

UCLA Clinical Microbiology Laboratory



800,000 tests per year

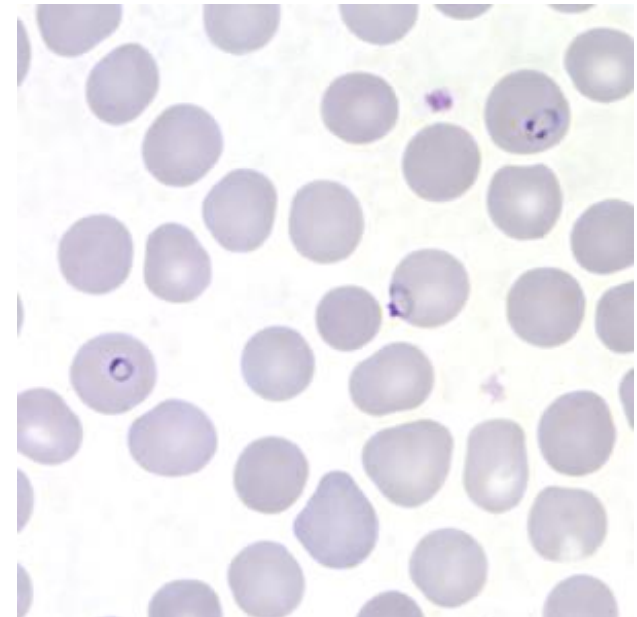
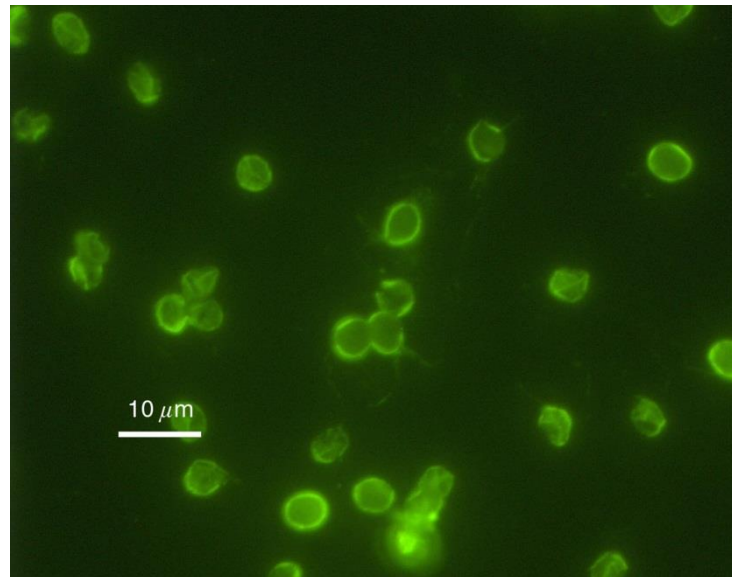
Bacteriology, Virology, Mycology,
Immunoserology, Parasitology

Sample Type: Respiratory, Wound,
Urine, Fecal, Blood, Tissue . . .

Pneumonia, blood stream infections,
Tissue infection (wounds), UTI, URI,
Diarrheal disease, STD testing, any
other disease of infectious origin

UCLA Clinical Microbiology Laboratory

Types of Tests



2008 CDC Case Definition for HIV Infection: AIDS-Defining Clinical Conditions

| AIDS-Defining Clinical Conditions | |
|--|--|
| Candidiasis (trachea, bronchia, or lung) | |
| Candidiasis (esophageal) | |
| Cervical cancer (invasive) | |
| Coccidioidomycosis (disseminated or extrapulmonary) | |
| Cryptococcosis (extrapulmonary) | |
| Cryptosporidiosis (intestinal, for longer than 1 month) | |
| Cytomegalovirus disease (other than liver, spleen, or nodes) | |
| Cytomegalovirus retinitis (with loss of vision) | |
| Encephalopathy (HIV-related) | |
| Herpes simplex: chronic ulcers (present for longer than 1 month) | |
| Herpes simplex: bronchitis, pneumonitis, or esophagitis | |
| Histoplasmosis (disseminated or extrapulmonary) | |
| Isosporiasis (intestinal, for longer than 1 month) | |
| Kaposi's sarcoma | |
| Lymphoma, Burkitt's (or equivalent term) | |
| Lymphoma, immunoblastic (or equivalent term) | |
| Lymphoma, primary of brain | |
| <i>Mycobacterium avium</i> complex, disseminated or extrapulmonary | |
| <i>Mycobacterium kansasii</i> , disseminated or extrapulmonary | |
| <i>Mycobacterium tuberculosis</i> ; any site (pulmonary or extrapulmonary) | |
| <i>Mycobacterium</i> , other species or unidentified species, disseminated or extrapulmonary | |
| <i>Pneumocystis carinii</i> pneumonia | |
| Recurrent pneumonia (two or more episodes in 1-year period) | |
| Progressive multifocal leukoencephalopathy | |
| Salmonella (recurrent septicemia) | |
| Toxoplasmosis (brain) | |
| Wasting syndrome due to HIV: greater than 10% involuntary weight loss plus either chronic diarrhea (2 or more stools per day for at least 30 days) or chronic weakness and documented fever (for at least 30 days) in the absence of a concurrent illness or condition other than HIV that could explain this finding. | |

Figure 2.3 2008 CDC Case Definition for HIV Infection: AIDS-Defining Clinical Conditions

Methods for Lab Diagnosing Infectious Disease

1. **Microscopy** (Parasitology, Bacteriology, Mycology)

Blood smear for malaria, Trichrome stain for Giardia, Gram stain of CSF for *N. meningitidis*, Chitin stain for *Candida*
Rapid turn around time, low sensitivity

2. **Culture** (Bacteriology, Mycology, Mycobacteria, Viral Culture)

Pseudomonas, *Aspergillus*, TB, HSV
Slow turn around, commensal organism contamination

3. **Antigen Recognition** (Nucleic Acid, Carbohydrates, Protein)

RT-PCR for *Bordetella*, *Mycoplasma*, HSV, Group A Strep Rapid test, *Trichomonas* antigen test
Rapid turn around time, little susceptibility information

4. **Serology** (Antibody response to a pathogen)

HIV, Hepatitis, *Toxoplasma*, Syphilis . . .
Hard to find any other way, provides diagnostic information about the course of the disease

What types of Microbes Exist?

4 Main Classes of Infectious Agents

Viruses

HIV (AIDS)
Ebola (Viral hemorrhagic fever)
Hepatitis A,B,C (Hepatitis)
Influenza (The flu)
Adenovirus (Common Cold)

Fungus

Candida albicans (Thrush)
Aspergillus fumigatus (Aspergillosis)
Dermatophytes (Athlete's Foot)
Coccidioides immitis (Valley Fever)
Cryptococcus neoformans (Meningitis)

Bacteria

Streptococcus pyogenes (Strep Throat)
Neisseria meningitidis (Meningitis)
Bordetella pertussis (Whooping Cough)
Clostridium perfringens (Gas gangrene)
Treponema pallidum (Syphilis)

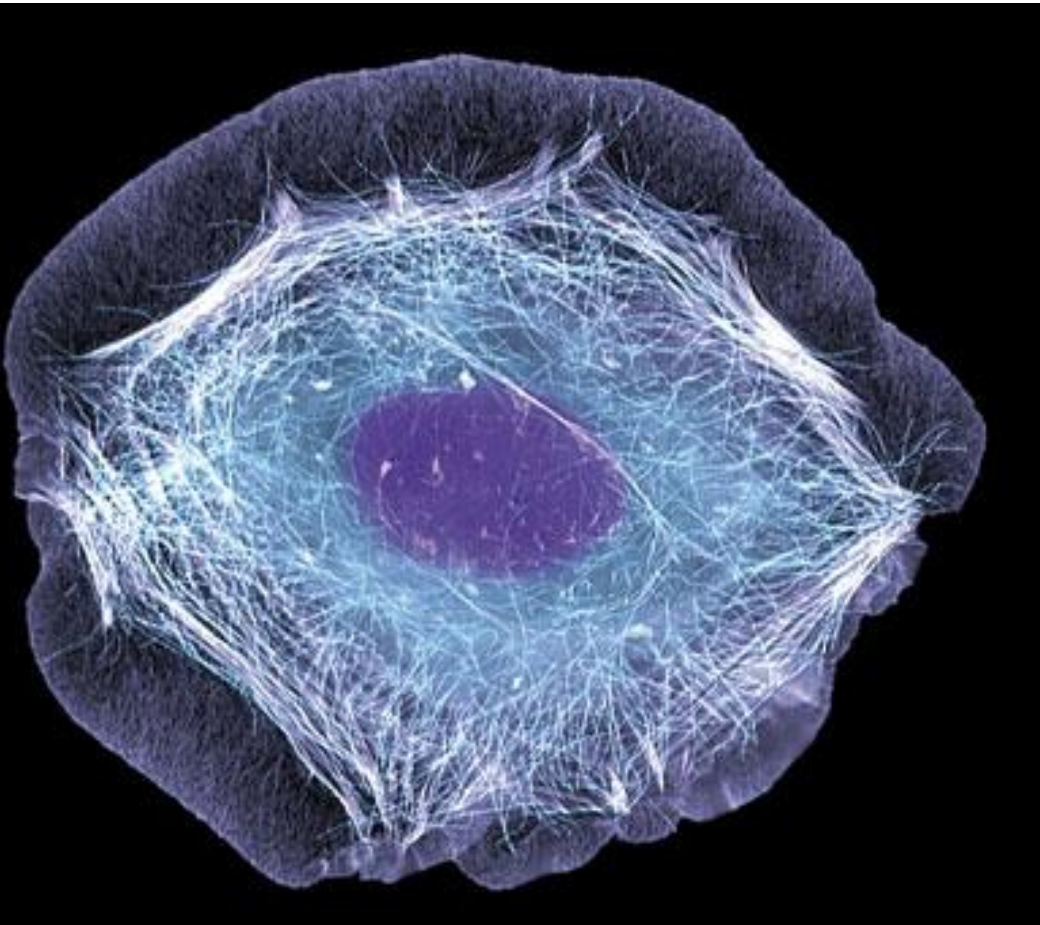
Parasites

Plasmodium falciparum (Malaria)
Trypanosoma cruzi (Chagas disease)
Ascaris lumbricoides (Intestinal roundworms)
Giardia lamblia (Giardiasis)
Trichomonas vaginalis (Trichomoniasis)

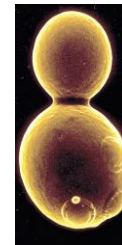
How big are microbes?

