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Overview of Microbiology

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James D. Dick, PhD

- Director, Bacteriology Section, Division of Medical Microbiology
- Associate Professor of Pathology, Molecular Microbiology and Immunology
- Researches biochemical and molecular techniques for the detection and identification of bacterial pathogens and antibiotic resistance mechanisms

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Section A

Overview of Microbiology

Classification of Infectious Disease

- Clinical
  - Major clinical manifestation
- Epidemiological
  - Transmission/reservoir
- Microbiological
  - Causative agent

Microbiological Classification of Infectious Disease

- Viral
- Bacterial
- Fungal
- Parasitic
- Prions

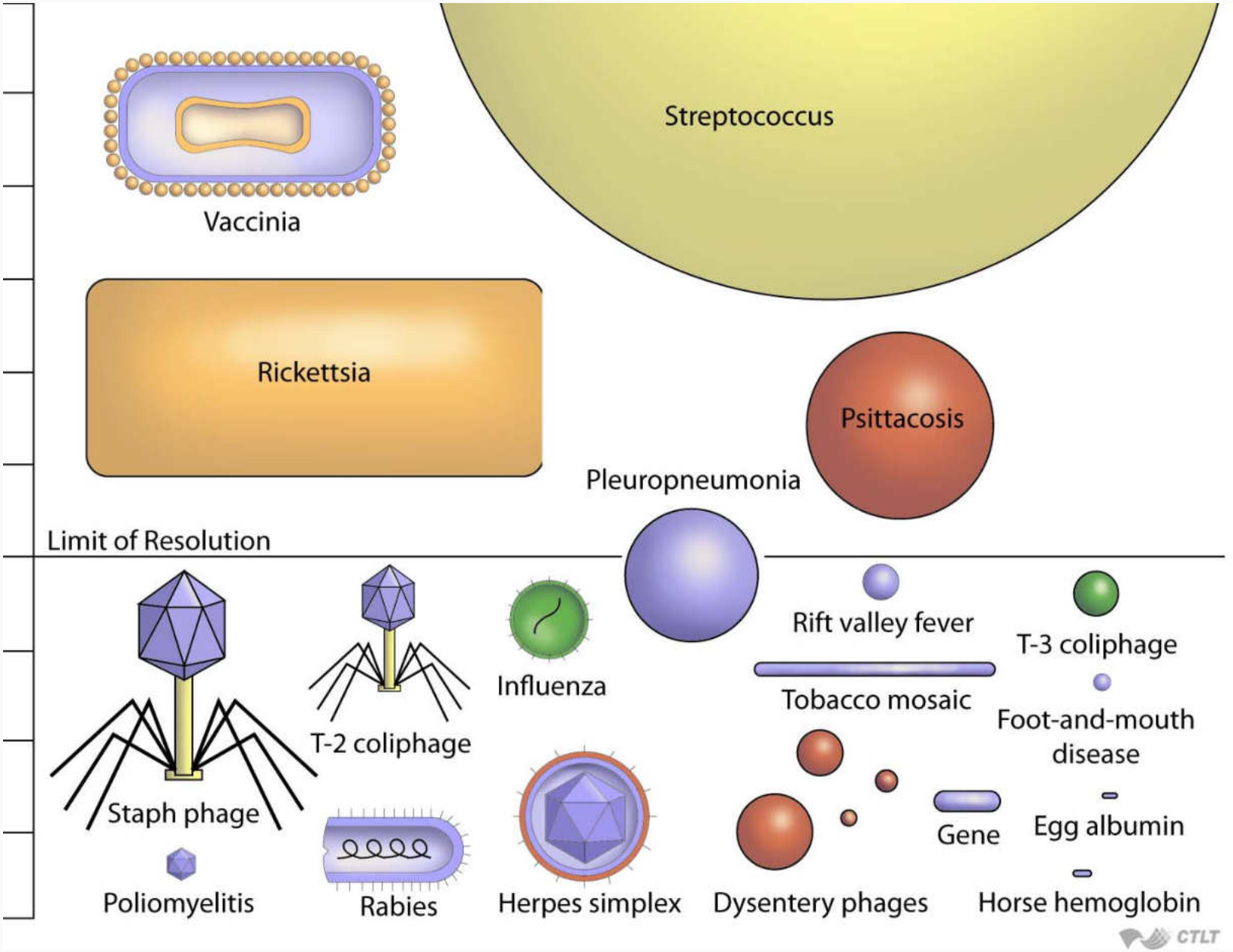
Diagnosis of Infectious Diseases

- Infection versus colonization
- Disease versus exposure
- Prevalence versus incidence

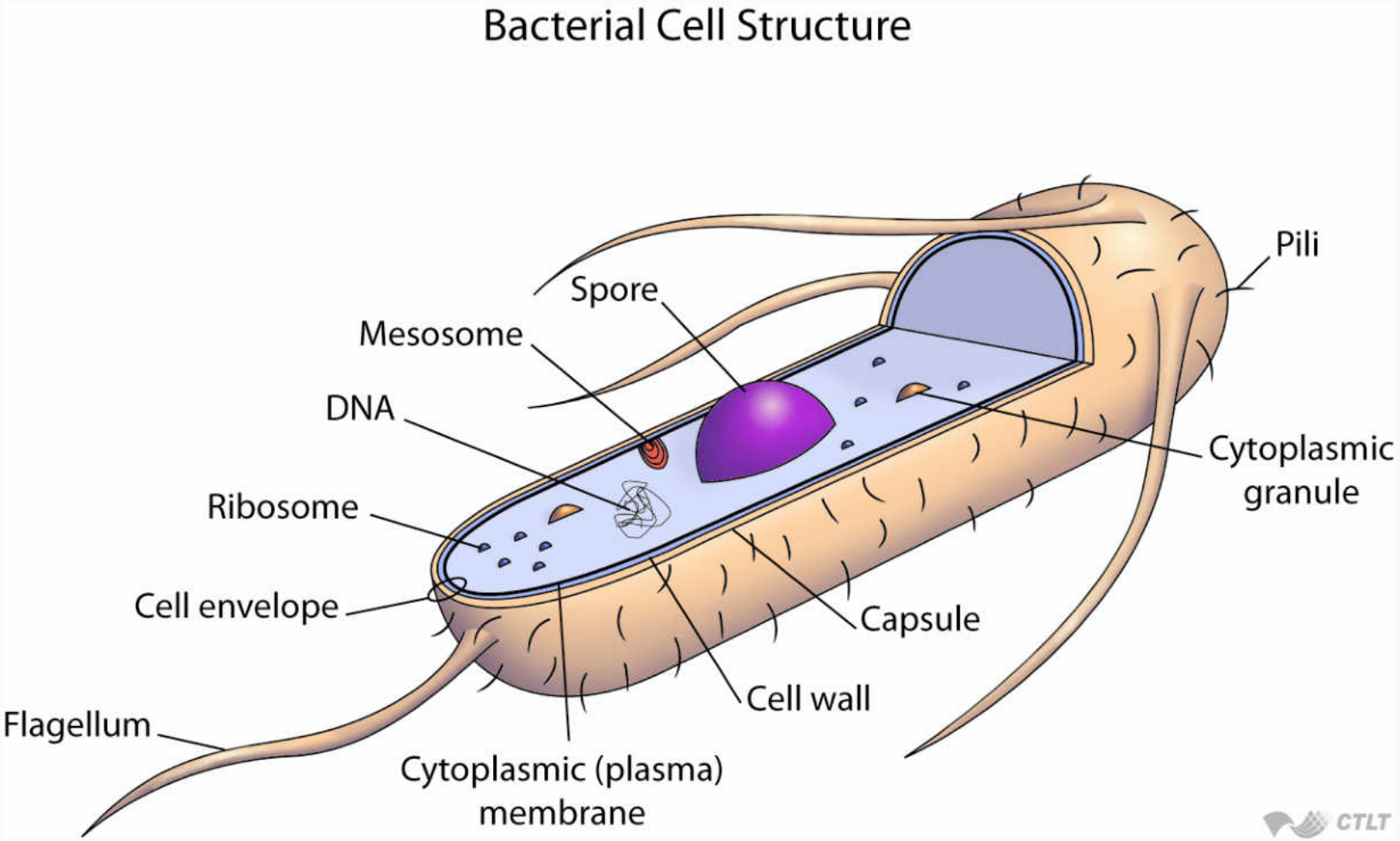
Comparison of Eukaryotic and Prokaryotic Cells

