Most commonly: seminal vesicle abnormality
  - Incomplete specimen collection

## pH

- Normal= 7.2-8.0 (slightly alkaline)
  - Balance between pH from acidic prostatic secretion and alkaline seminal vesicles secretion
- Measured within 1 hour of collection (if not loss of $CO_2$)
- Increased pH: Infection within the reproductive tract
- Decreased:
  - Increased prostatic fluid
  - Obstruction of ejaculatory duct
  - Poorly developed seminal vesicles
- Tested using a urine pH strip pH paper, or pH device

# Appearance

## Color

- Gray-white color, translucent
- Musty odor
- Increased turbidity indicates presence of WBCs, possible infection
- Red discoloration indicates RBCs
- Yellow discoloration can be due to
  - Urine contamination
    - Toxic to sperm, effects motility
  - Medications or infections

## Liquefaction

- Specimen is initially clotted, should liquefy within 30 to 60 minutes after collection
- Failure to liquefy within 60 minutes
  - Deficiency in prostatic enzymes
- Testing cannot begin until liquefaction of specimen
- After 2 hr. and no liquefaction
  - Equal volume of Dulbecco's phosphate-buffered saline or proteolytic enzymes added
    - Induce liquefaction
    - May affect biochemical testing

## Viscosity

- Consistency of the fluid
- Should be drawn into a pipette easily and form small discrete droplets
  - Should not not appear clumped or stringy when falling
- Threads longer than 2 cm= highly viscous (abnormal)
- Often rated 0 (watery) to 4 (gel-like)

# Sperm Count

- Counted using a Neubauer counting chamber
  - Counted using the small 5 squares in the large center square
  - Commonly performed with a 1:20 dilution using solution containing sodium bicarbonate and formalin
    - Solution will immobilize and preserve the cells to count
  - Sperm concentration(millions per mL)= Sperm counted multiplied by 1,000,000
- Normal Range: >20-250 million sperm per mL (10-20 million is borderline)
- Spermatids (immature sperm cells) are <u>not</u> counted
  - >1 million spermatids indicates spermatogenesis disruption
- Crystal violet could be used to aid in visualization

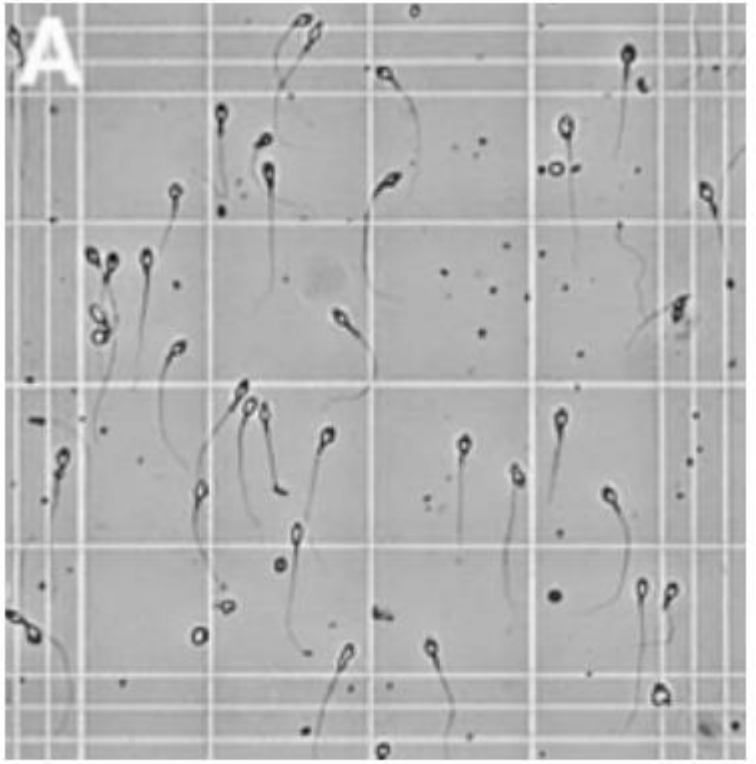



Figure 2. Sperm cells as seen in an Neubauer-improved counting chamber (Jackson Kirkman-Brown, source)

https://www.genderscilab.org/blog/what-does-sperm-count-count

# Sperm Motility

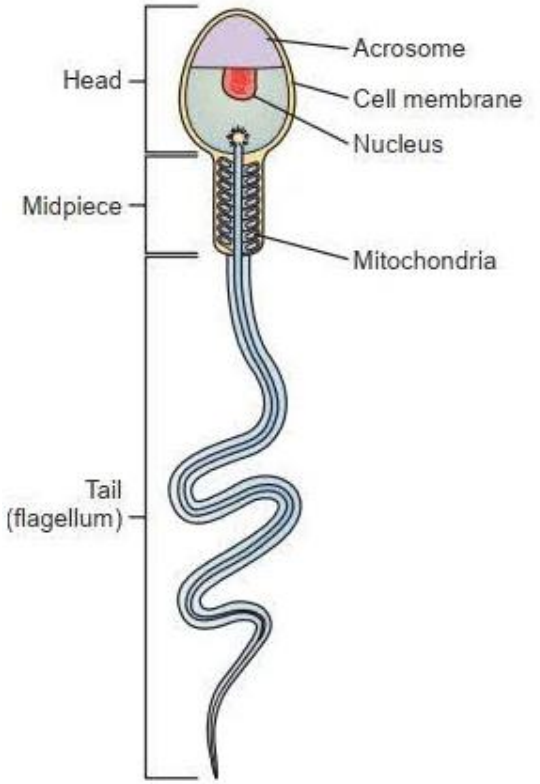
- Evaluated as a wet preparation on a cover slipped microscope slide using a calibrated positive-displacement pipette
- Cells allowed to settle for 1 minute
- % of sperm showing actual forward movement can be estimated after evaluating ~20 high power fields
  - Done in duplicate
- Motility evaluated by both speed and direction
- Grading can be done using a scale of 0 to 4

- WHO uses a rating scale of a, b, c, and d
  - Interpretation within 1 hour
    - 50% or more of the sperm should be motile in categories a, b, and c
    - 25% or more should show progressive motility (a and b)

| Grade | WHO Criteria | Sperm Motility Action |
|---|---|---|
| 4.0 | a | Motility with rapid, straight-line motility |
| 3.0 | b | Motile with slower speed, some lateral movement |
| 2.0 | b | Motile with slow forward progression, noticeable lateral movement |
| 1.0 | c | Motile without forward progression |
| 0 | d | No movement |

# Sperm Morphology

- Normal Sperm:
  - Oval-shaped head (5 µm long and 3 µm wide)
    - Acrosomal cap: critical to ovum penetration. Located on the tip of the head
  - Midpiece: neckpiece that attaches the head to the tail
    - Contains the mitochondria= energy for motility
  - Long, flagellar tail (approx. 45 µm long)




Normal Spermatozoa Structure (Source: Urinalysis and BodyFluids)

# Sperm Morphology


Abnormalities of sperm and tails (Source: Urinalysis and BodyFluids)

Examined on a stained slide

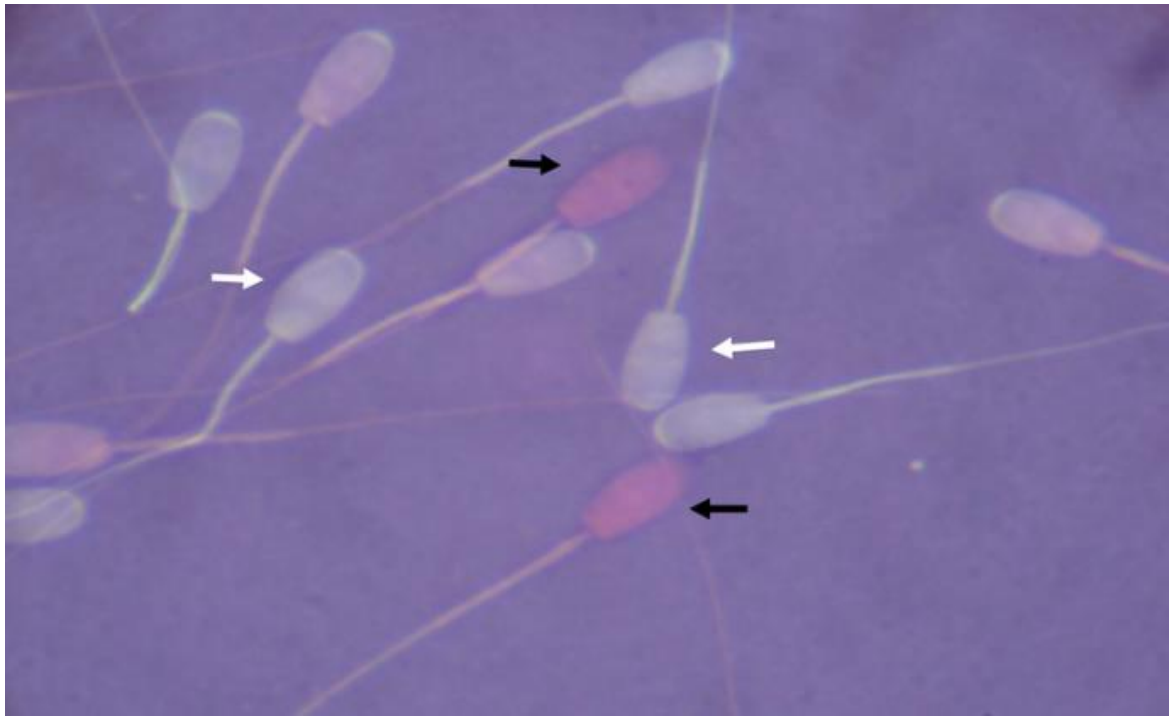
- Wright's
- Giemsa
- Shorr
- Papanicolaou stain