Human Cell
Size:
~20 μ M

1 μ M = 1/1,000,000 Meter



Fungus
Yeast
Size:
5 μ M



Bacteria
Size:
1-2 μ M

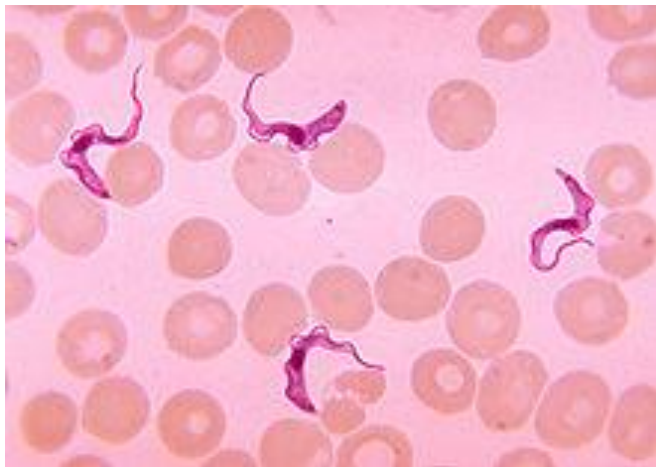
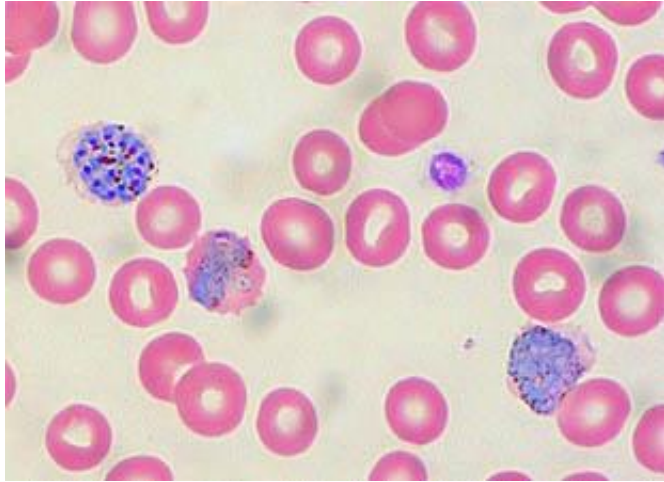


Virus
Size:
0.001 –
0.1 μ M



How big are microbes?

Parasites



Where do Microbes Live?

The Environment (Parasites, Fungus, Bacteria)



Clostridium tetani (Tetanus)
Aspergillus terreus (Aspergillosis)
Coccidioides immitis (Valley fever)
Bacillus anthracis (Anthrax)
Clostridium botulinum (Botulism)



Nagleria fowleri (Primary meningo-encephalitis)
Aeromonas hydrophila (Flesh-eating bacteria)
Vibrio parahaemolyticus (Sepsis)

Where do Microbes Live?

In/On Animals (Parasites, Fungus, Bacteria, Virus)



Plasmodium falciparum (Malaria)
Ehrlichia chaffeensis (Ehrlichiosis)
Borrelia burgdorferi (Lyme Disease)
Dengue Virus (Dengue Hemorrhagic Fever)
Chikungunya Virus (Chikungunya)

Influenza virus (Bird flu/swine flu)
Bartonella (Cat scratch disease)
Chlamydochila psittaci (Psittacosis)
Nipah Virus (Encephalitis)

Vector – Any agent that transmits an infectious agent into a living organism

Host – Organism that harbors an infectious agent

Where do Microbes Live?

On Humans (Fungus, Bacteria)

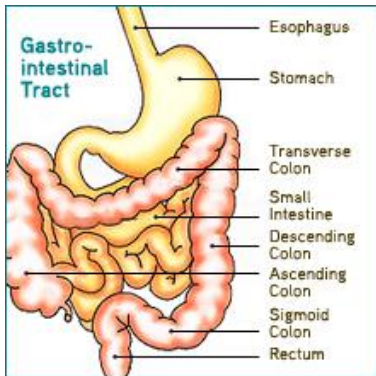
Commensal Organisms – Microbes that colonize the human body (normal microflora or human microbiome)

Symbiosis – Both the organism and the host have a mutually beneficial relationship

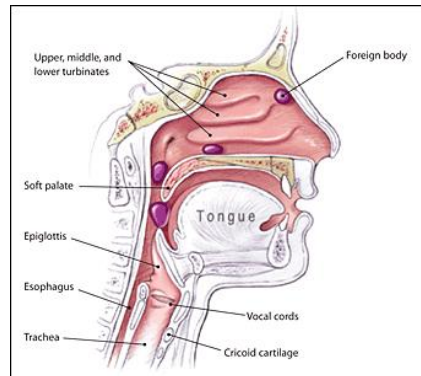
Number of Human cells in human body = 10^{13}

Number of bacterial cells in/on human body = 10^{14}

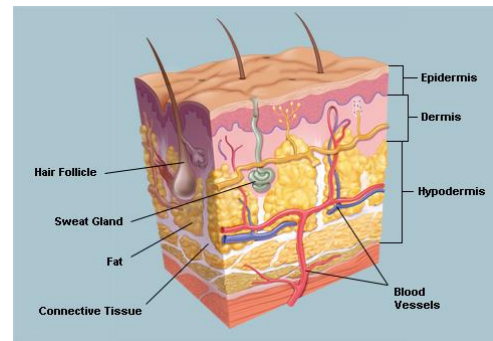
Gastrointestinal Tract



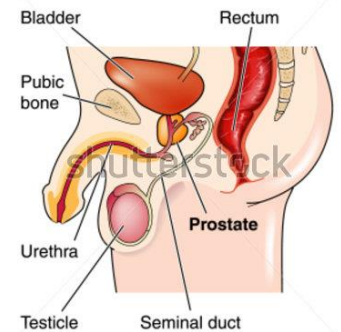
Mouth and Nasopharynx



Skin



Genitourinary Tract



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Escherichia coli, Viridans group streptococcus, *Staphylococcus*, *Lactobacillus*, *Candida*

Where do Microbes Live?

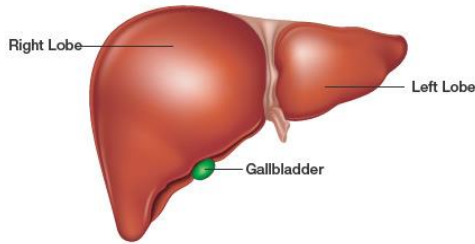
In Humans (Viruses, Bacteria, Parasites)

Blood



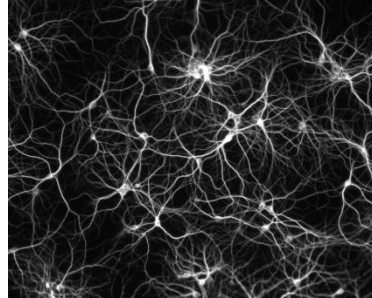
HIV (Immune cells)

Liver



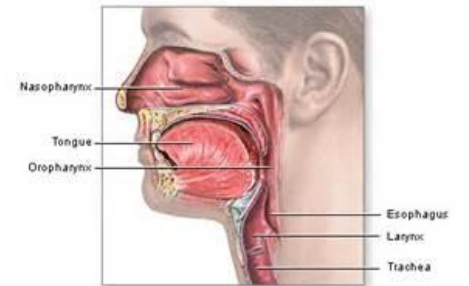
Hepatitis B

Neurons



Herpes

Oropharynx

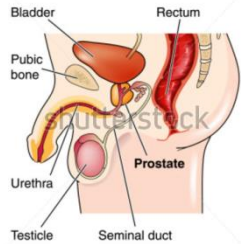


ADAM

Influenza

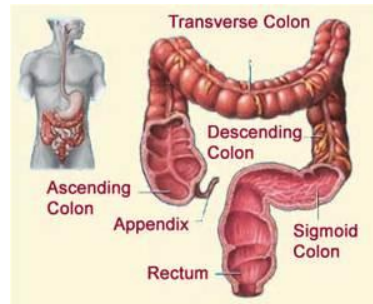
Bordetella pertussis
Mycoplasma pneumoniae

