

Warning Signs: A Practical Guide to Clinical Microbiology for the Infection Preventionist

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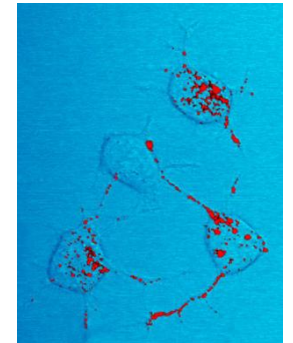
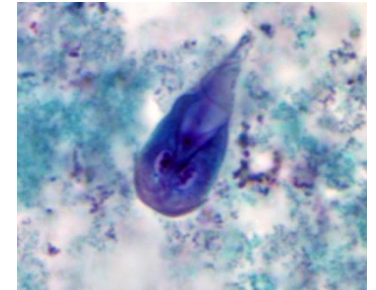
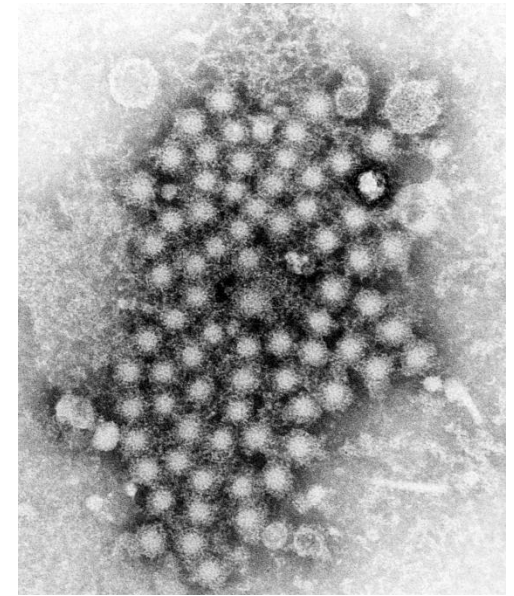
317-921-5894

Objectives

- To review categories of clinically-relevant microorganisms and their common reservoirs.
- To emphasize important points to consider when interpreting a microbiology report.
- To highlight microbiological characteristics that should trigger an epidemiological or infection control response.

Clinical Microbiology Overview

- Bacteria
- Viruses
- Fungi
- Parasites
- Prions*



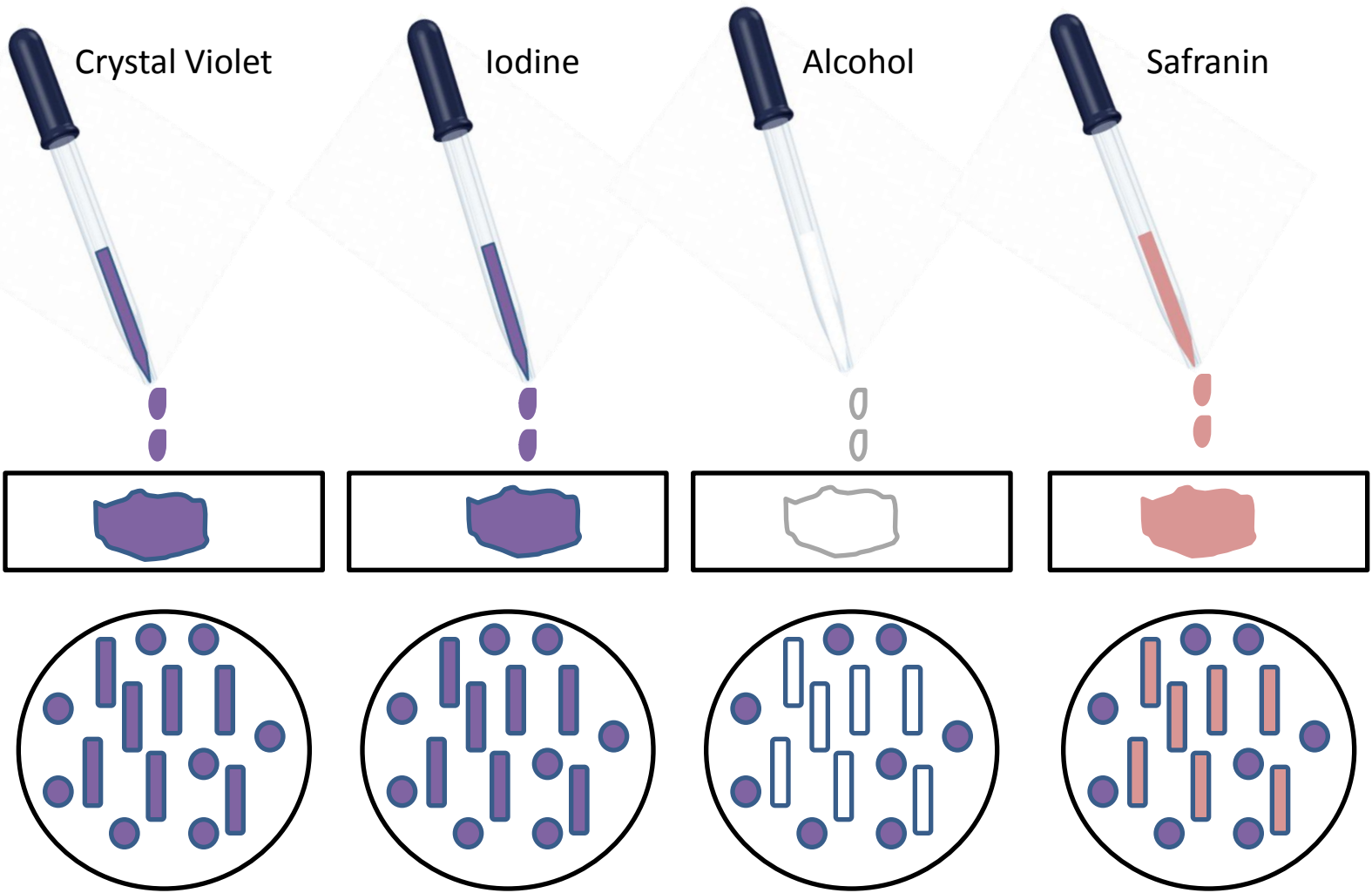


Bacteriology

Bacterial Identification Techniques: Culture

- Growth media are broths (liquid) or agar (solid or semisolid) that provide the appropriate nutrients for a microbe to grow
- Optimal incubation parameters are required for some organisms to grow (i.e. fastidious)
 - Temperature
 - Nutrients
 - Environment (oxygen, carbon dioxide, etc.)
 - Time

Gram Stain



Step 1 & 2: Crystal Violet and Iodine enters bacterial cells, staining them purple
Purple = gram positive
Pink = gram negative

Step 3: Alcohol wash rinses stain away from cells with low peptidoglycan content (destain)

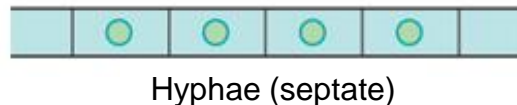
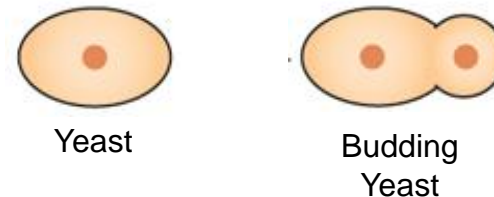
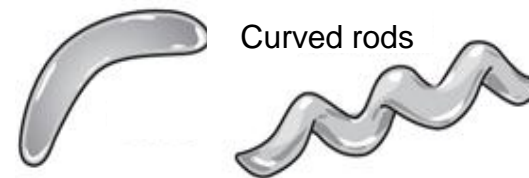
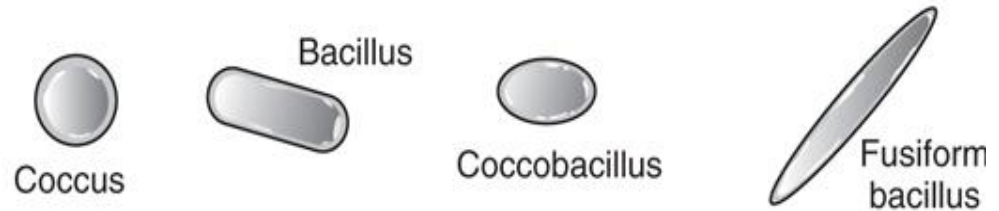
Step 4: Safranin counter stain turns cells decolorized in Step 2 pink



