MICROBIOLOGY NOTES

COLLECTION, TRANSPORT, PROCESSING, AND STAINING OF SPECIMENS

PRINCIPLES OF SPECIMEN COLLECTION

Major goal of microbiology laboratory – **aid diagnosis of infectious disease**

Goal of specimen collector **– maintain viability w/ minimal contamination**

FUNDAMENTALS

- Collect in _____ and _____ **antibiotics administration** (or within 2-3 days for viral infection)
- **Correct** anatomic site for collection
- **Proper technique and supplies** w/ minimal contamination
- **Appropriate quantity** of specimen
- Container or transport medium designed to maintain viability of organism & avoid hazard from leakage.
- **Accurate labeling** of spx. With:
  - Specific anatomic site
  - Patient's info
- **Immediate transport** of spx. to lab
- Provide environment that won't degrade suspected organism
- Notify lab if unusual / agents of bioterrorism are suspected

COLLECTION PROCEDURE

Spx. should be collected in **sterile containers**

- Except for **stool**

Swabs – not recommended

- Don't provide sufficient quantity
- Easily contaminated
- Can be dried out leading to organism loss
- Often vortexed in **0.5 – 1ml of saline or broth** for **10 – 20 secs.** to dislodge material from fibers.
- **Recommended for:**
  - Upper respiratory tract
  - External ear
  - Eye
  - Genital tract

"wound" is not appropriate specimen label (**exact site must be provided)**

PATIENT-COLLECTED SPECIMEN

Most effective method for instruction – _____

NOTED SPECIMEN COLLECTION GUIDELINES

Blood Culture

- Adult - **20 ml/ set**
- Children – **5 – 10ml/ set**

Body Fluids

- **≥1ml** (anaerobic transport system)

CSF

- Bacteria & virus - **≥ 1ml**
- Fungi & AFB - **≥ 2 ml**

Fungal Scrapings – wipe nails & skins with **alcohol**

- Hair – **10 – 12 hairs w/ intact shaft**
- Nails – **clip affected area**
- Skin – scrape at **outer edge of lesion**

Urethra

- Insert **2 – 4 cm** into urethra for **2 – 3 secs.**
- Or **collect discharge**

Nasopharynx

- Insert flexible swab thru nose – **rotate for 5 secs.**

Urine

- Preferred – _____

Sputum

- Preferred – **First Early Morning Specimen**

<u>PRESERVATION, STORAGE, AND TRANSPORT</u>

Primary Goal

- **Maintain specimen as near to its original state as possible w/ minimal deterioration**
- **Prevent risk to specimen handler**

Spx. should be transported to lab ideally within _____,
preferably within 2 hours

STORAGE

Specimens that can be maintained @ **4°C for 24 hours**

- Urine
- Stool
- Sputum
- Swabs (not for anaerobes)
- Catheters
- Viral specimens

CSF – if not processed, stored @ _____

SPECIMEN STORAGE GUIDELINES	
REFRIGERATED	**ROOM TEMPERATURE**
Catheter tip (IV)	Abscess, lesion, wound
CSF for virus	Body fluids
Ear: Outer	CSF for bacteria
Unpreserved feces	Ear: inner
Feces for *Clostridium difficile* toxin assay	Feces (unpreserved)
Sputum	Genital
Unpreserved urine	Nasal, N/P, throat
	Tissue
	Urine (preserved)
Suprapubic urine should be plate as soon as received.	

<u>PRESERVATION</u>

Specimens that can be preserved by preservatives

- Stool
- Urine

- **Boric acid** – used to maintain **accurate urine colony count**

Stool – can be refrigerated
- If delayed for **2 hours –** can be added to _____

Stool for ***Clostridium difficile* toxin assay**
  - Can be refrigerated
  - If delayed >48 hours:
    - **Frozen @ -70°C**

- _____/_____ – for ova and parasite (O&P) exam

ANTICOAGULANT

Sodium Polyanethol Sulfonate (SPS)
- Most common anticoagulant used for microbiology spx.
- must not exceed _____
  - _____ & certain anaerobes are inhibited by higher concentration

Heparin – used **for viral culture** and isolation of ***Mycobacterium* spp.** from blood

HOLDING OR TRANSPORT MEDIA
- Usually contains substances that do not promote multiplication of microorganisms but ensure their preservation
- Available in swab collection system

Stuart's and Amie's Transport Medium – commonly used

JEMBEC (James E. Martin Biological Environmental Chamber) System
- Used for specimens for _____

SPECIMEN PRIORITY

LEVELS OF SPECIMEN PRIORITIZATION		
LEVEL	**DESCRIPTION**	**SPECIMENS**
1	Critical / Invasive	Amniotic fluid
		Blood
		Brain
		CSF
		Heart valves
		Pericardial fluid
2	Unpreserved	Body fluids (not listed for level 1)
		Bone
		Drainage from wounds
		Feces
		Sputum
		Tissue
3	Quantitation required	Catheter tip
		Urine
		Tissue for quantitation
4	Preserved	Preserved feces
		Preserved urine
		Swabs in holding medium

		(aerobic and anaerobic)

Level 1 – critical; represent potentially life-threatening illness; from invasive source

Level 2 – unprotected; may quickly degrade or have overgrowth of contaminating flora.

Level 3 – require quantitation; may affect accuracy of quantitation if delayed.

Level 4 – spx. in holding or transport media

UNACCEPTABLE SPECIMENS

- Info on request form doesn't match to spx.
- Not submitted in appropriate transport container or leaking container
- Inadequate quantity of spx.
- Spx. transported >2 hours; not preserved
- Received in formalin
- Requesting anaerobic culture in spx. in w/c anaerobes are indigenous
- Dried up spx.
- More than 1 source was submitted from the same specimen. **Blood culture are exception**
- One swab was submitted with multiple requests for various organisms.

NOTE:

- Never discard an unacceptable spx. before contacting a member of health care team.
- Specimen that **impossible to recollect / require patient to undergo invasive procedure** may need to be processed regardless of the situation.

MACROSCOPIC EXAMINATION

- Swab or Aspirate
- Stool Consistency
- Blood or Mucus present – **part that is cultured and direct microscopic exam**
- Volume
- Fluid – clear or cloudy

If presence of **gas, foul smell, sulfur granules –** ANAEROBIC CULTURE

MICROSCOPIC EXAMINATION

Purpose:

- Determines quality of specimen
- Gives indication of infectious process involved.
- Guides routine culture workup based on the result of the smear
- Dictate the need for nonroutine or additional testing.

PRIMARY INOCULATION

TYPES OF CULTURE MEDIA

- **Nonselective media** – supports growth of most nonfastidious microbes
  - SBP
- **Selective media** – supports growth of one type or group of microbes but not another.
  - MAC
  - CNA
- **Differential media** – allows group of microbes based on **different characteristics** demonstrated on medium. Has dye or alcohol
  - SBA
- **Enriched media/nutritive media** – **contains growth enhancers** added to nonselective agar to allow fastidious organisms to flourish.
  - Blood agar, chocolate agar

- **Enrichment broth** – **liquid medium** designed to encourage growth of small numbers of particular organism while suppressing other flora.
    - LIM Broth
    - Todd-Hewitt w/ CNA

- **Broth media** – supplement to agar plates to detect small numbers of most aerobes, anaerobes and microaerophiles
    - Thioglycollate broth
    - Brain-Heart Infusion Agar
    - Tryptic Soy Broth

SPECIMEN PREPARATION

Forms of specimen arrive in lab

- Fluid
- Swab
- Tissue

FLUID

- **Inoculated directly** to selected media
    - Sterile body fluids
    - Pus
    - Urine
    - Sputum
- **Large volume of sterile body fluids**
    - If >1 ml – centrifuged @ **3000x g for 20 minutes**
    - If consistency is thin enough to avoid filter clogging
        - **Nalgene filter** can be used

SWAB – inoculated directly to culture media

- Should be submitted on _____
    - One – for **culture media**
    - Another swab – for **direct smear**

TISSUES

- Can be prepared thru **homogenization** for culture
    - It **can destroy certain organisms**

- Can be **minced** w/ sterile scissors and forceps into small pieces for culture.

ISOLATION TECHNIQUE

- **General purpose Isolation Streak**
    - Yields **semiquantitative estimate** of growth
    - Useful for most specimen

  Grading
    - 1st quadrant – **1+ (light growth)**
    - 2nd and 3rd quadrant – **2+ (moderate growth)**
    - 4th quadrant – **4+ (heavy growth)**

- **Quantitative isolation**
    - For **urine specimen & tissue from burn patients**
    - Uses calibrate loops
        - **0.01**
        - **0.001**

INCUBATION

- _____ **– bacteria, AFBs, & viruses**
- _____ – **fungi**
- Most routine bacterial cultures are held for **48 – 72 hours**
- **Anaerobes and broth cultures** – held for **5 – 7 days**

Aerobes – grow in ambient air

- **21% O_2**
- **0.03% CO_2**

Anaerobes – cannot grow in presence of _____

- Atmosphere in anaerobe jars
  - **5 – 10% H_2**
  - **_____% CO_2**
  - **80 – 90% N_2**
  - **0% O_2**

Capnophiles – requires
- **_____% CO_2**
- **15% O_2**

Candle Jar atmosphere – 3% CO_2
Examples: *H. influenzae; N. gonorrhoeae*

Microaerophiles – grows in
- reduced O_2 (**5 – 10%)**
- increased CO_2 (**8 – 10%)**

Example: *Campylobacter jejuni; Helicobacter pylori*

NONROUTINE SPECIMENS

- **Implant soak solution**
  - Requires large volume and
  - Concentration
- **Water sterility specimen**
  - Requires concentration
    - **Millipore Sampler**
      - Uses _____
- **Intrauterine device**
  - Cultured for detection of ***Actinomyces* spp.**
  - Inoculated into **THIO**
- **Vascular Catheter tips**
  - Use for **catheter-related infection**
  - Uses **Maki roll technique**

  Maki roll technique – **5-7cm** segment of catheter is rolled of a blood agar plate **4 times.**

Critical Values in Microbiology
Positive blood culture
Positive CSF gram stain or culture
Positive cryptococcal antigen test or culture
Positive blood smear for malaria
Streptococcus pyogenes from a sterile site
Positive acid-fast smears or positive *Mycobacterium* culture.
Streptococcus agalactiae or herpes simplex virus from genital site of pregnant woman at term
Detection of significant pathogen

SMEARS

Smears for Swab
- Should not be prepared from swab after used to inoculate culture media. (**2 swabs are submitted)**
- Prepared by **rolling the swab back and forth** over contiguous areas of the glass slide to deposit a thin layer of sample material.

Smears from thick liquid
- Swab method **–** swab is immersed in specimen for several seconds
  - Used to prepare thin spread of material in the glass slide

Smears from thick, granular, mucoid materials
- Opaque material must be thinly spread
- Most desirable to have both **thick and thin areas**
- Granules must be **crushed** – to assess their makeup
  - Too hard granules – probably don't represent infectious material

Smears from Thin Fluids
- Should be dropped but not spread on slide
- **Cytocentrifugation** - preferred for this type of specimen.

CYTOCENTRIFUGATION

- Excellent for _____ (CSF, BAL)

PROCEDURE

1. Small aliquots of fluid (0.1 – 0.2 ml) are placed into cytocentrifuge holder
2. Material is spun for **10 minutes**
3. Slide is removed. If deposit of cells is too heavy, a portion of cellular deposition can be smeared
4. Fixed and decontaminated in **70% alcohol for 5 mins.**

MICROSCOPY

MAGNIFICATION

100,000x – for viruses

_____ – for bacteria, fungi, parasites

RESOLUTION – extent to which **detail in the magnified object is maintained**

CONTRAST – needed to make **objects stand out from the background;** achieved by **staining technique** – higlights organisms and allow them to be differentiated.

- If staining is absent – **reduce diameter of microscope aperture diaphragm, increasing contrast at the expense of the resolution**.

FLUORESCENT MICROSCOPY

- Uses **fluorescent microscope**
- Uses certain dyes – _____

Color of fluorescent light depends on:

- Dye
- Light filters

Acridine orange, auramine, FITC

- Requires **blue excitation light** (450- 490λ)

Calcofluor White

- Requires **violet excitation light** (355-425λ)

TECHNIQUES

- **FLUOROCHROMING**
  - fluorescent dye is used alone
  - **direct** chemical interaction between dye and component of bacterial cell
  - Most Common Methods:
    - **acridine orange**
      - binds to _____(bright orange)
      - used in **blood cultures**
      - used also in *Mycoplasma*
    - **auramine-rhodamine stain**
      - for _____ in cell walls of mycobacteria (bright yellow/ orange in greenish background)
    - **calcofluor white**
      - binds to _____ of fungi
      - also used to visualize _____

- **IMMUNOFLUORESCENCE**
  - fluorescent dyes have been conjugated to specific antibodies
  - **Fluorescein isothiocyanate (FITC)** – most commonly used for conjugation to antibodies (_____)

DARK-FIELD MICROSCOPY

- Condenser does **not allow light to pass directly through the specimen** but directs the light to hit the specimen at an oblique angle.
- Used to detect bacteria that has:
  - Thin dimensions
  - Can't be seen in light microscopy

- Difficult to grow in culture

Example -*Treponema pallidum*

ELECTRON MICROSCOPY

- Uses
  - **Electron** - visualize small objects
  - Focused on **electromagnetic fields** to form an image on fluorescent screen
  - Powerful research tools.
  - Not needed for laboratory diagnosis of most infectious disease.
- Allows magnification in **excess of 100,000x**

- **GENERAL TYPES**
  - **Transmission electron microscope (TEM)** – allows visualization of **internal structures**
  - **Scanning Electron Microscope (SEM)** – scan surface of objects; provides _____ of surface structures.

SMEARING

Reasons why organisms grow in culture that was not seen in Direct Smear

- Slow-growing organism was present
- Patient receiving antibiotic treatment – prevents growth of organism
- Specimen was not appropriately processed
- Organism is no longer viable
- Organism requires special media for growth.