Genital Tract



Chlamydia trachomatis
Treponema pallidum
Neisseria gonorrhoea

Feces



Salmonella typhi
Norovirus
Vibrio cholera

Definitions

Pathogen - A disease producing microorganism

Host – Organism that harbors a pathogen

Obligate Pathogen – A microbe whose presence signifies a disease
HIV, Influenza, Giardia, Bordetella pertussis, Hepatitis B, Salmonella

Opportunistic Pathogen – A commensal or environmental microbe that only causes disease under certain conditions (immunocompromised, breach of barrier)

Candida albicans, Escherichia coli, Staphylococcus, Streptococcus

Transmission – How a microbe comes into contact with a host

Virulence – How likely is it that a microbe will cause disease

Virulence factors – Biological properties of a microbe that contribute to virulence

Definitions

Communicable - transmitted from external source, animate or inanimate

Contagious - transmitted from patient to patient

Iatrogenic Infection - produced by medical interventions

Nosocomial Infection - acquired in health care facility

Opportunistic Infection- infection caused by an agent of low virulence in an immunocompromised patient

Subclinical or Asymptomatic Infection- infection with no clinical symptoms

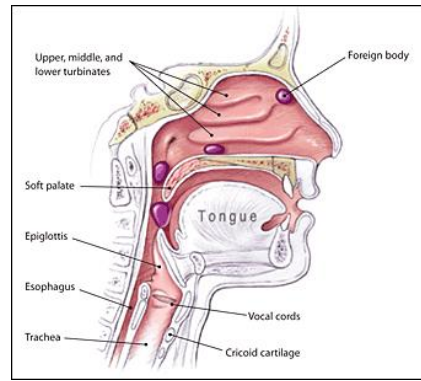
How We Come into Contact with Pathogens

Barriers to Infection

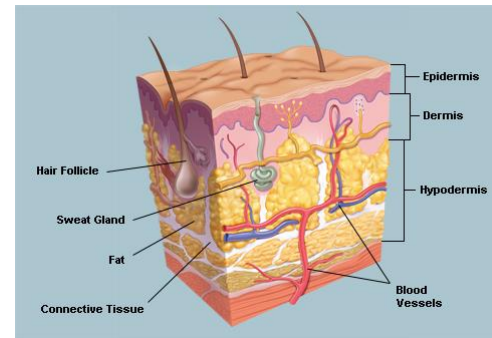
Gastrointestinal Tract



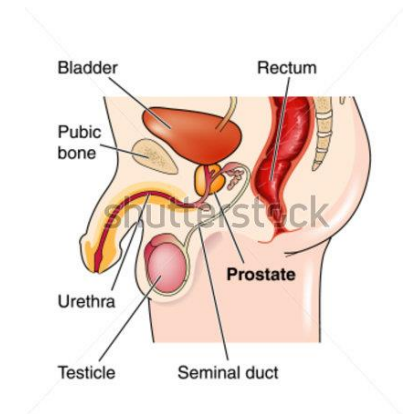
Respiratory Tract



Skin



Genitourinary Tract



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- Epithelial Barrier
- Acidic Secretions
- Bile and Pancreatic enzymes
- Commensal GI microbes

- Mucociliary clearance
- Resident alveolar macrophages

- Epidermal Barrier
- Commensal skin Microbes

- Urination
- Commensal vaginal microbes
- Intact epidermal and epithelial barrier

Modes of Transmission

Fecal to oral

Causes Diarrheal Illness



Basis for Failure of Barrier to Infection (GI Tract)

Epithelial Barrier
(Attachment and local proliferation of microbes)

- *Vibrio cholerae*
- *Giardia*

Epithelial Barrier
(Attachment and local invasion of microbes)

- *Shigella*
- *Salmonella*
- *Campylobacter*

Acidic Secretions
(Acid-resistant cysts and eggs)

- *Entamoebae*
- *Cryptosporidium*

Commensal GI Microbes
(Broad Spectrum Antibiotic use)

- *Clostridium difficile*

Bile and Pancreatic Enzymes
(Resistant Microbial external coats)

- Hepatitis A
- Rotavirus
- Norovirus

Modes of Transmission

Person to person by respiratory droplets

Causes Respiratory Illness



Basis for Failure of Barrier to Infection (Respiratory Tract)

Mucociliary Clearance

(Attachment and local proliferation of microbes)

- Influenza virus
- Cold Virus

Mucociliary Clearance

(Ciliary paralysis by toxins)

- *Haemophilus influenzae*
- *Mycoplasma pneumoniae*
- *Bordetella pertussis*

Resident alveolar

macrophages

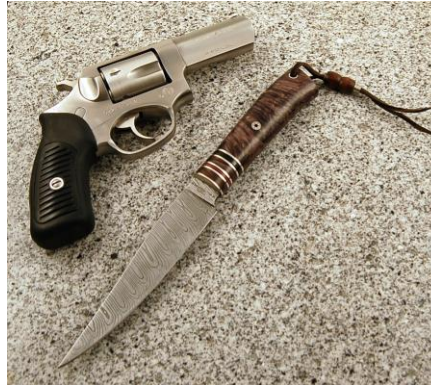
(Resistance to killing by phagocytes)

- *Mycobacterium tuberculosis*

Modes of Transmission

Blood borne

Causes skin and soft tissue infections, and systemic illness



Basis for Failure of Barrier to Infection (Skin)

Epidermal barrier
(Mechanical defects,
punctures, burns, ulcers)

- *Staphylococcus aureus*
- *Candida albicans*
- *Pseudomonas aeruginosa*

Epidermal barrier
(Needle sticks)

- HIV,
- hepatitis viruses

Epidermal barrier
(Arthropod and
animal bites)

- Yellow fever
- Plague
- Lyme disease
- Malaria
- Rabies

Epidermal barrier
(Direct
infection/local
invasion)

- Hookworm
- Strongyloides

Modes of Transmission

Sexual/Body Fluids/Contact with Commensal Organisms

Causes localized urogenital infections



Basis for Failure of Barrier to Infection (Genitourinary Tract)

Urination
(Obstruction,
attachment
and local
proliferation)

- *Escherichia coli*

Commensal vaginal microbes
(Antibiotic use)

- *Candida albicans*

Epithelial barrier
(Microbial attachment and local proliferation)

- *Neisseria gonorrhoea*

Epithelial barrier
(Direct infection local invasion)

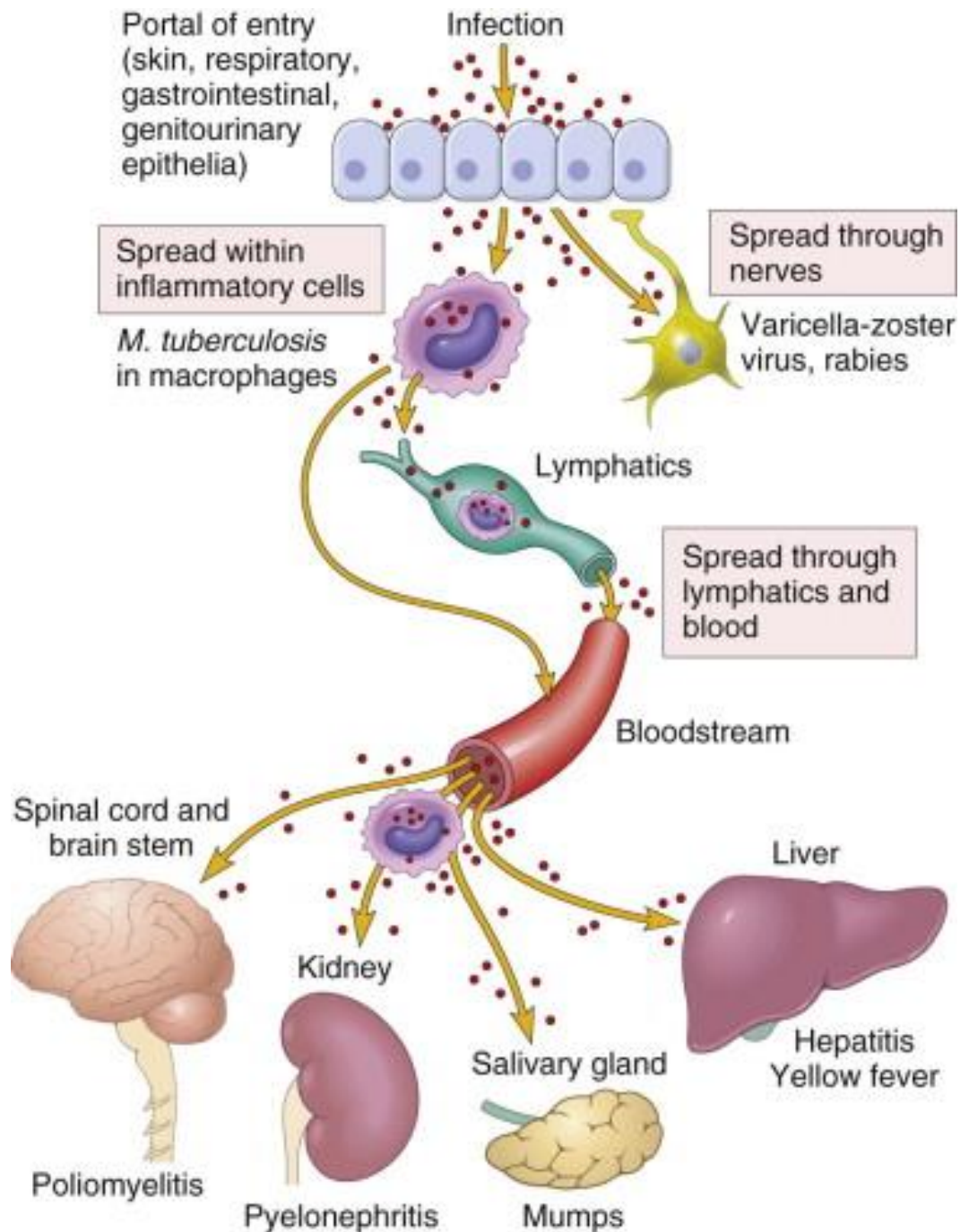
- Herpes viruses
- Syphilis

Epithelial barrier
(Local trauma)

- HPV
- HIV

How do Organisms Cause Disease?