**Normal:**

- >30% when using routine criteria
- >14% normal forms when using strict criteria

# Sperm Vitality

- Assessed within 1 hour of ejaculation
- Mixing specimen with an eosin-nigrosine stain
  - Will count the number of dead cells in 100 sperm
    - Live sperm: unstained
    - Dead sperm: stain red/pink
- Vitality = # of dead cells in 100 sperm
  - Normal = 50% or more living cells
- Should correspond to motility
  - Large # vital cells but immobile
    - Defective flagellum
  - Large # immotile and nonviable cells
    - Epididymal pathology



https://www.researchgate.net/figure/The-viability-of-sperm-was-evaluated-under-a-light-microscope-1000X-following_fig1_282222961

# Additional Testing

- **Seminal Fluid Fructose**
  - Low sperm concentration may be caused by lack of support medium produced in the seminal vesicles which can be indicated by low or absent fructose level
  - Screened using resorcinol test
    - Produces an orange color when fructose is present
  - Normal levels: (= or >) 13 µmol per ejaculate (spectrophotometric method)


Fig. 10.3 No color change observed [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]


Fig. 10.4 Color change observed [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

https://www.clevelandclinic.org/reproductiveresearchcenter/docs/publications/132_Agarwal_et_al_Qualitative_Seminal_Fructose.pdf

# Additional Testing

- **Antisperm Antibodies**
  - Can be present in both men and women
  - Normally, Blood-testes barrier separates sperm from male immune system
    - Barrier can be disrupted and antigens on sperm produce an immune response that damages the sperm. The damaged sperm may cause the production of antibodies in female partner
      - Disruptions include- Vasectomy, Trauma, Infection
  - Suspected when clumps of sperm are presented

- Testing:
  - MAR (Mixed agglutination Reaction)
    - Detects IgG antibodies
    - Semen incubated with AHG (antihuman globulin) and suspension of latex particles of RBCs coated with IgG
    - <10% of sperm attached to particles is normal
  - Immunobead test:
    - Detects IgG, IgM, and IgA
    - Demonstrates what area of sperm is affected (head, neck-piece, mid-piece, or tail)
    - Presence of beads on <50% of sperm is normal

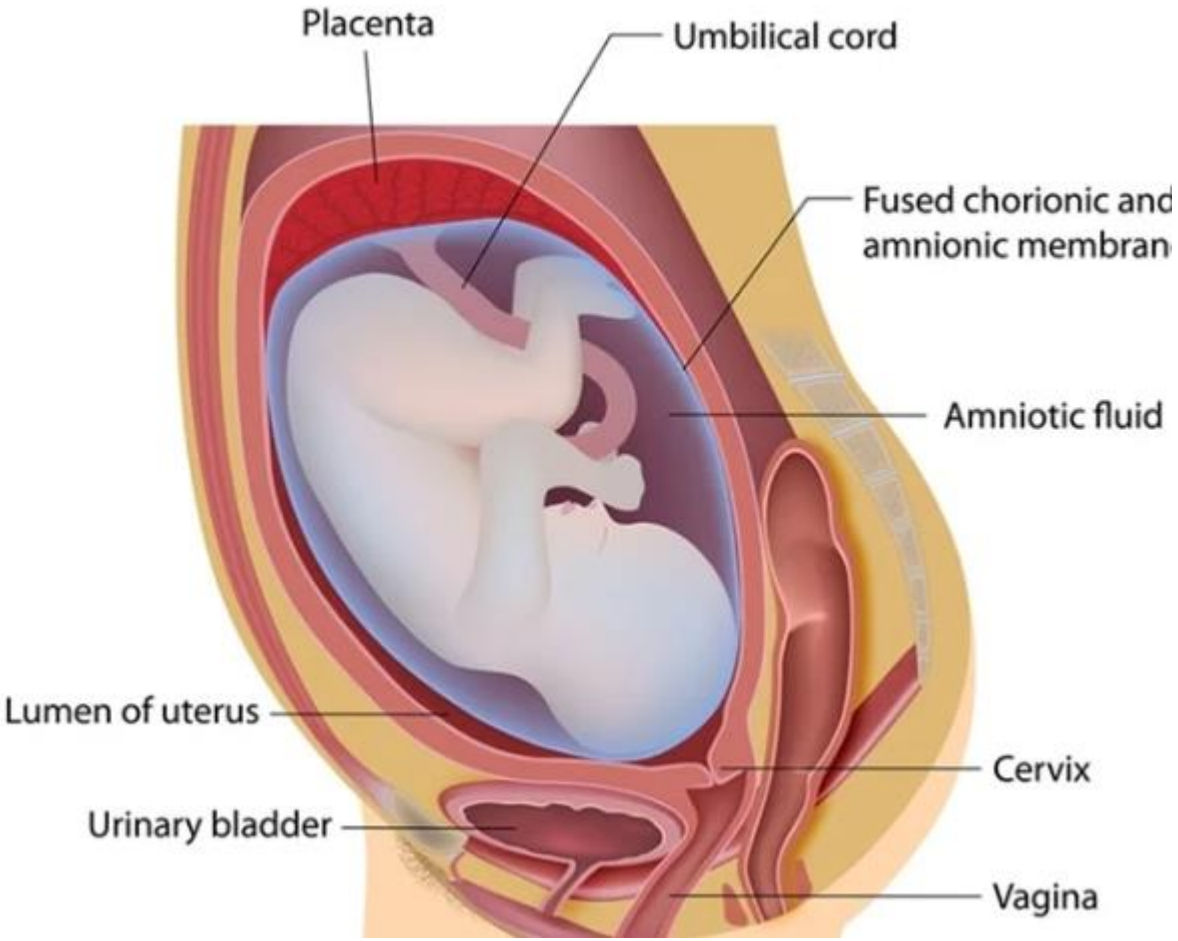
# Additional Testing

- Chemical Testing
  - Fructose
    - Decreased when lack of seminal fluid
  - Neutral alpha-glucosidease
    - Decreased in disorders of epididymis
  - Zinc, citric acid, glutamyl transpeptidase and acid phosphatase
    - Decreased when lack of prostatic fluid
  - Spectrophotometric methods are used to quantitate citric acid and zinc
- Legal Issues
  - Microscopically examining vaginal fluid for sperm by enhancing specimen with xylene
    - Non-motile sperm detected up to 3 days (mobile 24 hr) and head detected up to 7 days
  - Most specific test: measuring seminal PSA (Prostatic specific antigen)

# Sperm Function Tests

- Hamster egg penetration
  - Sperm incubated with species non-specific hamster eggs and penetration observed microscopically
- Cervical mucus penetration
  - Partner's midcycle cervical mucus
- Hypo-osmotic swelling
  - Low sodium environment and monitored for membrane integrity and sperm viability
- In-vitro acrosome reaction
  - Evaluate acrosome ability to produce enzymes