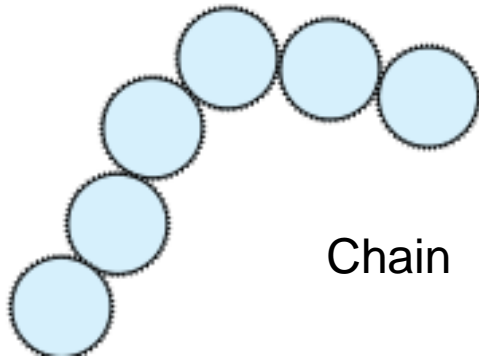
Gram Stain Morphology: Shape

- Rods
- Cocci
- Coccobacillus
- Curved Rod
- Fusiform bacillus

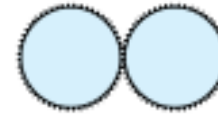
- Yeast
- Hyphae or Pseudohyphae



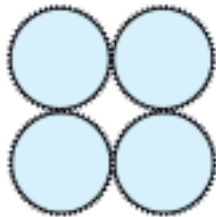
Gram Stain Morphology: Shape & Arrangement of GPCs



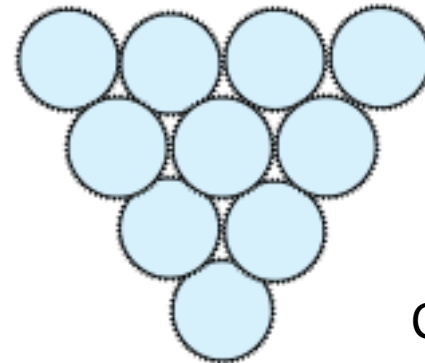
Chain



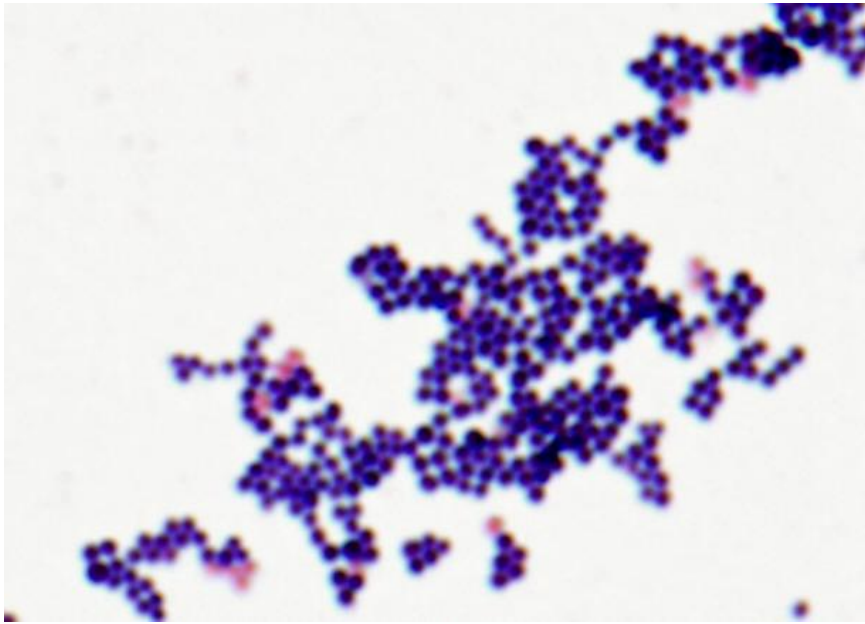
Pair



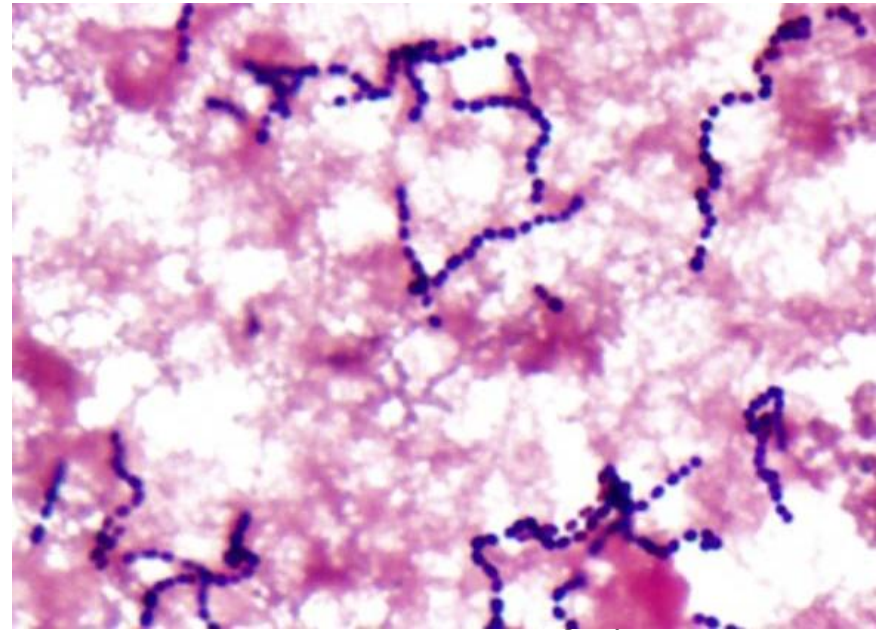
Tetrad



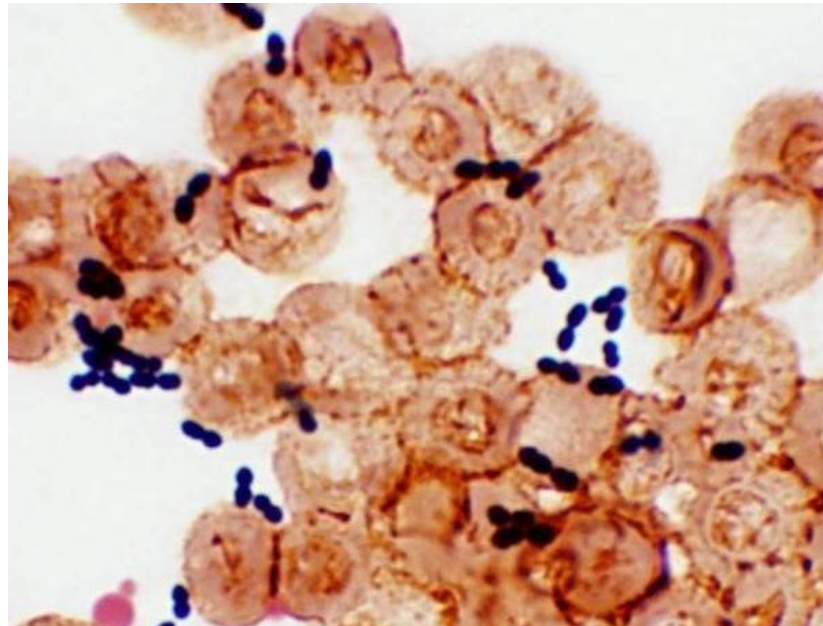
Cluster



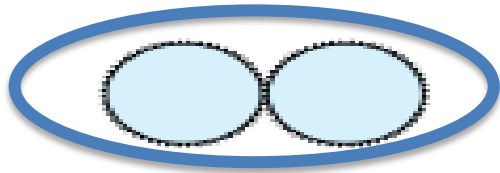
Staphylococcus - clusters



Streptococcus – pairs & chains



Enterococcus- pairs

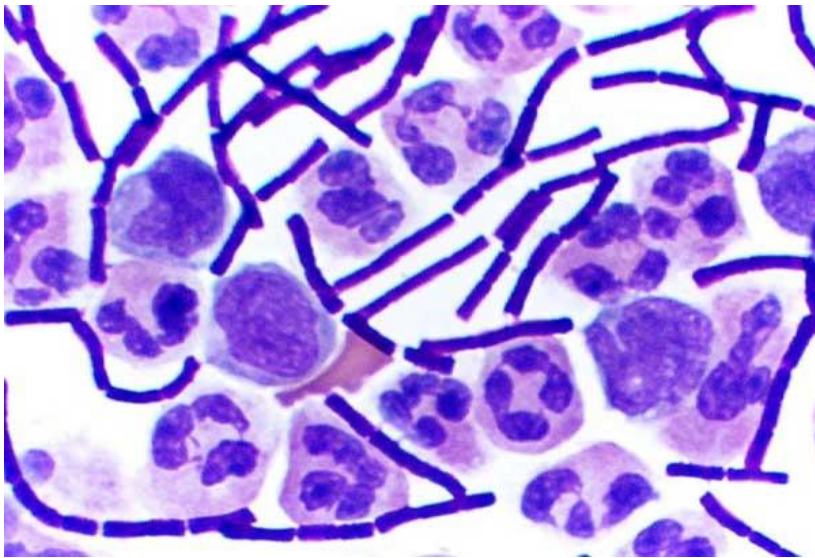


GPC in pairs with capsule

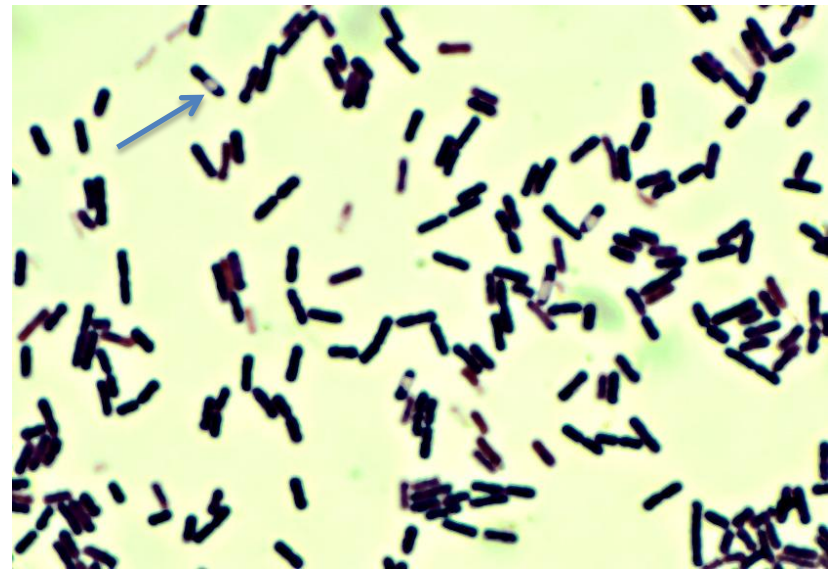