Characteristic	Eukaryotes	Prokaryotes
Form	Multicellular	Single cells
Nucleus	Nuclear membrane	DNA in contact with cytoplasm
Organelles	Membrane-bound organelles present	No organelles
Sterols	Always	Only in *Mycoplasma*
Ribosomes	80s = 40s + 60s	70s = 30s + 50s
Cell wall	Absent or cellulose/chitin	Peptidoglycan
Mitosis	Yes	No

Size Comparisons of Microorganisms



Bacterial Cell Structure



Bacterial Classification

- Gram-positive
- Gram-negative
- Acid-fast

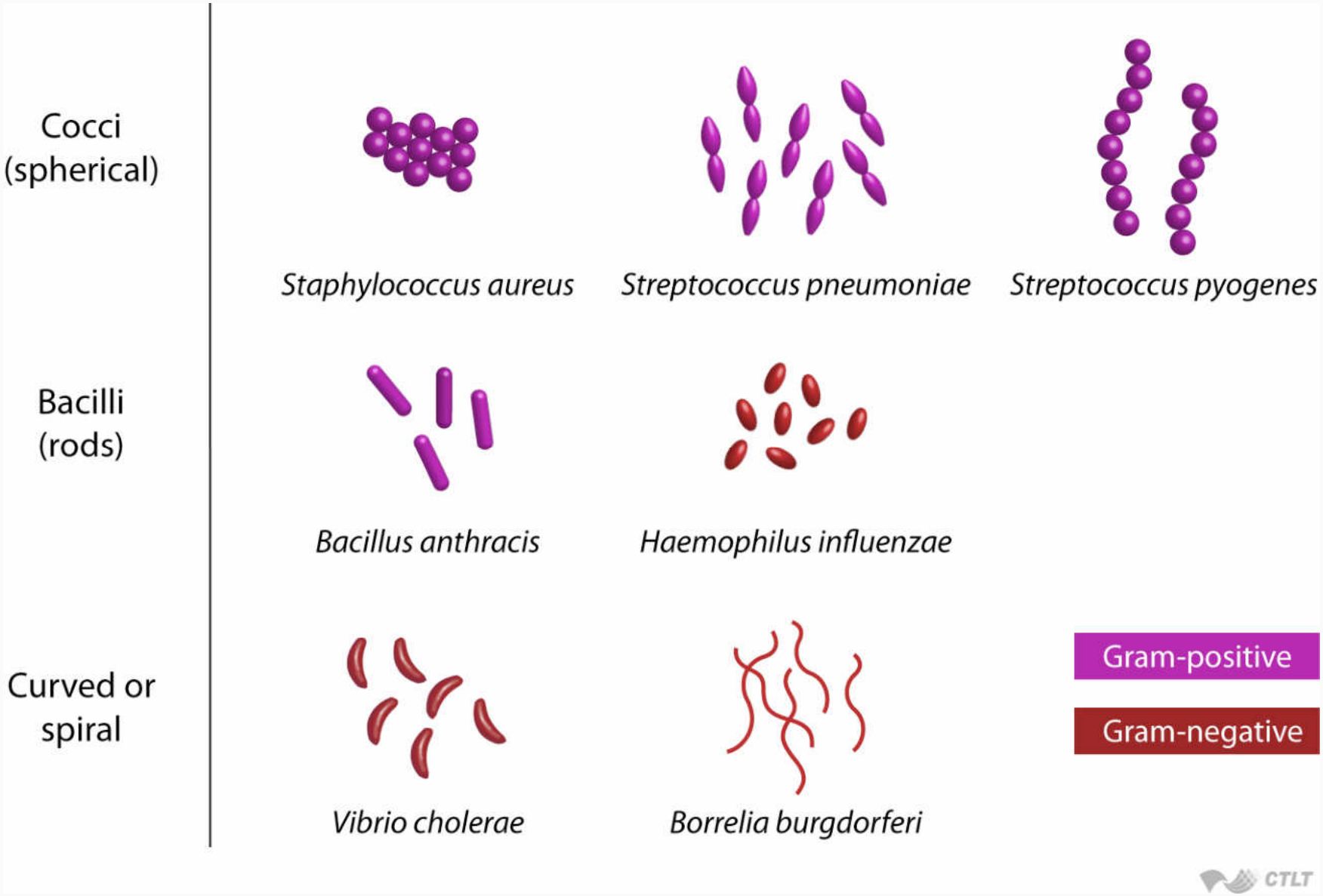
Bacterial Shapes

- Cocci-round or spherical cells
- Bacilli-rod-shaped cells
- Curved, spiral forms