DIRECT SMEAR – preparation of primary clinical cample received in the laboratory for processing.

- Provides mechanism to identify _____ **present in specimen**.

INDIRECT SMEAR – when

- **Primary sample has been processed in culture**
- smear contains organisms following **purification or growth on artificial media**.

STAINS

- **Simple stains –** directed toward coloring the forms and shapes present

- **Differential stains** – directed toward coloring **specific components** present

- **Diagnostic antibody or DNA probe-mediated stain**
  - Specific at identification of organism

Gram stain (by_____, 1884)

- Fixative – **heat / methanol**
- Primary stain – **crystal violet** (hexamethyl-p-rosanaline chloride) – (30 secs.)
- Mordant – _____ (no water rinse employed; 30 – 60 secs.)
- Decolorizer – **alcohol-acetone** (quick)
- Counterstain – **Safranin** (1 minute)

Quantitation of Organisms in Gram Stain		
Many	4+	10-20 / OIO
Moderate	3+	6-10 / OIO
Few	2+	3-5 / OIO
Rare	1+	<10 on complete smear

Quantitation of Cells in Gram Stain		
Many	4+	≥25 / LPO
Moderate	3+	10-25 / LPO
Few	2+	2-10 / LPO
Rare	1+	<2 / LPO
None		

Precaution

- If crystal violet rinsed too vigorously before complexed with iodine
  - **wash away and leave poor/no staining of gram-negative organism**
- If decolorizer is too vigorous or prolonged
  - **Gram-positive complex will be removed; gram-positive organism will not stain.**

- Decolorizer is insufficient
  - **False gram-positive organisms in thicker areas of sample**
- **Presence of inflammatory cells** – key indicator of infectious process.

BASIC FUCHSIN – alternative counterstain for **faintly-staining gram negative organisms** (ex. *Campylobacter; Helicobacter*)

ACID FAST STAINING

Most common Acid-Fast staining methods

- Auramine-rhodamine
- Ziehl-Neelsen
- Kinyoun

- **Fluorescent Stain**
  - Primary stain – **auramine-rhodamine T stain** (25 mins.)
  - Decolorizer – _____ (2 mins)
    - (0.5% HCl in 70% alcohol)
  - Counterstain – **potassium permanganate** (4 mins)

  POSITIVE RESULT **– BRIGHT YELLOW/ ORANGE against GREENISH BACKGROUND**

REPORTING	
No. of acid-fast bacilli	**Report**
1 – 20	Number seen
21 – 80	Few
81 – 300	Moderate
>300	Numerous

- **Ziehl-Neelsen Method** (_____)
  - **Heat** – allows penetration of stain into waxy surface of microorganism

  - Primary stain – **carbolfuchsin** (5 minutes)
  - Decolorizer – **acid-alcohol** (3% HCl in 95% ethanol)
  - Counterstain – **methylene blue** (1 min)

- **Kinyoun Method** (_____)
  - Primary stain – **carbolfuchsin** (5 mins.)
  - Decolorizer – **acid-alcohol**
  - Counterstain – **methylene blue** (1 min)

NOTE

- **has higher concentration of phenol in primary stain,** therefore heat is not required.
- Identification of a single **acid-fast bacillus** in a single sputum is **considered diagnostic.**

- **Modified Kinyoun Method** (for partial acid-fast)
  - Primary stain – **carbolfuchsin** (5 mins.)
  - Decolorizer – _____
  - Counterstain – **methylene blue** (30 secs)

FUNGAL STAIN

Most common fungal stains are:

- KOH
- PAS
- Grocott's Methenamine Silver Stain
- Calcofluor White

Calcofluor White

- colorless dye
- binds to _____ **and** _____
- for **fungal elements**
- fluoresce maximally at **440 nm**

Evans Blue – counterstain

RESULT: fungi appears **bright apple-green / blue-white fluorescence**

SPECIMEN COLLECTION & PROCESSING (PARASITOLOGY)

STOOL COLLECTION (TYPICAL)
- one spx. collected every other day
- total of _____ collected in _____

Diagnosis of Amoebiasis
- _____

If patient in therapy of BISMUTH, BARIUM, & MINERAL OIL
- collect **PRIOR TO THERAPY**
- not until **5-7 or 4 – 5 DAYS** after completion of therapy

DELAYED FOR 2 WEEKS – if patient's in **antibiotic/antimalarials**
_____ – acceptable amount of stool (**walnut-size)**

Stool
- Bacterial infection – **1 a day for 3 days**
- Parasitic infection - _____
- Stool to preservative ratio – _____

FOR TROPHOZOITE MOTILITY DEMONSTRATION
- **FRESH SPX. IS REQRUIED**
- Examined in _____

STOOL FIXATIVES – 3:1 fixative : stool ratio

FORMALIN
- **5%** - protozoan cyst
- **10%** - helminth eggs & larvae
- must be fixed within **30 MINS.**

ADVANTAGES
- easy to prepare
- preserves spx. for up to several years
- long shelf-life

POLYVINYL ALCOHOL – for **permanent-stained smear**
- has **plastic powder**
- most often combined with **schaudinn solution**

SCHAUDINN SOLUTION
- **Zinc sulfate**
- **Copper sulfate**
- **Mercuric chloride (base)**

SODIUM ACETATE FORMALIN – for **concentration technique & permanent stained smear**
- Can be used for modified-acid fast stain for coccidian oocysts

PROCESSING

MACROSCOPIC- must be **FRESH, UNPRESERVED.**
- Consistency
  - _____ **–** possible to see cyst
  - _____ - trophozoite
- Color – brown (normal color)

MICROSCOPIC EXAM
- **DIRECT WET PREP**
  - To detect motile trophozoite
  - Used **unfixed specimen**

- 0.85% saline
- Glass slide – **3 x 2 inch-size**
- **22-mm square** cover slip
- _____– temporary seal

- **DIRECT WET PREP**
  - **Enhance details of cyst**
  - Drop of **lugol's or D' Antoni's Formula**

- **BUFFY COAT SLIDES**
  - Oxalated / citrated blood
  - Placed in **wintrobe tubes**
  - **30 mins. @ 100 x g**
  - For _____

CONCENTRATION TECHNIQUE

- Detects small no. of parasite
- Best to detect helminth eggs and larvae
- **Types**
  - **Floatation**
  - **Sedimentation**
- **FECT (FORMALIN – ETHYL ACETATE SEDIMENTATION)**
  - **Most widely used**
- **ZINC SULFATE FLOATATION TECHNIQUE**
  - Zn sulfate SG – _____
- **KNOTT TECHNIQUE**
  - **1 ml blood**
  - **10 ml 2% formalin**
  - **1 min @ 500 x g**

PERMANENT STAINS
- Sample of choice – _____

Wheatly Trichrome – most widely used
Iron Hematoxylin – for excellent morphology of intestinal protozoa

OTHER SPECIMENS ASIDE FROM STOOL

- **DUODENAL MATERIAL**
  - _____
  - *Cryptosporidium*
  - *Isospora belli*
  - *S. stercoralis*
  - *Fasciola hepatica*
  - *C. sinensis*

- **SIGMOIDAL MATERIAL (COLON)**
  - *E. histolytica*
  - Coccidian parasite
  - *Microsporidia*

- **CELLOPHANE TAPE PREP**
  - _____
  - *Taenia* spp.

- **BLOOD – Giemsa stain** is preferred.
  - *L. donovani*
  - *Trypanosoma* spp.
  - *Plasmodium* spp.
  - *Babesia* spp.
  - Microfilaria

- **CSF**
  - *Naegleria* spp.
  - *Acanthamoeba*
  - *T. gondii*
  - Microsporidia
  - *T. solium* cysticercus

- *Echinococcus* spp.

- **TISSUE**
  - ***Leishmania***
  - ***T. gondii***
  - ***Trypanosoma***
  - ***T. spiralis***

- **SPUTUM**
  - _____
  - *S. stercoralis*
  - Microporidia
  - *E. histolytica*
  - *E. gingivalis*
  - *A. lumbricoides*
  - Hookworm

- **URINE**
  - *S. haematobium*
  - *T. vaginalis*
  - Microfilaria

- **EYE SPECIMENS**
  - *Acanthamoeba* spp.
  - *T. gondii*
  - *Loa loa*

- **SKIN SNIPS**
  - *O. volvulus*

- **NASAL DISCHARGE**
  - *N. fowleri*

- **MOUTH SCRAPINGS**
  - *E. gingivalis*
  - *T. tenax*

CULTURE MEDIA

ACETATE AGAR
Purpose – differentiate *E. coli* from *Shigella* spp.

Components:
- **Acetate** – carbon source
- **Bromthymol blue** – pH indicator

RESULT:
- Green – negative (didn't utilized acetate)
- Blue – positive (utilized acetate)

ALKALINE PEPTONE WATER
Purpose – for recovery of _____ And ***Aeromonas***

spp. Components:
- **0.5 – 1.0% NaCl –** to recover *Vibrio* spp.

BACTEROIDES BILE ESCULIN AGAR
Purpose – for isolation of ***Bacteroides fragilis*** **group Components:**

- **Oxgall –** separates bile-resistant & bile-sensitive species.
- **1% esculin & ferric ammonium citrate** –
  - to visualize esculin hydrolysis
  - **(+)** reaction – **dark brown or black**

BILE ESCULIN AGAR

Purpose – used to isolate & identify **group D streptococci and enterococci.**

Components:
- **Oxgall** - inhibits most gram (+) organisms
- **Esculin** – differential component

Esculin ---Bacteria---> Esculetin

Esculetin + **Ferric citrate** □ insoluble iron salts (black)

NOTES:
- Addition of **vancomycin –** used to detect vancomycin-resistant streptococci & enterococci
- Addition of **azide** – inhibits gram-negative organism

BISMUTH SULFITE AGAR
Purpose – isolation of _____

Components:

- **Selective ingredients –** inhibits gram (+) bacteria
  - Bismuth sulfite
  - Brilliant green
- **Ferrous sulfate** – reacts to H2S to produce **black ppt.**

- **COLONIES**
  - *Salmonella* serotype Typhi **- black surrounded with metallic sheen**
  - Serotype Gallinarum, Cholerasuis, Paratyphi – **light green**

KANAMYCIN AND VANCOMYCIN BLOOD AGAR

Purpose – for isolation of obligate gram (-) anaerobes particularly ***Bacteroides* spp.**

Components:
- **Antimicrobials**
  - Kanamycin
  - Vancomycin

LAKED BLOOD AGAR W/ KANAMYCIN & VANCOMYCIN & VITAMIN K
Purpose – for isolation of ***Bacteroides* and *Prevotella***

spp. Components:
- **Antimicrobials**
  - Kanamycin
  - Vancomycin
- **Laked erythrocytes** (lysed by freezing)
- **Vitamin K**

NOTE: helpful in isolation of ***Prevotella melaninogenica***

RABBIT BLOOD AGAR
Purpose – for recovery and demonstration of beta- hemolysis of ***Haemophilus* spp. & *Gardnerella vaginalis***

BORDET – GENGOU BLOOD AGAR
Purpose – for isolation _____ ***& B. parapertussis***

Components:
- **Selective agents:**
  - Penicillin
  - Methicillin
  - cephalexin
- **Peptone**
- **Glycerol**
- **Potato infusion**
- Defibrinated **sheep blood** (sterile)
- **15 – 30% Blood enrichment** (3-6 ml/20-ml tube)

NOTE: plate must be held for **5 days, but not more than 7 days**, before regarded as negative

BRAIN-HEART INFUSION BROTH

Purpose – recommended for **cultivation of pneumococci for bile solubility test**

Components:

- **Brain & Beef heart** – provide nutrients
- **Peptone**
- **Glucose**
- **NaCl**
- **Buffers**

NOTE: 6.5% NaCl can be added – to differentiate salt- tolerant enterococci from streptococci.

BUFFERED CHARCOAL YEAST EXTRACT (BCYE) AGAR

Purpose – for isolation of ***Legionella* spp. Components:**

- **Ferric pyrophosphate** – provides Iron
- **Enhances growth of *Legionella***
  - Yeast extract
  - Alpha-ketoglutarate
  - L-cysteine
- **Activated charcoal** – absorb toxic compounds from organism's metabolism

NOTE:

- can be used to isolate ***Francisella* & *Nocardia* spp.**
- ***Legionella* spp.** Not be visible til **3-5 days after inoculation**

WADOWSKY – MODIFICATION

Components:

- **Inhibitors of Gram (-) organism**
  - Glycine
  - Polymyxin B
- **Vancomycin –** inhibits gram (+) cocci
- **Anisomycin –** inhibits fungi
- **Differential components:**
  - Bromcresol purple
  - Bromthymol blue

RESULT: *L. pneumophila* colonies – **light blue w/ pale green tin**

BURKHOLDERIA CEPACIA AGAR

Purpose – isolate *B. cepacian* from respiratory spx. of patients with cystic fibrosis

Components:

- **Inhibitors –** inhibits gram (+) & gram (-)
  - Crystal violet
  - Bile salts
  - Polymyxin B
  - Ticarcillin
- **Inorganic salts**
- **Peptones**
- **Pyruvate**
- **Phenol red –** pH indicator

CAMPYLOBACTER BLOOD AGAR

Purpose – for isolation of ***Campylobacter* spp. Components:**

- **Brucella agar –** base medium
- **Sodium bisulfite** – lowers redox potential, enhancing recovery of microaerophilic organism.
- **10% sheep blood**
- **Inhibitors**
  - Vancmycin
  - Trimethoprim – prevents *Proteus*
  - Polymyxin B

- Amphotericin B – prevents fungi
- Cefoperazone – antipseudomonal

Colony characteristic

- *Campylobacter* spp.
  - **Flat, gray, nonhemolytic, raised or mucoid**
  - Some may be **tan or slightly pink**
  - May appear **swarming / spreading across surface of plate**

CETRIMIDE AGAR (pseudosel agar / *Psedomonas*- selective agar)

Purpose – for *Pseudomonas* spp. (**except for *P. fluorescens)***

Components:

- **Inhibitor**
  - **Cetrimide** (cetyl trimethyl ammonium bromide / hexamethyltrimethyl ammonium bromide
  - **Pyocyanin production Stimulator – Magnesium chloride**
  - Potassium sulfate
- **Low Iron content** – stimulates **pyoverdin** prod.