ASM MicrobeLibrary.org © Tomalty

Streptococcus pneumoniae – cocci in capsule

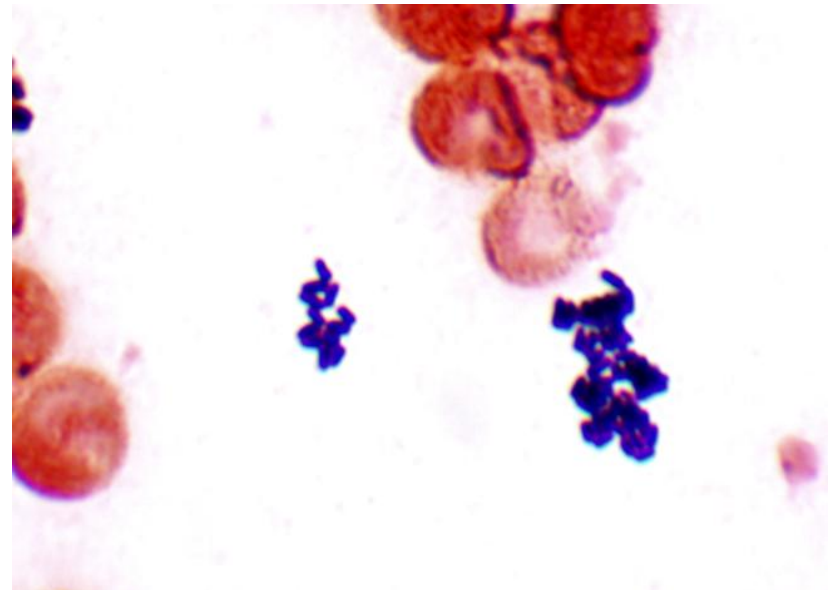


Chains, or 'end-to-end'



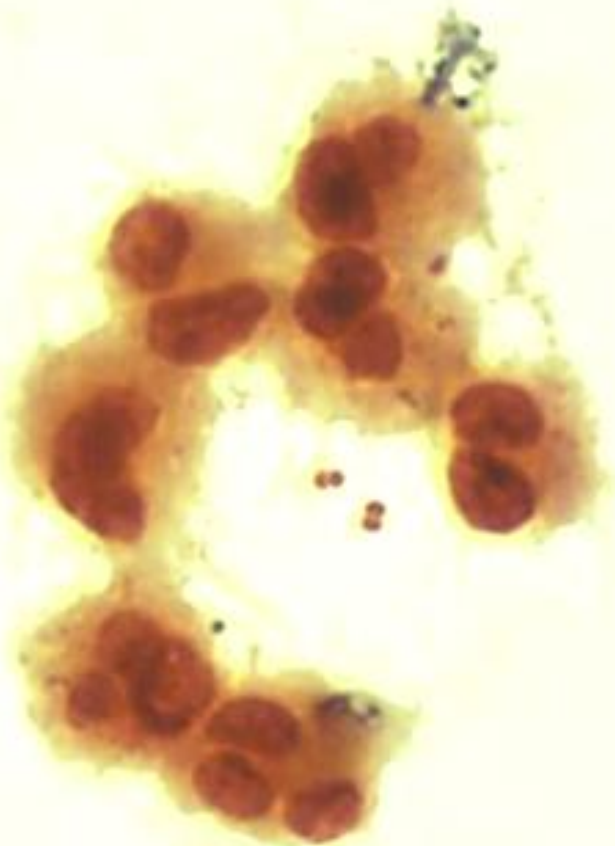
Spore-forming

Gram Stain
Morphology: Shape &
Arrangement of Gram
Positive Rods (GPRs)

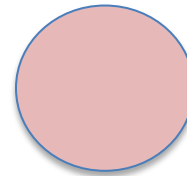


Coryneform

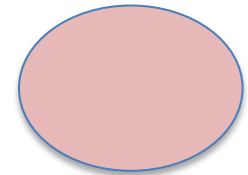
Gram Stain Morphology: Shape and Arrangement of Gram Negative Cocci and Coccobacilli



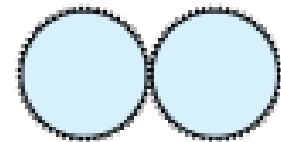
Diplococcus



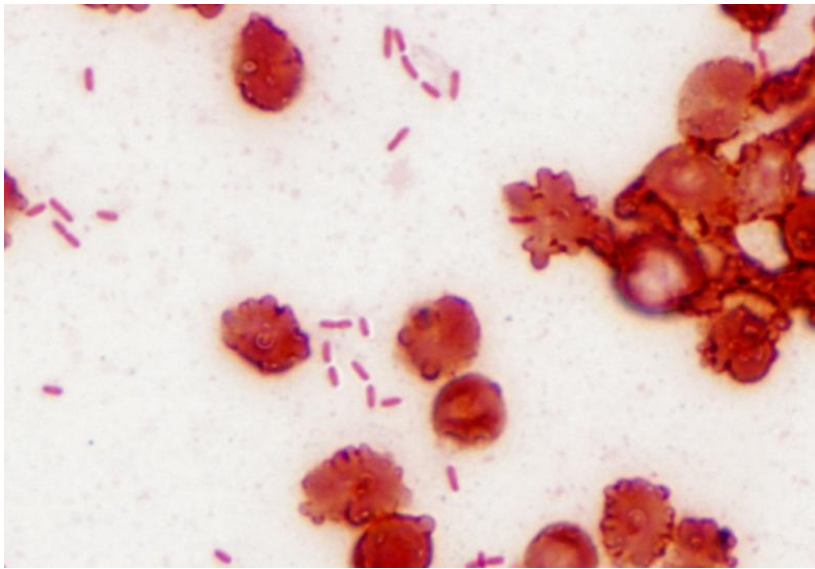
Cocci



Coccobacillus



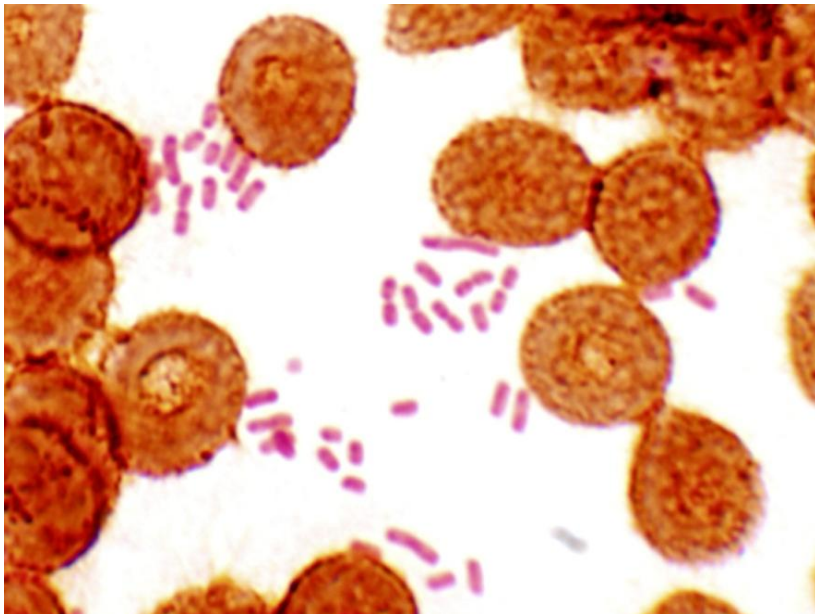
Pair =
'diplococci'
GNDC



Long, Thin GNR
(ex. *Pseudomonas*, *Stenotrophomonas*)