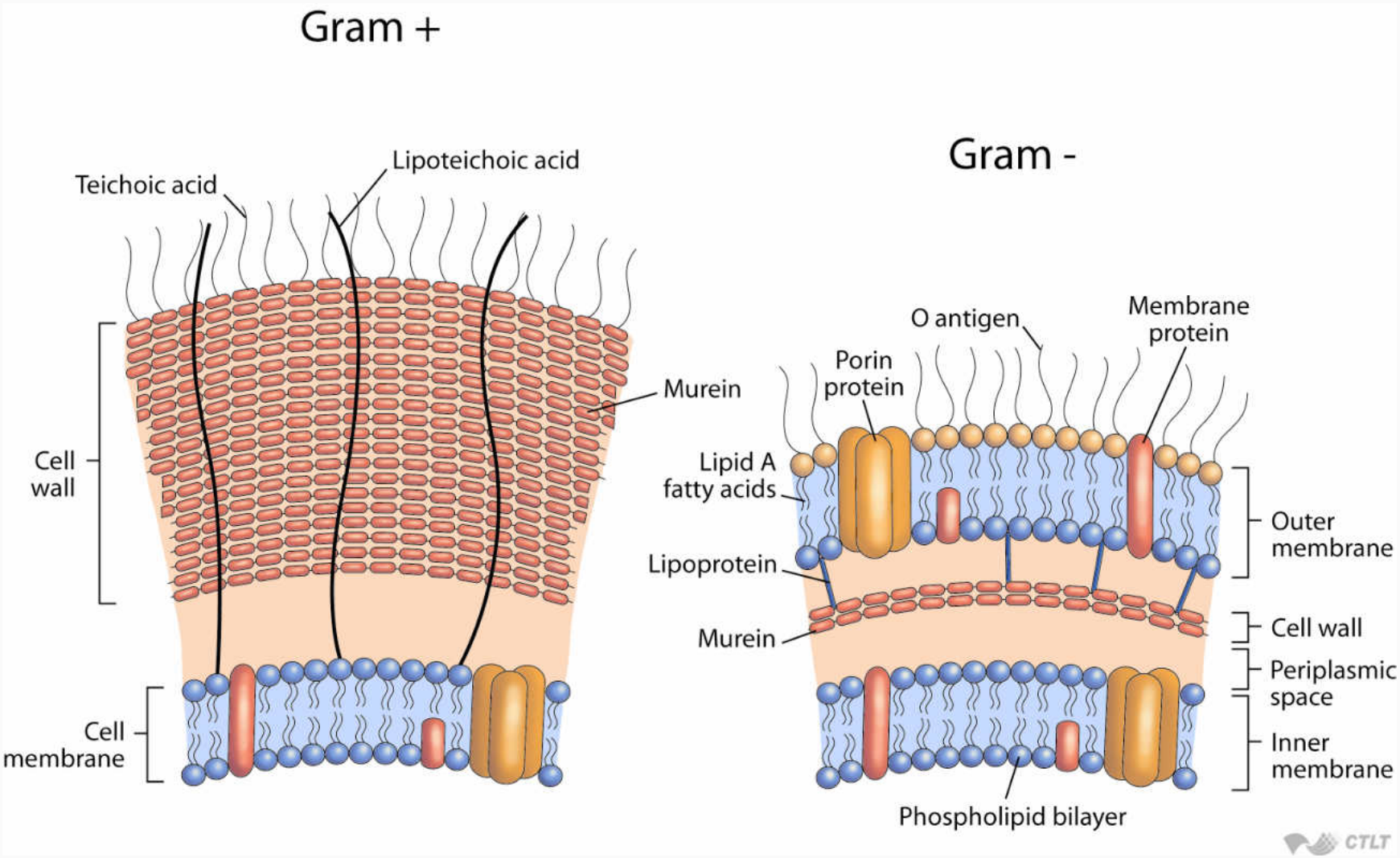
Biological Stains

- Bacteria—gram stain
- Fungi—KOH, lactophenol blue, India ink, silver stains in tissue
- Mycobacteria—acid fast stains
- Parasites—trichrome stain, Wright's stain
- Viruses—antibody conjugated dyes

Spherical, Rod-Shaped, and Spiral



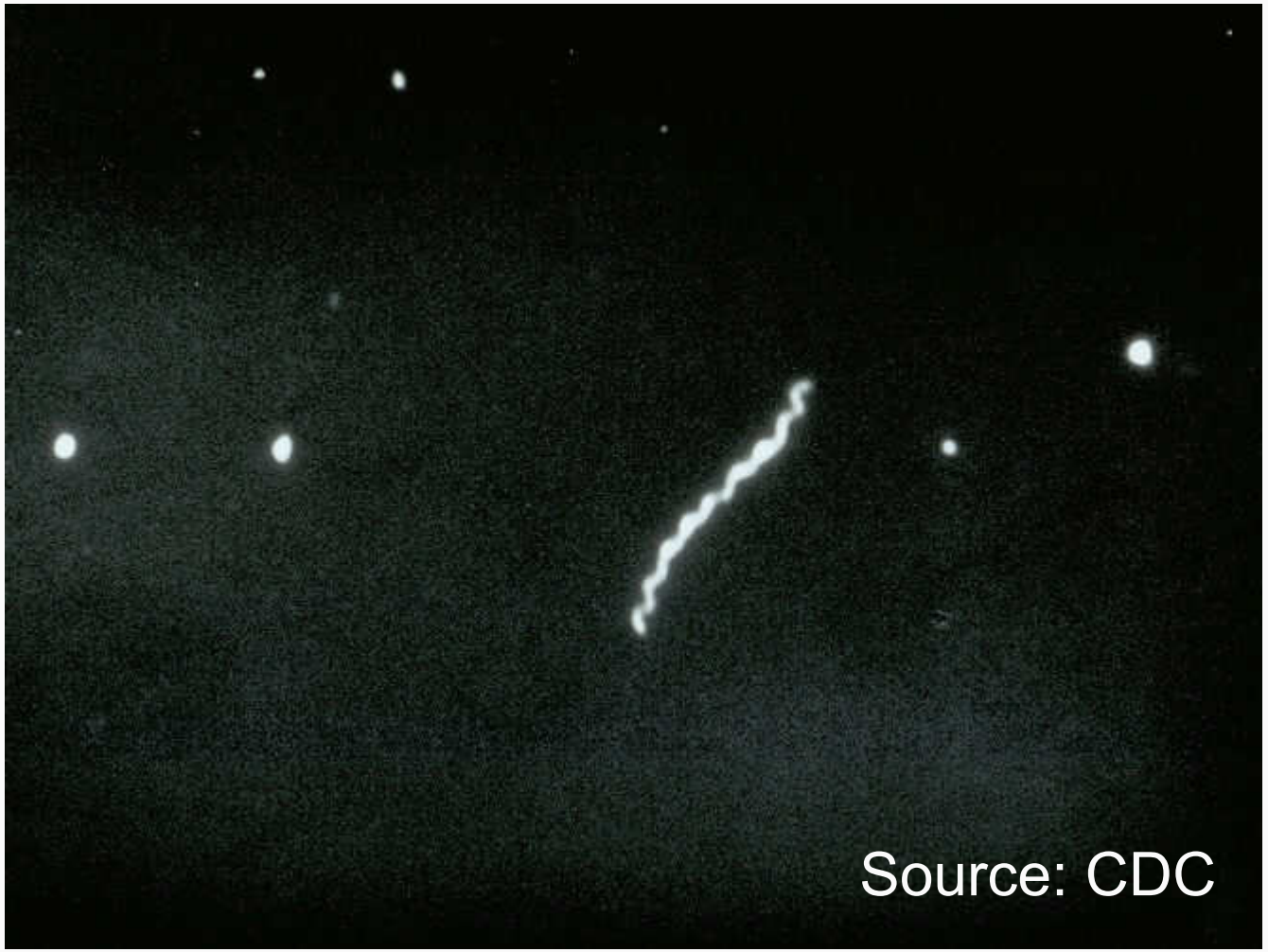
Bacterial Cell Walls



Pappenheim Stain of Streptococcus pyogenes

Public Domain

Dark Field Microscopy of Syphilis

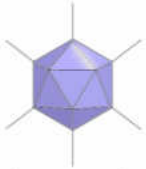


Classification of Viruses

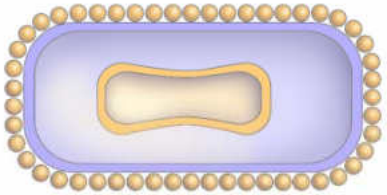
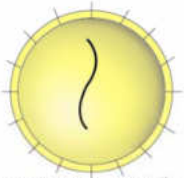
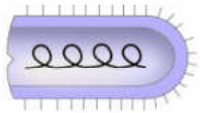
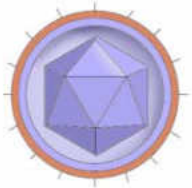
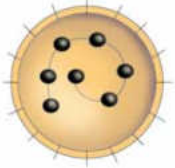
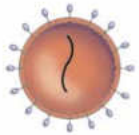
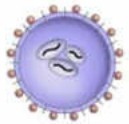
Family	Example	Genome Size, Kilobases of Kilobase Pairs	Envelope
RNA Viruses			
Single-stranded			
Picornaviridae	Poliovirus	7.2-8.4	No
Togaviridae	Rubella virus	12	Yes
Flaviviridae	Yellow fever virus	10	Yes
Coronaviridae	SARS	16-21	Yes
Rhabdoviridae	Rabies virus	13-16	Yes
Paramyxoviridae	Measles virus	16-20	Yes
Orthomyxoviridae	Influenza virus	14	Yes
Bunyaviridae	California encephalitis virus	13-21	Yes
Arenaviridae	Lymphocytic choriomeningitis virus	10-14	Yes
Retroviridae	HIV	319	Yes
Double-stranded			
Reoviridae	Rotaviruses	16-27	No
DNA Viruses			
Single-stranded			
Parvoviridae	Human parvovirus B-19	5	No
Partially double-stranded			
Hepadnaviridae	Hepatitis B	3	Yes
Double-stranded			
Polyomaviridae	JC virus	8	No
Adenoviridae	Human adenovirus	36-38	No
Herpesviridae	Herpes simplex virus	120-220	Yes
Poxviridae	Vaccinia, smallpox	130-280	Yes