# Amniotic Fluid



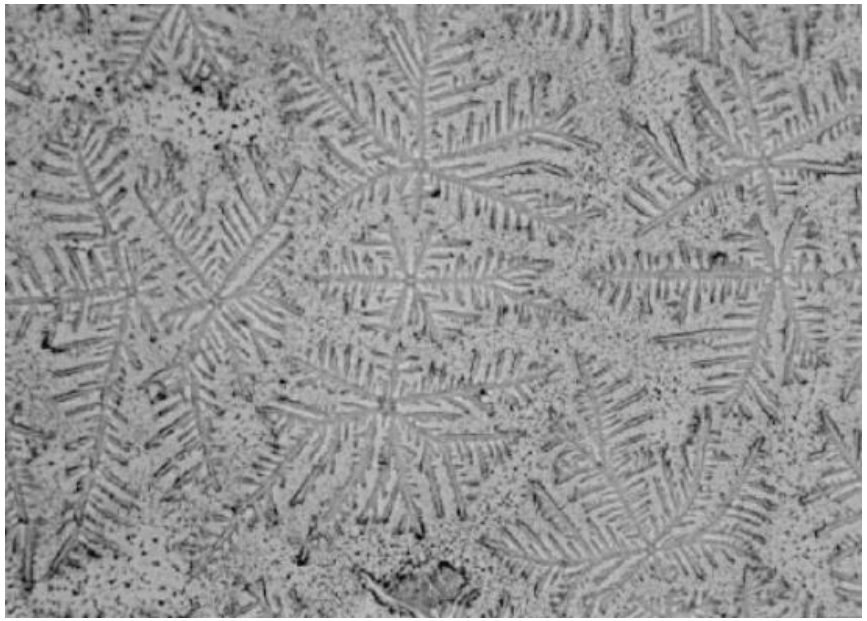

https://www.news-medical.net/health/What-is-Amniotic-Fluid.aspx

# Amniotic fluid

- Basics:
  - Metabolically active fluid inside the amnion
  - Exchanges $H_2O$ and other chemicals between fluid/maternal blood
  - Provides protective cushion for fetus and allows fetal movement
  - Stabilizes temperature and permits proper lung development
- Volume:
  - Regulated balance between production of fetal urine and lung fluid and the absorption from fetal swallowing and intramembranous flow
  - Increases throughout pregnancy
    - Peaks 3rd trimester: ~1L (800-1200 mL)
      - <800 mL= oligohydramnios
      - >1200 mL= polyhydramnios

# Amniotic Fluid

- Placenta is the ultimate source of amniotic fluid water and solutes
- Similar composition to maternal plasma and contains small amount of sloughed fetal cells
- Composition changes after 1st trimester when fetal urine production begins
  - Increase:
    - Creatinine: <3.5 mg/dL (maternal urine -10 mg/dL)
    - Urea: <30 mg/dL (maternal urine -300 mg/dL)
    - Uric acid
  - Decrease
    - Glucose and protein

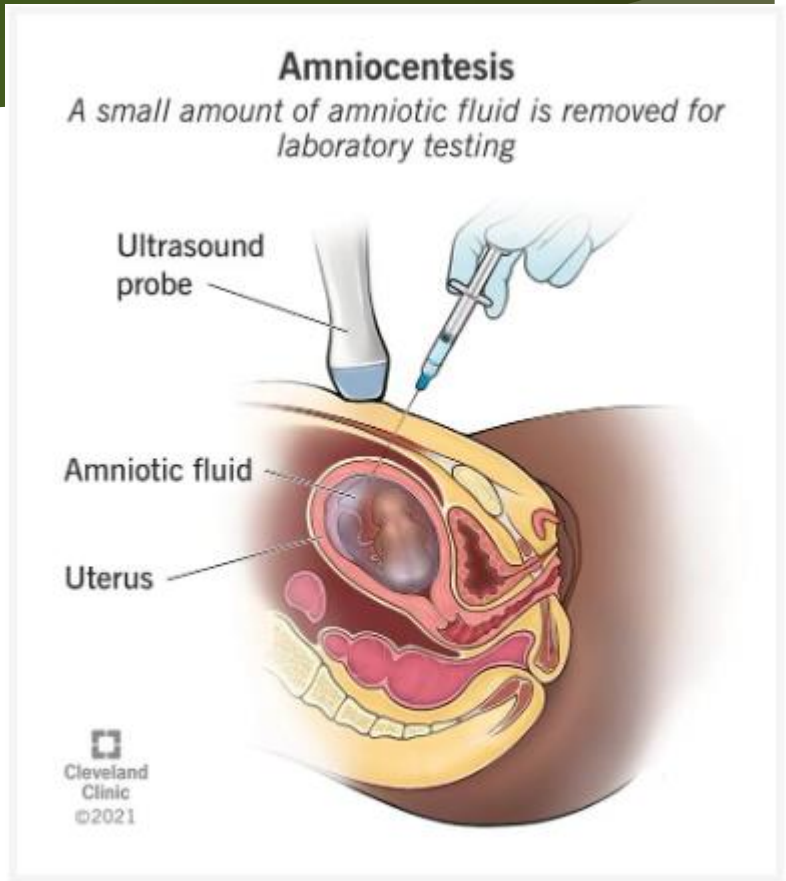


- Fern test: differentiate amniotic fluid from mother's urine
  - presence of fern-like crystals due to protein and NaCl indicates fluid is amniotic

https://www.researchgate.net/figure/Dried-amniotic-fluid-on-a-slide-demonstrating-a-positive-fern-test-with-an-arborization_fig12_232198182

# Amniocentesis

- Generally considered safe if done at 15 to 18 weeks
- Need for early treatment or early intervention
  - Genetic testing (16 weeks)
    - Mature mother (>35)
    - Past history (3 or more miscarriages)
    - Elevated alpha fetoprotein (trisomy 21)
- Evaluation of fetus maturity (done in 3rd trimester)
  - Fetal Lung Maturity
  - Fetal Distress Test
    - Hemolytic Disease of Fetus and Newborn (HDFN)
    - Neural Tube Defects
  - Infection



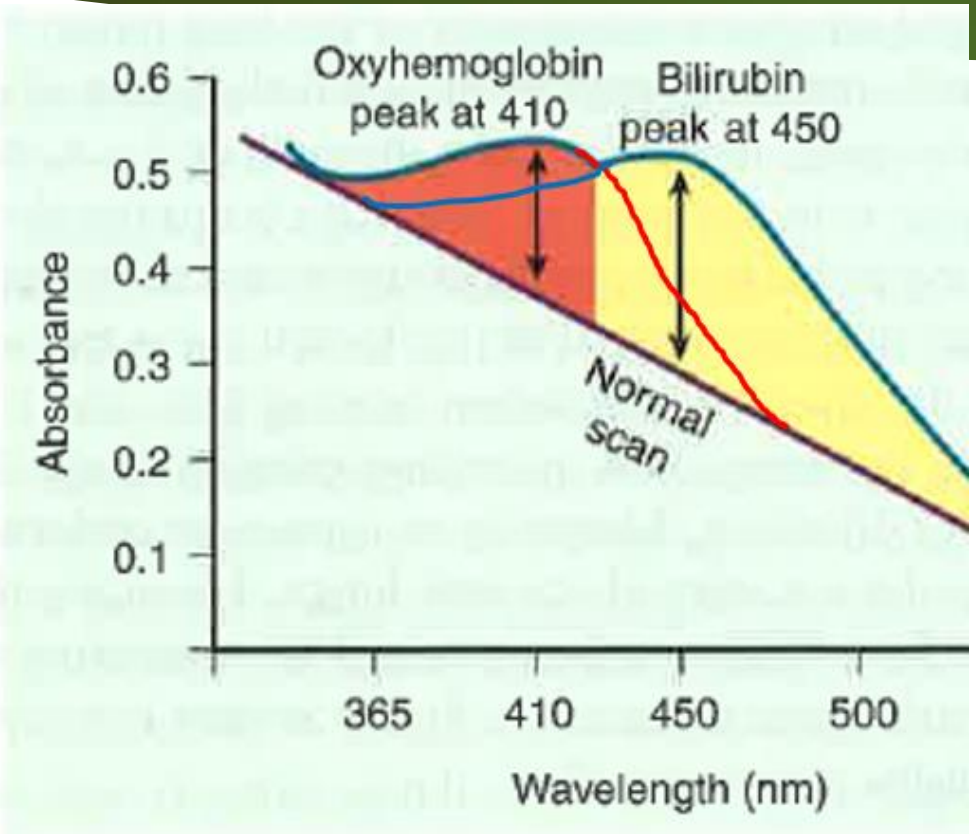

Amniocentesis is a prenatal test that diagnoses certain congenital disorders during pregnancy.