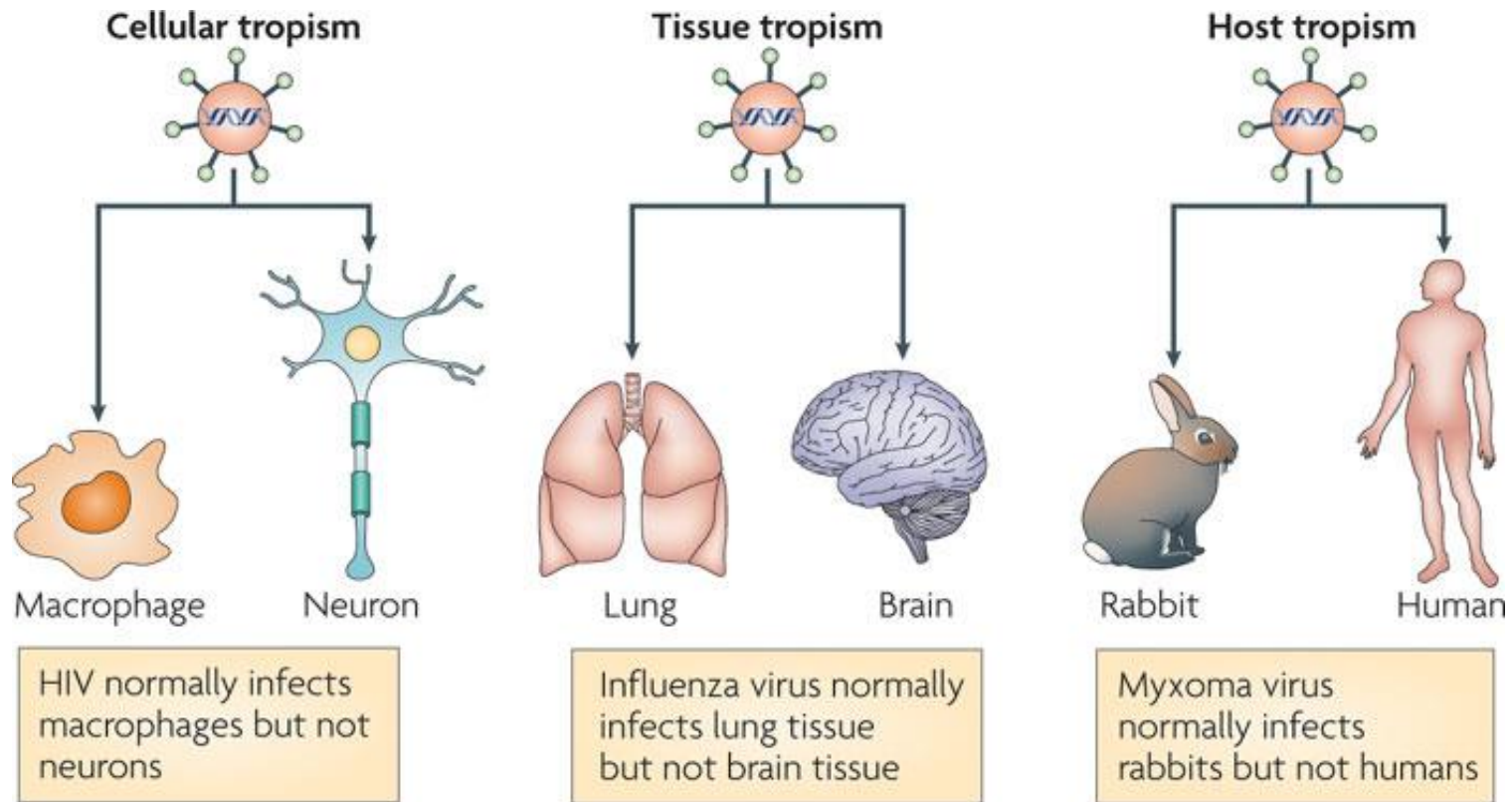
(Which Microbe goes Where, and Why?)



How do Organisms Cause Disease?

Tropism – The specificity of a microbe for a host, host tissue, or cell type

Determined by ligand and receptor binding for the microbe and the host



How do Organisms Cause Disease?

| Virus | Transmission | Tropism | Disease |
|------------|----------------------------------|------------------------------|---|
| Influenza | Respiratory Droplets | Respiratory Epithelial Cells | Upper Respiratory Tract Infections |
| Norovirus | Fecal-Oral | Intestinal Epithelial Cells | Gastroenteritis |
| HIV | Blood, Sexual Contact | Immune Cells | Acquired Immune Deficiency Syndrome |
| Hantavirus | Animal Contact | Vascular endothelium | Hemorrhagic fever with renal syndrome or cardiopulmonary syndrome |
| Ebola | Animal Contact/ Human Contact | Endothelial Cells | Viral Hemorrhagic fever |

Microbe tropism helps determine transmission and disease

What types of Microbes Exist?

4 Main Classes of Infectious Agents

Viruses

HIV (AIDS)
Ebola (Viral hemorrhagic fever)
Hepatitis A,B,C (Hepatitis)
Influenza (The flu)
Adenovirus (Common Cold)