Viruses

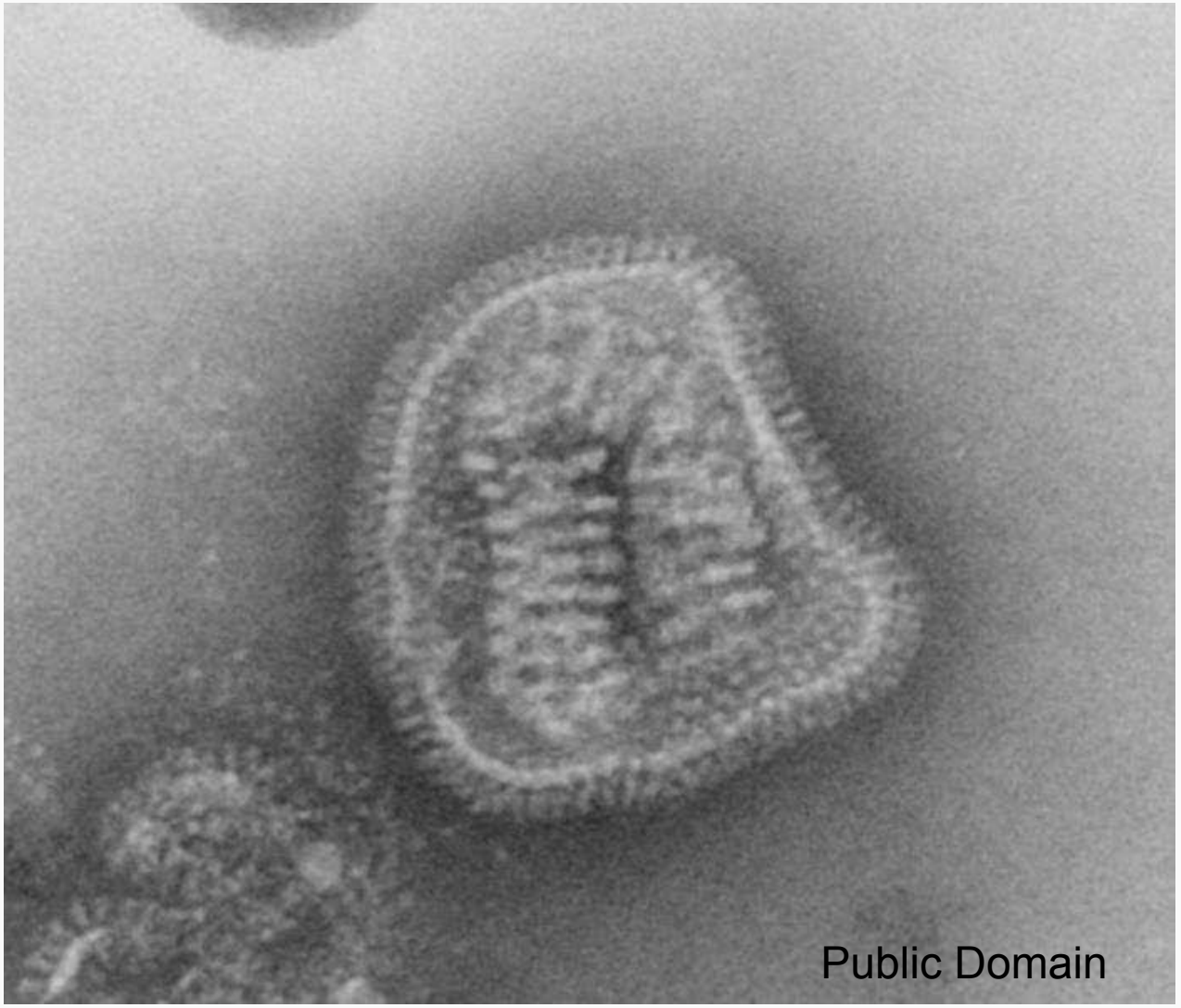
Nonenveloped

Double-stranded DNA		Double-stranded RNA	Single-stranded RNA		Single-stranded DNA
					
Papovaviridae (Papilloma)	Adenoviridae (Adeno)	Reoviridae (Reo)	Calicivirus (Norwalk?)	Picornaviridae (Polio)	Parvoviridae (Erythema infectiosum)

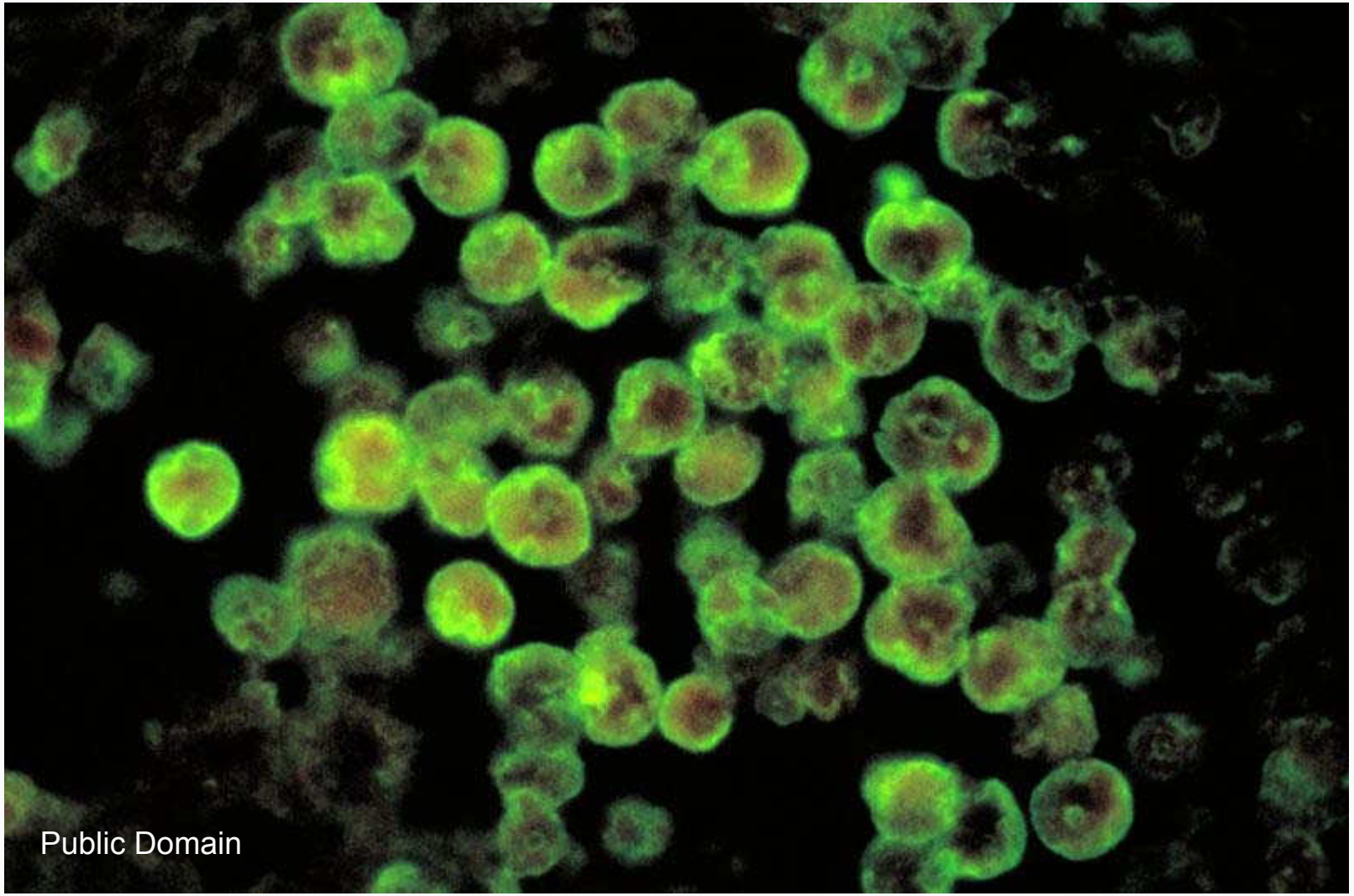
Enveloped

Double-stranded DNA	Single-stranded RNA			
				
Poxviridae (Vaccinia)	Paramyxoviridae (Measles)	Orthomyxo-viridae (Influenza)	Rhabdoviridae (Rabies)	Retroviridae (Human immunodeficiency)
				