Fusiform
(ex. *Fusobacterium*, *Capnocytophaga*)

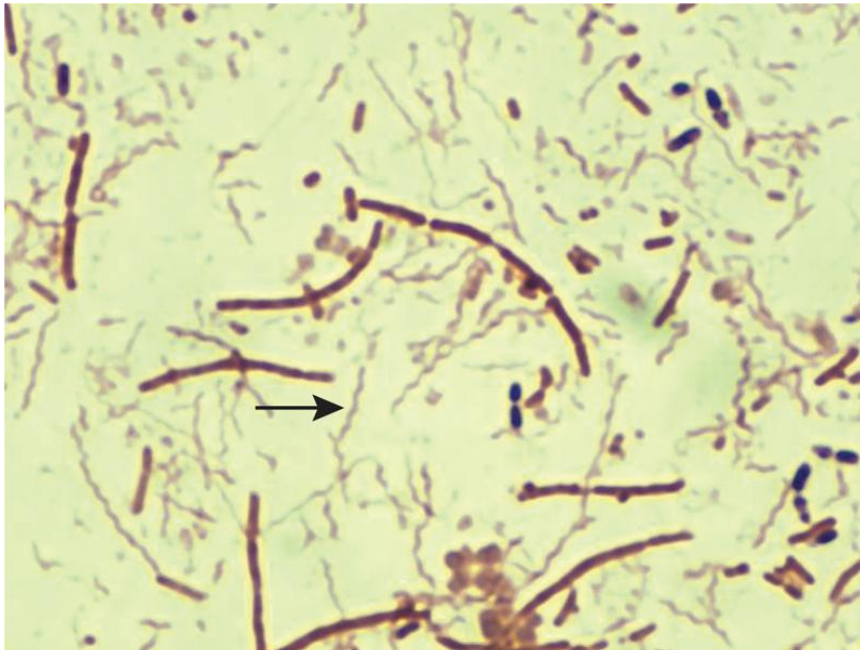
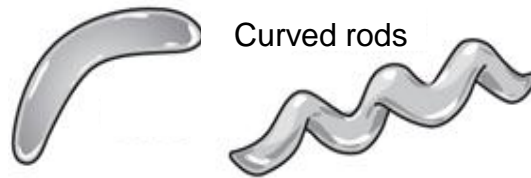


Bipolar Staining
(ex. *Enterobacteriaceae*)

Gram Stain Morphology: Shape & Arrangement of Gram Negative Rods (GNRs)

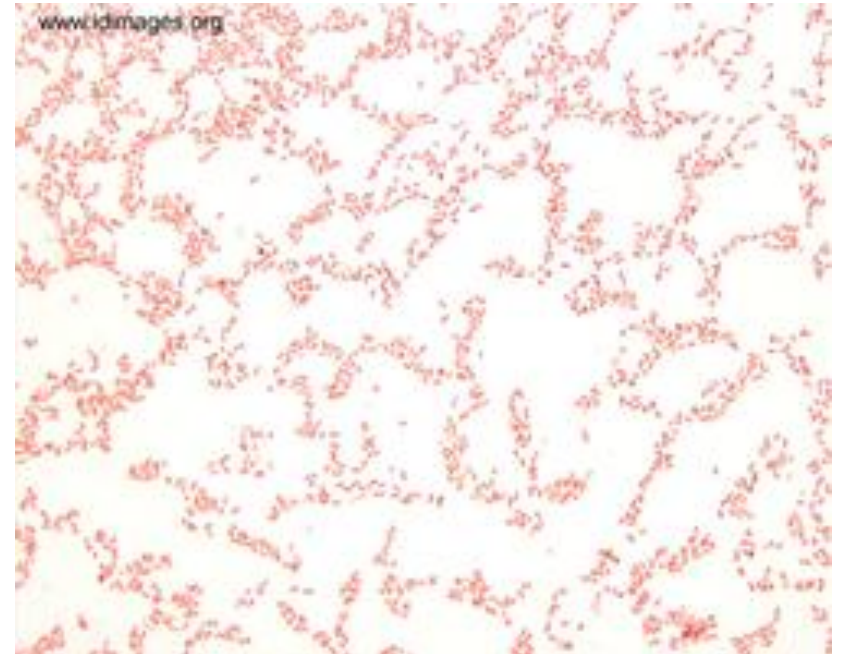
Curved Gram Negative Rods

Gram Stain Morphology: Shape & Arrangement of Curved Gram Negative Rods



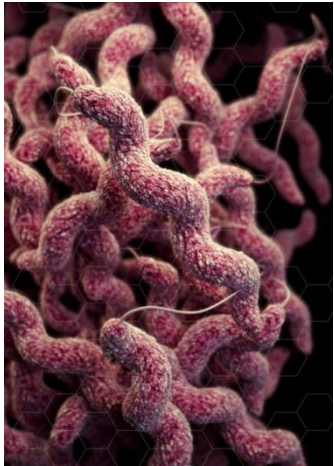
Murray et al: Medical Microbiology, 7e
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ex. *Campylobacter*



ex. *Vibrio*

Bacteria of Greatest Concern Epidemiologically



GPCs

S. aureus
S. pneumoniae
Group A Strep
Group B Strep

GNRs

Acinetobacter sp.
Bordetella pertussis
Brucella sp.
Campylobacter sp.
E. Coli O157
*Enterobacteriaceae**
STEC
Francisella tularensis
Haemophilus influenzae (invasive)
Legionella sp.
Pseudomonas aeruginosa
Salmonella sp.
Shigella sp.
Vibrio
Yersinia sp.

GPRs

Bacillus anthracis
Clostridium botulinum
Clostridium difficile
Clostridium tetani
Cornebacterium diphtheriae
Listeria monocytogenes
Mycobacterium tuberculosis

Gram Negative Cocci

Neisseria meningitidis

Non-Culturable

Coxiella burnetii
Bartonella sp.

Important Clinical Bacteria by Gram Stain

GPCs

Staphylococcus
Streptococcus
Enterococcus
Abiotrophia
Granulicatella
Micrococcus
Rothia
Leuconostoc
Aerococcus

Gram Negative

Cocci

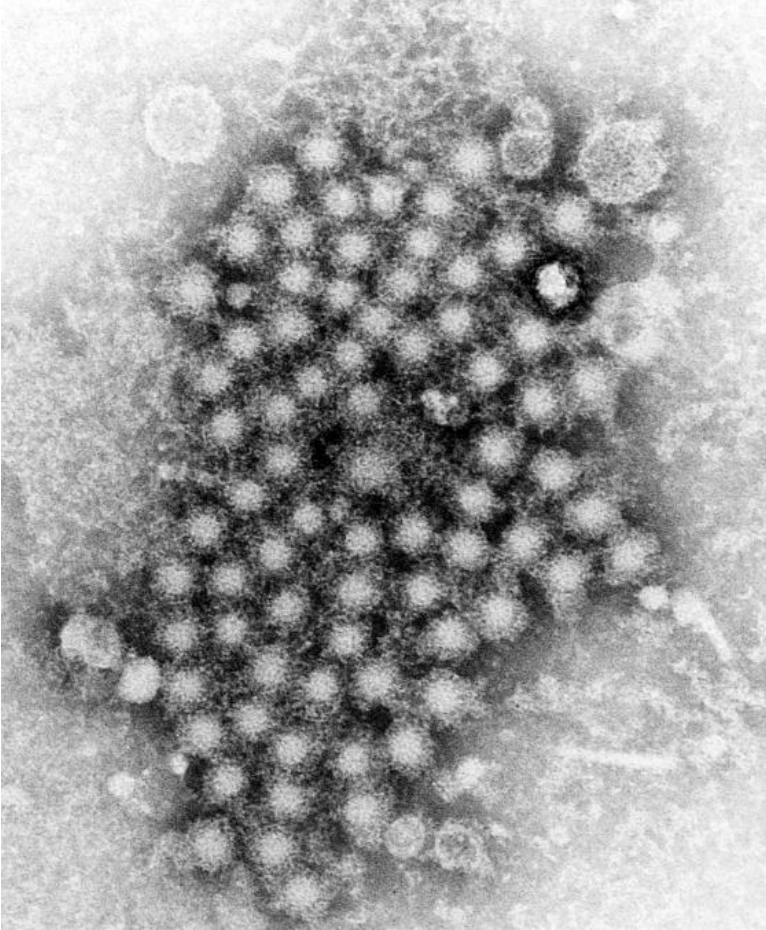
Moraxella
Neisseria
*Acinetobacter**
*Haemophilus**