COOKED MEAT (CHOPPED MEAT GLUCOSE) MEDIUM

Purpose – useful in cultivation of anaerobes esp. ***Clostridium* spp.**

Components:

- **Solid Meat Particles –** initiates growth from very small inoculum
- **Peptone**
- **Beef heart**
- **Dextrose**

CYCLOSERINE CEFOXITIN FRUCTOSE AGAR (CCFA)

Purpose – for isolation and identification of ***Clostridium difficile***

Component:

- **Inhibitors –** inhibits intestinal normal flora
  - Cycloserine
  - Cefoxitin
- **Fructose**
- **Neutral red** – pH indicator

OTHER VARIATIONS

- **1st variation**
  - **Mannitol** (instead of fructose)
  - **Bromthymol blue** – pH indicator
- **2nd variation**
  - Addition of **egg yolk suspension**
    - Detection of **lipase and lecithinase activity**
  - **COLONY CHARACTERISTIC**
    - *C. difficile* **– yellow colony**
    - In UV light – **gold- yellow**

0.4% DILUTE GELATIN MEDIUM

Purpose – useful in differentiation of:

- ***Nocardia* spp.** from one another
- ***Streptomyces* spp.**

COLONY CHARACTERISTICS

- ***N. asteroids*** – doesn't grow / grows poor
- ***N. brasiliensis*** – compact, rounded colonies
- ***Streptomyces* spp.**
  - Poor to good growth
  - With stringy or flaky morphology

EGG YOLK AGAR (McClung Toabe Agar)

Purpose – for detection of **lecithinase, lipase, protease activity**

Component – Egg emulsion

- Provides lecithin, lipids, and proteins

RESULTS:

- Lecithinase activity – **zone of opacity**
- Lipase activity – **iridescent sheen** around surface of colonies

Protease activity – **clearing of medium**

HAEMOPHILUS TEST MEDIUM

Purpose

- for **susceptibility testing of *Haemophilus***
- also for broth minimal inhibitory concentration (MIC)

Components:

- Beef
- Yeast
- Casein extract
- Hematin
- NAD

FLETCHER SEMISOLID MEDIUM **Purpose –** for *Leptospira* spp.

Component: Rabbit serum w/ hemoglobin – **enrichment Growth:** turbidity (examined in dark-field microscope)

HEKTOEN ENTERIC AGAR

Purpose

- for direct isolation of enteric pathogens
- for indirect isolation from selective enrichment broth

Components:

- bile salt – selective component
- lactose
- salicin
- sucrose
- bromthymol blue – pH indicator
- **for detection of H_2S gas**
  - sodium thiosulfate
  - ferric ammonium citrate

NOTE:

- SHOULD NOT BE AUTOCLAVED
- AVOID OVERHEATING

COLONY CHARACTERISTICS

- Most nonpathogen
  - **bright orange to salmon-pink**
- *Salmonella* & *Shigella* spp.
  - **Green to blue-green colonies**

LIM BROTH (Modified Todd-Hewitt Broth)

Purpose – for isolation of ***Streptococcus agalactiae***

Component:

- Peptone
- Yeast extract
- Dextrose
- **Inhibitors for gram (-)**
  - Colistin
  - Nalidixic acid

LOEFFLER COAGULATED SERUM SLANT

Purpose – for primary recovery of ***C. diphtheriae***

Component:

- Serum (high content)
- Animal heart muscle
- Dextrose
- Egg
- NaCl

MacCONKEY AGAR

Purpose – selects for Enterobacteriaceae & other gram (-) rods

Components:

- **Inhibitors**
  - Bile salt

    - Crystal violet
- **Lactose –** sole carbohydrate source
- **Neutral red** – pH indicators

NOTE:
- ***Enterococcus* spp**. may produce tiny colonies
- **MAC w/out crystal violet** – used to help identify mycobacteria

MacCONKEY SORBITOL AGAR
Purpose – used to isolate ***E. coli* O157:H7 D-sorbitol** is substituted for lactose

MALONATE BROTH
Purpose – identification of *Salmonella* spp.

Component:
- Sodium malonate – carbon source
- Glucose
- Yeast extract
- Bromthymol blue – pH indicator

REACTION
- **Prussian blue color** – utilized malonate
- **green (no change of color)** - no growth

MANNITOL SALT AGAR
Purpose – for recovery & identification of staphylococci

Components:
- **7.5% NaCl** – inhibits gram (-) & (+) except staphylococci
- **Mannitol** – carbohydrate source
- _____ – pH indicator

COLONY CHARACTERISTICS
- *S. aureus* **– yellow zone in colonies**

NOTE: *Enterococcus* spp.- may able to grow & weak mannitol fermenter

MOTILITY TEST MEDIUM
Purpose – to determine if organism is motile or nonmotile

NOTE: add **1% triphenyltetrazolium chloride** – to enhance detection of motility

MODIFIED THAYER-MARTIN AGAR

Purpose – for recovery of *N. gonorrhoeae* & *N. meningitidis*

COMPONENTS
- Hemoglobin
- Vitamins
- Diphosphopyridine nucleotide
- L-cysteine
- NAD
- Glutamine
- Cornstarch – absorb inhibitory substances
- **Inhibitors**
    - Vancomycin
    - Colistin
    - Nystatin – prevents fungal growth
    - Trimethoprim – prevents *Proteus* swarm

Martin-Lewis Agar (Components)

- **Anisomycin (20ug / mL)** – substitute for nystatin
- **Vancomycin (4ug/mL)** – higher conc. Than MTM

MUELLER-HINTON AGAR
Purpose – for susceptibility testing of organisms in antimicrobial agents.
COMPONENTS:
- Animal infusion
- Casein extract
- Starch

NOTE:

- Add **5% sheep blood** – to perform susceptibility testing on **streptococci**
- Add **heated / chocolatized SRBC** – for fastidious organism (*Haemophilus* & *Neisseria*)
- **Ca^{2+} & Mg^{2+} concentration** – critical in testing of ***Pseudomonas*** isolates w/ aminoglycoside antibiotics

MUELLER-HINTON AGAR W/ 2% NaCl
Purpose – for detection of **MRSA**

Cefoxitin & oxacillin – used for detection of MRSA (in Kirby-Bauer or Etest)

MHA w/ 4% NaCl & 6ug OXACILLIN
Purpose – to screen ***S. aureus*** isolates selectively for resistance to **oxacillin or nafcillin**

NEW YORK CITY MEDIUM

Purpose – used also for *N. gonorrhoeae* & *N. meningitidis*

COMPONENTS:

- Hemoglobin- from lysed horse RBC
- Yeast dialysate
- Horse plasma
- **Inhibitors**
  - Vancomycin
  - Colistin
  - Amphotericin B
  - Trimethoprim

NOTE: also supports growth for ***Mycoplasma*** as well as *Ureaplasma urealyticum*

O-F POLYMYXIN B-BACITRACIN-LACTOSE AGAR
Purpose – for isolation of ***Burkholderia cepacia***

INHIBITORS:

- Polymyxin B
- Bacitracin

PHENYLETHYL ALCOHOL AGAR

Purpose – for isolation of **gram (+) cocci & rods COMPONENT:**

- **Phenylethyl alcohol** – inhibits facultative gram neg. rods

NOTE: ***Bacillus anthracis*** will not grow in this medium

POTASSIUM TELLURITE BLOOD AGAR
Purpose – for isolation of ***C. diphtheriae***

COMPONENTS:

- **Cystine**
- **Potassium tellurite –** inhibits gram (-) organisms, staph, strep, while allowing growth of *C. diphtheriae*

NOTE:

- Some *Staphyloccocus*, gram (-) bacilli, yeast **will overcome inhibition**

COLONY CHARACTERISTICS

- ***C. diphtheriae* –** dull, gray black (reduction of tellurite)
- **Diphtheroids** – light gray-green
- ***Staphylococcus* –** large, glistening, jet black
- **Gram (-) bacilli & yeast** – dull, gray-black (larger)

PPLO (Pleuropneumonia-like organism) Agar

Purpose – used to isolate *Mycoplasma* spp.

Component:
- NaCl
- Agar
- Antimicrobials

REGAN-LOWE MEDIUM
Purpose – for isolation of *B. pertussis* & *B. parapertussis*

Components:
- Beef extract
- Horse blood
- Niacin
- Pancreatic digest
- **Neutralizers**
  - Charcoal
  - Starch
- Cephalexin – selective agent

COLONY CHARACTERISTICS
- *B. pertussis* **–** domed, shiny, transparent, and tiny; **mercury droplet appearance**

SALMONELLA-SHIGELLA AGAR
Purpose – selection of *Salmonella* & some strains of *Shigella* spp. from stool

Components:
- **Inhibitors:**
  - Bile salts
  - NaCl
  - Brilliant green
- Lactose – carbohydrate source
- Neutral red – pH indicator
- **For detection of H_2S gas**
  - Sodium thiosulfate
  - Ferric ammonium citrate

NOTE: HEAVY INOCULUM OF STOOL should be plated on SS agar – because medium is very inhibitory

SELENITE BROTH
Purpose – recovery of **low numbers** of *Salmonella* and some strains of *Shigella* spp. from stool

Component:
- Sodium selenite **–** inhibitor; effective at **neutral pH**
- **Maintains neutral pH**
  - Lactose
  - Phosphate buffers

NOTE: 1-2g of stool should be inoculated

SP-4 BROTH & AGAR
Purpose – primary isolation media for _____

Components:
- Yeast
- Preformed nucleic acid
- Fetal bovine serum – supplies cholesterol
- **Inhibitors**
  - Penicillin
  - Amphotericin B
  - Polymyxin B

STREPTOCOCCUS-SELECTIVE AGAR
Purpose – for isolation primarily for **beta-hemolytic streptococci**

Component:
- **Columbia agar** – base
- **Maltose** – enhances prod. Of streptolysin
- **Inhibitors**
  - Polymyxin B
  - Neomycin

In order formulation, with:
- Oxolinic acid
- Colistin

TETRATHIONATE BROTH
Purpose – recovery of *Salmonella* **except serotypes**

- **Typhi**
- **Arizonae**

Components:

- **Inhibitors**
  - Iodine-potassium iodide solution (**added**)
  - Bile salt in conjunction with thiosulfate
  - Brilliant green
  - Crystal violet

THIOSULFATE CITRATE BILE SALT SUCROSE AGAR
Purpose – isolation of *Vibrio* spp.

Components:
- **Inhibitors**
  - Sodium citrate
  - Sodium thiosulfate
  - Oxgall
- Bromthymol or thymol blue – pH indicator
- **For H_2S detection**
  - Sodium thiosulfate
  - Ferric citrate
- Sucrose – carbohydrate source

COLONY CHARACTERISTICS
- Sucrose fermenters – **yellow**
  - *Vibrio cholerae*
  - ***Vibrio alginolyticus***
- Non-sucrose fermenters – **blue-green colonies**
  - *Vibrio parahaemolyticus*
  - ***Vibrio vulnificus***
- Other organism – **blue colonies**
  - *Pseudomonas*
  - ***Plesiomonas***
  - ***Aeromonas***

NOTE: Heavy inoculum should be applied

TINSDALE AGAR
Purpose – used for isolation of ***C. diphtheriae***

COMPONENTS:
- **Inhibitors**
  - Potassium tellurite (high concentration)
- Cystine
- Thiosulfate

COLONY CHARACTERISTICS
- *Corynebacterium* spp. **– gray to black colonies**
  - *C. diphtheriae* – with **brown halo**
  - *C. ulcerans & pseudodiphthericum*
    - **Dark halo**
- *Proteus* – **mucoid**
- Staphylococci & Streptococci (rare)
  - **Dark colonies**

TODD-HEWITT BROTH W/ GENTAMICIN & NALIDIXIC ACID

Purpose – used to grow streptococci from vaginal & rectal swab **for serotyping**

COMPONENT:
- Peptone
- Beef heart infusion
- Glucose
- **Inhibitors**
  - Gentamicin
  - Nalidixic acid

VAGINALIS AGAR

Purpose – isolation of *Gardnerella vaginalis*

Components:

- Columbia agar – base
- **Inhibitors**
  - Colistin
  - Nalidixic acid
  - Nystatin

XYLOSE-LYSINE-DESOXYCHOLATE AGAR

Purpose – used to isolate *Salmonella* and *Shigella*

Components:

- Sodium desoxycholate – inhibitor
- Sucrose
- Lactose
- Xylose – high concentration
- Phenol red – pH indicator
- **For H2S detection**
  - Sodium thiosulfate – sulfur source
  - Ferric ammonium citrate

COLONY CHARACTERISTICS

- **Yellow colonies –** ferments excess carbohydrates
  - *E. coli*
- **Yellow colonies w/ black centers** – ferments excess carbohydrates; H2S producers
  - *Citrobacter* spp.
  - *Proteus* spp.
- **Colorless or red colonies**
  - *Shigella* spp.
- **Red colonies**
  - *Salmonella* spp.
  - *Edwardsiella* spp.

MEDIA FOR MYCOBACTERIA

AMERICAN TRUDEAU SOCIETY MEDIUM

- Egg-based

Purpose – isolation of *M. tuberculosis*

Component:

- Eggs – provides fatty acid
- Potatoes – carbon source
- **Malachite green** – inhibitor

LOWENSTEIN-JENSEN MEDIUM

Purpose – for cultivation of *Mycobacterium* spp.

Component:

- Potato flour
- Egg
- Glycerol
- **Asparagine** – for **max. production of niacin** by certain *Mycobacterium* spp.
- **Malachite green** – inhibitor

MODIFICATIONS

- **LJ medium w/ 5% NaCl** – to aid in identifying **rapid growers**
- **Gruft modification** – more selective
  Components:
  - **Selective agents**
    - Penicillin (50 U/ml)
    - Nalidixic acid (35 ug/ mL)
  - **Ribonucleic acid** (0.05 ug/ml)
    - Increases rate of mycobacterium isolation

- **Petran and Vera modification** – permits gentler decontamination or digestion procedures
  Components:
  - **Selective agents (added):**
    - **Cyclohexamide**
    - **Lincomycin**
    - **Nalidixic acid** **MIDDLEBROOK 7H10 & 7H11**

AGARS Purpose – used to cultivate *Mycobacterium* spp.