https://my.clevelandclinic.org/health/treatments/4206-genetic-amniocentesis

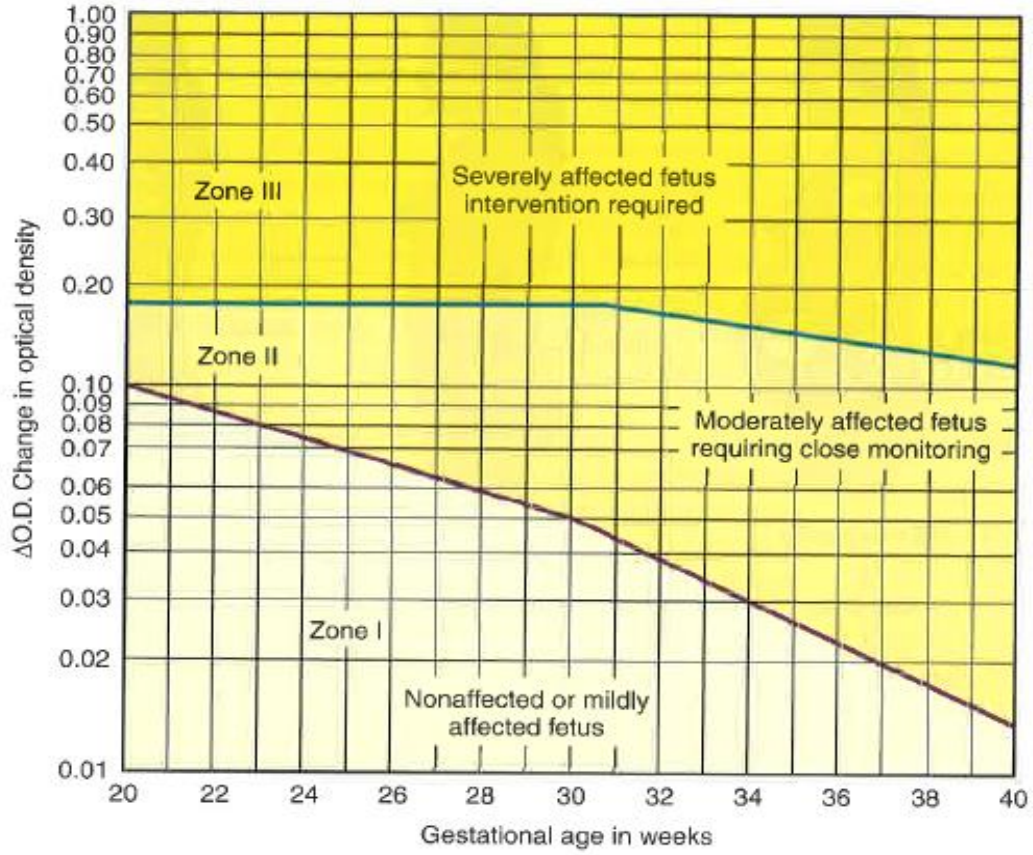
# Fetal Distress Tests: HDFN

- Hemolytic Disease of the Fetus and Newborn
  - Causes Anti-Rh antibody production from the mother
  - Treated with RhoGam
- HDFN results in the destruction of fetus red blood cells that will result in unconjugated bilirubin in the amniotic fluid
- The amount of bilirubin in the amniotic fluid is measured to determine severity of anemia
- Methods
  - Spectrophotometric analysis of optical density (365- 550 nm)
    - Rise at 450 nm when bilirubin present
    - Extremely sensitive to light exposure (decreased)
    - Blood can interfere with measurement (OxyHb)
  - Lilley Graph (Δ A 450)
    - Difference in OD at 450
      - Meconium can cause falsely low result

# Bilirubin Graphs




Spectrophotometric bilirubin



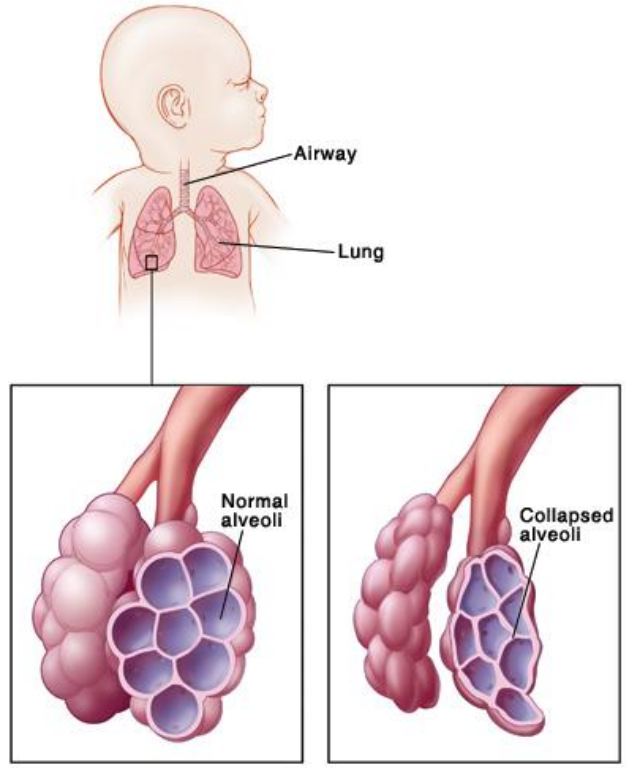

Liley Graph

# Fetal Distress Tests: NTD- Neural Tube Defects

- Increased alpha-fetoprotein (AFP) can be indicative of fetal neural tube defects
  - Can be measured in both maternal serum and amniotic fluid
  - Neural tube defects associated
    - Anencephaly
    - Spina bifida
- Alpha fetoprotein (AFP)
  - Major protein produced by fetal liver during early gestation (prior to 18 wks, max between 12-15 weeks)
  - Reported in MoM (Multiples of Median)
    - Value > 2 MoM considered abnormal
- If elevated MoM, **amniotic acetylcholinesterase (AChE)** is measured
  - Confirmatory test
  - More specific for neural tube defects
  - Not performed on blood
    - Blood contains AChE so contamination will alter these results

# Tests for Fetal Maturity

- When considering preterm delivery
- Respiratory distress syndrome
  - Frequent complication of early delivery
  - Insufficiency of lung surfactant
- Testing used:
  - Lecithin- Sphingomyelin Ratio
  - Phosphatidyl glycerol
  - Foam stability
  - Lamellar bodies




Air travels through the airways (tubes in the lungs) to the alveoli (air sacs). Normally, alveoli stay open after each breath. RDS occurs when alveoli collapse after each breath. This means the baby has to work harder to breathe.

https://mountnittany.org/wellness-article/premature-infant-respiratory-distress-syndrome

# Lecithin/Sphingomyelin Ratio

- **Lecithin** is the primary component of the surfactants (phospholipids, neutral lipids, and proteins)
  - Makes up the alveolar lining and account for stability
  - Produced at low levels until 45 weeks gestation
- **Sphingomyelin** is a lipid that is produced at a constant rate after 26 weeks of gestation
- Both can be measured using TLC
- Before 35 weeks gestation, the L/S ratio is usually <1.6
  - Due to large amount of lecithin not being produced
- After 35 weeks gestation, lecithin concentration increases while sphingomyelin remains constant
  - L/S ratio will rise to 2.0 or higher (at this time only, preterm delivery can be considered safe)

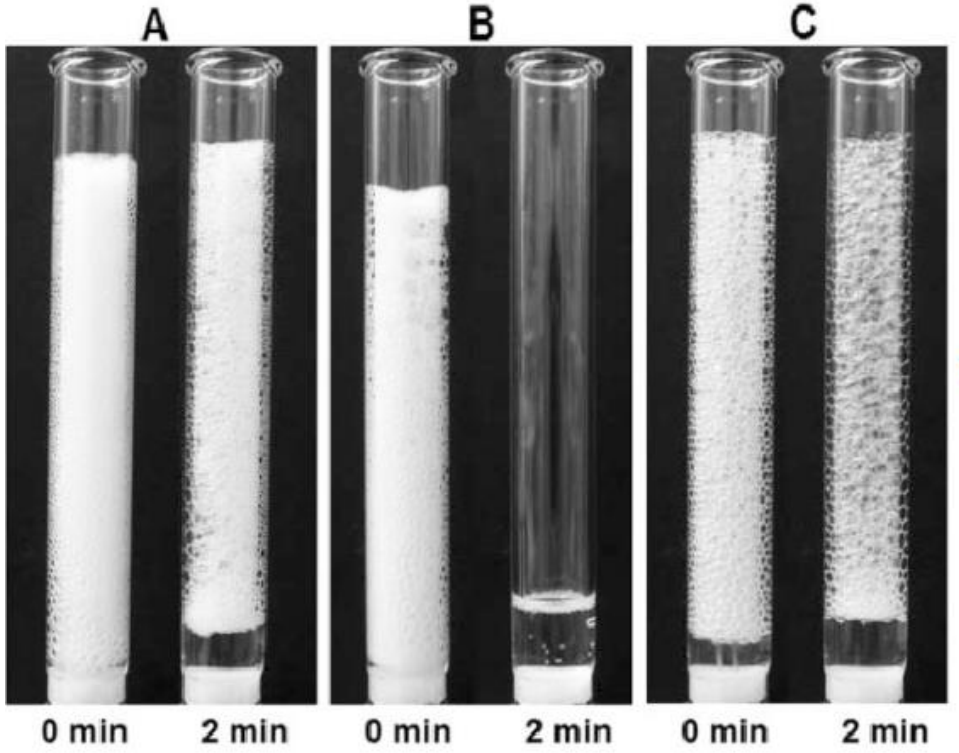
# Phosphatidyl Glycerol- PG

- Another crucial lung surface lipid
  - Parallels lecithin production (after 35 weeks) except it is delayed in cases of maternal diabetes