GNRs

<i>E. coli</i>	<i>Morganella</i>
<i>Klebsiella</i>	<i>Providencia</i>
<i>Serratia</i>	<i>Proteus</i>
<i>Enterobacter</i>	<i>B. fragilis</i>
<i>Citrobacter</i>	<i>Campylobacter</i>
<i>Salmonella</i>	<i>Helicobacter</i>
<i>Shigella</i>	<i>Vibrio</i>
<i>Pseudomonas</i>	<i>Aeromonas</i>
<i>Acinetobacter</i> *	<i>Brucella</i>
<i>Haemophilus</i> *	<i>Pasturella</i>
<i>Capnocytophaga</i>	<i>Cardiobacterium</i>
<i>Kingella</i>	
<i>Legionella</i>	
<i>Eikenella</i>	
<i>Yersinia</i>	

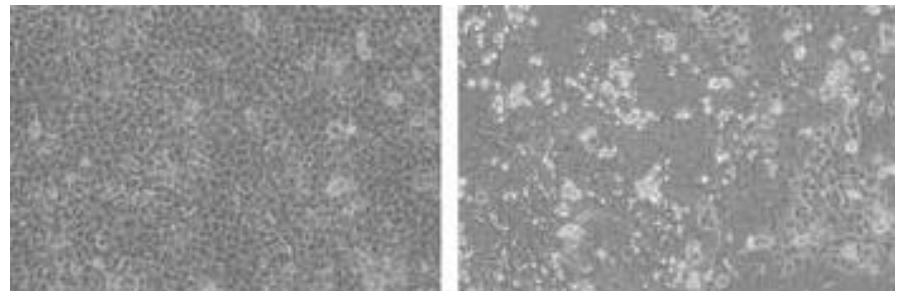
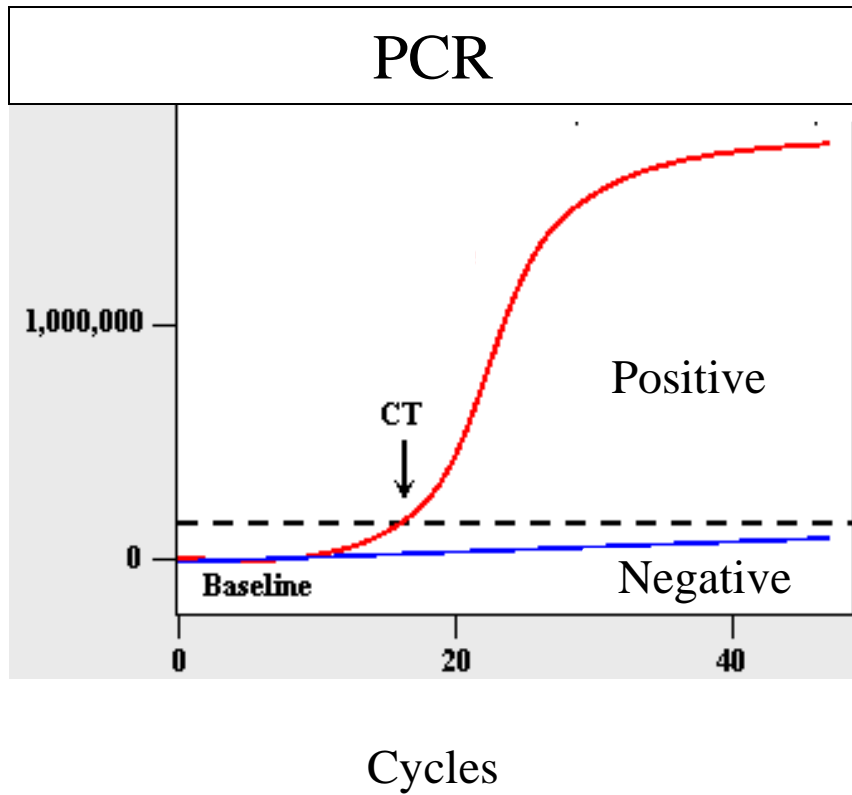
GPRs

Bacillus
Clostridium
Lactobacillus
Corynebacterium
Listeria
Propionibacterium
*Mycobacterium**
*Nocardia**
Erisipelothrix
Arcanobacterium
Actinomyces



Virology

Viral Diagnostics



Uninfected

Infected

Viruses by Primary Source of Isolation

Diarrhea

Astrovirus
Norovirus
Sapovirus
Rotavirus

Respiratory

Rhinovirus
Coronavirus
Parainfluenza (I-IV)
Respiratory syncytial virus (RSV)
Influenza (A/B)
Adenovirus *
Metapneumonvirus
Enteroviruses*
MERS-CoV

Transplant

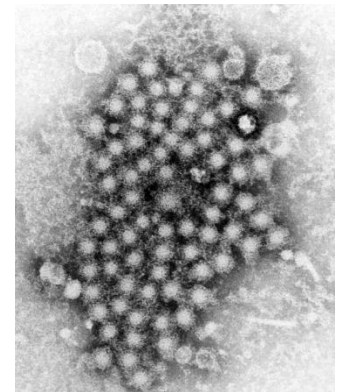
Cytomegalovirus
(CMV)
HSV 6
HSV 8
JC Virus
BK Virus
Epstein-Barr Virus
(EBV)*

Vaccine-Preventable

Measles
Mumps
Rubella
Poliovirus
VZV
Hepatitis A
Hepatitis B

Chronic

HIV
HTLV
Hepatitis C
Hepatitis Delta Virus
Human Papillomavirus (HPV)
Herpes Simplex Virus 1/2



Viruses 2

Zoonotic/Arboviruses

Rabies

Hantavirus

Lymphocytic choriomeningitis virus (LCMV)

Herpes B virus Yellow fever

Encephalitis Viruses (many)

Chikungunya

Dengue

Many others

Other

Parvovirus

Viruses of Immediate Concern

Vaccinia virus (smallpox vaccine)

Variola virus (smallpox)

Monkeypox

SARS

Avian Influenza (H5 or H7)^Z

Ebolavirus^Z

Crimean-Congo^Z

Eastern Equine Encephalitis (EEE)^Z

Lassa fever virus^Z

Lujo virus^Z

Marburg Virus^Z

1918 Influenza Virus*

S. American Hemorrhagic Fever
viruses^Z

Kyasanur Forest disease virus^Z

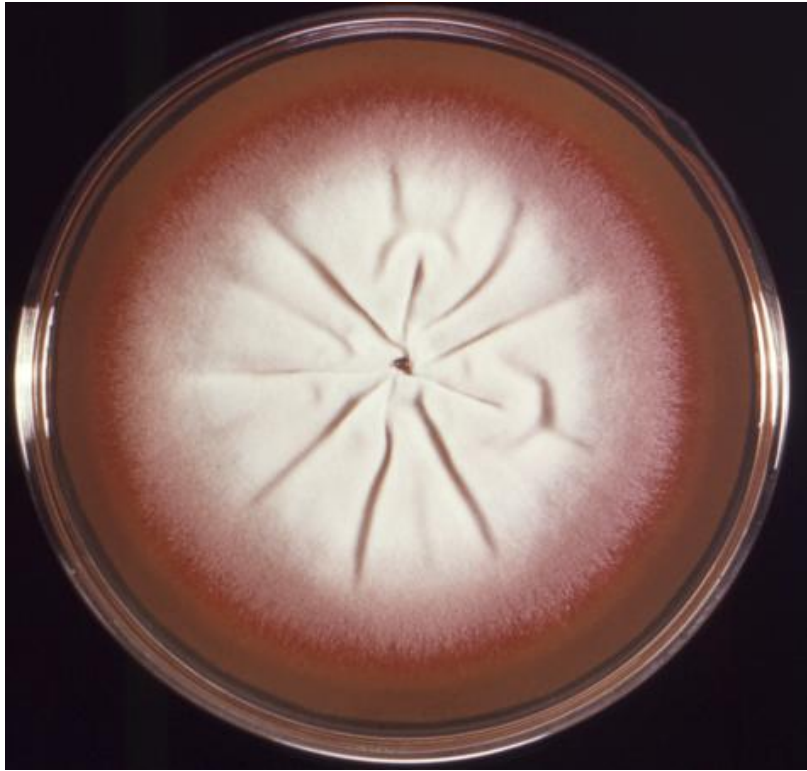
Omsk hemorrhagic fever virus^Z

Hendra virus^Z

Nipah virus^Z

Rift Valley fever virus^Z

Venezuelan equine encephalitis virus^Z



Fungi

Fungal Wet Mount

1st Clinical Decision-Making Point = Fungal Wet Mount

Is it...