Herpesviridae (Herpes simplex)	Arenaviridae (Lymphocytic choriomeningitis)	Coronaviridae (Coronavirus)	Bunyaviridae (Hantavirus)	Togaviridae (Rubella)
				
Hepadnaviridiae (Hepatitis B)	Flaviviridae (St. Louis encephalitis)			

Electron Micrograph of Viral Particle (Influenza)



Fluorescent Antibody Stain (Meningoencephalitis)



Characteristics of Fungi

- Pathogenic fungi have two forms: yeasts (unicellular) and molds (multicellular)
- Some fungi are dimorphic
- Molds grow as filamentous, branching strands of connected cells known as hyphae

Characteristics of Fungi

- Fungi classified by type/method of reproduction
- Asexual—conidia; sexual—spores
- “Micro” or “macro” refers to size of spores

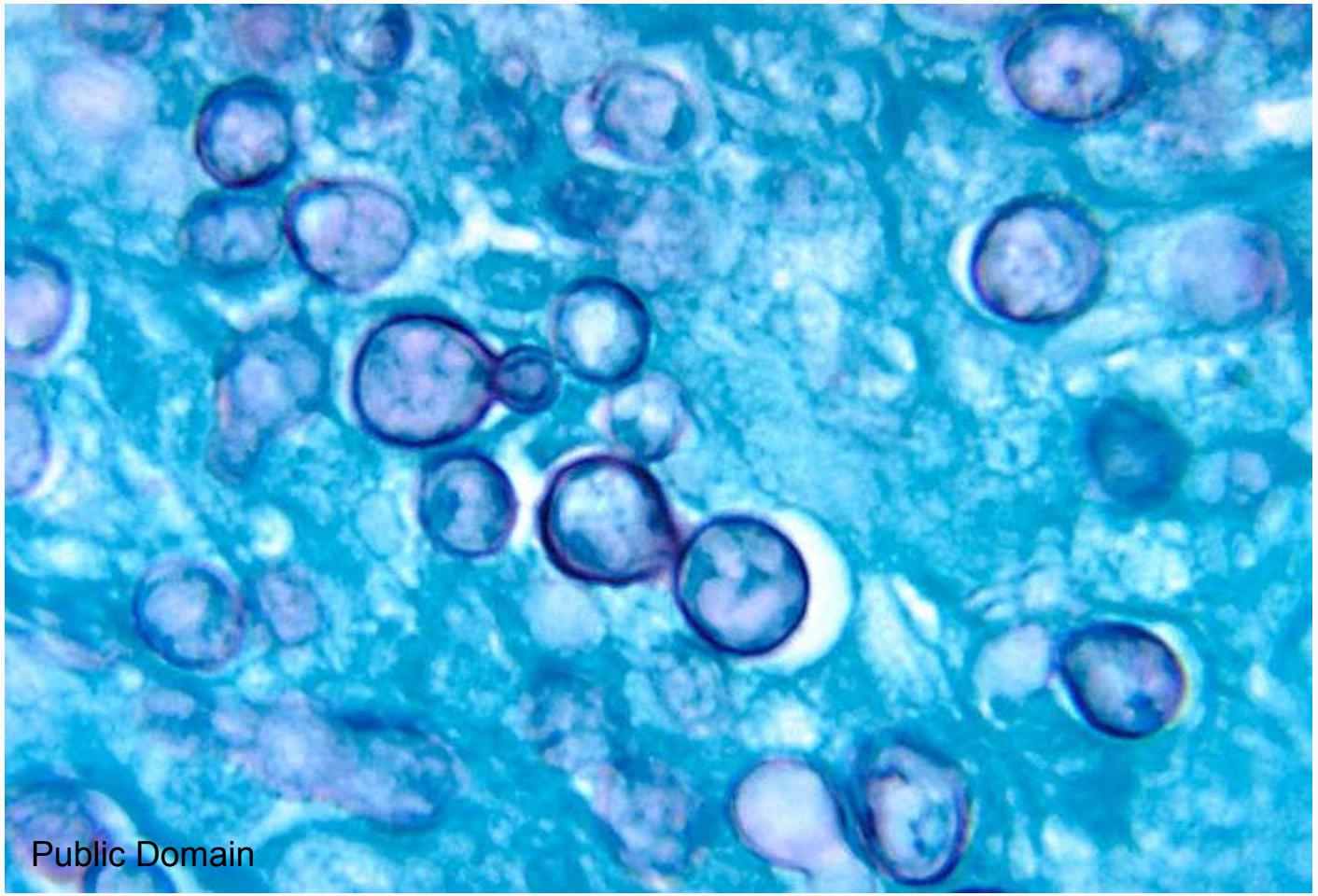
Grouping of Fungi of Medical Importance

- Superficial mycoses—outermost layers of skin and hair
- Cutaneous mycoses—epidermis
- Subcutaneous mycoses—dermis and subcutaneous tissues
- Systemic mycoses—internal organ systems

Fungi of Medical Importance

Fungus	Classification	Disease
Malassezia furfur	Yeast	Superficial mycoses
Trichophyton rubrum	Filamentous	Tinea, cutaneous mycoses
Microsporum audouinii	Filamentous	Tinea, cutaneous mycoses
Epidermophyton floccosum	Filamentous	Tinea, cutaneous mycoses
Candida albicans	Yeast	Mucocutaneous and systemic mycoses
Sporothrix schenckii	Dimorphic	Subcutaneous mycoses
Histoplasma capsulatum	Dimorphic	Systemic mycoses, histoplasmosis
Blastomyces dermatitidis	Dimorphic	Systemic mycoses, blastomycosis
Coccidioides immitis	Dimorphic	Systemic mycoses, coccidioidomycosis
Paracoccidioides brasiliensis	Dimorphic	Systemic mycoses, paracoccidioidomycosis
Penicillium marneffei	Dimorphic	Systemic mycoses, penicilliosis
Cryptococcus neoformans	Yeast	Systemic mycoses, opportunistic cryptococcosis
Candida species	Yeast	Opportunistic infections
Aspergillus fumigatus	Filamentous	Opportunistic infections
Aspergillus flavus	Filamentous	Opportunistic infections
Fusarium species	Filamentous	Opportunistic infections
Rhizopus species	Filamentous	Opportunistic infections
Mucor species	Filamentous	Opportunistic infections
Absidia species	Filamentous	Opportunistic infections
Pseudallesheria species	Filamentous	Opportunistic infections
Pneumocystis carinii	Dimorphic, cysts	Opportunistic infections

Histoplasma capsulatum



Medical Parasitology

- Protozoa
  - Amebiasis
  - Leishmaniasis
  - Trypanosomiasis
  - Malaria
- Helminths—adult stage in worm

Hookworm in stool

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Section B

Diagnostic Microbiology

Diagnostic Microbiology

- Microscopy
- Culture
- Immunology
- Molecular methods

Diagnostic Microbiology

- Microscopy
  - Light microscopy
  - Fluorescence microscopy
  - Electron microscopy

Bacteria

- Staining characteristics
  - Gram negative/gram positive
- Shape
  - Cocci-round or spherical cells
  - Bacilli-rod-shaped cells
  - Curved, spiral forms
  - Coccobacilli
  - Pleomorphic cells
- Size