NOTE: Isoniazid-resistant strains grows better in this medium

Components (7H11)

- Casein hydrolysate – stimulates growth of **drug- resistant** *Mycobacterium tuberculosis*

Components (Both)

- **Oleic-acid-dextrose-catalase (OADC)** –simulates egg components
  - **Oleic acid**– fatty acid used by mycobacteria
  - **Dextrose** – for energy production
  - **Catalase** – neutralize toxic peroxidase
- **Albumin –** inhibits toxic agents; source of CHON
- **Malachite green** - inhibitor

MITCHISON 7H11 SELECTIVE AGAR

Component:

- **Inhibitors:**
  - Amphotericin B
  - Carbenicillin
  - Polymyxin B
  - Trimethoprim

MEDIA w/ BROMTHYMOL BLUE

- **Acetate agar**
- **BCYE**
- **CCFA**
- **Hektoen Enteric Agar**
- **Malonate Broth**

w/ BROMCRESOL PURPLE

- **Wadowsky-Yee BCYE**
- **CCFA**
- **Moeller**
- **LIA**

w/ NEUTRAL RED

- **CIN**
- **MacConkey**
- **Sorbitol McConkey**

w/ PHENOL RED

- **Burkholderia cepacia Agar**
- **TSI**
- **Kligler**
- **MSA**

MOLECULAR DIAGNOSTICS

POLYMERASE CHAIN REACTION

- **DENATURATION – 94 – 95 degree Celsius (15 – 30 secs)**
  - For dsDNA separation
- **PRIMER ANNEALING – 45 – 65 degree Celsius (30 secs. – 2 mins.)**
  - Anneals primer to target DNA

- **PRIMER EXTENSION – 68 – 72 degree Celsius**
  - Synthesis of new strands of DNA

PCR COMPONENTS

- **Template DNA** – target for PCR
- **Oligonucleotide Primers** – starts synthesis new strands of DNA
- **Thermostable DNA** – synthesizes new strands of polymerase of DNA
- **Magnesium** – required by DNA polymerase for proper reaction
- **Buffer** – ensures proper conditions and pH for DNA polymerase
- **Deoxynucleotides** – used by polymerase to synthesize new DNA
- **Thermal Cycler** – heats and cools PCR cycle steps.

ANTIMICROBIAL SUSCEPTIBILITY TEST

AST STANDARDIZATION

- **McFarland Turbidity Standards**
  - **1% H_2SO_4**
  - **1.175% $BaCl_2$**

0.5 McFarland – most commonly used

Growth Medium –

- pH – **7.2 – 7.4**
- cation concentration
- blood and serum components
- thymidine content

TESTING MEDIUM FOR DIFFERENT ORGANISM

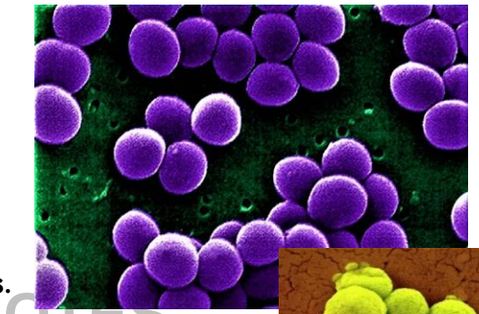
Organism	Media	Inoculum size			Incubation
		Broth Dilution	Agar dilution	Disk diffusion	
Enterobacteriaceae P. aeruginosa Enterococci	Mueller-Hinton	**5 x 10^5 cfu/Ml**	**1 x 10^4 cfu/spot**	**1.5 x 10^8 cfu/ml**	35°C; air 16 – 20 hrs.
Staphylococci	MH w/ 2% NaCl				30-35° C; 5-10% CO_2
Streptococcus	MHA w/ 5% Sheep's Blood		Not needed for S. pneumoniae		35°C 5-10% CO_2
H. influenzae	HTM		**1 x 10^4 cfu/spot**		20 – 24 hrs
N. meningitidis	MH w/ 2-5% lysed horse blood				35°C; 5-7% CO_2; 24 hrs.
N. gonorrhoeae	GC agar + supplements	None			35°C; 5% CO_2; 24 hrs.
Anaerobes	Brucella BA w/ Hemin	**1 x 10^6 cfu/ml**	**1 x 10^5 cfu/spot**	------	Anaerobic, 35-37°C; 48 hrs.

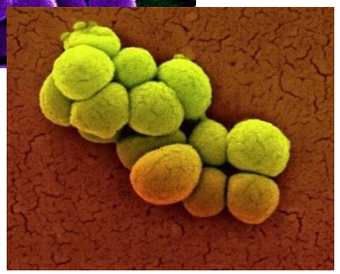
AUTOMATED ANTIMICROBIAL SUSCEPTIBILITY SYSTEMS	
Vitek 2	• 64-well; has specific concentration of antibiotics • Advanced Expert System (AES)
MicroScan WalkAway	• Microdilution manullary inoculated with **multiprong device**
Phoenix System	• Convenient, albeit manual, **gravity-based** inoculation process • Growth monitoring – based on **redox indicator system**

SUPPLEMENTAL METHODS FOR ANTIMICROBIAL RESISTANCE DETECTION	
Oxacillin Agar screen	For staphylococcal resistance to penicillinase-resistant penicillin
Vancomycin agar screen	For enterococcal resistance to vancomycin
Aminoglycoside screens	For acquired enterococcal high-level resistance to aminoglycosides that would compromise synergy with a cell wall-active agent
Oxacillin disk screen	For streptococcus resistance among S. aureus resulting from efflux
Cefoxitin disk test 30 ug	To improve detection of oxacillin-resistant CoNS
Aminoglycosides	For serious enterococcal infections, and acquired high-level resistance

STAPHYLOCOCCUS & MICROCOCCUS

- **Gram (+) cocci**
- **Facultatively anaerobes** (except for **S. saccharolyticus – OBLIGATE ANAEROBE)**
- **In tetrads or in clusters**
- **Catalase (+)**
- **Oxidase (+)**
- **Non-motile**
- Grows in **7.5 – 10% NaCl**





Characteristics:

COLONY

- Produced after **18-24 hrs.**
- Medium-sized (4-8 um)
- COLORS:
  - **Cream-colored**
  - **White**
  - **Rarely light gold**
  - "_____"

HUMAN NARIS (NOSTRILS) – PRIMARY RESERVOIR FOR STAPHYLOCOCCI

***S. aureus* Disease Association**

- Folliculitis
- Furuncles (Boil)
- Carbuncles
- Bullous impetigo
- Scalded skin syndrome / Ritter's Disease / Pemphigus neonatorum
- Toxic Shock Syndrome
- Toxic Epidermal Necrolysis
- Food poisoning
- Staphylococcal pneumonia
- Osteomyelitis

- Septic arthritis (children)

<u>*S. aureus* Virulence Factors:</u>

- **STAPHYLOCOCCAL ENTEROTOXINS**
  - Stable @ **100°C**

Enterotoxin	Disease Association
B (10%), **A** (78%), **D** (38%)	FOOD POISONING
B, C, G, I, F	TOXIC SHOCK SYNDROME

Enterotoxin B – assoc. w/ **staphylococcal pseudomembranous colitis**
Enterotoxin F – former name for **TSST-1** (assoc. w/ **using of tampons)**

- **Other Virulence Factors**

Virulence Factor	Function and role in disease
Alpha-hemolysin	Lyses: **RBCs, platelets, Macrophages** Causes: **Severe Tissue Damage**
Beta-hemolysin (Sphingolmeylinase C)	• "_____" • Enhance hemolysis @ **37°C & 4°C** • Exhibited in **CAMP test** • Acts on **sphingomyelinase of RBC**
Staphylococcal enzymes	Enzymes: • Protease • Lipase • Hyaluronidase (Duran-Reynal Factor) • Staphylocoagulase Facilitates **spread of infection** (protease, lipase, hyaluronidase)
Panton-Valentine Leukocidin (PVL)	• Exotoxin lethal to PMNs • **Assoc. w/ gamma-hemolysin** • Causes: **-severe cutaneous infection** **- necrotizing pneumonia** • Assoc. w/: **-community-acquired staph infection**
	antiphagocytic

OTHER STAPHYLOCOCCI		
Organism	**Virulence factor**	**Disease association**
S. epidermidis	Biofilm Delta toxin Poly-γ-glutamic acid	• Prosthetic valve endocarditis (**most common)** • Nosocomial infection.
S. saprophyticus	Adheres to epithelial lining	UTI;**(_____)** **– significant**
S. lugdunensis	mecA gene for oxacillin resistance	UTI and endocarditis **Catheter-related bacteremia**
S. haemolyticus	Vancomycin resistance	UTI and endocarditis

TESTS TO DIFFERENTIATE STAPHYLOCCOCUS & MICROCOCCUS

TEST	STAPHYLOCCOCUS	MICROCOCCUS
Furoxone-Tween 80-ORO Agar (growth)	-	+
Lysosome (50-mg disk)	Resistant	Susceptible
Anaerobic acid prod. From glycerol in presence of erythromycin	+	-
O/F Test	Fermenter	Oxidizer
Modified oxidase	-	+
Bacitracin (_____)	Resistant <10mm	Susceptible >10mm
Furazolidone (100 ug)	Susceptible	Resistant
Lysostaphin (200ug/ml)	Susceptible	Resistant

Organism	PYR	VP Test

S. aureus	-	+
S. lugdunensis	+	+
S. intermedius	+	-
S. schleiferi	+	+

COAGULASE TEST: differentiates *S. aureus from CoNS;* uses **rabbit or pig plasma**

- **Slide method –** for **clumping factor (_____)**
- **Tube method –** for **staphylocoagulase (_____)**

Coagulase (+) Staphylococci:

- *S.* ***d****elphini*
- *S.* ***a****ureus*
- *S.* ***h****yicus*
- *S.* ***i****ntermedius*
- *S.* ***l****uteus*

Contains CLUMPING FACTOR

- *S. lugdunensis* - confused w/ S. aureus in slide method
- *S. schleiferi*

SMALL COLONY VARIANTS STAPHYLOCOCCI

- **Fastidious**
- Requires: **CO_2, Hemin, Menadione**
- Grows on media containing **blood.**

STREPTOCOCCUS & ENTEROCOCCUS

- **Gram (+) cocci**
- **In pairs or chains**
- **Aerotolerant anaerobes**
- Some are **capnophilic**

Characteristics

COLONY

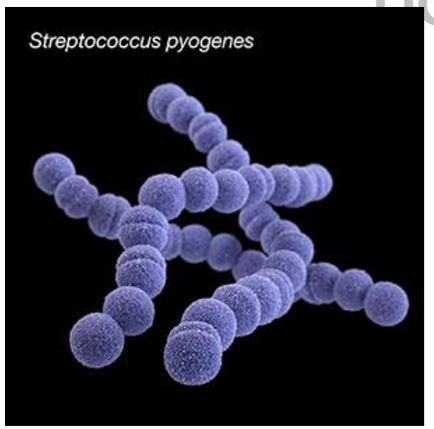
- Usually **small and transparent**

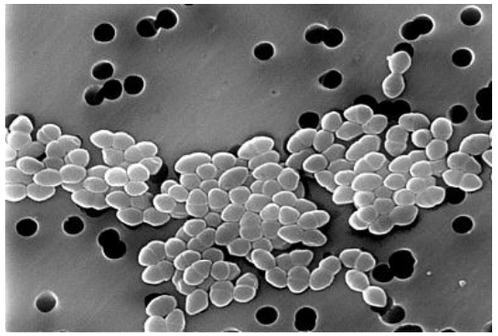
CATEGORIES OF NECROTIZING FASCIITIS	
Type	**Description**
1	Polymicrobial infection (aerobic & anaerobic)
2	Consist of **Group A Streptococci**
3	Clostridial myonecrosis
Saltwater Necrotizing Fasciitis	Caused by ***Vibrio* spp.**

Organism	Lancefield	Smith and Brown's
S. pyogenes	A	Beta
S. agalactiae	B	Beta
S. dysgalactiae, S. equi	C	Beta
***S. bovis* group**	D	Alpha, Gamma
E. faecalis, E. faecium	D	Alpha, Beta, Gamma
S. pneumoniae	None (has _____)	Alpha
S. anginosus, mutans, mitis	A, C, F, G, N	Alpha, Beta, Gamma

Organism	Virulence Factor	Disease Assoc. / Characteristics

S. pyogenes		Antiphagocytic	• Necrotizing fasciitis / galloping gangrene • Erysipelas (St. Anthony's Fire) • Impetigo • Puerperal Sepsis • Childbed Fever • Scarlet Fever • Post-streptococcal acute glomerulonephritis (Bright's Disease)
	Protein F	Fibronectin-binding protein	
	Hyaluronic acid	Capsule, **prevents opsonization**	
	Streptolysin O	O_2 labile, antigenic, **subsurface hemolysis**	
	Streptolysin S	O_2 stable, non-antigenic, **surface hemolysis**	
	Streptokinase	Thrombolytic drug, fibrinolysis	
	Hyaluronidase (_____)	Spreading factor	





	Erythrogenic Toxins (Streptococcal Pyrogenic Exotoxins)	SPEs **A, B, C, F** **A** – Scarlet Fever & Toxic shock sydrome	
S. agalactiae	**Sialic acid**	Critical virulence determinant	Meningitis, obstetric complications, mastitis in cattles
S. pneumoniae	**Capsular polysaccharide**	Serotypes **1, 2, 3** – common cause of **lobar pneumonia**	Meningitis, _____, otitis media, sinusitis, bacteremia, **2° atypical HUS**
Enterococci	**cytolysin**	Capable of producing **pseudoperoxidase reaction (weak bubbling)**	
Viridans Streptococci	• Common cause of **subacute bacterial endocarditis** • Gingivitis • Dental caries (_____)		
***S. bovis* group (*S. gallolyticus* subsp. *Gallolyticus*)**	• Associated with **gastrointestinal carcinoma (colorectal tumors)**		

TESTS FOR STREPTOCOCCI

Bacitracin Disk Test / Taxo A (0.04 U)	Differentiates *S. pyogenes* from other Beta-hemolytic groups Result: Group C and G are susceptible
Sulfamethoxazole & Trimethoprim (SXT) test	Result: Group B – **resistant** to SXT Group C – **sensitive** to SXT
Pyrrolidonyl Arylamidase (PYR) test	More specific than bacitracin test *S. pyogenes* is the only Beta-hemolytic strep that is positive.
CAMP (Christie, Atkins, Munch-Petersen) test	Used to differentiate *S. agalactiae* from other Beta-hemolytic streptococci. Result: (+) Beta-hemolysis.
Hippurate Hydrolysis Test	Used to differentiate *S. agalactiae* from other Beta-hemolytic streptococci. Result: **(+) purple color**
Dick's Test	Skin test for
Schultz-Charlton Test	Immunity test for Scarlet fever
	Capsular swelling test for *S. pneumoniae* & other bacteria that has capsule.
Francis Skin Test	Detection of presence of antibodies against pneumococci.
Bile solubility test	Evaluates the ability of *S. pneumoniae* to lyse in the presence of bile salt.