Budding yeast??

OR

Hyphae??



Budding Yeast

With Pseudohyphae:

Candida albicans
C. tropicalis
Trichosporon



Without Pseudohyphae:

Candida glabrata
Cryptococcus
Malassezia

Or

Dimorphic Fungi

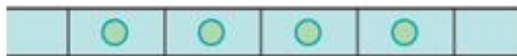


Hyphae

With Septa:

Aspergillus
Fusarium
Alternaria
Exserohilium

(Hyaline Molds,
Dematiaceous Molds,
Dimorphic Fungi)

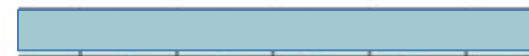


Hyphae (septate)

Without Septa:

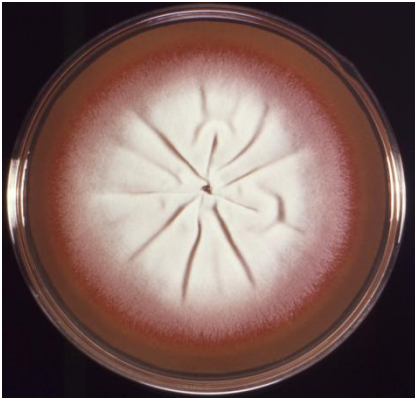
Mucor
Rhizopus
Rhizomucor
Apophysomyces

(Mucorales)



Hyphae (non-septate)

Fungal Overview



Hyaline Molds

Aspergillus
Penicillium
Fusarium
Scedosporium
Many Others

Yeasts

Candida
Cryptococcus
Trichosporon
Malassezia

Dimorphic Fungi

Sporothrix
Histoplasma
Blastomyces
Coccidioides
Paracoccidioides
Penicillium marneffeii

Dermatophytes

Trichophyton
Microsporum
Epidermophyton

Dematiaceous Molds

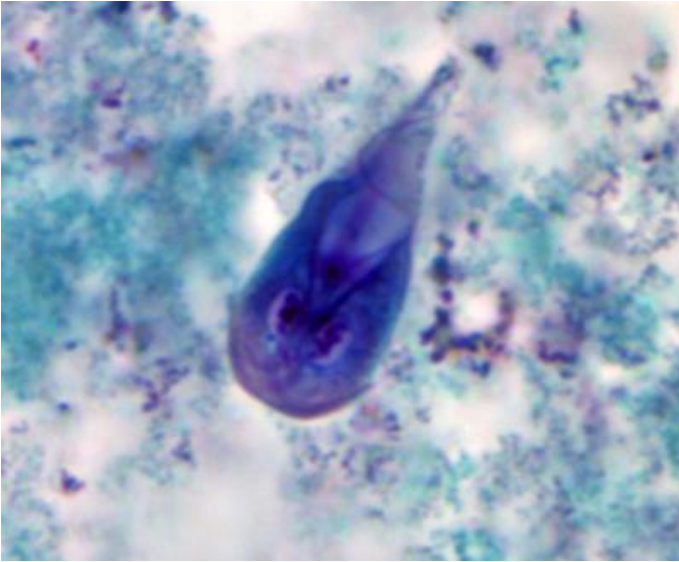
Alternaria
Exserohilium
Others

Other

Pneumocystis

Mucorales

Rhizopus
Mucor
Rhizomucor
Apophysomyces



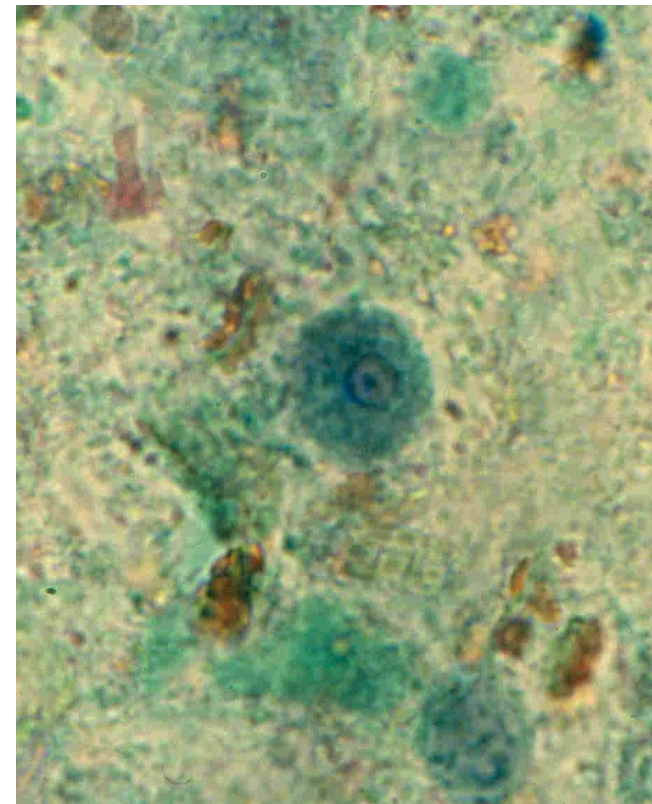
Parasitology

Parasitology Techniques

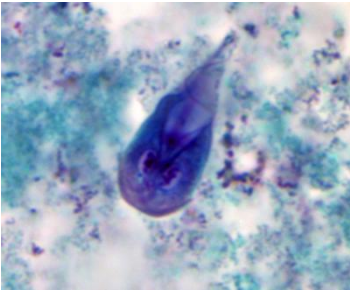
Stool Culture



Microscopy



Entamoeba histolytica, Trichrome stain



Parasitology Overview

Blood

Plasmodium
Babesia
Leishmania
Trypanosoma
Microfilaria

Lungs

Paragonimus

Muscle

Trichinella

Urogenital

Trichomonas

Bone Marrow

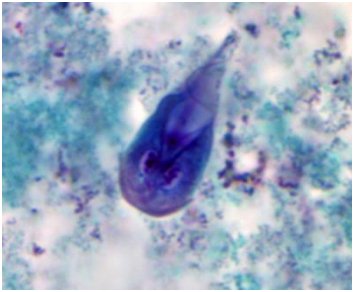
*Leishmania**
*Plasmodium**
*Trypanosoma**

Liver/Spleen

Echinococcus

Brain/CSF

Taenia solium
Echinococcus
Naegleria
Acanthamoeba
Balamuthia
Toxoplasma
Microsporidia