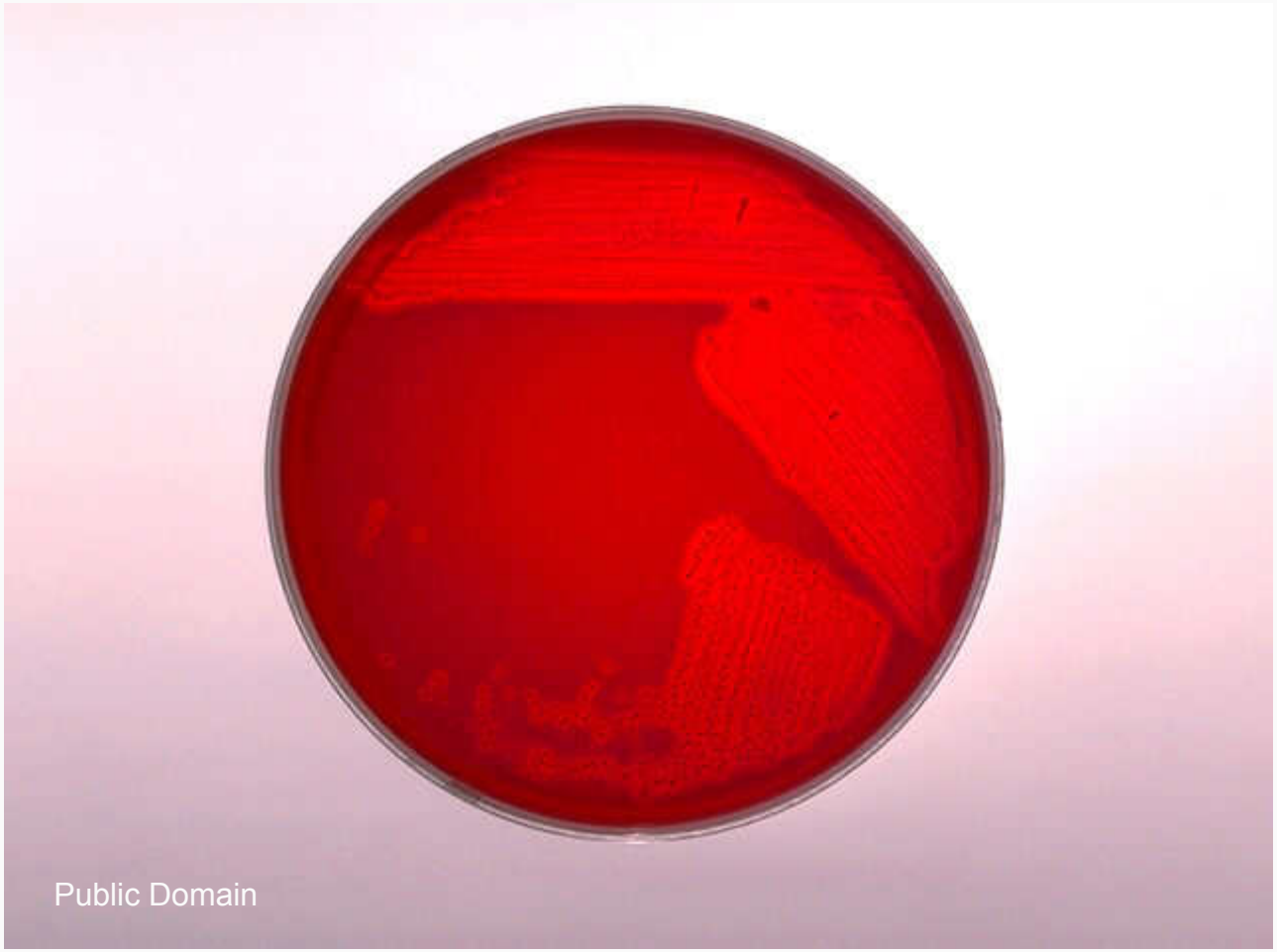
Microscopy

- Sensitivity
  - Relatively poor, one bacterial cell per high-powered field is equivalent to 100,000–1,000,000 bacteria per ml
  - This can be improved through fluorescence
- Specificity
  - Poor for bacteria, high for microorganisms exhibiting distinctive morphology—filamentous fungi, parasites, viruses by EM

Diagnostic Microbiology

- Culture
  - Defined versus undefined media
  - Enrichment media
  - Selective media
  - Specialized media

Blood Agar (Corynebacterium haemolyticum)



MacConkey's Agar (Proteus Vulgaris)

Culture

- Sensitivity—usually considered the gold standard
- Specificity—excellent when used in conjunction with phenotypic, immunologic, and molecular techniques

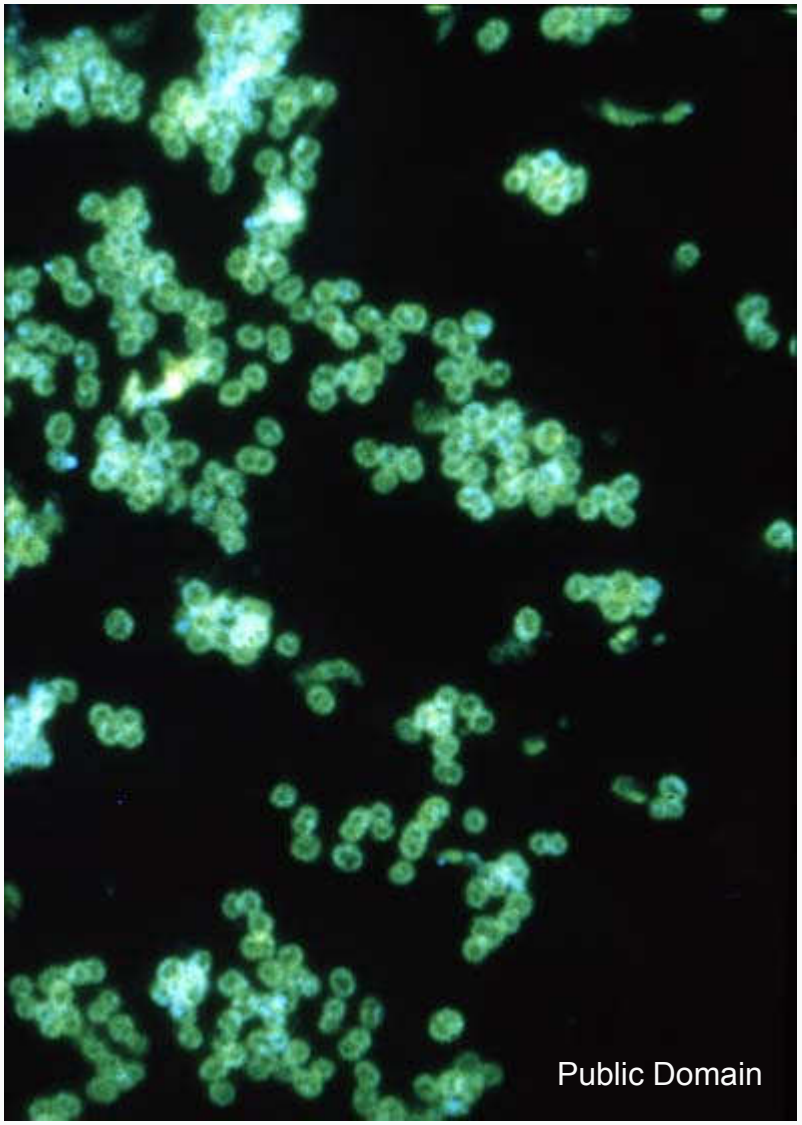
Diagnostic Immunology

- Testing for specific microbial antigens
  - Direct detection from clinical specimens
  - Characterization of a cultured organism
- Testing for antibody to specific microbial antigens
  - Detection of a particular isotope, usually IgM or IgG
  - IgA and IgE not usually used