- **Amniostat FLM**
  - Immunologic agglutination test for PG (prevents the need of TLC)
  - Polyclonal anti-sera specific for phosphatidyl glycerol containing lamellar bodies in amniotic fluid
    - Lamellar bodies: layered storage granules containing surfactant
  - Negative= indicates pulmonary immaturity
  - Positive= indicates pulmonary maturity

# Foam Stability Index

- Screening test (could be performed at the bedside)
- Measures presence of lung-surface surfactants
- Test:
  - Add 95% ethanol to the amniotic fluid and shake for 15 seconds
  - Sit undisturbed for 15 minutes
  - Presence of bubbles on the outside edge
    - Indicates sufficient amount of phospholipids to reduce the surface tension of the fluid allowing the bubbles to remain
- Slightly modified procedure
  - 0.5 mL of amniotic fluid added to increasing amounts of 95% ethanol
  - Ratio of ethanol/fluid >47 indicates fetal lung maturity
  - Falsely high with blood or meconium contamination




https://www.researchgate.net/figure/Comparative-foamability-and-foam-stability-of-015-mg-mL-21-solutions-of-AM1-SDS-and_fig3_43455732

# Lamellar Bodies

- Densely packed layers of phospholipids that represent storage granules for pulmonary surfactant
  - Composed of 90% phospholipid and 10% protein
- Increase in concentration after 26 weeks gestation (50,000) to almost 200,000 per microliter by the end of the 3rd trimester
- As fetal lung matures, increased lamellar bodies production is reflected by increased in phospholipids and L/S ratio
- Presence of these lamellar bodies increases the amniotic fluid OD
  - Specimens are centrifuged and examined using a wavelength of 650 nm
  - An OD of 0.150 correlates with an L/S ratio of greater than or equal to 2 and the presence of PG

# Lamellar Body Counts

- Similar in size to platelets (1-5 um)
- Can be obtained using platelet channel on automated hematology analyzers
- Depending on method used (optical/impedance) values for FLM differ
- General consensus:
  - LBC greater than 50,000/uL= FLM
  - LBC less than 15,000/ul= immature FL

# Fecal Analysis

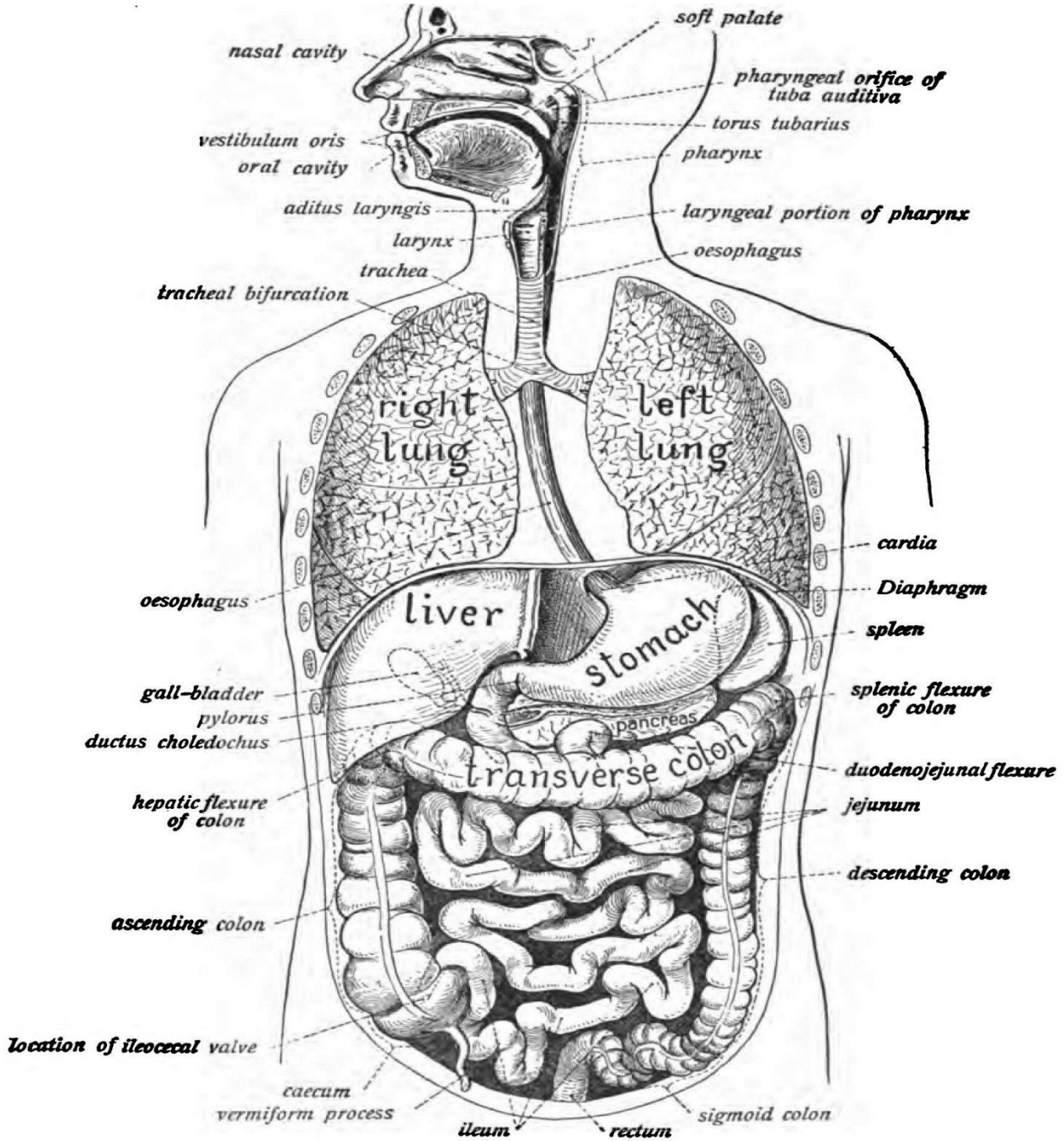

https://www.reddit.com/r/funny/comments/j6fu9/stool_sample/

# Fecal Analysis

- Macroscopic, microscopic and chemical analysis
- Early detection of:
  - GI bleeding
  - Liver/biliary duct disorders
  - Maldigestion/ malabsorption syndromes
  - Pancreatic diseases
  - Inflammation
  - Other causes of diarrhea and steatorrhea

# Physiology

- Normal fecal specimen contains:
  - Bacteria, cellulose, undigested foodstuffs, GI secretions, bile pigments, cells from the intestinal walls, electrolytes, and water
- Approximately 100 to 200 g of feces excreted in 24-hour period
- Digestive enzymes released by the pancreas (trypsin, chymotrypsin, amino peptidase, and lipase)- secreted into the small intestine
  - Small intestine- the primary site for final breakdown of proteins, carbs and fats
- Large intestine can absorb approximately 3,000 mL of water
  - >3,000 mL of water = diarrhea
  - < 3,000 mL of water= constipation




https://en.wikipedia.org/wiki/Human_digestive_system

# Diarrhea

- >200 g of stool weight a day, increased liquidity of stools and >3 movements a day
- >4 weeks is chronic and <4 weeks is acute
- Measure by fecal electrolytes (Na and K), Osmolality and Fecal pH
- Normal Stool:
  - pH: between 7-8
  - Osmolality: 290 mOsm/kg
  - Fecal Na: 30 mmol/L
  - Fecal K: 75 mmol/L
  - Osmotic Gap = 290 – [2(fecal Na +fecal K)]