Stool Parasitology Overview

Entamoeba histolytica

Blastocystis hominis

Giardia lamblia

Dientamoeba fragilis

Cryptosporidium

Cyclospora cayentanensis

Cystoisospora belli

Enterobius vermicularis

Hookworm

Strongyloides

Taenia

Trichuris tricurua

Sarcocystis

Ascaris lumbricoides

Diphyllobothrium latum

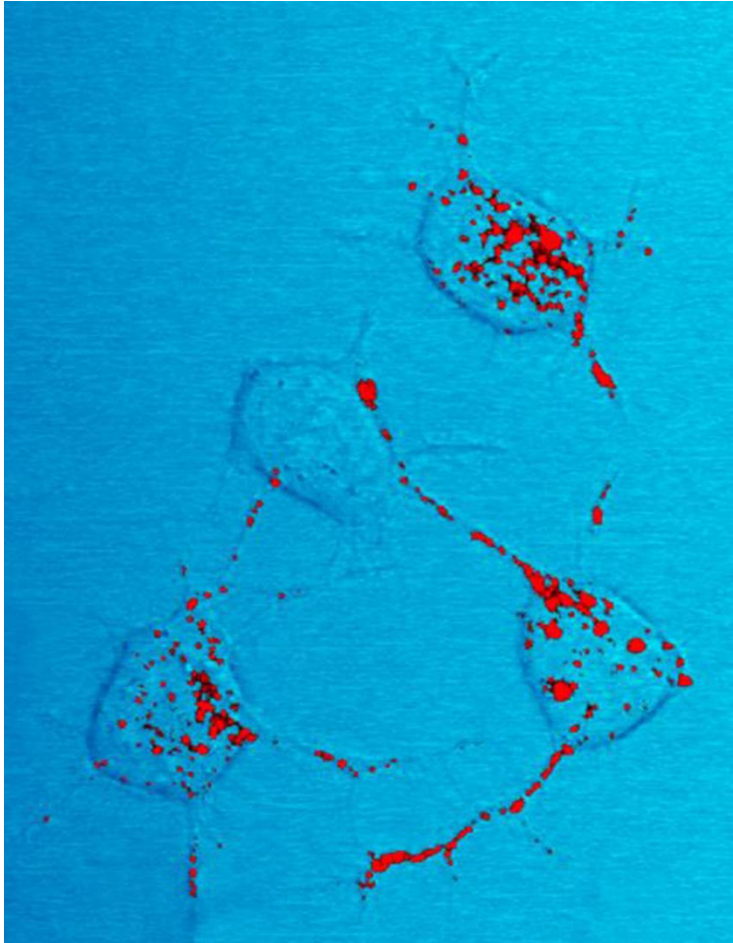
Clonorchis sinensis

Paragonimus

Schistosoma

Fasciolopsis

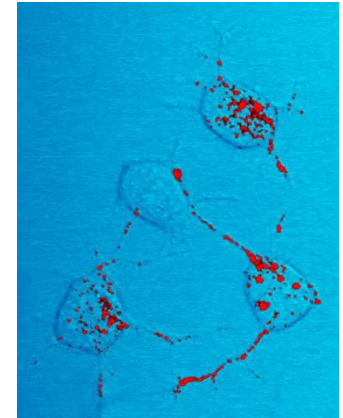
*Enterobius**



Prions

phil.cdc.gov

- Transmissible spongiform encephalopathies
- Rare, progressive neurodegenerative disorders that affect both humans and animals
- Prion disease is caused by the accumulation of misfolded proteins



Human Prion Diseases:

Creutzfeldt-Jakob Disease (CJD)

Variant CJD (vCJD)

Fatal Familial Insomnia

Kuru

Animal Prion Diseases:

Bovine Spongiform
Encephalopathy (BSE)

Chronic Wasting Disease (CWD)

Scrapie

Important points to consider when interpreting a microbiology report.

A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)^a

Ellen Jo Baron,^{1,2} J. Michael Miller,³ Melvin P. Weinstein,⁴ Sandra S. Richter,⁵ Peter H. Gilligan,⁶ Richard B. Thomson Jr.,⁷ Paul Bourbeau,⁸ Karen C. Carroll,⁹ Sue C. Kehl,¹⁰ W. Michael Dunne,¹¹ Barbara Robinson-Dunn,¹² Joseph D. Schwartzman,¹³ Kimberle C. Chapin,¹⁴ James W. Snyder,¹⁵ Betty A. Forbes,¹⁶ Robin Patel,¹⁷ Jon E. Rosenblatt,¹⁷ and Bobbi S. Pritt¹⁷

Microbiology Pearl #1

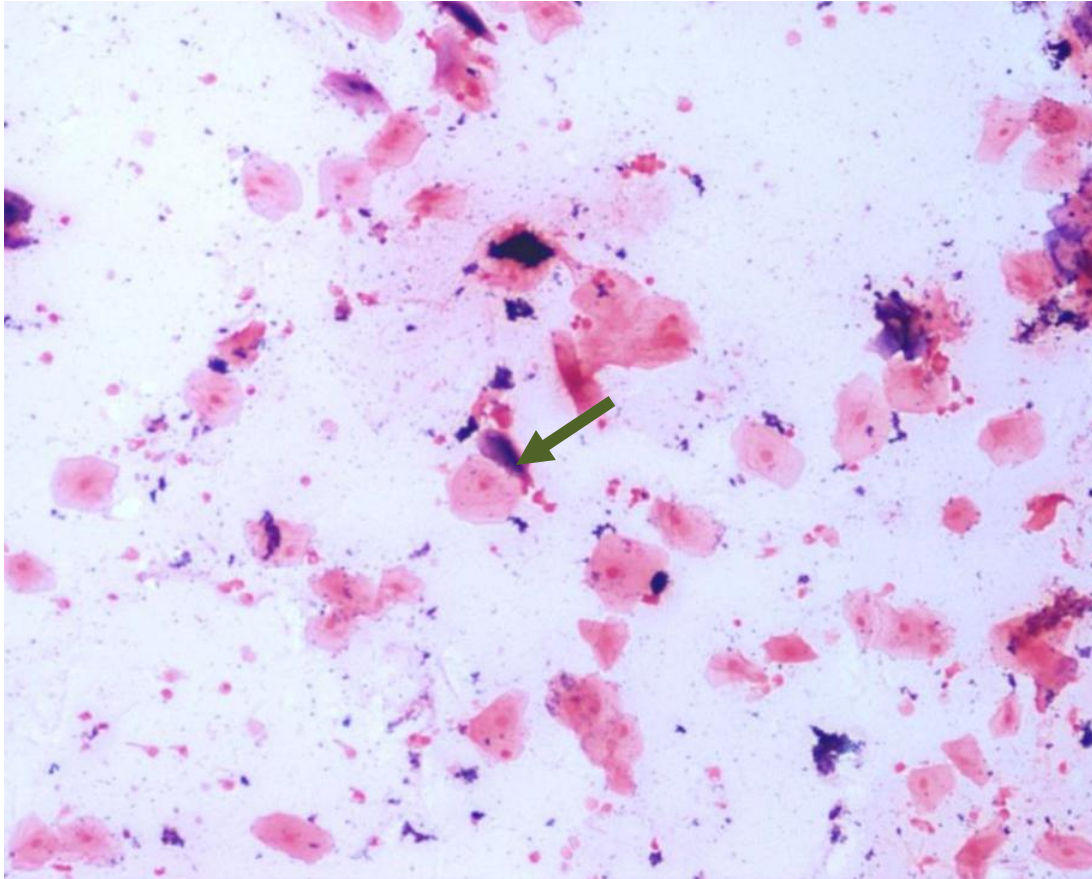
A test is only as good as the specimen submitted.

Microbiology Standard

For Labs that perform direct Gram stains:

“The laboratory has protocols in place to use Gram stain results to provide a preliminary identification of organisms, evaluate specimen quality when appropriate, and to guide work-up of cultures.”

Respiratory Specimens



- Assessment of specimen quality using low-powered magnification
- Generally this is based on the ratio of PMNs (neutrophils) to epithelial cells

Read: >10 SEC/LPF or “Many squamous epithelial cells”

Respiratory Specimens

- If a respiratory specimen is considered “good quality”
 - Generally up to 3 “potential pathogens” are worked up.
 - Workup may be guided by the gram stain result.
- Respiratory gram stains report only predominant morphologies above the background of normal oral flora.
 - Moderate mixed flora
 - Many Gram positive diplococci.
 - Few Gram negative rods.
 - Many Gram positive cocci in clusters.

Microbiology Pearl #2

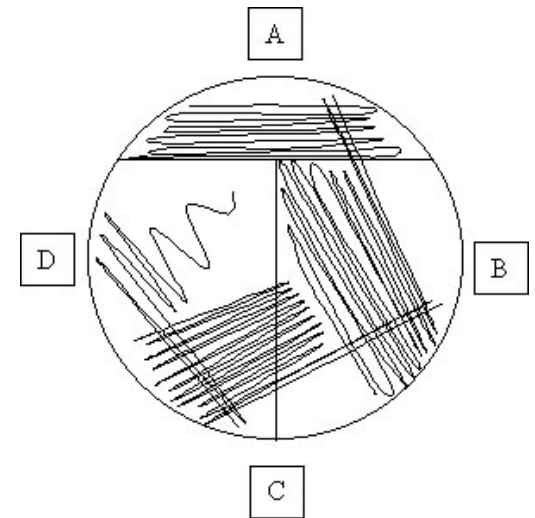
The Laboratory report will not list everything that grows. Specimen source dictates workup.

Microbiology Standard

With the exception of a quantitative culture (ex. Urine) labs that perform culture will report a qualitative assessment of the number of colonies growing for a particular organism.

Example

- **1 COLONY**
- **SCANT** [2 colonies]
- **LIGHT** [3-10 colonies]
- **MODERATE** [>10 colonies in the 1st and 2nd streak quadrants]
- **HEAVY** [>10 colonies into the 3rd and 4th streak quadrants]



Example #1: Physician requests gram stain and culture on a patient suspected of having bacterial pneumonia.