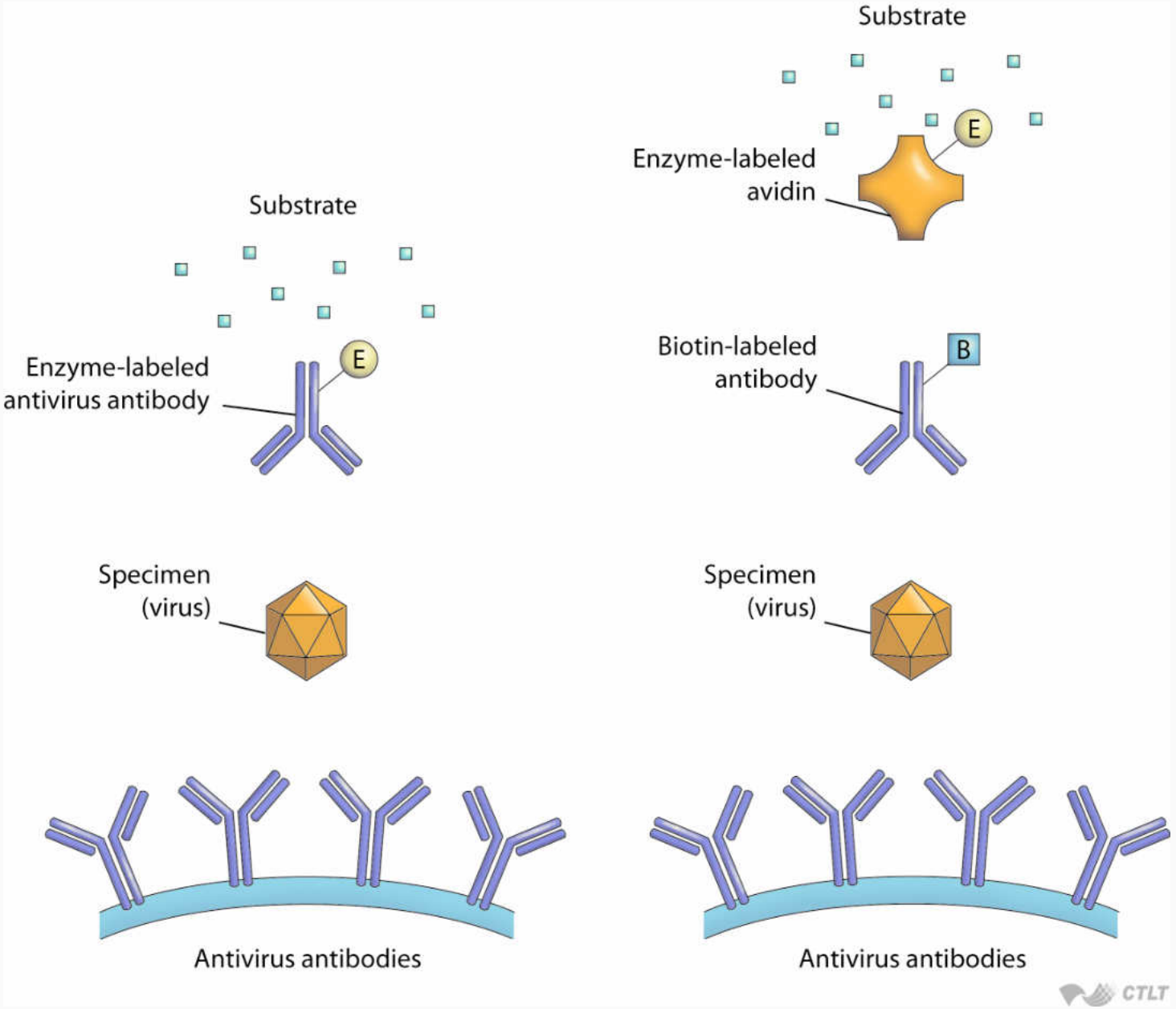
Diagnostic Immunology

- Complement fixation
- Agglutination assays
- Neutralization/hemagglutination assays
- Enzyme immunoassays (EIAs, ELISAs)
- Radioimmunoassays (RIA)
- Fluorescent antibody techniques

Fluorescent Antibody Stain (Neisseria gonorrhoeae)



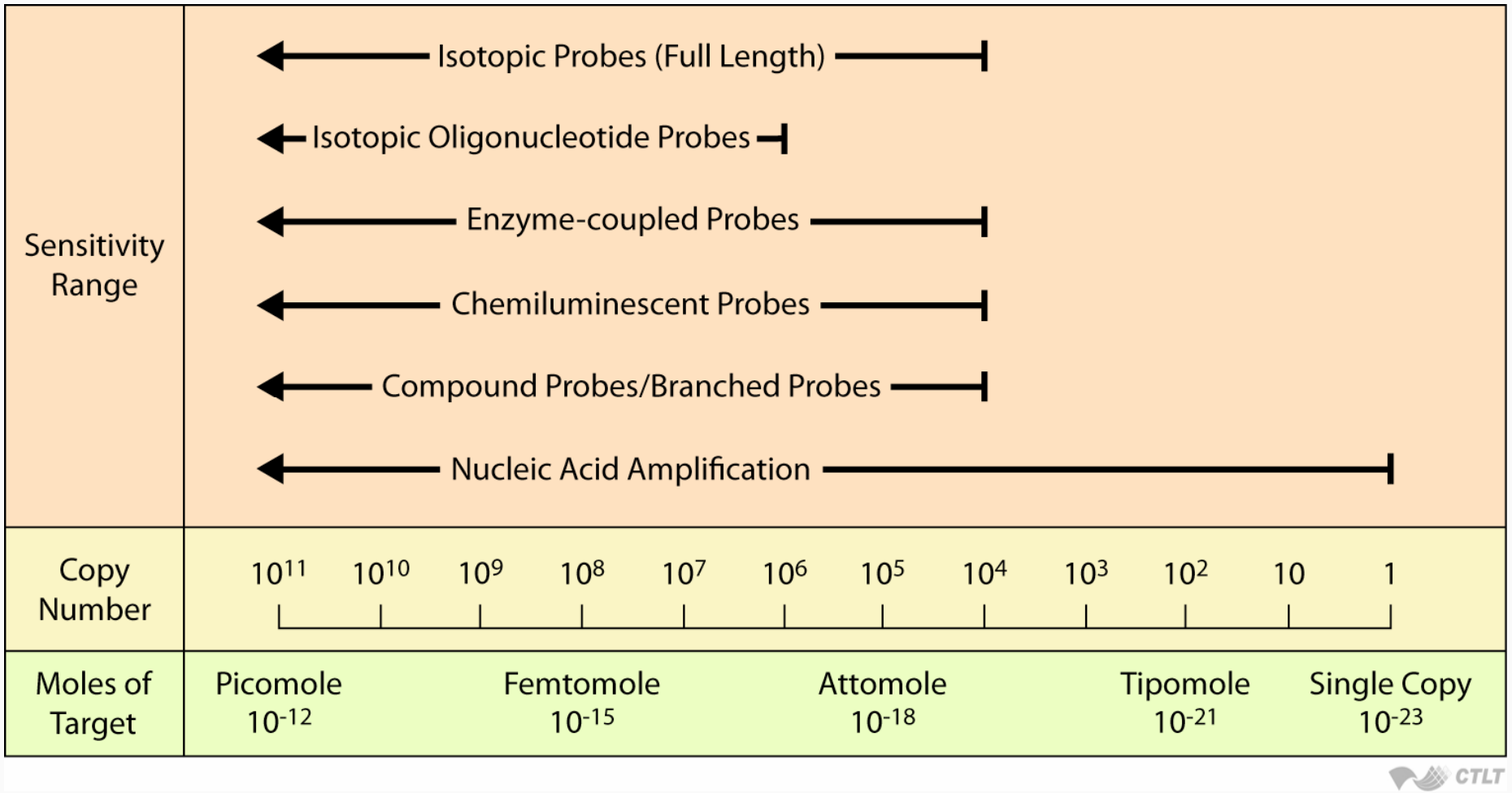
Microbiology Tools for the Epidemiologist



Molecular Diagnostics

- Nucleic acid probes
- Signal amplification methods
  - PCR
  - RT-PCR
  - Nested PCR
  - Multiplex PCR