# Diarrhea

## Osmotic Diarrhea

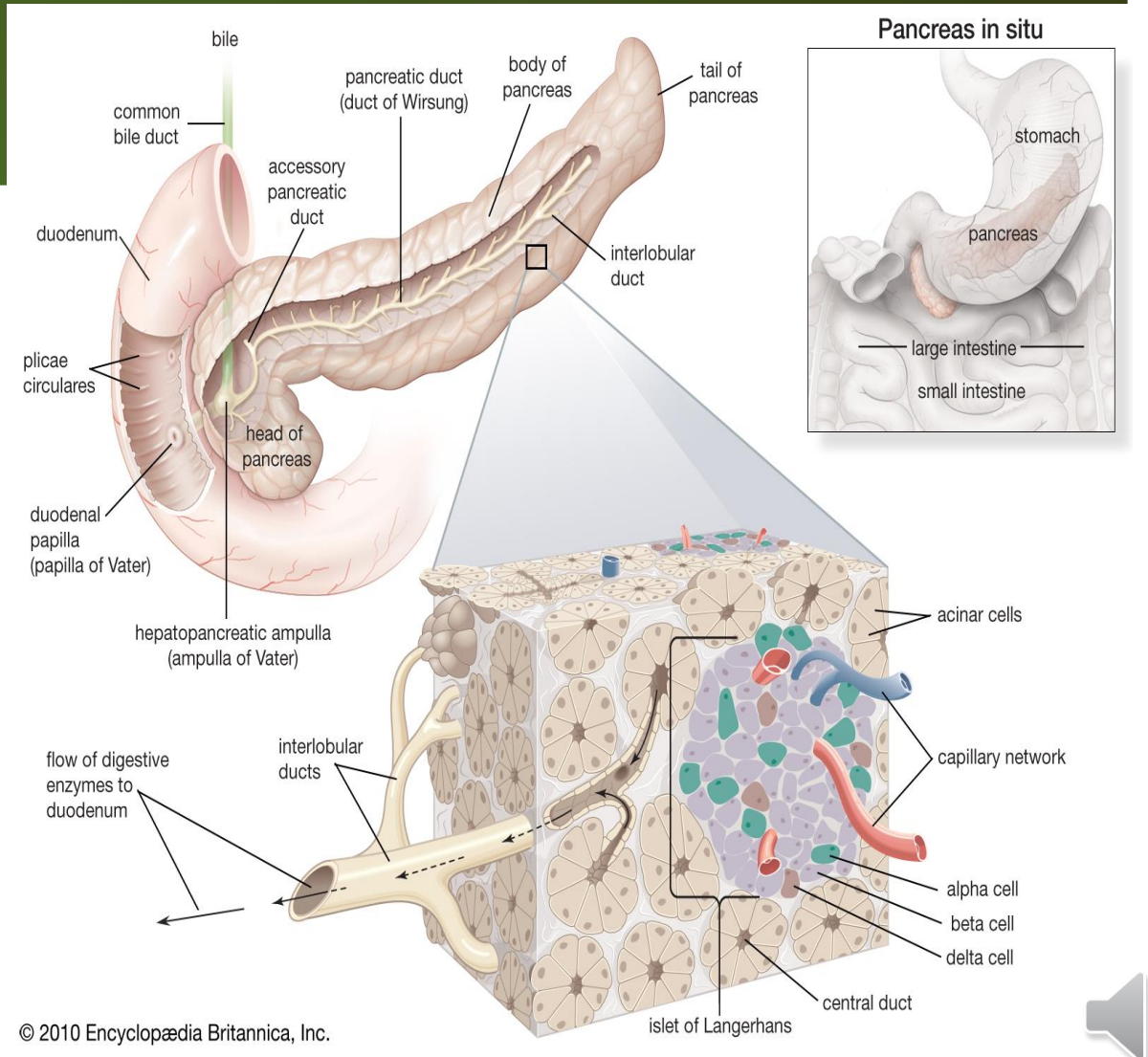
- Caused by poor absorption exerting osmotic pressure across intestinal mucosa
- Water and electrolyte retention in large intestine = watery stools
- Maldigestion and malabsorption
- Test results
  - Osmotic Gap = >50 Osm/kg
  - Na = <60 mmol/L
  - pH = <5.3
  - Stool Output in 24 hours = <200g

## Secretory Diarrhea

- Caused by increased secretion of water
- Bacterial, viral and protozoan infections
  - Enterotoxin-producing organisms:
    - E.coli O157, Shigella, Vibrio cholera …
- Other causes: drugs, laxatives, hormones, IBS (Crohns, Ulcerative Colitis, Diverticulitis), endocrine disorders (Zollinger-Ellison syndrome)
- Test results
  - Osmotic Gap = <50 Osm/kg
  - Na = >90 mmol/L
  - pH = >5.6
  - Stool Output in 24 hours = >200g

# Steatorrhea

- A.k.a Fecal fat
- Important in detecting when diagnosing pancreatic insufficiencies and small bowel disorders that cause malabsorption
- Absence of bile salts that assist pancreatic lipase in the breakdown and subsequent reabsorption of dietary fat produces an increase in stool fat > 6g per day
- Pancreatic disorders: cystic fibrosis, chronic pancreatitis, and carcinoma can decrease production of pancreatic enzyme and cause steatorrhea

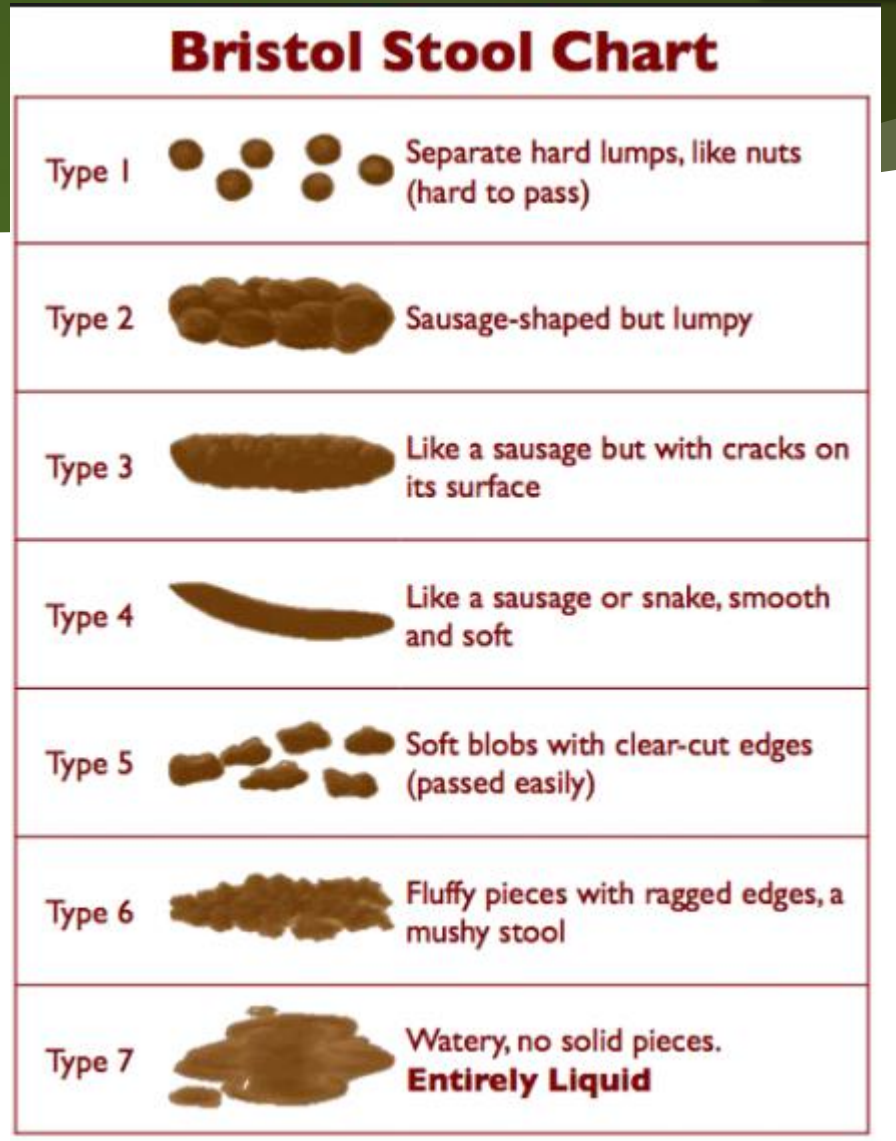


# Stool Collection

- Sample collection:
  - Clean container – transferred to provided lab container depending on specific testing
    - Avoid contamination with urine or toilet water
- Qualitative testing: (blood or certain microscopic observations)
  - Plastic or glass screw top container
- Quantitative testing: (fecal fats)
  - Timed specimen usually required
  - (Fecal fats) most representative sample is 3-day collection

# Macroscopic Screening

- Brown Color – from intestinal oxidation of stercobilinogen to urobilin
- Pale stools – acholic stools – signify blockage of bile duct or barium sulfate
- Green stools – oral antibiotics or increased green veggies
- Bloody stools:
  - Bright Red = bleed in lower GI, beets
  - Black = upper GI bleed, charcoal or bismuth
- Watery – Diarrhea
- Small, hard – constipation
- Slender, ribbon-like – obstruction
- Bulky/frothy – biliary obstruction and steatorrhea
- Mucus-coated – inflammation or irritation