Fungus

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Bacteria

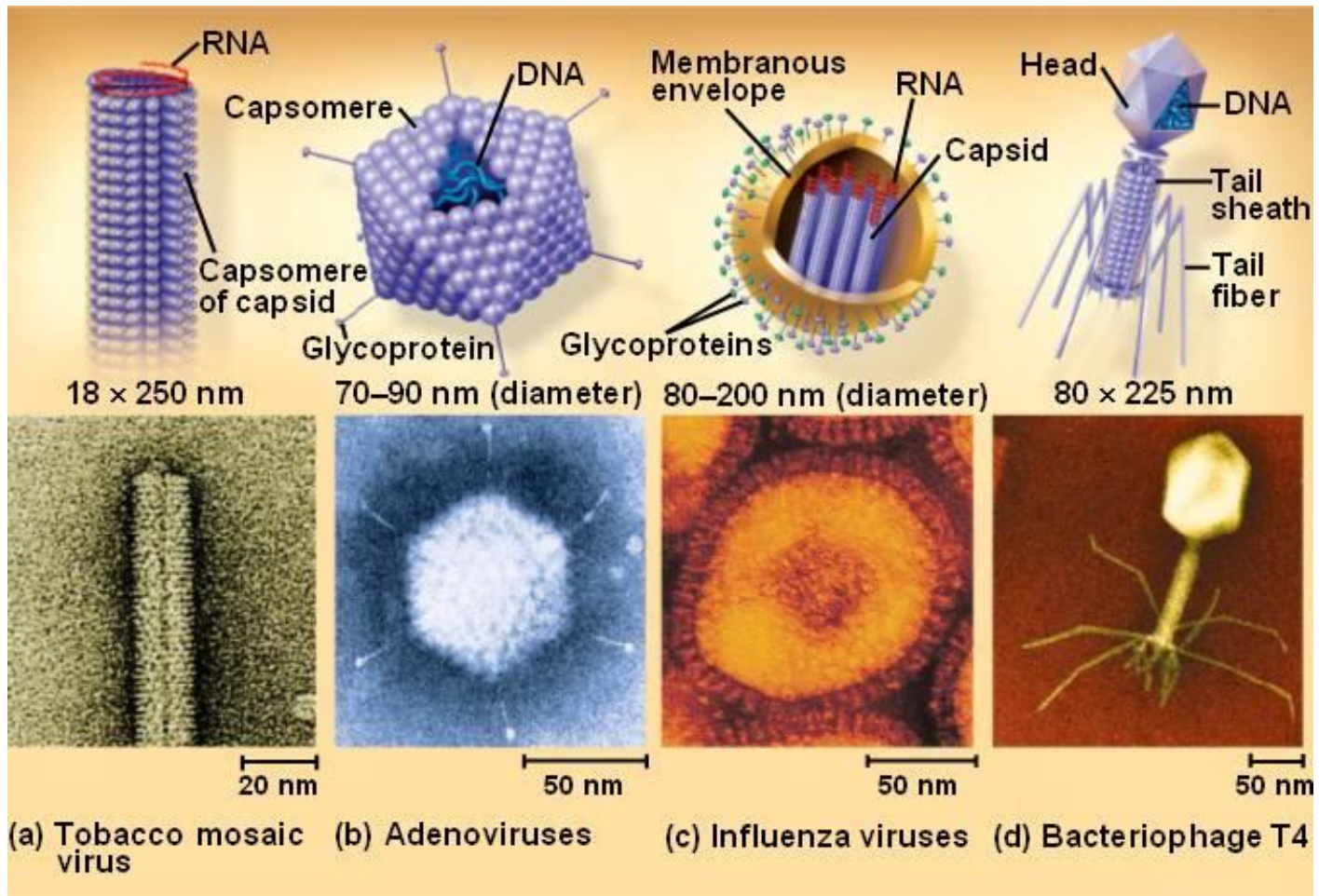
Streptococcus pyogenes (Strep Throat)
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Bordetella pertussis (Whooping Cough)
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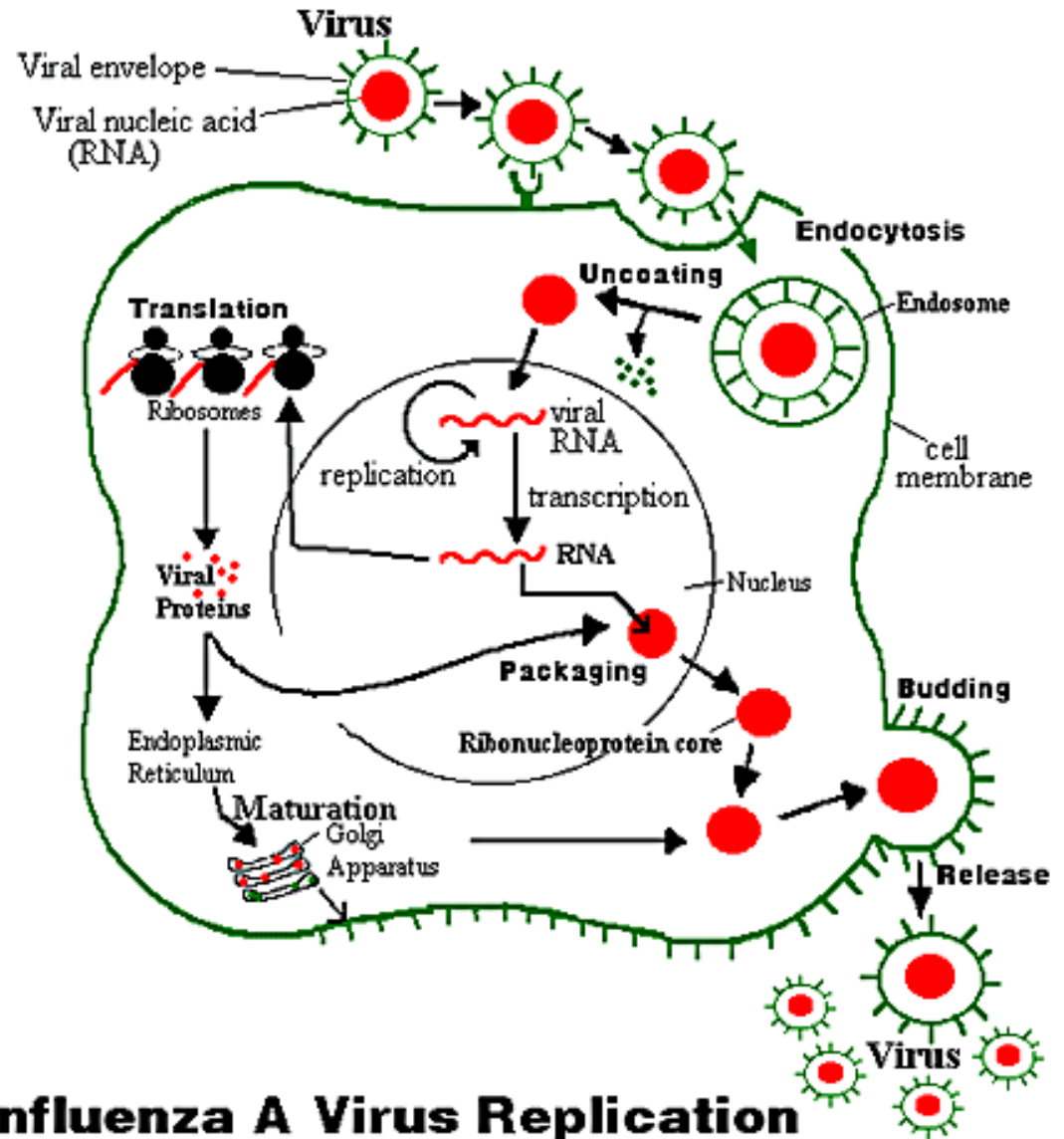
Class #1: Viruses (Structure)

- The smallest infectious agents (20-300 nm)
- Composed of nucleic acid genome surrounded by a protein coat called capsid
- May contain DNA or RNA but not both
- Some viruses are enveloped – Capsid is surrounded by plasma membrane
- Composed of virus specific proteins and nucleic acid



Viral Replication

- Viruses do not have the biological machinery to replicate
- They have to infect cells, and hijack cellular machinery for replication
- Viral replication is damaging to cells



Influenza A Virus Replication

Case Report #1

46 year old woman, fever (103.1) and severe headache

Recent travel to Sierra Leone where as a missionary, she administered health care to sick locals

Liver enzymes (AST and ALT) elevated

Thrombocytopenia and leukopenia

Day 6 - Renal function declined to the point that dialysis was necessary

Day 12 – Hematemesis and melena associated with hemorrhagic gastritis

Day 17 – Scattered petechiae on lower limbs, sacral hematoma, gingival bleeding

Large intracranial hemorrhage

Day 23 – Death

Blood samples from day 7 confirmed to contain Ebola virus by PCR

IgM antibodies specific to Ebola virus also confirmed the diagnosis



Ebola Virus

What class of microbe is causing disease?



Where does the microbe live?



How did the patient contract the disease?



VIRUS EBOLA

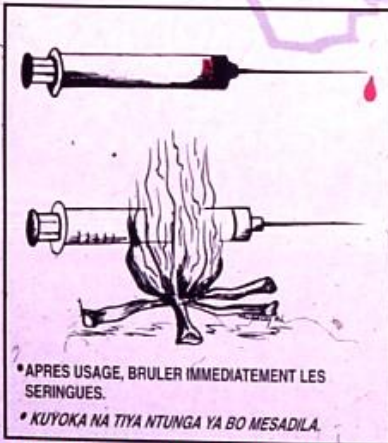
FIEVRE HEMORRAGIQUE DE KIKWIT



- KINSHASA**
- EVITER TOUT CONTACT AVEC LE SANG, LES URINES ET LES VOMIQUES DU MALADE.
 - *BIKA KUSIMBA MENGA, MASUBA NA BILUKA YA MUNTU YA IKELE NA KIMBEVO.*



- INTERDICTION DE LAVER LES CADAVRES.
- *BIKA KUYOMBISA MVUMBI.*



- APRES USAGE, BRULER IMMEDIATEMENT LES SERINGUES.
- *KUYOKA NA TIYA NTUNGA YA BO MESADILA.*



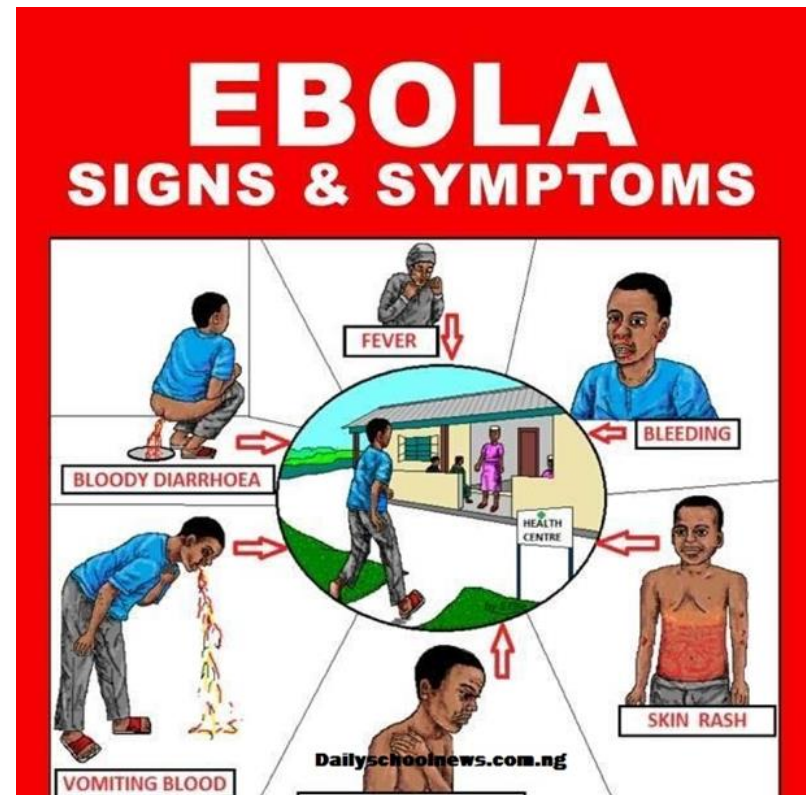
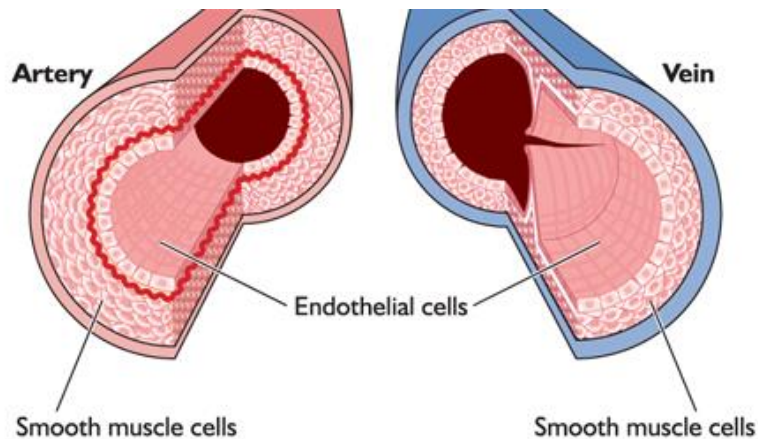
- MANIPULER LES VETEMENTS DES MALADES AVEC DES GANTS ET LES FAIRE BOUILLIR AVANT DE LES LAVER.
- *KUTOKISA NTETE BILELE YA MBEKŌ WA NTWALA YA KUYOBISA YAU.*

UN SEUL CONSEIL: Tout malade doit être dirigé vers l'Hôpital ou un dispensaire

How does Ebola Virus cause Disease?