Sensitivity Comparisons of Molecular Methods



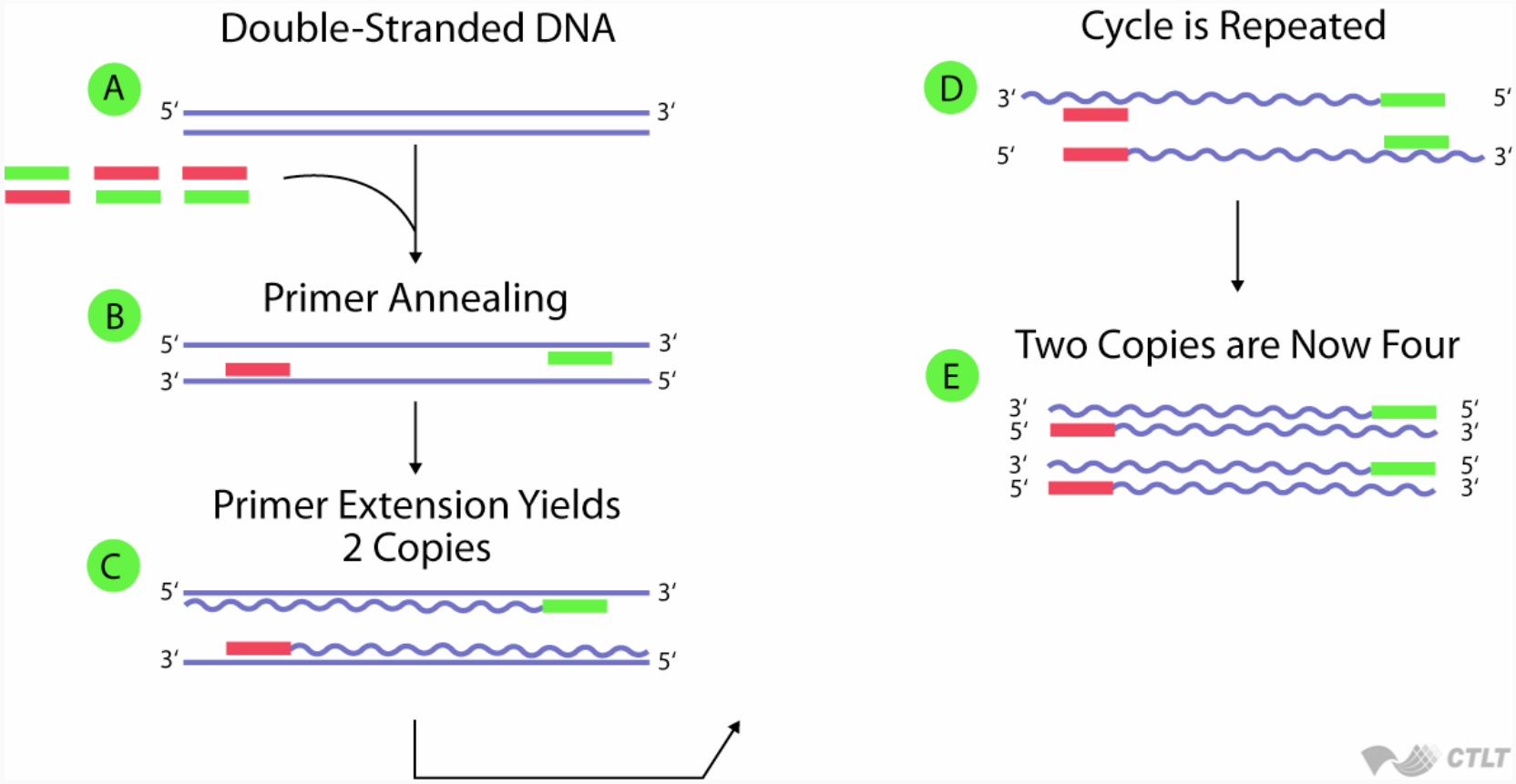
Probe Technology

- Detection of fastidious, slow-growing, or nonculturable organisms as well as antibiotic resistance genes and phenotypically difficult organisms

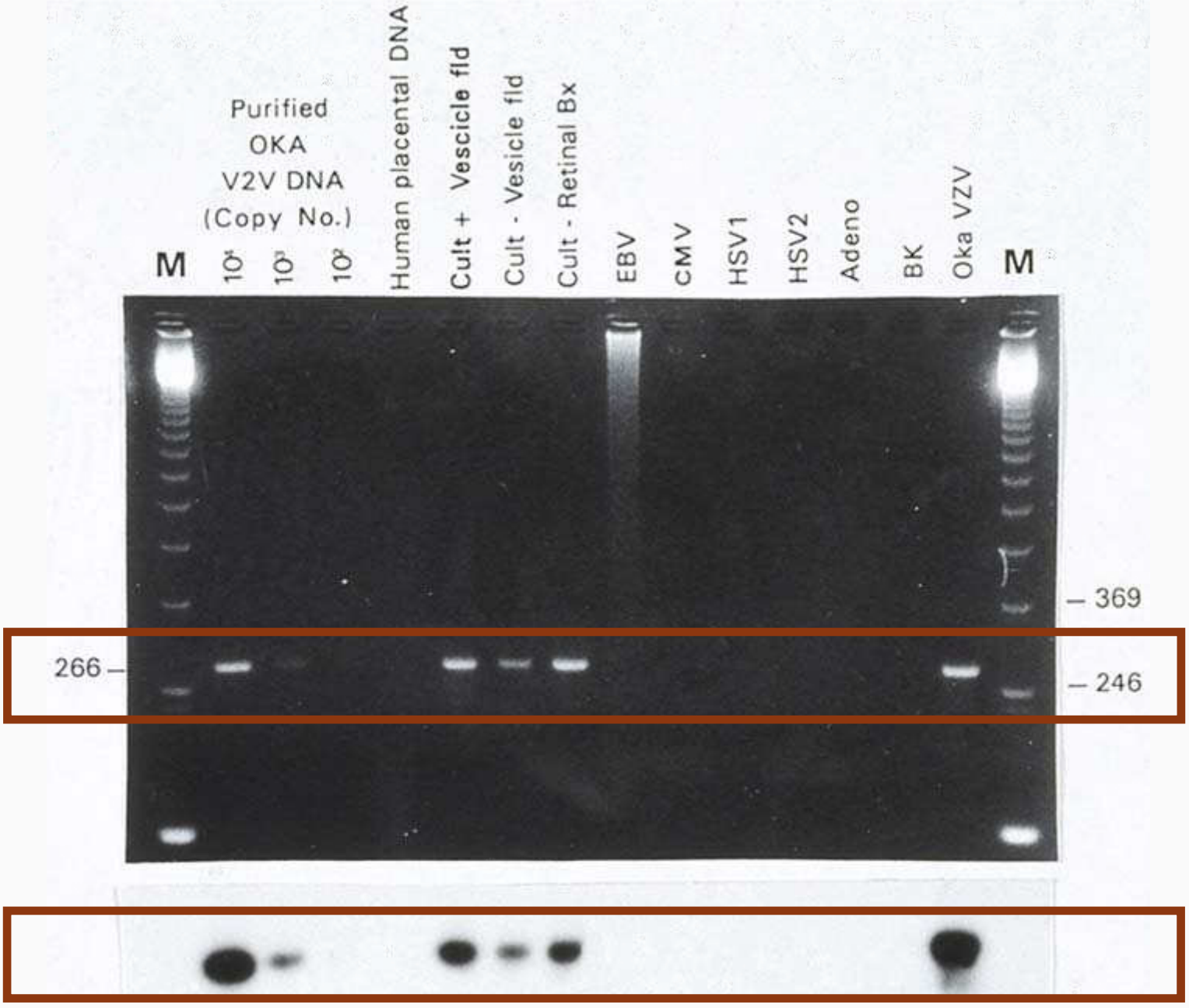
Hybridization Techniques

- Solid-phase
- Liquid phase
- *In situ*

Polymerase Chain Reaction (PCR)



Gel and Hybridizations of PCR Reactions



Limitations of Molecular Technology

- False positive results
  - Contamination
- False negative results
  - Presence of inhibitors
- Narrow spectrum of detection
  - Detection of a single pathogen
- Cost