Organism	SXT (1.26 ug) Group A and B vs. others	CAMP for Group B	Hippurate Hydrolysis	CAMP	PYR
Group A	S	R	-	-	+
Group B	R	R	+ (Enhance hemolysis)	+	-
Group C, F, G	R	S	-	-	-

Organism	Bile Esculin hydrolysis	6.5% NaCl	Optochin	PYR
Enterococcus	+	+	R <14mm	+
Non-Enterococcus	+	-	R <14mm	-
S. pneumoniae	-	-	S >14mm	-

Organism	Bile Esculin	6.5% NaCl	PYR	LAP	MRS broth
Enterococcus	+	+	+	+	-
Pediococcus	+	+	-	+	-
Leuconostoc	+	+	-	-	+

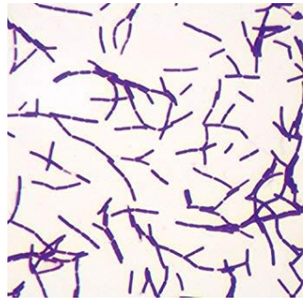
NUTRITIONALLY VARIANT STREPTOCOCCI

- requires **SULFHYDRYL COMPOUNDS**

-causes **hard-to-treat endocarditis (surgery is required for cure)**

- **Abiotrophia**
- **Granulicatella**

BACILLUS



- **Aerobic and facultatively anaerobic**
- **Gram (+)**
- **Catalase (+)**
- **Spore-forming bacilli**

Organism	characteristics	Virulence factors	Disease association
B. anthracis	• **non-motile** • "_____" appearance • Produces **endospores** Appearance in **5% SBA**: • _____ • Ground-glass appearance • Beaten egg-whites In MHA: • String of Pearls	• **Protective Antigen –** facilitates transport of two other protein into the cell. • **Edema factor (adenylate cyclase) -** responsible for edema • **Lethal factor (protease)** – primarily responsible for death. • **D-glutamic acid capsule** – resistant to hydrolysis	• **Cutaneous anthrax –** most common **Eschar (malignant pustule)** – black necrotic lesion. • **Gastrointestinal Anthrax** – most severe • **Pulmonary Anthrax (Woolsorter's / Ragpicker's Disease)** • **Injectional Anthrax** – assoc. w/ "skin popping"
B. cereus	• **Penicillin-resistant** • **Beta-hemolytic** • **MOTILE** • **Frosted glass-appearing colony** • **Feathery, spreading, beta-hemolytic colonies**	**Diarrheal** - responsible for most symptoms. • **Hemolysin BL** • **Nonhemolytic enterotoxin** • **Cytotoxin K** **Emetic** • **Cereulide** – heat-stable, proteolysis, acid-resistant	• Opportunistic • **Assoc. w/ food-borne disease** • **Causes PROGRESSIVE ENDOPHTHALMITIS** • **Some strains can carry *B. anthracis* toxin genes.**
B. thuringensis	Prduces **parasporal crystals**	Harbors genes of *B. cereus*-associated enterotoxins.	

DIFFERENTIATION		
Test	***B. anthracis***	***B. cereus***
Capsule	-	+
Growth @ 45°C	-	+
Salicin Fermentation	-	+
Hemolysis	-	+
Motility	-	+
Penicillin susceptibility	S	R
Growth in Penicillin (10 U/ml) agar	-	+
"string of pearls" reaction	+	-
Gelatin hydrolysis	-	+
Growth in PEA agar	-	+

BIOLOGICAL INDICATORS	
	Autoclave
	Ionizing radiation
	Ethylene oxide sterilization

LISTERIA, CORYNEBACTERIUM

- **Gram (+) bacilli**
- **Catalase (+)**
- **Non-spore forming**
- **Non-branching**

Organism	Virulence Factors	Disease Assoc.	Characteristics
Corynebacterium diphtheriae	**Diptheria Toxin** - blocks protein synthesis - causes **demyelinating peripheral neuritis** Bacteria is infected by **lysogenic Beta-phage.** **TONSIL / PHARYNX** – most common site of infection.	**Respiratory Diphtheria** – development of **pseudomembrane** (gray to white) **Cutaneous Diphtheria** – non-healing ulcers (dirty gray)	• Pleomorphic **(club-shaped)** • Irregular staining.

C. minutissimum – causes **erythrasma**
C. pseudotuberculosis – causes **granulomatous lymphadenitis**
- also produces **dermonecrotic toxin**

Babe's-Ernst Granules / Volutin Granules – accumulation of nutrient reserves
_____– for visualization of Babe's-Ernst Granules

Cardiac Failure – often cause of death of diphtheria
Antitoxin – treatment for the toxin.

Organism	Virulence Factor	Disease Assoc.	Characteristic
Listeria monocytogenes	**Hemolysin (Listeriolysin O)** - damages the phagosome **Intermalin (Protein p60)** - induces phagocytosis; increase adhesion, penetration into mammalian cells	**Listeriosis** - Newborn (bacteremia and meningitis) - Pregnant Women (spontaneous abortion – **3rd trimester**) Has highest tropism in **CNS.**	Small, round, smooth translucent forms narrow zone of Beta-hemolysis. Optimal growth: **30 – 35°C** but growth occurs @ **0.5 – 45°C** **Tumbling or end-over-end motility** **Umbrella-shaped or** _____ (semi-solid tube @ 22-25°C)

Test	*Listeria*	*Corynebacterium*
CAMP	+	-
Hippurate Hydrolysis	+	-
Esculin Hydrolysis	+	-

Motility	+	-
Salicin	+	-

Erysipelothrix rhusiopathiae

- **Gram (+) bacilli**
- **Catalase (-)**
- **Non-branching**
- **H_2S (+) in TSI**

Erysipelothrix rhusiopathiae		
Disease Association	**Colony Appearance on BAP**	**ID**
• **Erysipeloid** • bacteremia • cutaneous infection	Large, rough, or small, smooth and translucent Shows **alpha-hemolysis** after prolonged incubation.	_____ in gelatin stab culture.

Gardnerella vaginalis

- **gram variable / gram (-)**
- **beta-hemolytic (HBT agar) / nonhemolytic (BAP)**
- causes **bacterial vaginosis**

Causes Bacterial Vaginosis

- ***Gardnerella vaginalis***
- ***Prevotella* spp.**
- ***Peptostreptococcus* spp.**
- ***Porphyromonas* spp.**
- ***Mobiluncus* spp.**
- ***Mycoplasma hominis***

_____ **–** most accurate means of diagnosing bacterial vaginosis than culture.

NUGENT SCORING SYSTEM					
***Lactobacillus* morphotypes** (boxy, gram (+) bacilli		***Gardnerella* & *Bacteroides*** (pleomorphic, gram-variable, gram (-), short bacilli)		***Mobiluncus* morphotypes** (curved, gram-variable bacilli)	
Quantity	**Points**	**Quantity**	**Points**	**Quantity**	**Points**
4+	0	0	0	0	0
3+	1	1+	1	1+ to 2+	1
2+	2	2+	2	3+ to 4+	2
1+	3	3+	3		
0	4	4+	4		

NUGENT SCORING INTERPRETATION

0 – 3 – NORMAL VAGINAL MICROBIOTA

4 – 6 – INDETERMINATE FOR BACTERIAL VAGINOSIS

_____ – BACTERIAL VAGINOSIS

NOCARDIA, RHODOCOCCUS

- **Gram (+)**
- **Branching**
- **Partially acid-fast**

Organism	**Characteristics**	**Disease Assoc.**

Nocardia	• Beaded appearance • **Strictly aerobic** • Presence of DAP • Produces **Nocobactin** – iron-chelating compound • **Urease (+)**	• Cutaneous infection • Actinomycotic mycetoma

Nocardia brasiliensis – most common cause of cutaneous infection and actinomycotic mycetoma.

Nocardia asteroides – causes **pulmonary infection**

Organism	Casein Hydrolysis
Nocardia brasilisiensis	+
Nocardia asteroides	-

Organism	Characteristics	Disease Assoc.
Rhodococcus equi	• Facultative intracellular organism; replicate within macrophage.	Infections in immunocompromised patients such as patients with HIV.

	• **Salmon-pink pigment** • **Cocci to rods – 24 hours** • **CAMP (+) w/ *S. aureus***	

Test	Nocardia	Actinomyces spp.
O_2 requirement	Aerobic	Anaerobic
AFS	Acid-fast	Non acid-fast
Catalase	+	-
Urease	+	-
Sulfur Granules	+	+

ENTEROBACTERIACEAE

- **Non-spore forming**
- **Facultatively anaerobes**
- **Glucose fermenters**
- **Oxidase (-) (exc. *Plesiomonas)***
- **Catalase (+) (exc. *Shigella dysenteriae)***
- **Commensal flora except *Salmonella, Shigella, Yersinia* (true pathogen)**
- **Nonencapsulated except (*Klebsiella & Enterobacter*)**

Organism	Disease Association	Description
E. coli	Most common cause of nosocomial infections	Primary marker for fecal contamination in water.

***E. coli* BIOTYPES**		
Strain	**Infection**	**Virulence Factors**
Meningitis/sepsis-associated *E. coli*	Meningitis	**K1 antigen** – identical capsule to *N. meningitis*
Enteropathogenic *E. coli* (EPEC)	Infantile diarrhea (without blood) – large amounts of mucus	**Adhesive properties (pili and intimin)** – no exotoxins.
Enterohemorrhagic *E. coli* (EHEC) (Serotoxigenic/verotoxigenic)	Bloody diarrhea (NO WBCs) Assoc. w/	**Cytotoxin** **- Verotoxins I & II** – produces damage to vero cells. Most common serotypes – **O157:H7**
	Traveler's diarrhea / Montezuma's revenge Watery diarrhea	**Cholera-like toxin** **Heat-labile enterotoxin (LT);** **Heat-stable (ST) toxin**
Enteroinvasive *E. coli* (EIEC)	**Dysenrery-like / shigella-like infection** Watery Diarrhea (**w/ WBCs**)	Direct invasion **HEp-2 cells** – used to detect invasiveness **(stacked-brick pattern)**
Enteroaggregative *E. coli* (EAEC)	**Watery diarrhea**	Global aggregative regular gene, **AggR**, responsible for cellular adherence.
Uropathogenic *E. coli* (UPEC)	MOST COMMON CAUSE OF UTI Considered cause of diarrhea in HIV patients.	**Pili** (primary virulence factor to cause UTI) **Cytolysins** – kill phagocytes. **Aerobactin** – chelates iron

Escherichia albertii – newest species to the genus
- Assoc. w/ diarrhea in children.