Viral Hemorrhagic Fever

Ebola virus tropism – Human vascular endothelial cell (blood vessel) infection



Early symptoms – fever, severe headache, muscle pain, weakness, diarrhea, vomiting, unexplained bleeding and bruising, rash

Late symptoms – blood in diarrhea in vomitus, internal hemorrhaging, organ failure

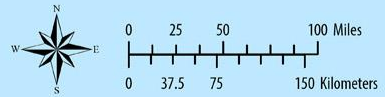


2014 – Ebola virus outbreak

Suspected and Confirmed Case Count: **2473**
Suspected Case Deaths: **1350**
Laboratory Confirmed Cases: **1460**



- Ebola treatment center
- Field laboratory
- National laboratory
- National capital
- No active virus transmission
- Areas with confirmed and probable cases
- Areas reporting suspect cases



Class #2: Bacteria

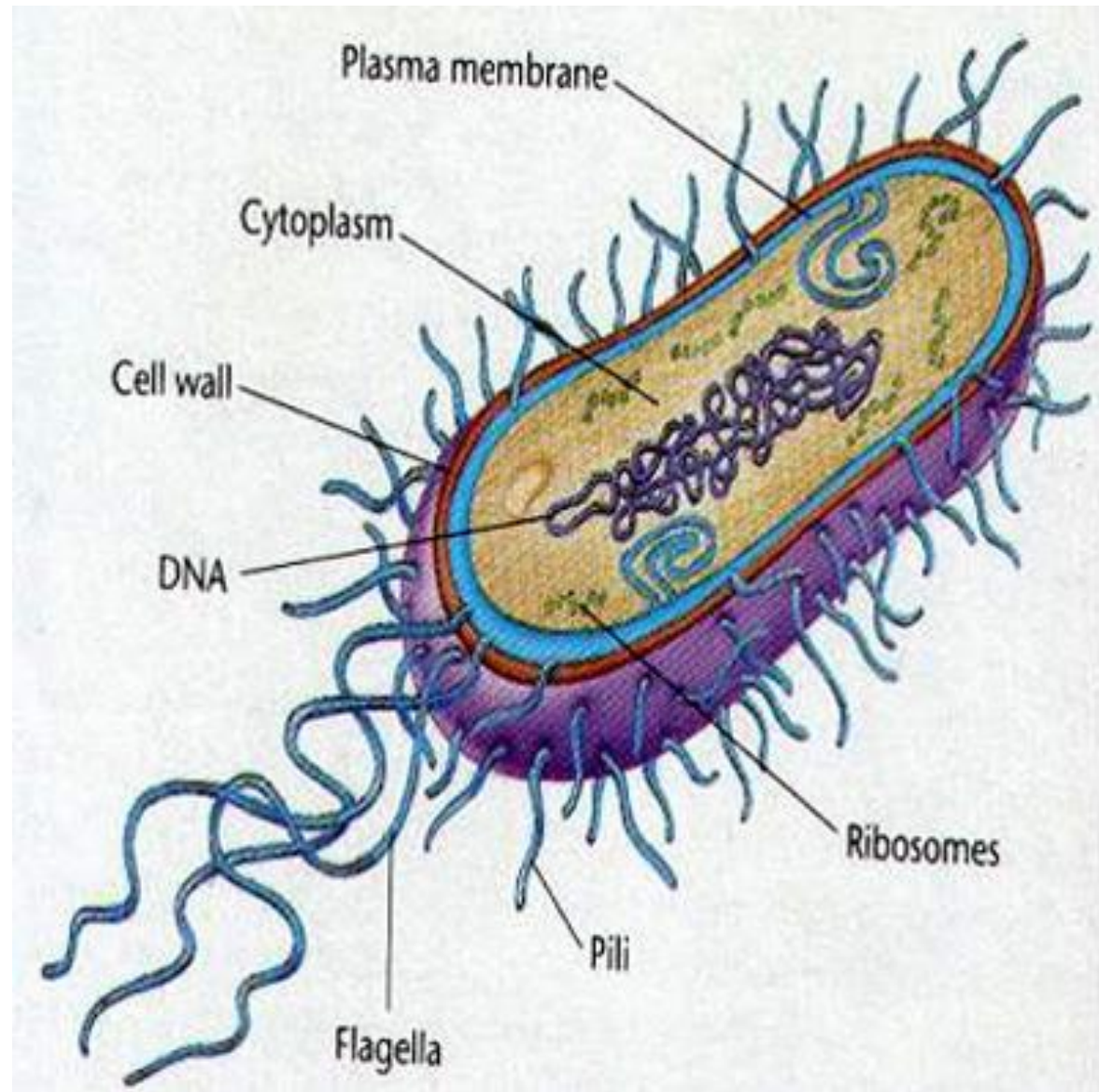
Single Celled prokaryotic organism

Contain both DNA and RNA

Have No Nucleus or any other membrane bound organelles (golgi, mitochondria . . .)

Reproduce by binary fission

Have bacterial specific nucleic acid, proteins, carbohydrates and lipids

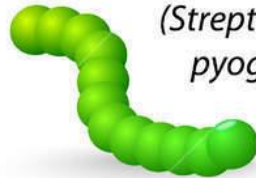


SHAPES OF BACTERIA

COCCI



Diplococci
(*Streptococcus pneumoniae*)



Streptococci
(*Streptococcus pyogenes*)

Tetrad



Staphylococci
(*Staphylococcus aureus*)

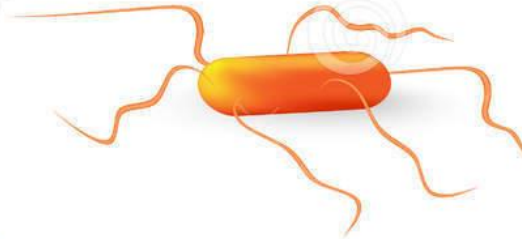


Sarcina
(*Sarcina ventriculi*)

BACILLI



Chain of bacilli
(*Bacillus anthracis*)



Flagellate rods
(*Salmonella typhi*)



Spore-former
(*Clostridium botulinum*)

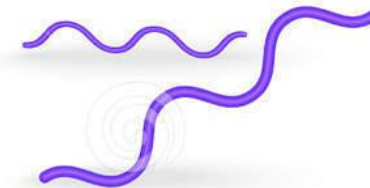
OTHERS



Vibrios
(*Vibrio cholerae*)



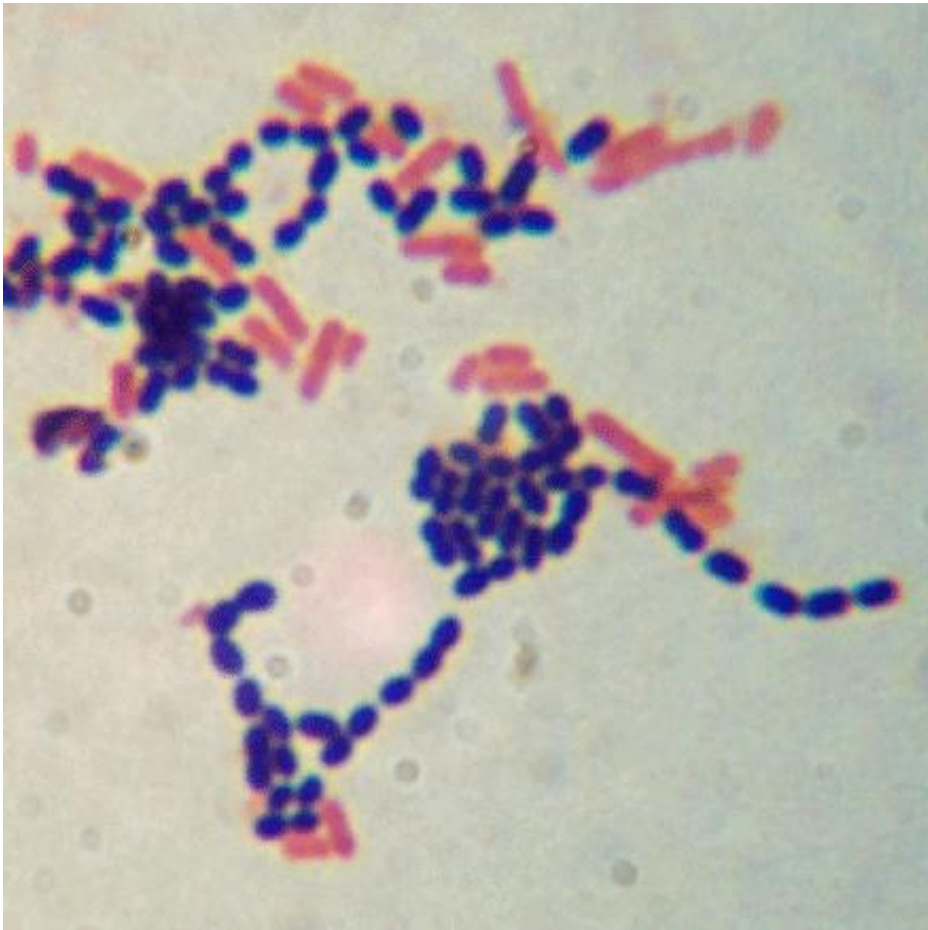
Spirilla
(*Helicobacter pylori*)