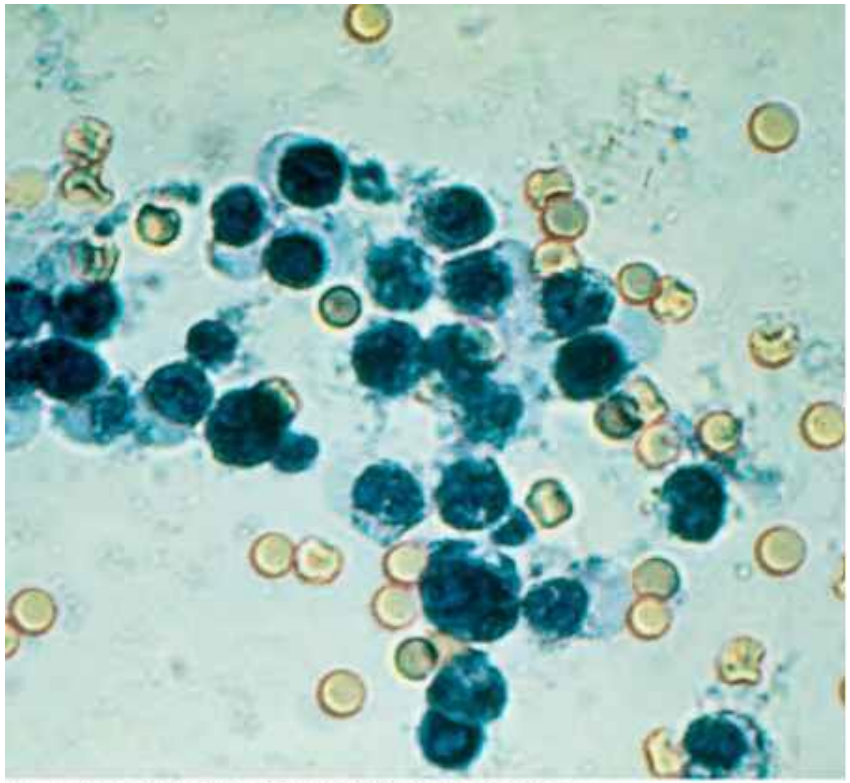

https://gpnotebook.com/simplepage.cfm?ID=x20100606160522260465

# Microscopic Evaluation

- Leukocytes
- Undigested muscle fiber
- Fecal Fats

# Leukocytes

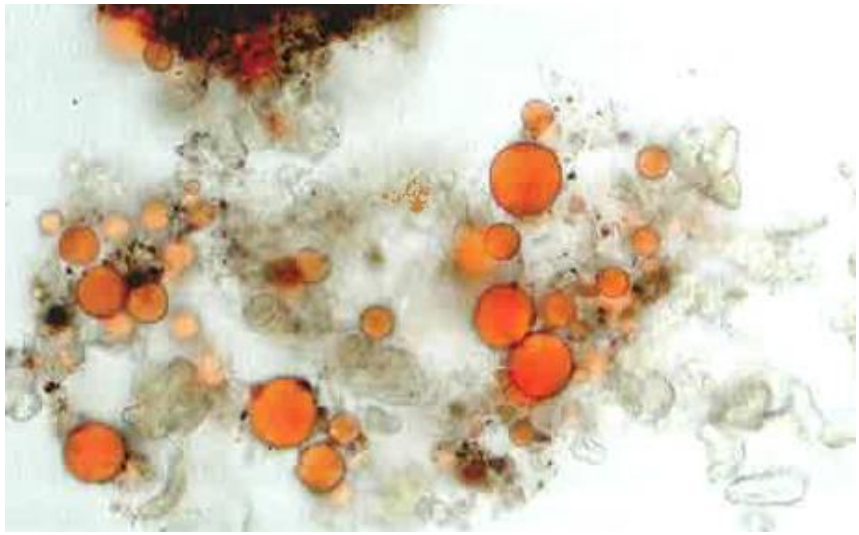
- Primarily neutrophils
  - Seen in conditions affecting intestinal mucosa
- Preliminary test to determine if diarrhea is being caused by invasive bacterial pathogens (leukocytes present)
  - Salmonella, shigella, campylobacter, yersinia, E.coli
- Bacteria causing diarrhea by toxin production **DO NOT** cause leukocytes (S. aureus, Vibrio spp., viruses, parasites)
- WBCs examined as wet prep stained with methylene blue or dried smears using gram stain
  - High power scope – few as 3 neutrophils per field = invasive condition
  - Oil immersion – any neutrophils = invasive condition
- Lactoferrin latex agglutination is available for detecting leukocytes and remains sensitive for frozen and refrigerated specimens



Source: Knoop KJ, Stack LB, Storrow AB, Thurman RJ: *The Atlas of Emergency Medicine, 3rd Edition:* http://www.accessmedicine.com

# Fecal Fats

- Qualitative
- Lipids included in microscopic exam of feces are:
  - Neutral fats (triglycerides)- stained by Sudan III
    - More than 60 droplets/high power field = steatorrhea
    - Can use a split fat stain for better indication
  - Fatty acid salts (soaps) – do not stain with Sudan III
    - Second slide must then be examined after specimen mixed with acetic acid/heated
      - Size of droplets important as well
      - High number (over 100 droplets) and larger size = steatorrhea
  - Cholesterol – stained by Sudan III after heating, as specimen cools crystals form
- Increased neutral fat on first slide = maldigestion
- Increased amount of total fat on split fat stain (second slide) with normal fat on first slide = malabsorption



Courtesy of Urinalysis and Body Fluids, 7th Edition

# Muscle Fibers

- Undigested muscle fibers – patients with pancreatic insufficiency (cystic fibrosis)
- Detected by prepared emulsion with 10% alcoholic eosin (enhance striations)
  - Examined for exactly 5 minutes (only undigested striations fibers counted)
  - Undigested fibers – vertical and horizontal striations
  - Partially digested fibers – striations in one direction
  - Digested fibers – no visible striations


https://docplayer.es/31845086-Fibras-musculares-y-granos-de-almidon-sin-digerir-en-las-heces-de-un-paciente-diabetico-con-diarrea.html

# Fecal Chemical Testing

- Occult Blood
- Bleeding in excess of 2.5mL/150g of stool is considered pathologically significant
  - (may not be visible) -(hidden) = occult
- FOBT = fecal occult blood testing – high predictive value for colorectal cancer

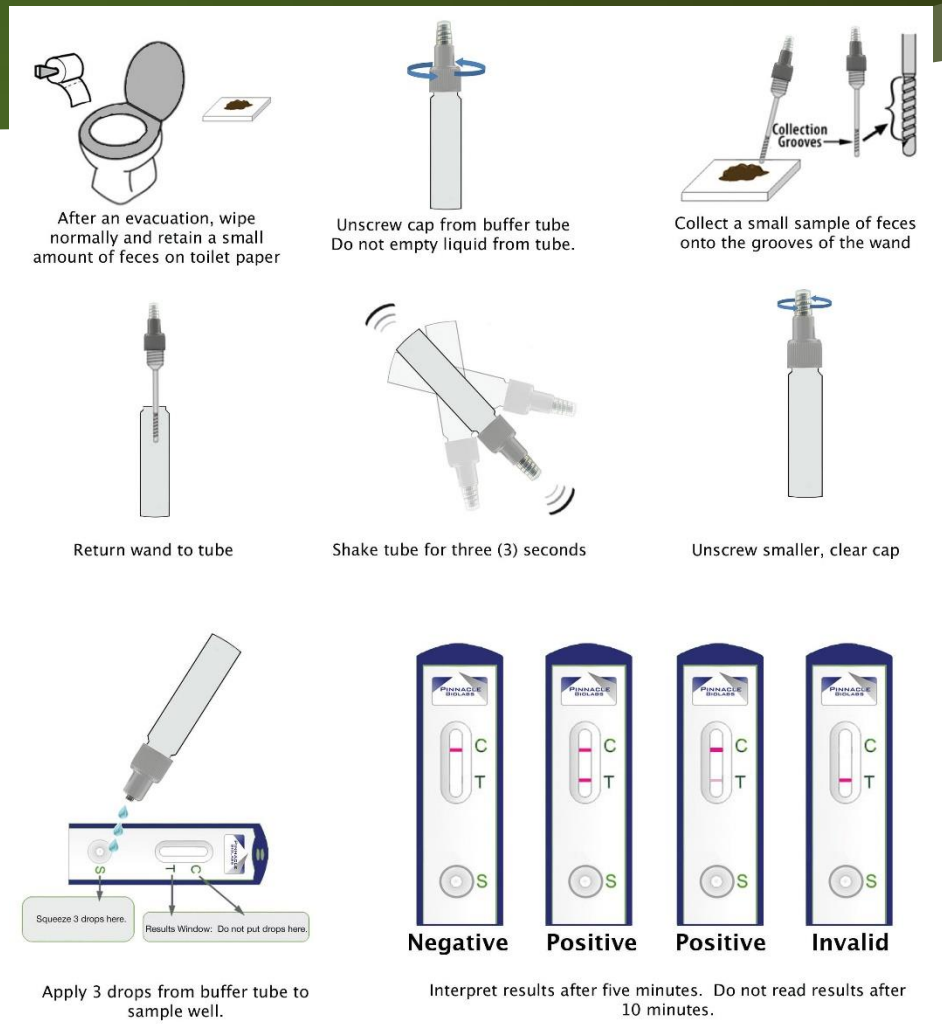
  1) Guaiac –Based Fecal Occult Blood Test (gFOBT)
  - Same as reagent urinary strip with different indicator
  - Measures pseudoperoxidase activity of hemoglobin
  - 3 consecutive day sample tested within 6 days
  - Should avoid meat/veggies/NSAIDS and Vitamin C before collection
    - Meat/veggies – dietary pseudoperoxidase
    - NSAIDs – intestinal irritation
    - Vit. C – strong reducing agent
  - -Guaiac is least sensitive reagent (small amount of blood can be normal)