YELLOW-PIGMENTED
- ***E. hermannii***
- ***E. vulneris***

Organism	Disease Assoc.	Description
***Citroacter* spp.**	Septicemia, menigitis, brain abscesses	*C. freundii* – may harbors inducible _____genes (encode resistance to ampicillin and first-generation cephalosporin) - endocarditis in intravenous drug abusers
Cronobacter sakazakii	Bacteremia, causes neonatal meningitis from powdered infant formula, necrotizing colitis in neonates;	Produces yellow pigment that is enhanced by incubation @ 25°C

Edwardsiella tarda	Gastroenteritis	Assoc. w/ harboring fish or turtles
***Enterobacter* spp.**	Healthcare – associated infection (contaminated medical device)	
Hafnia alvei	Gastrointestinal infection	Motile; non-lactose fermenter **DELAYED CITRATE POSITIVE REACTION**
***Klebsiella pneumoniae* (Friedlander's Bacillus)**	Lobar pneumoniae – _____	K1 capsular-containing Mucoid colonies – tends to string
***Morganella* spp.**	Normal inhabitants of gastrointestinal tract; neonatal sepsis	Resembles *E.coli* in IMVIC patter
Pantoea agglomerans	Sporadic infections can occur due to trauma	Yellow-pigmented colony; Lysine, arginine, ornithine and arginine
***Proteus* spp.**	Assoc. w/ UTI ***P. mirabilis* -** most common isolate _____ - due to urease activity	Swarming Odor: **chocolate cake or burnt chocolate smell** **Swimmers** – standard vegetative cells **Swarmers** – hyperflagellated; capable of coordinated movement
***Providencia* spp.**	Most commonly associated w/ UTI (*P. retgerii)* and the feces of children w/ diarrhea	***P. stuartii*** – outbreaks in **burn units**
***Serratia* spp.**	Colonization and cause of pathogenic infection in healthcare setting	_____ – red pigment produced by *Serratia*.
***Salmonella* spp.**	-Acute gastroenteritis or food poisoning -Enteric fever (Typhoid Fever) assoc. w/ Typhi & Paratyphi Isolated in: - blood (1 – 2 weeks) - urine (3 – 4 weeks) - stool (2 – 3 weeks)	Diagnosed with _____ **Fimbriae** – initiates intestinal infection -Ability to traverse intestinal mucosa -enterotoxin
***Shigella* spp.**	Bacterial dysentrery • Blood • Pus • Mucus	Non-motile; low infectious dose (100-200)
Yersinia pestis	Plague Only species that is transmitted from animals by bite of an insect vector *(Xenopsylla cheopis)*	- non-motile - Grows best @ **25°C – 30°C** - Colonies: **pinpoint** @ 24 hrs. but resemble those of other Enterobacteriaceae after 48 hrs. - **cauliflower appearance** @ 48 hrs in SBA

		- _____ in broth culture
Yersinia enterocolitica	Acute enteritis (enterocolitis) – **most common form** Arthritis & Erythema nodosum – **mimics appendicitis**	48 hrs. incubation @ RT in CIN – develops _____

Other *Klebsiella* spp.	
Organism	**Disease Assoc. / Characteristics**
K. oxytoca	Assoc. w/ antibiotic-associated hemorrhagic colitis **Indole (+)**
K. pneumoniae* subsp. *Rhinoscleromatis	Isolated from patient's with **rhinoscleroma**
***K. pneumoniae* subsp. Ozaenae**	- Causes **atrophic rhinitis** - Assoc. w/ presence of **plasmid-mediated ESBLs**
K. granulomatis	Causes **donovanosis**

FACTS ABOUT *Salmonella* spp.	
SPECIES	
Salmonella enterica	
Salmonella bongori	
***Salmonella enterica* SUBSPECIES**	
I	Enterica
II	Salamae
IIIa	Arizonae
IIIb	Diarizonae
IV	Houtenae
VI	Indica
***Salmonella enterica* SEROTYPES**	
Typhi	
Choleraesuis	
Paratyphi	
OTHER FACTS	
Causes Typhoid fever	Typhi
Causes Enteric fever	Choleraesuis & Paratyphi
Salmonellosis infective dose	10^6 bacteria
Development of typhoid fever	9-14 days
Gallbladder	Site of CHRONIC CARRIAGE
Vi antigen	Important in identifying Salmonella Typhi

FACTS ABOUT *Shigella* spp.	
SEROGROUP	
A	Dysenteriae
B	Flexneri
C	Boydii
D	Sonnei
Kiyoshi Shiga	First man who isolated *Shigella*

FORMS OF PLAGUE	
Bubonic/Glandular – most common; high fever w/ BUBOES	
Septicemic	
Pneumonic	
Y. pseudotuberculosis	- 1° pathogen of rodents - causes caseous swelling (pseudotubercles) - typically looks like plague bacillus

BIPOLAR STAINING

- Wayson stain
- Methylene blue

PSEUDOMONAS, BURKHOLDERIA

Organism	Disease Assoc.	Virulence factor	Appearance on BAP	Odor	Motility
P. *aeruginosa*	- Primary cause of pneumonia in Cystic Fibrosis patients. -Swimmer's ears - contact lens infection - _____ _____ - erythema gangrenosum	• Exotoxin A – most important • Hemolysins • Pili • Alginate – mucoid • Pyocyanin – specific • Biofilm production	Spreading and flat; serrated edges, silver metallic sheen, bluish green, red or brown pigmentation Beta-hemolytic MAC – colorless w/ green	Rubber-like Grape-like Corn-tortilla like	+
B. mallei	Glander's disease		N/A	N/A	-
B. pseudomallei	-Melioidosis -_____ _____	Capable of survivial in human macrophage	Smooth; mucoid to dry and wrinkled	Earthy odor	+
B. cepacia	Infections in patients w/ CF	Can survive hospitals due to intrinsic resistance to antibiotics	Smooth and raised MAC **–** pink colonies (lactose oxidizer)	Dirtlike/earthy odor	+

Organism	Growth @ 42°C	Lysine Decarboxylase	Glucose oxidation
B. mallei	-	-	+
B. pseudomallei	+	-	+
B. cepacia	Variable	+	+
P. aeruginosa	+	-	+

FLUORESCENT PSEUDOMONADS GROUP			
Organism	**Growth @ 37°C**	**Growth @ 42°C**	**Pyocyanin**
P. fluorescens	+	+	+
P. putida	+	-	-
P. aeruginosa	+	-	-

VIBRIO, AEROMONAS, CHROMOBACTERIUM

Vibrio

- Motile (monotrichous) –_____
- Oxidase (+), except for *V. metschnikovii*
- Halophilic except *V. cholerae* and *V. mimicus*
- LOA = ++-
- 0129 Susceptible vs. Aeromonas (R)

Vibrio cholerae

- Agent of _____
- Hallmark: _____ **(caused by cholera toxin)**
- Somatic antigens – **O1 & O139**
  - Assoc. w/ *V. cholerae* envelope
  - Positive markers for spread of pandemic and epidemic cholera
  - **Cholera toxin / Choleragen** – increase cAMP → dehydration, loss of water, Na and K.

CLASSIFICATION OF VIBRIO CHOLERAE O1 (PANDEMIC TYPE)		
Biotype	**Classical**	**El Tor (Common Type)**
Polymyxin Susceptibility	S	R
Lysis BY bacteriophage	+	-
Chicken RBC Agglutination	-	+
Hemolysis of Sheep RBC	-	+
Vogues-Proskauer Test	-	+

Serotype	Ogawa	Inaba	Hikojima
Anti – Ogawa	+	-	+
Anti – Inaba	-	+	+

Organism	Vibrio	Aeromonas	Plesiomonas
TSI	A/A (*V. cholerae)* K/A (*V. parahaemolyticus*)	A/A gas+	K/A or A/A (glu + inositol)
NaCl	+ (vs. Aero and Plesio)	-	-
Oxidase	+	+	+
O129 Sensitivity / Vibrio Static Test	S (vs. Aero and Plesio)	R	S/R
Motility	+	+	+
LOA	++-	+-+	+++
DNAse	-	+	-
Esculin hydrolysis	-	+	-

	Disease	8% NaCl	TCBS	Other
V. cholerae	Cholera (rice watery)	-	Yellow	String test + (0.5% Na desoxycholate)
V. alginolyticus	Wound and ear infection **LEAST PATHOGENIC** Most frequently isolated	+	Yellow	Strict halophilic (1% NaCl; can tolerate up to 10%)
***V. parahaemolyticus* (O3:K6)**	Gastroenteritis **2nd most common cause of gastroenteritis**	+	Green	Arabinose + Kanagawa + Beta-hemolytic in **Wagatsuma agar**)

V. vulnificus	Primary septicemia, wound infection Seen in blood cultures **2nd most serious type of infection**	+	Green	Lactose +

***Aeromonas* spp.** – Beta – hemolytic
- grows on Modified Cefsulodin-Irgasan-Novobiocin (CIN)

C. violaceum
- **Violacein** – ethanol soluble, _____ (Room Temp.)
- Beta-hemolytic with _____

EIKENELLA

	Eikenella corrodens	***Methylobacterium* spp.**
Normal flora of human	+	-
Spectrum of Disease and Infections		Bacteremia and peritonitis in patients undergoing chronic ambulatory peritoneal dialysis
Gram Staining	Slender, medium length gram (-) straight rod with rounded ends.	Short medium-length gram (-) bacillus **vacuolated, pale staining, may resist decolorization**
Colonial appearance and characteristic	Hallmark characteristic:	• Pink to coral pigment
Medium: BA	Improved detection: Selective media + clindamycin	• Optimal growth occurs: 15°C – 30°C • Temperature-sensitive • Chlorine-resistant
TESTS		
Catalase	-	+
Xylose-oxidizing	-	+
Indole	-	-

PASTEURELLA

- **Gram (-)**
- **Non-motile**
- **Oxidase (+)**
- **Ferments glucose**
- **Most are susceptible to penicillin**
- **Catalase (+)** exc. *P. bettyae* and *P.caballi*
- **Reduce nitrate to nitrite**

	Disease Assoc.	**Gram Stain**	**BAP**
P. multocida	• Focal soft tissue infection • Respiratory disease • Systemic disease _____ – risk factor for systemic disease	Coccobacilli; frequent **bipolar staining**	Convex, smooth, gray, **nonhemolytic**, some are rough and mucoid; some have _____
P. pneumotropica	Rare systemic infection	Short, straight bacilli	Convex, smooth nonhemolytic

P. bettyae	Genital tract-associated disease; neonatal infection	Thinner, short, straight bacilli	Convex, smooth, nonhemolytic

***Aggregaatibacter actinomycetemcomitans* – STAR-SHAPE W/ FOUR TO SIX POINTS COLONIES.**

Capnocytophaga – _____

HAEMOPHILUS

- **Gram (-) coccobacilli**
- **Pleomorphic**
- **Requires X and V factor**
- **Facultative anaerobes**

	X	V	Porphyrin	Others
H. haemolyticus	+	+	-	Beta-hemolysis in Horse BAP
H. aegypticus (Koch-Weeks Bacillus)	+	+	-	Pink-eye conjunctivitis; Brazilian Purpuric fever
H. influenzae (Pfeiffer's bacillus)	+	+	-	Virulence factor: Type B capsule, IgA protease, pili, **LPS** – has paralyzing effect on ciliated respiratory epithelium. **Major cause of epiglottitis** Otitis media, pneumonia, cellulitis.
H. parainfluenzae	-	+	+	Primary site of infection – **mitral valve**
H. parahaemolyticus	-	+	+	Beta-hemolysis on Horse BAP
H. paraphrophilus	-	+	+	
H. ducreyi	+	-	-	_____ **school of fish, grows well @ 33°C**
H. aphrophilus	-	-	+	

SPECIMEN COLLECTION

- *Haemophilus* spp. are **susceptible to DRYING and TEMP. EXTREMES.**
- **Lower RT spx: bronchoalveolar lavage**
- **Pneumonia and CSF infection – STERILE FLUID AND BLOOD**
- *H. ducreyi* **- genital ulcers**

BARTONELLA

- Negative to
  - Catalase
  - Urease
  - Nitrate reductase
  - Oxidase
- Facultatively intracellular bacterium
- Multiply and persist in the RBCs
- Angioproliferation, can inhibit endothelial cell apoptosis

Organism	Disease Assoc.
B. alsatica	Human accidental host
B. bacilliformis	Carrion's disease
B. Quintana (form. *Rochalimea Quintana*)	Bacillary angiomatosis
B. henselae	1° cause of cat-scratch disease Peliosis hepatitis
B. clarridgeiae	
B. elizabethae	Endocarditis

CAMPYLOBACTER, HELICOBACTER

- Gram (-) bacilli
- Microaerophilic (5-10% O_2)

	Campylobacter	Helicobacter
Disease Assoc.	**Most common cause of** _____ • Febrile systemic disease • Periodontal disease • Gastroenteritis **Postinfection complication:** • Reactive arthritis • **Guillain-Barre Syndrome**	***H. pylori*** • Can cause peptic ulcer disease & gastric carcinoma, gastritis • **Major cause of Type B gastritis**
Laboratory Diagnosis	Blood, feces, rectal swabs are acceptable	Tissue biopsies Placed in stuart's Refrigerated for 24 hrs.
Direct Detection	_____, **S-shaped ; DARTING MOTILITY** (Hanging drop)	Warthin-Starry or Silver stain and giemsa stain on biopsy specimen.
Media and Cultivation	• Skirrows • Medium V • Butzler Medium • Campy-CVA • Charcoal Cefoperazone Deoxycholate Agar (CCDA)	• Brucella agar w/ 5% SB • Selective Media • Skirrow's Media • Modified Thayer Martin Agar

	C. jejuni subsp. jejuni	*C. coli*	*C. fetus*	*H. pylori*
Hippurate hydrolysis	+	-	-	-
Growth in 25°C	-	-	-/+	-
Growth @ 42°C	+	+	-	+
Catalase	+	+	+	+
Urease	-	-	-	+
Nitrate to nitrite	+	+	+	+/-
H_2S in TSI	-	-	-	-
Nalidixic acid	S	S	R	R
Cephalotin	R	R	S	S

LEGIONELLA

- **Gram (-) fastidious bacilli**
- **Mesophilic (20-45°C)**
- **Obligate aerobe, motile**

Legionella pneumophila	
Disease Association	• Legionnaires Disease • Pontiac Fever • Wound abscesses, encephalitis, or endocarditis
Direct Directions	**0.1% Fuchsin** substituted for safranin in the gram stain • Tissue sections use silver or giemsa stains
Media and Cultivation	Two agar plates (atleast one BCYE)

BRUCELLA

- Poorly stained by conventional gram stain
- Resembles **fine grain of sand**
- Requires erythritol
- Urease (+), catalase (+)

Brucella	
Disease association	Brucellosis-zoonosis, systemic infection
Lab Diagnosis	Blood (routine), Bone marrow (preferred), CSF, pleural, synovial, urine, abscesses, other tissues.
Media and cultivation	• Brucella agar or infusion base-spx other than blood • 5% heated horse or rabbit serum enhances growth on all media • 5-10% CO_2 in a humidified atmosphere (BHI,TSB) • **Incubated 3 weeks** before considered negative

Species	**CO_2 required for growth**	**Time to positive in urease**	**H_2S produced**	**INHIBITION BY DYE**	
				Thionine	**Fuchsin**
B. abortus	±	2 hrs (rare 24hrs)	+	+	-
B. melitensis	-	2 hrs (rare 24hrs)	+	-	-
B. suis	-	15 mins.	±	-	+
B. canis	-	15 mins.	-	-	+

BORDETELLA

- **PERTUSSIS/ WHOOPING COUGH**
  - Usually disease of children
  - Has 3 symptomatic stages
    - **Catarrhal** - mild cold; runny nose
    - **Paroxysmal** – vomiting and with "whooping"
    - **Convalescent**

Lab Diagnosis	
Culture	• Most sensitive early in the illness • Traditional diagnostic standard for pertussis • May become undetectable by culture 2 weeks after start of paroxysms
	Nasopharyngeal aspirates or nasopharyngeal swab; Calcium alginate or Dacron **COTTON SWABS – INHIBITORY** **THROAT, SPUTUM – UNACCEPTABLE** **ANTERIOR NOSE – SITES ARE NOT LINED WITH CILIATED EPITHELIUM**
Cultivation	Regan – Lowe – with **charcoal supplemented w/ horse blood**
	Bordet-Gengou – potato fusion base
	Cephalexin
Colony Appearance	small and shiny; resembles _____; becomes whitish gray w/ age.