- **Gram Stain:** Many neutrophils, many gram negative rods.
- **Culture grows:** moderate *Pseudomonas aeruginosa*, moderate *E. coli*, moderate *Proteus* sp., few Diptheroids
- **Workup:** *P. aeruginosa*, *E. coli*, *Proteus* sp.

Example #2: Physician requests gram stain and culture on a patient suspected of having bacterial pneumonia.

- **Gram Stain:** Many neutrophils, few epithelial cells, many GPCs in clusters, few GNRs, many mixed oral flora.
- **Culture grows:** moderate coagulase-negative *Staphylococcus*, moderate Diptheroids, few *E. coli*, rare *Staphylococcus aureus*
- **Workup:** *E. coli*, *S. aureus*

Case:

A central venous catheter tip is received in the microbiology laboratory for culture. It is plated to blood agar plate using the roll plate method. The catheter tip culture is growing a total of 4 colonies.

Two sets of blood cultures collected within 24 hours of the catheter tip culture were received in the laboratory; these are negative.

How should the lab proceed with work up of the catheter tip culture?

- A. Identify the organism and perform susceptibility testing.
- B. Perform susceptibility testing if the organism identified is *Staphylococcus hominis*.
- C. Perform susceptibility testing if the organism identified is *Candida albicans*.
- D. Report that the catheter tip culture is clinically insignificant.

Microbiology Pearl #3

Be specific about the body site and type of wounds or fluids.

Wounds

In the case of wounds, the specimen of choice is a biopsy of the advancing margin of the lesion. A swab of a wound is not as optimal for diagnostic purposes.

Burns

Human Bites

Animal Bites

Trauma-Associated

Surgical Site

Interventional Radiology/Drain Device Associated

Microbiology Pearl #4

“A strong, ongoing partnership between microbiology laboratory professionals and infection preventionists should remain a top priority in all infection prevention programs to ensure maximum patient safety and positive patient outcomes.”

- The Infection Preventionist's Guide to the Lab

Bacterial Resistance

GNRs

MDR *Acinetobacter baumannii* complex

*Enterobacteriaceae**

1.) ESBL

2.) CRE

3.) CP-CRE: KPC, NDM-1

MDR *Pseudomonas aeruginosa*

Staphylococcus aureus

MRSA

ViSA

VRSA

Enterococcus faecium or

Enterococcus faecalis

VRE

GPRs

Mycobacterium tuberculosis

(MDR, XDR)

Appendix A. Suggestions for Confirmation of Resistant (R), Intermediate (I), or Nonsusceptible (NS) Antimicrobial Susceptibility Test Results and Organism Identification

Organism or Organism Group	Resistance Phenotype Detected ^a	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results ^a		
		Category I	Category II	Category III
		Not reported or only rarely reported to date	Uncommon in most institutions	May be common, but is generally considered of epidemiological concern
		Action Steps:		
		<ul style="list-style-type: none"> • Confirm ID and susceptibility.^a • Report to infection control. • Send to public health laboratory. • Save isolate. <p><i>NOTE: May be appropriate to notify infection control of preliminary findings before confirmation of results.</i></p>	<ul style="list-style-type: none"> • Confirm ID and susceptibility if uncommon in your institution.^a • Check with infection control in your facility to determine if special reporting procedures or further action are needed. • Check with your local public health department to determine which isolates should be reported to them and when isolates should be sent to the public health laboratory. 	<ul style="list-style-type: none"> • Confirm ID and susceptibility if uncommon in your institution.^a • Check with infection control in your facility to determine if special reporting procedures or further action are needed.
Any <i>Enterobacteriaceae</i>	Carbapenem – I or R ^b		x	
	Amikacin, gentamicin, and tobramycin – R			x
<i>Escherichia coli</i> <i>Klebsiella</i> spp. <i>Proteus mirabilis</i>	Extended-spectrum cephalosporin ^c – I or R			x
<i>Salmonella</i> and <i>Shigella</i> spp. ^d	Cephalosporin III – I or R		x	
	Fluoroquinolone – I or R		x	
<i>Acinetobacter baumannii</i>	Colistin/polymyxin – R		x	
	Carbapenem – I or R			x
<i>Pseudomonas aeruginosa</i>	Colistin/polymyxin – I or R		x	
	Amikacin, gentamicin, and tobramycin – R Carbapenem – I or R			x



FEDERAL SELECT AGENT PROGRAM



HOME

REGULATIONS

SELECT AGENTS AND TOXINS

RESOURCES

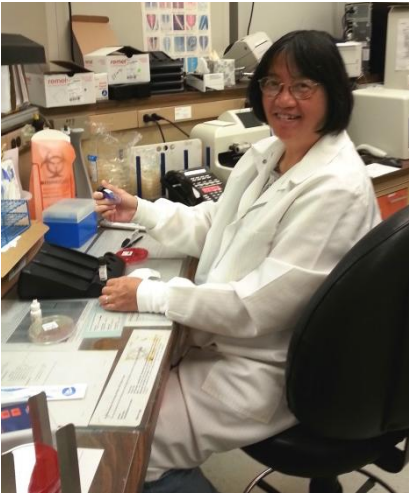
FORMS

SELECT AGENTS AND TOXINS LIST

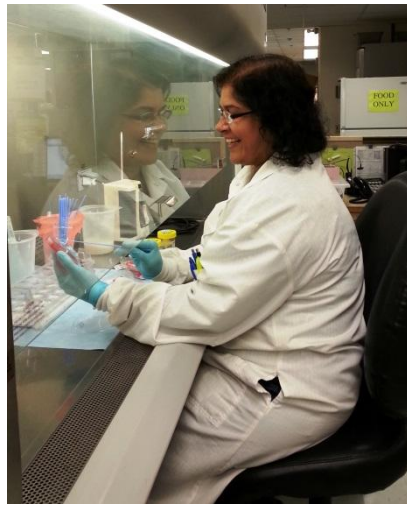
The following biological agents and toxins have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the Select Agent Regulations. Here is a list of [excluded agents and toxins](#).

HHS and USDA Select Agents and Toxins
7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73





BSL-1



BSL-2

BSL-3



BSL-4



Classify the Microorganisms by Risk Group and Biosafety Level

- | | |
|-------------------------------|-------|
| A. HSV | BSL-1 |
| B. E. coli K12 | |
| C. Candida albicans | BSL-2 |
| D. Ebola virus | |
| E. Brucella abortus | BSL-3 |
| F. Salmonella | |
| G. Mycobacterium tuberculosis | BSL-4 |
| H. Marburg virus | |
| I. Coccidioides immitis | |

Answers

- **BSL-1:**
 - *E. coli* K12
- **BSL-2:**
 - *Candida albicans*
 - HSV
 - *Salmonella*
- **BSL-3:**
 - *Brucella abortus*
 - *Mycobacterium tuberculosis*
 - *Coccidioides immitis*
- **BSL-4:**
 - Marburg Virus
 - Ebola Virus

**IN CASE OF
EMERGENCY**

Call **THE
MICRO LAB**

When to always call the laboratory...

... Gram Stains

Specimen ...	You Read	Your concern ...
CSF	GNDCs	<i>Neisseria meningitidis</i>
	GPRs	<i>Bacillus anthracis</i> <i>Listeria</i>
	GNRs	<i>Haemophilus influenzae</i>
Blood Culture	GPR with spores	<i>Bacillus anthracis</i> (and others)

... Culture Results

Source	You Read ...	The Concern...
ANY	Bacillus anthracis	Anthrax
	Yersinia pestis	The Plague
	Burkholderia pseudomallei	
	Burkholderia mallei	
	Franciscella tularensis	Rabbit Fever
	Brucella sp.	Brucellosis
	Corynebacterium diphtheriae	Diphtheria
	Listeria	
Wound	Clostridium perfringens	
Sputum	Mycobacterium tuberculosis	TB
Vaginal	β -hemolytic Strep	Group B Strep (pregnancy)

.... Ordered Tests

Source	You Read ...
ANY	Rule out for Agent of Bioterrorism
	Parvovirus B19
	VZV PCR or Serology
	Measles PCR or Culture

What is the Role of the Microbiology Laboratory?

1. Clarify the presence of infection. Clinical presentation *must* guide the microbiological workup and all results *must* be interpreted clinically!
2. Specify the etiology of infection.
3. Motivate the appropriate selection of antimicrobial agents
4. Assist in identification, control, and prevention of nosocomial or public health infections

**WHEN IN
DOUBT**

Call **THE
MICRO LAB**