Spirochaetes
(*Treponema pallidum*)



Gram Stain



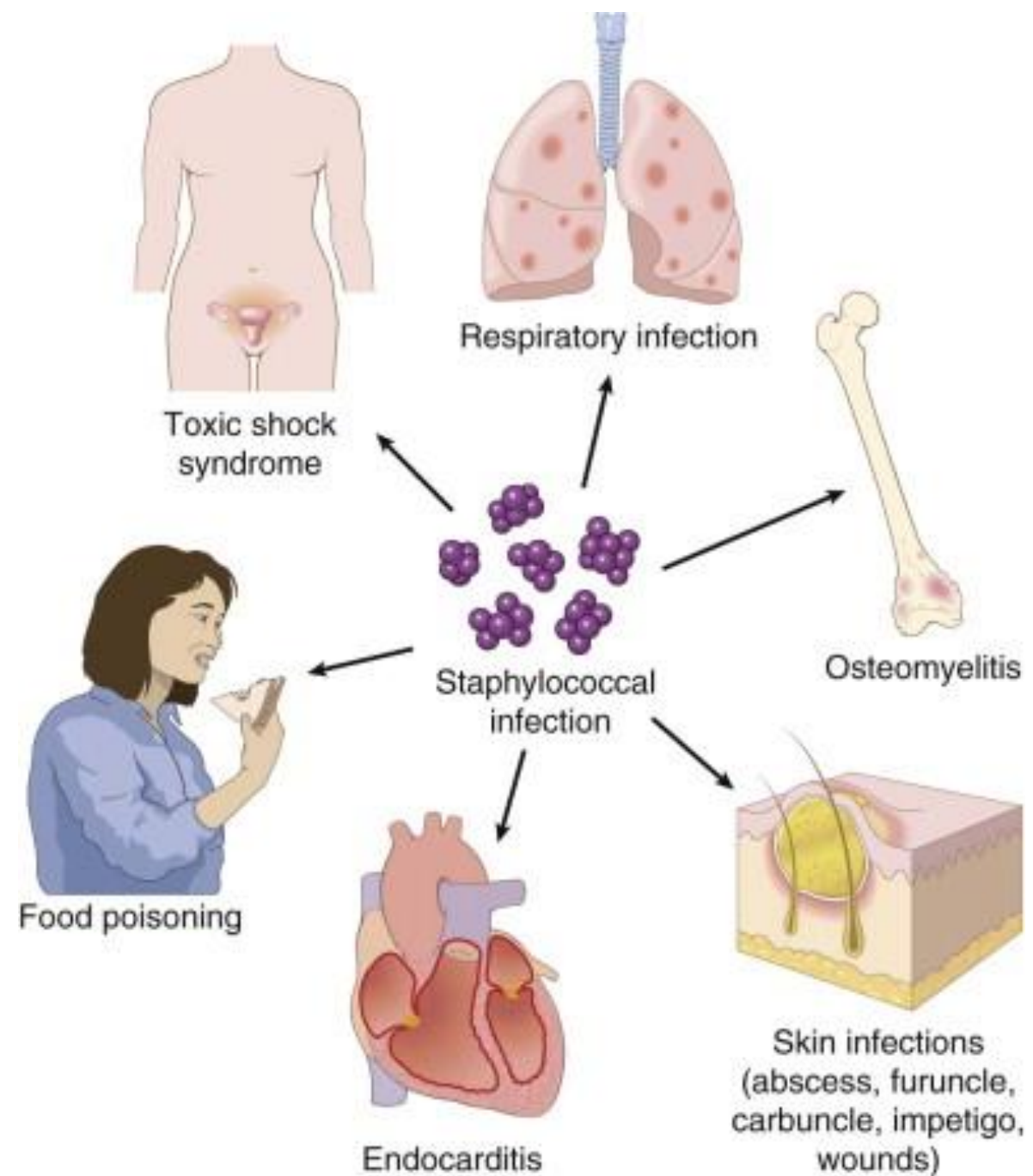
Culture Plate



Some bacteria can cause a wide variety of infections

Tropism for certain bacteria is very broad, can infect a wide variety of cell types

Many bacteria can produce toxins that can also cause disease



Case Report #2

9 yr old, fever 102, severe throat pain

Difficulty swallowing

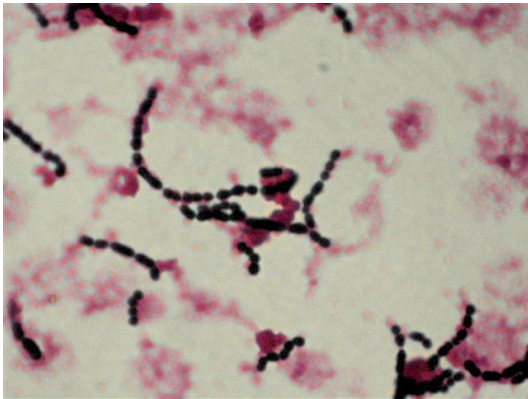
Red swollen tonsils with white patches

Lab cultures *Streptococcus pyogenes* (Group A strep) from a swab of the throat

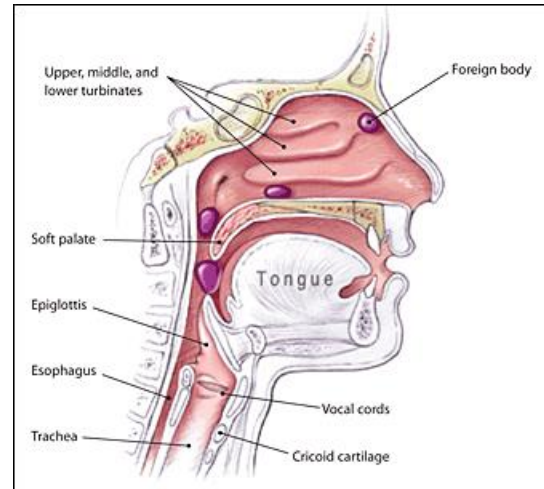


Strep Throat

What class of microbe is causing disease?



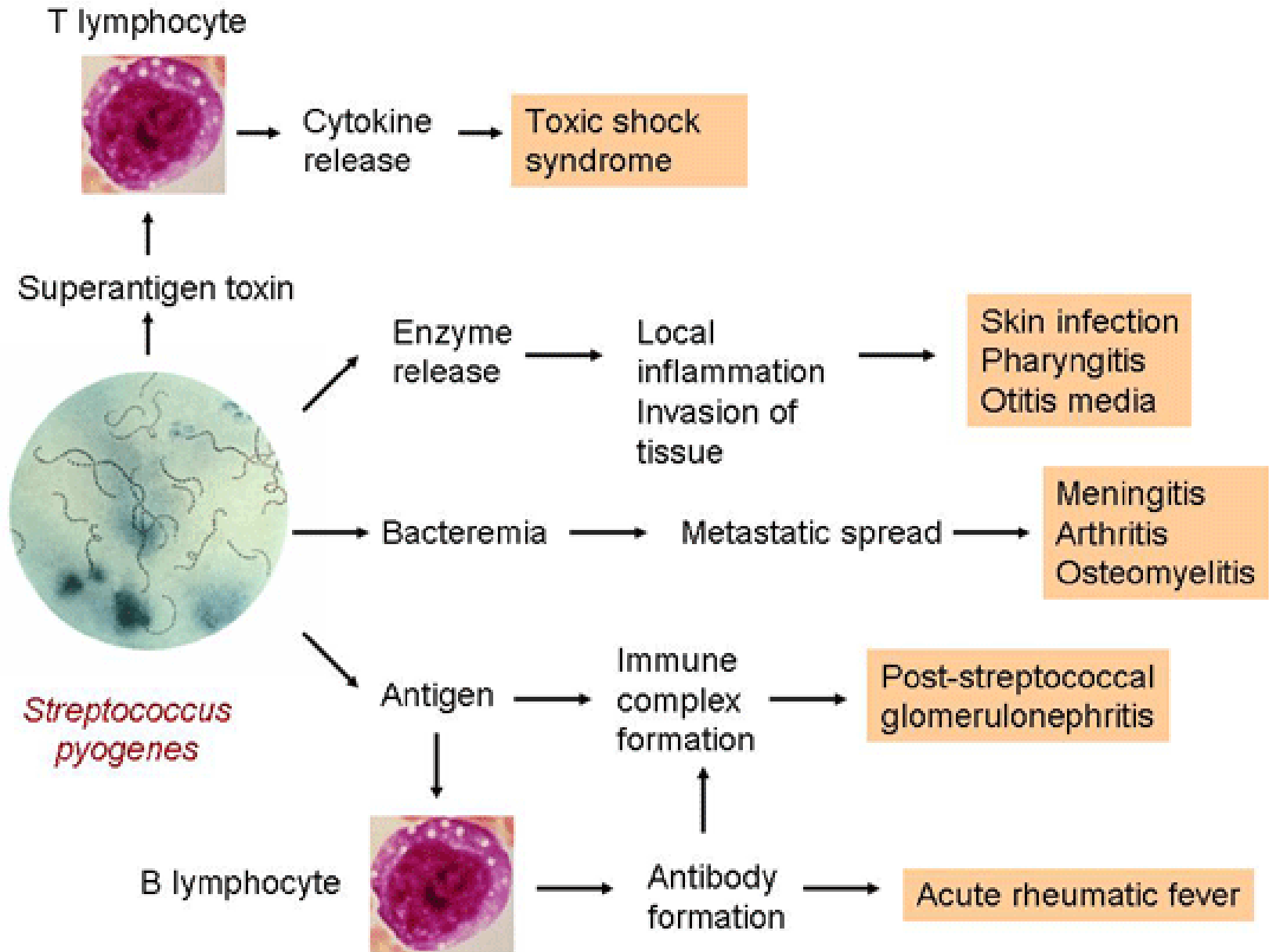
Where does the microbe live?