Characteristics	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*
Catalase	+	+	+
Oxidase	+	-	+
Motility	-	-	+
Nitrate	-	-	+
Urease	-	+ (24 hrs.)	+ (4 hrs.)
Growth Regan-Lowe agar	3-6 days	2-3 days	1-2 days
Blood agar	-	+	+
McConkey Agar	+	+\-	+

VIRULENCE FACTORS OF *Bordetella pertussis*

- **Pertussis toxin** – exotoxin; interferes signal transduction
- **Adenylate Cyclase toxin** – inhibits immune effector cells; induces supraphysiologic conc. of cAMP
- **Tracheal toxin** – causes ciliostasis; inhibits DNA synthesis; promotes cell death
- **Filamentous hemagglutinin**

FRANCISELLA

- Gram (-) coccobacilli
- Strict aerobes
- Urease (+), motility (-), oxidase (-)
- MAJOR VIRULENCE FACTOR – CAPSULE

	***Francisella* spp.**
Disease association	**Tularemia** – one of the most common lab acquired infection -Rabbit fever -Deer fly fever -Market men's disease
Lab diagnosis	BSL Level 2 Pathogen Specimen: - scrapings from infected ulcers - lymph node biopsies - sputum **Whole blood –** acceptable specimen for all types of tularemia
Direct Directions	Gram stain – little use with primary specimen. **Basic fuchsin-** used as counterstain for better staining.
Media and Cultivation	Media with **sulfhydryl compounds (cysteine, _____, thiosulfate, or IsoVitaleX) – for primary isolation**

STREPTOBACILLUS AND SPIRILLUM

	Streptobacillus moniliformis	***Spirillum minus***
General characteristics	• Requires blood, serum or ascite fluid in the medium and incubation under CO_2 • Facultative, **nonmotile anaerobe** • **Highly pleomorphic**	Gram (-), **helical**, strictly aerobic
Disease Association	Haverhill fever	Ratbite fever (SODOKU)
Lab diagnosis	Blood	Blood, exudate, or lymph node tissues
Direct Detections	Pus or exudates – stained with gram or giemsa stain	Characteristic spirochetes – using Giemsa or Wright stain / dark-field microscopy

Media and cultivation	Broth cultures – "_____"	nonculturable

NEISSERIA & MORAXELLA

- Gram (-) cocci
- Positive for
  - **Catalase**
  - **Oxidase**
  - **Superoxol (Neisseria)**
  - **Glucose fermenters** exc. For Moraxella (asaccharolytic)
- **Gonorrhea**
  - Flow of seeds
  - "Clap" – "clapoir" (French) – brothel

	N. gonorrhoeae	***N. meningitidis***
Disease Association	• Leading cause of sexually transmitted disease • always pathogenic; not a normal flora • Opthlamia neonatorum	• Leading cause of fatal bacterial meningitis • Waterhouse-friderichsen syndrome • Endemic & epidemic meningitis • Meningococcemia • Pneumonia • Purulent arthritis • Endophthalmitis
Lab diagnosis	• Specimen: **rectal, pus, throat swab, gastric washing, joint fluid** • Very sensitive to drying and extreme temp. • Swabs are acceptable • _____ – inhibits *N. gonorrhoeae*	
	• Dacron/Rayon – recommended • JEMBEC plates **ENDOCERVIX** – most common site of infection in women **URETHRA –** for men	

Media	
Thayer-Martin Agar	V+C+N
Modified thayer-martin agar	V+C+N+ Trimethoprim lactate
Martin-Lewis Agar	V + C + Anisomycin + T
New York City Agar	V + C + Amphotericin B + T
GC – LECT	Lincomycin + V + C + Amphotericin B + T

	Glucose	**Maltose**	**Lactose**	**Sucrose**	**DNAse, Nitrate, Butyrate Disk**	**Others**
N. meningitidis	+	+	-	-	-	
N. gonorrhoeae	+	-	-	-	-	
N. sicca	+	+	-	+	-	Wrinkled colony / breadcrumb
N. lactamica	+	+	+	-	-	ONPG +
M. catarrhalis	-	-	-	-	+	

	Beta-galactosidase	Gamma-glutamyl aminopeptidase	Prolyl-hydroxylprolyl aminopeptidase
N. meningitidis	-	+	-
N. gonorrhoeae	-	-	+
M. catarrhalis	-	-	-

***Neisseria gonorrhoeae* VIRULENCE FACTORS**

- **LOS** – endotoxin; major in-vivo virulence factor; protective device
- **Pili** (fimbriae) – inhibits phagocytosis
- **IgA protease** – cleaves IgA
- **Cell Membrane Proteins** – protective device for organism
  - **Protein I** (por) – channels nutrients to pass into waste products to exit cells.
  - **Protein II** (opa) – adherence to phagocyte and epithelial cells
  - **Protein III** (rmp) – blocks host IgG against organism

Specimen collection

- ***N. gonorrhoeae***
  - **Urethra** – insert 2 cm swab in anterior
  - **Rectal culture** – 4-5 cm in and canal
- ***N. meningitidis***
  - **CSF**
    - 1ml
    - 1000 x g for 10 mins
    - Cytocentrifuge (recommended)

ANAEROBIC ORGANISM

- **Gram positive SPORE-FORMING BACILLI**
  - *Clostridium perfringens*
  - *Clostridium botulinum*
  - *Clostridium tetani*
  - *Clostridium difficile*
  - *Clostridium septicum*
- **Gram positive Bacilli**
  - *Actinomyces* spp.
  - *Propionibacterium* spp.
  - *Bifidobacterium* spp.
- **Gram negative bacilli**
  - *Bacteroides fragilis*
  - *Porphyromonas* spp.
  - *Prevotella* spp.
  - *Fusobacterium* spp.
- **Gram negative cocci**
  - *Veillonella* spp.

	Virulence Factor	Disease Association	Others
Clostridium perfringens	• Alpha & Beta toxins -Type A (mild) -Type C -food poisoning (enteritis necrotans) • Enterotoxin	• _____ – myonecrosis / eating sore • **Pig-bel-necrotic enteritis**	• Encapsulated, nonmotile • _____ _____, **DOUBLE ZONE HEMOLYSIS** • **reverse CAMP +** • stormy milk formation • _____ _____
C. botulinum	**Botulinum toxin**	• flaccid paralysis • wound botulism	• Toxin used to treat **strabismus**

	• most potent toxin • neurotoxin	• infant botulism (floppy baby syndrome) • SIDS • Crib death	• Tennis racket spores • Terminal spore .
C. tetani	**Tetanospasmin** • Neurotoxin that causes spastic paralysis with continuous muscle spasm	• Tetanus • Tetanus neonatorum	• Drumstick, lollipop • Narrow zone of hemolysis • _____ _____ vs. *C. ramosum* – terminal spore but **glucose fermenter**
C. difficile	**Toxin A** • Toxic to cells of intestinal mucosa **Toxin B** (cytotoxin) • Necrosis of colonic mucosa	• Antibiotic-associated diarrhea • Pseudomembranous colitis • Associated with **CLINDAMYCIN**	• **Horse-stable, barnyard odor** • **Yellow "ground-glass" in CCFA**
C. septicum		Associated with **colorectal cancer**	• Subterminal spore • Beta-hemolytic

			• Medusa head in Anaerobic BAP

	Swarming	Motility	Glucose	Lactose	Lecithinase	Lipase	Spore Formation
C. perfringens	-	-	+	+	+	-	ST
C. botulinum	-	+	+	-	-	+	ST
C. tetani	+	+	-	-	-	-	T
C. difficile	-	+	+	-	-	-	ST
C. septicum	+	+	+	-	-	-	ST

MYCOBACTERIA

- Slender, rod-shaped (0.2- 0.6 um x 1- 10um in size)
- Nonmotile; non-spore formers
- Strictly aerobic
- Increased CO2 – enhances growth

RAPID GROWERS

- Grows in simple media
- Grows 2-3 days
- 20-40°C

DISEASE-ASSOCIATED MYCOBACTERIA

- Requires **2-6 weeks**
- Requires complex media
- Has specific optimal temp.

MTB COMPLEX

- ***M. microti* –** TB in immunocompetent and compromised
- _____ **–** TB in tropical africa
- ***M. tuberculosis***

- _____– TB in cattle and other ruminants
- ***M. canettii***

_____ – size of droplet that can transmit MTB

Decreased antigen; Increased Hypersensitivity rxn. – granuloma formation
Increased antigen and hypersensitivity rxn – tissue necrosis

MOST COMMON SITES OF SPREAD OF MTB (in-order)

- Spleen
- Liver
- Lungs
- Bone marrow
- Kidney
- Adrenal glands
- Eyes

MOST COMMON EXTRAPULMONARY SITES IN HIV PATIENTS

- Lymph nodes
- Genitourinary tract
- Abdominal cavity

Miliary TB

- Most cases is in _____
- Common form of TB in HIV-infected people

_____– skeletal TB of the spine
_____ of the deformed spine in Pott's Disease

MDR-TB – resistant to atleast **Isoniazid & rifampin (1° treatment)**
XDR-TB – resistant to **1° treatment, fluoroquinolone, atleast 1 of 3 injectable 2nd-line anti-TB drugs**

Mycobacterial Test

- _____ – primary diagnostic method
- **Chest X-ray** – used to complement bacteriologic testing
- **TB culture & DST (Ogawa & LJ)** – routine diagnostic test for DR – TB
- **Tuberculin Skin test / Mantoux Test / PPD Test** – basic screening tool for TB infection
- **Xpert MTB / RIF & Line Probe Assay** -rapid test that detects MTB and rifampicin resistance

DSSM Results & Interpretation	
IUATLD / WHO Scale	**Conventional Light Microscope**
0	No AFB seen in 300 OIO fields
+n	1 – 9 AFB / 100 OIF
1+	10 – 99 AFB / 100 OIF
2+	1 – 10 AFB/ OIF in 50 fields
3+	>10 AFB / OIF in atleast 20 fields

Xpert MTB / RIF Results & Interpretation	
T	MTB detected; rifampicin resistance not detected
RR	MTB detected; rifampicin resistance detected
TI	MTB detected; rifampicin resistance intermediate
N	MTB not detected
I	Invalid/ no result/ error

PARASITOLOGY

NEMATODES

Characteristics

- Cylindrical, elongated, & bilaterally symmetrical
- Anterior end equipped with hook, teeth, plates and papillae
- Alimentary tract is simple, extending from mouth to anus. NO CIRCULATORY SYSTEM

UNHOLY THREE
Hookworm
Ascaris lumbricoides
Trichuris trichiura
HEART-LUNG MIGRATION
Ascaris lumbricoides
Strongyloides stercoralis
Hookworm
SMALL INTESTINE
Capillaria philippinensis
Hookworm
Ascaris lumbricoides
Trichuris trichiura
Strongyloides stercoralis
LARGE INTESTINE
Enterobius vermicularis
Trichuris trichiura

Ascaris lumbricoides

- Largest intestinal worm
- Anterior end has **3 lips** and **triangular buccal cavity** with sensory papillae
    - INFECTIVE STAGE – **EMBRYONATED EGGS**
    - MODE OF TRANSMISSION – **INGESTION**
    - DIAGNOSIS – **(+) EGG IN THE FECES**

PATHOLOGY & MANIFESTATION

- "worm ball" / bolus formation in heavy infection
- Ascaris pneumonitis
- Eosinophilia
- Abdominal pain
- Loeffler's syndrome

VECTORS

- *Periplaneta Americana*
- *Blatella germanica*

TREATMENT

- Benzimidazole
- Pyrantel pamoate

Trichuris trichiura

- Whipworm; holomyrian
- Anterior resembles "_____"
- Adult worm inhabits the cecum and colon
    - INFECTIVE STAGE – **EMBRYONATED EGG (lemon/football shaped)** "_____"
    - MODE OF TRANSMISSION – **INGESTION**

PATHOLOGY & CLINICAL MANIFESTATION

- Petechial hemorrhage – may predispose amebic dysentery ulcers and invasion of *E. histolytica*

- Cause anemia
- Rectal prolapse
- Adult worm produces pore-forming protein caplled TT47

TREATMENT
- Mebendazole
- Albendazole

HOOKWORMS: *Necator americanus & Ancylostoma duodenale*

- INFECTIVE STAGE – **FILARIFORM LARVAE**
- MODE OF TRANSMISSION – _____
  - Egg – **resembles** _____

PATHOLOGY AND CLINICAL PRESENTATION
- Mazza mora, ground itch, dew itch, water sore
- Wakana Disease
- Iron Deficiency Anemia
- Hypoalbuminemia

	A. Duodenale	*N. americanus*
Position of the head	Anterior head continuous in the same curve as the body	Anterior and strongly reflexed dorsally
Buccal cavity	2 pairs of teeth	1 pair semilunar cutting plates
Copulatory bursa	Large tripartite	Small, tripartite
Copulatory spicules	2 hair-like spicules	Spicules fuse at tip into a barb
Vulva	Posterior half of the body	Anterior half of the body
Cervical Curvature	C-shaped	S-shaped
Remarks	"_____" • Percutaneous & fecal oral route with **transmammary transmission**	"_____" • Purely percutaneous • Predominant in Philippines

Animal Hookworms
- ***A. braziliense* & *A. caninum* –** causes "creeping eruption" or cutaneous larva migrans (CLM)
- ***A. ceylanicum* –** first case was recorded in Ilocus Norte in 1968