2) Immunochemical Fecal Occult Blood Test (iFOBT)
- Specific for globin portion of human Hb
- Uses polyclonal anti-human Hb antibodies
- No dietary or drug restrictions
- Hb from upper GI bleed broken down by bacteria – immunochemically inactive

3)Porphyrin-Based Fecal Occult Blood Test
- Fluorometric test measuring conversion of heme to fluorescent porphyrins
- More sensitive to upper GI bleed
- Avoid red meat for 3 days

# Fecal Chemical Testing


https://retroflexions.com/endoscopy/occult-gastrointestinal-bleeding-and-the-stool-guaiac-test/




https://www.pblabs.com/pages/what-is-a-fecal-immunochemical-test

# Additional Testing

- Quantitative Fecal Fat Testing
  - 3-day specimen with regulated fat intake
  - Primary method is Van de Kamer titration (gold standard)
  - More rapid test is hydrogen nuclear magnetic resonance spectroscopy ($^1$H NMR) or Near-infared reflectance spectroscopy (NIRS)
- APT Test (Fetal Hemoglobin)
  - Grossly bloody stills or vomitus seen in neonates
  - Material to be tested is emulsified in water to release Hb and after centrifugation, 1% sodium hydroxide added to the pink Hb supernatant
  - Presence of alkali-resistant fetal Hb - solution remains pink
  - Denaturation of maternal hemoglobin (Hb A) produces a yellow-brown color
- Fecal Enzymes
  - Proteolytic enzymes produced by pancreas include trypsin, chymotrypsin and elastase I
  - Measured by spectrophotometric methods (chymotrypsin), immunoassay (elastase I)
- Carbohydrates
  - Presence of increased carbs = osmotic diarrhea from osmotic pressure of unabsorbed sugar in intestine drawing in fluid and electrolytes
  - Increased carbohydrate – detected by serum/urine tests but also copper reduction test

# Bronchial Lavage (BAL)

# Clinical Significance

- Method for obtaining cellular, immunologic, and microbiological information from the lining fluid of the lower respiratory tract

- Partially useful in evaluating patients who are immunocompromised, interstitial lung disease, airway disease, and suspected alveolar hemorrhage

# Specimen Collection and Handling

- Bronchoscopy
  - Fiber-optic bronchoscope is guided into a selected bronchopulmonary segment of the lung
  - Aliquots of sterile normal saline are instilled into the alveolar spaces and mix with the bronchial contents
  - Contents are aspirated for cellular examination and culture
- Handing and transport
  - Should be kept at room temperature during transport and processed immediately
  - If delayed longer than 30 minutes, transport on ice

Figure 1. Bronchoscopy. (Illustration © 2018 Chris Gralapp.)

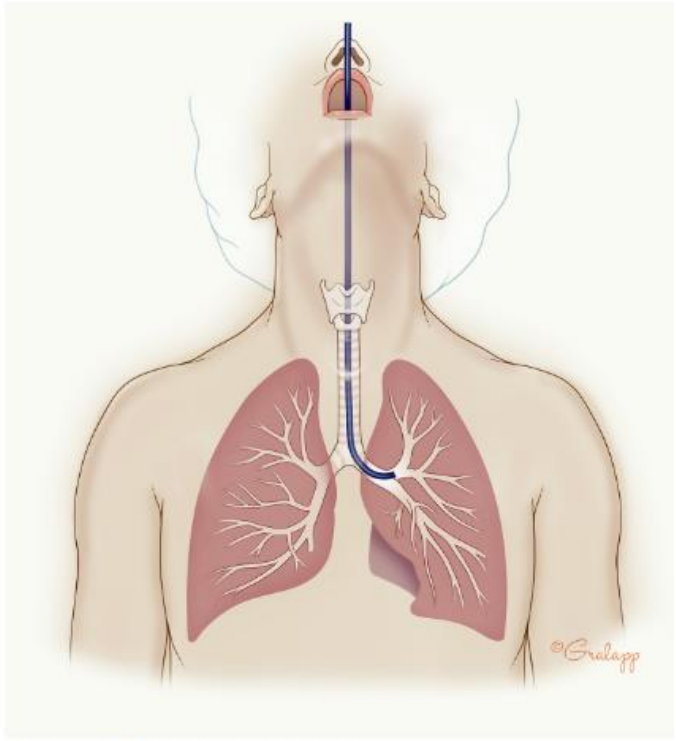
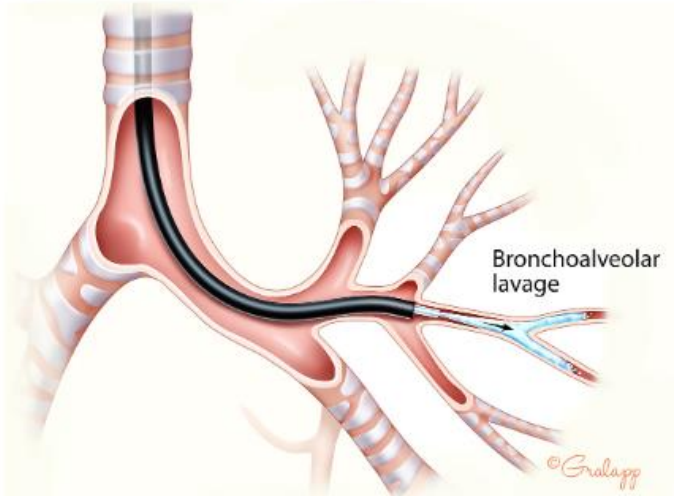



Figure 2. Bronchoalveolar Lavage. (Illustration © 2018 Chris Gralapp.)




https://psnet.ahrq.gov/web-mm/one-bronchoscopy-two-errors#references

# Diagnostic Testing

- Diagnostic tests on a BAL fluid include cell count with differential, microbiological studies, and cytopathology
- Color and clarity is noted
  - Color: colorless, milky white, light brown-beige, grey beige, red
  - Clarity: clear, hazy, cloudy, turbid

# Cell Counts and Differential

- Counts for white blood cells (WBCs) and red blood cells (RBCs) are performed on a BAL and may be diluted to facilitate counting using a hemocyocytometer
- Normal differential
  - Macrophages (56-80%): can have phagocytized material including hemosiderin
    - Pigment-laden macrophages (contain hemosiderin)
  - Lymphocytes (1-15%): increased in cases of interstitial lung disease, drug reactions, pulmonary lymphoma, and nonbacterial infections
  - Neutrophils (<3%): elevated in patients who are cigarette smokers, bronchopneumonia, and toxic exposure
  - Eosinophils (<1-2%): elevated in cases of asthma, drug-induced lung disease, infections, hypersensitivity, pneumonitis, and eosinophilic pneumonia
  - Erythrocytes: presence indicate alveolar hemorrhage
  - Epithelial cells (4-17%): more commonly seen in bronchial wash specimen than in bronchial lavage specimens
  - Mast Cells: can be present in hypersensitivity pneumonitis

# Microbiological and Cytology Tests

- Microbiology
  - Quantitative or semi-quantitative culture are useful for diagnosis in cases of ventilator-associated pneumonia and can diagnose the infection if the organism is identified

- Cytology
  - Include observing sulfur granules (actinomycetes), hemosiderin-laden macrophages, Langerhans cells, cytomegalic cells, fat droplets (oil red O stain), and lipid laden alveolar macrophages (Sudan III)

# References


- Urinalysis and Body Fluids, 7th Edition, Susan King Strasinger and Marjorie Schaub Di Lorenzo