Diagnosis
- **Harada-Mori culture method**
- **PCR based**
- **ELISA**

TREATMENT
- Albendazole
- Iron supplement & adequate diet

Strongyloides stercoralis

- Threadworm
- Free-living
- Capable of parthenogenesis
  - INFECTIVE STAGE – **FILARIFORM LARVAE**
  - MODE OF TRANSMISSION – **SKIN / MUCOSAL PENETRATION**

PATHOLOGY & CLINICAL MANIFESTATION
- Cochin-china diarrhea / Vietnamese diarrhea
- Autoinfection → hyperinfection

DIAGNOSIS

- Baermann funnel gauze method
- Harada-Mori Culture
- Beale's String test
- Duodenal aspiration

TREATMENT

- Ivermectin

Enterobius vermicularis

- Pinworm; seatworm; society/social worm
- Adult worm: anterior end with lateral wings or **cephalic alae**
- Egg: flattened on one side: **D-shaped**; **Italian bread egg;** embryonated after **6 hrs.**
  - INFECTIVE STAGE – **EMBRYONATED EGG**
  - MODE OF TRANSMISSION – **INGESTION/INHALATION**

PATHOLOGY & CLINICAL MANIFESTATION

- Pruritus ani
- Oxyuriasis
- Insomnia
- Extraintestinal enterobiasis
- External autoinfection

DIAGNOSIS

- Graham's scotch adhesive tape swab (perianal cellulose tape swab)

TREATMENT

- Mebendazole
- Albendazole
- Pyrantel Pamoate

Capillaria philippinensis

- Pudoc worm
- Mystery worm
- Capable of _____ and _____

CHARACTERISTICS

- NATURAL HOST – _____
- PEANUT – SHAPED w/ striated shells and flattened bipolar plugs
  - INFECTIVE STAGE – **INFECTIVE LARVAE**
  - MODE OF TRANSMISSION – **INGESTION OF INFECTED FISH WITH LARVAE**
  - INTERMEDIATE HOST – **FRESHWATER FISH / BRACKISH WATER FISH: BAGSIT**

PATHOLOGY & CLINICAL MANIFESTATION

- Malabsorption syndrome
- Borborygmus and abdominal pain

DIAGNOSIS

- Direct smear/ wet mount / stool concentration technique
- ELISA (coproantigens)
- Duodenal Aspiration

Blood & Tissue Nematodes

SUBCUTANEOUS

- ***Loa loa***
- ***Mansonella streptocerca***
- ***Onchocerca volvulus***

SEROUS CAVITY

- ***Mansonella* spp.**

LYMPHATIC

- ***Wuchereria bancrofti***
- ***Brugia malayi***
- ***Brugia timori***

	W. bancrofti	***B. malayi***
Mean length (um)	290	222

Cephalic space: breadth	1:1	2:1
Sheath in giemsa	Unstained	Pink
Nuclei	Regularly spaced; separate	Irregularly spaced; overlapping
Tail	Single row of nuclei; does not reach tail end	Single row of nuclei reaches the tail
Terminal nuclei	NONE	2 nuclei; bulge at cuticle
Appearance in blood film	Smoothly curved	Kinky

Filarial worm	Periodicity	Diagnostic test	Interediate host	Specimen	Microfilariae
W. bancrofti	Nocturnal (8 pm – 4 am)	Microfilariae	*Culex, Aedes, Anopheles*	Blood	Sheathed; absent nuclei at tail
B. malayi	Nocturnal	Microfilariae	*Anopheles, Mansonia*	Blood	Sheathed, 2 separate nuclei at tail
Loa loa		Microfilarie	Chrysops fly, tabanid or mango fly	Blood	Sheathed, nuclei continuous up to the tip of the tail.
O. volvulus	None	Adult worm in excised tissue	Simulium (black fly)		NO SHEATH

Parastrongylus cantonensis

- Rat lungworm
- _____– spiral arrangement of uterine tubules
    - DEFINITIVE HOST – **RATS**
    - INTERMEDIATE HOSTS IN MOLLUSC – **1ST LARVA STAGE**
        - INTERMEDIATE HOST – **SLUGS & SNAILS**
        - MODE OF TRANSMISSION – Ingestion or penetration
    - INTERMEDIATE HOST IN HUMANS – **3RD STAGE LARVA**
    - MODE OF TRANSMISSION (HUMANS)
        - Ingestion of raw mollusk
        - Ingestion of contaminated food
        - Ingestion of paratenic host
        - Drinking contaminated water

DIAGNOSIS

- DOT-BLOT ELISA
- Immuno-PCR detection

TREATMENT

- Surgical removal
- Prednisone

Trichinella spiralis

- INFECTIVE STAGE: **ENCYSTED LARVA IN STRIATED MUSCLE**
- MODE OF TRANSMISSION: **INGESTION OF UNDERCOOKED OR RAW MEAT**

PATHOLOGY & CLINICAL MANIFESTATION

- **Stages**
    - **ENTERIC** – invasion of intestine and incubation
    - **INVASION** – larval migration and intestinal invasion
    - **CONVALESCENT** – encystment and encapsulation

DIAGNOSIS

- Muscle Biopsy
- ELISA
- Latex Agglutination
- Bachmann Intradermal Test

TREATMENT

- Mebendazole

- Albendazole

PREVENTION AND CONTROL

- Cook meat at minimum of 77°C
- FREEZING
  - -15°C for 20 days
  - – 30°C for 6 days

Anisakis

- Parasite of marine animals
  - INFECTIVE STAGE – **3RD STAGE LARVA**
  - MODE OF TRANSMISSION – **INGESTION OF UNDERCOOKED OR RAW SQUID OR FISH**

DIAGNOSIS

- Gastroscopic / endoscopic exam
- ELISA
- RAST

TREATMENT

- Mechanically remove larva using endoscopic forceps

CONTROL & PREVENTION

- Freezing

Dracunculus medinensis

- Longest nematode of man
- "guinea worm", "_____"
  - INFECTIVE STAGE – **INFECTIVE LARVAE**
  - MODE OF TRANSMISSION – **INGESTION OF CONTAMINATED CRUSTACEANS**
  - INTERMEDIATE HOST – **AQUATIC CRUSTACEANS (COPEPODS / CYCLOPS)**

Toxocara canis & Toxocara cati

- Clinical forms of Toxocariasis
  - **Visceral Larva Migrans (VLM)** -
  - **Ocular Larva Migrans**
  - **Covert Toxocariasis**

DIAGNOSIS

- Tissue biopsy
- IgG ELISA
- Western blot
- PCR

TREATMENT

- Albendazole
- Mebendazole w/ anti-inflammatory drugs

CESTODES

- Tapeworm; flat and ribbon-like
- Hermaphrodite; lack digestive organs

Body

- Scolex (head)
- Neck (region of growth)
- Proglottids (strobila)

Taenia spp.

	Taenia solium	***Taenia saginata***
Common name	Pork Tapeworm	Beef tapeworm
Intermediate host	Pig; man	Cattle
Scolex	w/ rostellum armed with 2 rows of large & small hooklets	No rostellar hooks 4 prominent acetabula
Length		<25 meters
No. of proglottids	8000-10,000	1000-4000
Gravid proglottids	Finger-like (dendritic) 7-13 lateral branches (less active)	Tree-like (dichotomous) _____ Genitals: irregularly alternate

	w/ accessory ovarian lobe; w/o vaginal sphincter	
Infective stage	_____ Infected meat: "measly pork"	
Eggs	Indistinguishable: spherical, striated inside with oncosphere and 6 hooklets	

CLINICAL MANIFESTATION (*T. solium*)

- Intestinal infection
- Cysticercosis
- Neurocysticercosis
  - Parenchymal
  - Extraparenchymal

DIAGNOSIS

- CAT
- CSF-ELISA
- Electroimmuno transfer blot
- Western blot
- DOT ELISA

TREATMENT

- Praziquantel
- Niclosamide

Taenia asiatica

- _____
- Misidentified as *T. saginata*
- INFECTIVE STAGE – **CYSTICERCUS VISCEROTROPICA**

Hyemenolepis

	H. nana	***H. diminuta***
Common Name	"dwarf tapeworm"	"rat tapeworm"
Length	25-45 mm	60 cm
Scolex	4 cup-shaped suckers with rostellum & Y-shaped hooklets	Rudimentary unarmed rostellum
Egg	Spherical/subspherical with thin outer layer and thick inner layer w/ **bipolar thinking & 4 hairlike polar filament**	w/ bipolar thickening; absent bipolar filaments Hooklets: fan-like arrangement
Infective stage	Direct: eggs Indirect: Cysticercoid larvae	Cysticercoid larvae
Remarks	ONLY human tapeworm which can complete its entire cycle in 1 host	Requires intermediate host

Diphyllobothrium latum

- Fish tapeworm; broad tapeworm
- INFECTIVE STAGE – _____
-
- Scolex – **2 bothria**
- Proglottids – 4000
- Egg – with inspicuous operculum

CLINICAL MANIFESTATION & PATHOGENESIS

- _____
- w/ thrombocytopenia and leukopenia

DIAGNOSIS

- Finding eggs and proglottids in stool

TREATMENT

- Praziquantel

Echinococcus spp.

- Scolex – pyriform w/ 4 acetabula; armed
- Proglottids: 3 (immature, short neck, & 1 gravid proglottid)
- INFECTIVE STAGE **–** egg
- LARVAL STAGE – _____ w/ protolices inside

CLINICAL MANIFESTATION AND PATHOGENESIS

- Human cystic echinococcus

DIAGNOSIS

- Radiographic findings / ultrasonography
- Positive serologic tests
  - Indirect hemagglutination
  - Indirect fluorescent antibody (IFA)
  - Enzyme immunoassay
- **GOLD STANDARD** – IgE detection hydatid cyst fluid derived native or recombinant antigen B through ELISA or immunoblot

TREATMENT

- Surgical resection
- Albendazole; mebendazole
- Percutaneous aspiration, injection, re-aspiration

Diphylidium caninum

- Dog tapeworm; double-pored dog tapeworm
- Mature and gravid proglottid "pumpkin seed" shaped
- Infective stage – cysticercoid larvae

TREMATODES

- Known as flukes
- Requires **2 intermediate hosts** exc. Schistosomes
  - 1st – snail
  - 2nd :

FISH	H. heterophyes, C. sinensis O. felineus
	P. westermani
PLANT	F. hepatica F. gigantica F. buski
SNAIL	E. ilocanum

BLOOD FLUKES

- *S. japonicum* – _____**; oriental blood fluke;** _____ **swimmer's itch; snail fever**
- *S. mansoni* – _____**;** smallest blood fluke
- *S. haematobium* – _____**; bladder fluke; bilharziasis; urinary schistosomiasis**
- *S. mekongi*
- *S. intercalatum*

GENERAL DIAGNOSIS

- Presence of ova
- Liver or rectal biopsies
- **Faust and Meleney's Egg hatching technique**
- **Circumoval Precipitin Test (COP) of Oliver & Gonzales**

LUNG FLUKES

Paragonimus westermani

- Oriental lung fluke
- Disease: LUNG FLUKE DISEASE; ENDEMIC HEMOPTYSIS
- Egg resembles **coffee bean**

- Specimen: **stool or sputum**

INTESTINAL FLUKES

Fasciolopsis buski

- Giant intestinal fluke
- Largest fluke parasitizing human
- _____; resembles eggs of Fasciola

Echinostoma ilocanum

- Garrison's fluke
- Intermediate hosts are SNAILS

Heterophyes heterophyes

- Von Siebod's fluke
- Smallest fluke but deadliest fluke of man
- With _____ genital suckers

LIVER FLUKES

Fasciola hepatica

- Sheep liver fluke

Fasciola gigantica

- Giant Liver Fluke
- Infects cattle in Philippines

Clonorchis sinensis

- _____
- Most important liver fluke
- Egg resembles _____

PROTOZOANS

Protozoa	Transmission	Morphology	Clinical Findings	Diagnosis
E. histolytica	Fecal-oral	• Oocyst • Troph: bullseye shaped nucleus; RBC in cytoplasm	Asymptomatic carrier Bloody diarrhea Liver abscess	Fecal smear Serology CT Scan
G. lamblia	Fecal-oral	• Oocyst • Flagellated trophozoite	**Foul-smelling, greasy diarrhea**	Fecal smear Immunoassay
***Isospora* spp.**	Fecal-oral	Oocyst	Severe diarrhea and malabsorption in AIDS patient	Fecal exam Biopsy Eosinophilia
C. cayetanensis	Oocyst from stool	Oocyst	Watery diarrhea Nausea and vomiting	Oocyst fluoresce under UV light
Cryptosporidium	Fecal-oral	Oocyst	Watery diarrhea	Fecal exam Biopsy in small intestine

			Abdominal pain & vomiting	
T. vaginalis	Sexually-transmitted	Trophozoite ONLY	Vaginal itching Burning on urination Yelloe-green, frothy vaginal discharge	Urinalysis Vaginal discharge examination
N. fowleri	Lives in freshwater lake	Amoeba	Acute meningitis	CSF examination
***Acanthamoeba* spp.**	Lives in freshwater lake Eye infection from dirty contact lenses	Amoeba and cyst stage in brain	Chronic granulomato-us brain abscess Corneal infection	CSF; brain tissue; corneal scrapping exam
T. gondii	Ingestion of oocyst in raw pork Inhalation of oocyst	Oocyst (infectious) Trophozoite	Congenital Dissemination infection	Serology CT Scan

	Congenital			
***Leishmania* spp.**	**Sandfly bite** Blood transfusion Zoonotic	• Promastigote • Amastigote	Cutaneous leishmaniasis _____	Blood smear Biopsy of skin, spleen or liver
African Trypanosomes: • ***T. rhodesiense*** • ***T. gambiense***	**Tsetse fly** Blood transfusion	• Motile trypomastigote • Epimastigote	African sleeping sickness	Trypomastigote in blood, spinal fluid and lymph fluid Serology
T. cruzi	**Kissing Bug** Blood transfusion	• Trypomastigote • Amastigote • Epimastigote		Trypomastigote in blood _____