How did the patient contract the disease?



How Does Group A Strep cause disease?



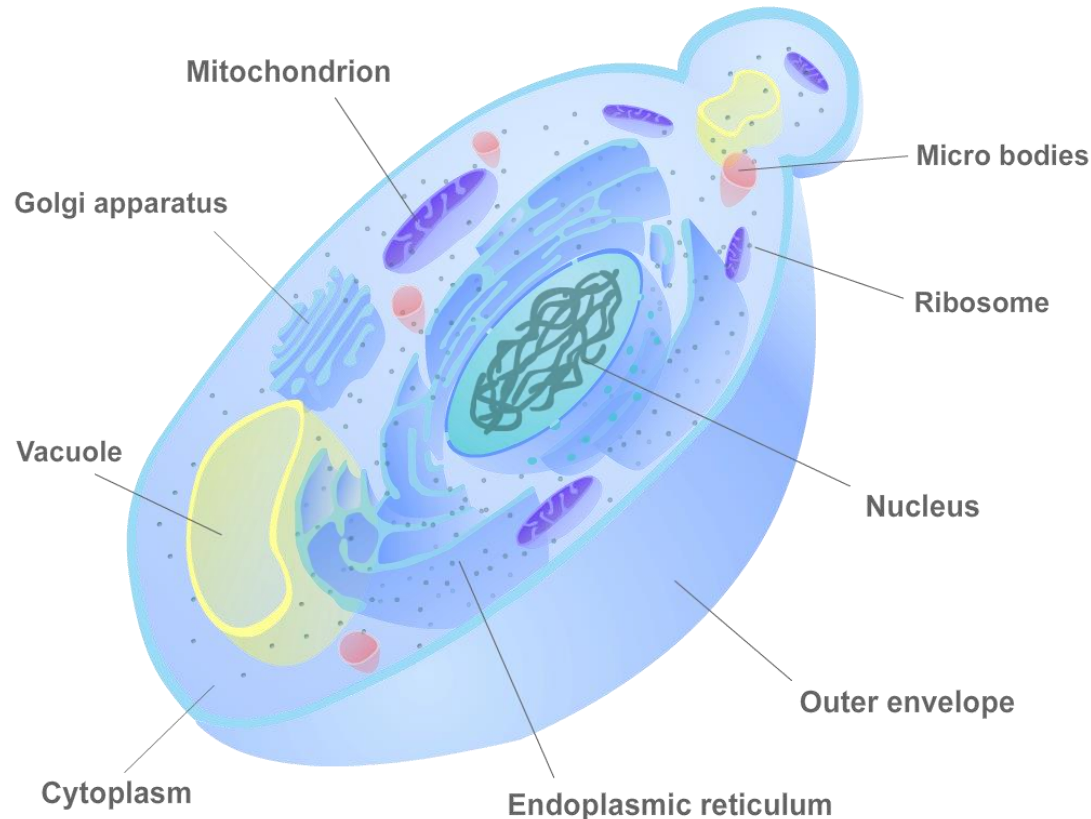
Class #3: Fungus

Eukaryotic organism

Contains a cell wall, nucleus and membrane bound organelles

Both DNA and RNA

Has fungus specific nucleic acid, proteins, carbohydrates and lipids

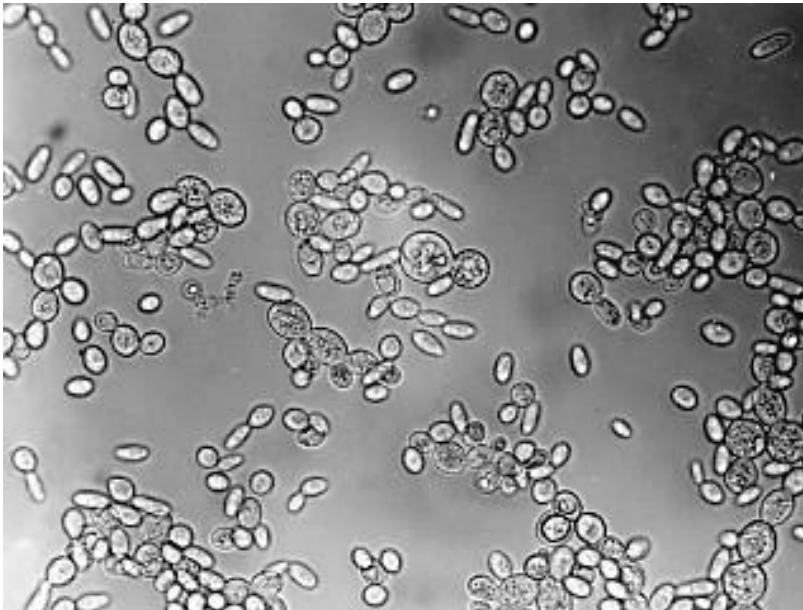


Two types of Fungus

Yeast (single celled)

Asexual reproduction by blastoconidia formation (budding)

Sexual reproduction by production of ascospores or basidiospores.



Molds (multicellular)

Hyphae- tubelike, basic structure

Mycelium- intertwined hyphae



Yeast

A soft, pasty, smooth colony; usually no filamentous (fuzzy) growth can be observed macroscopically

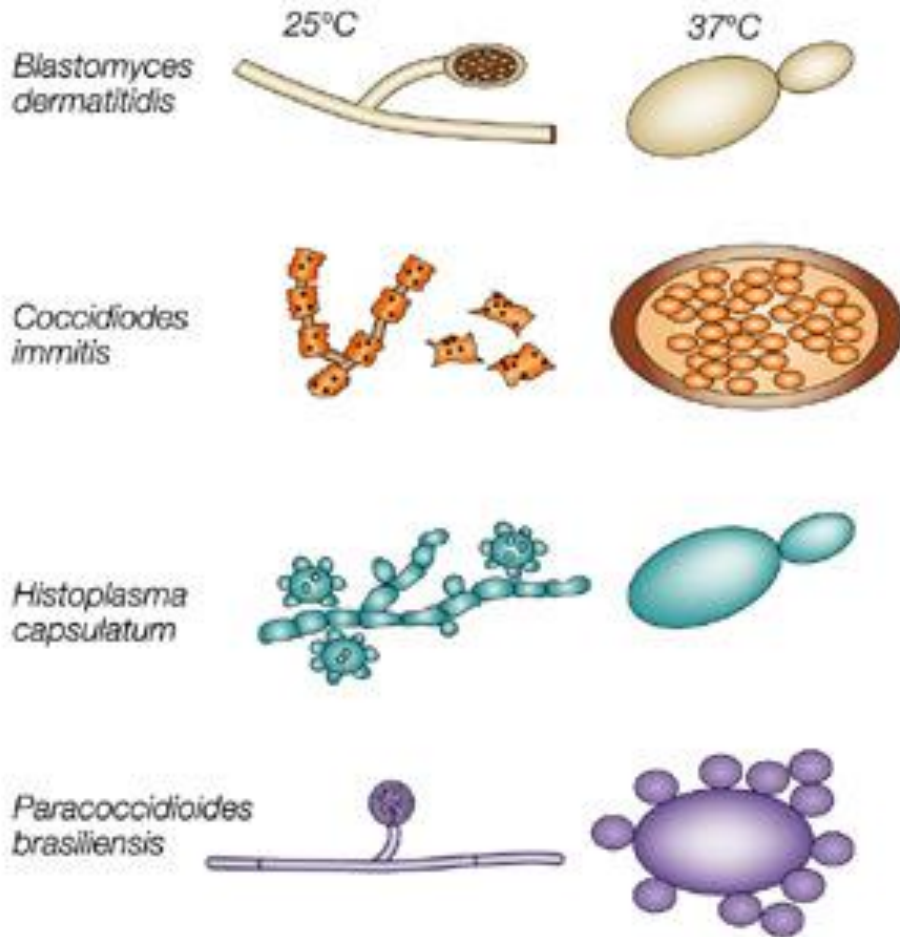


Mold

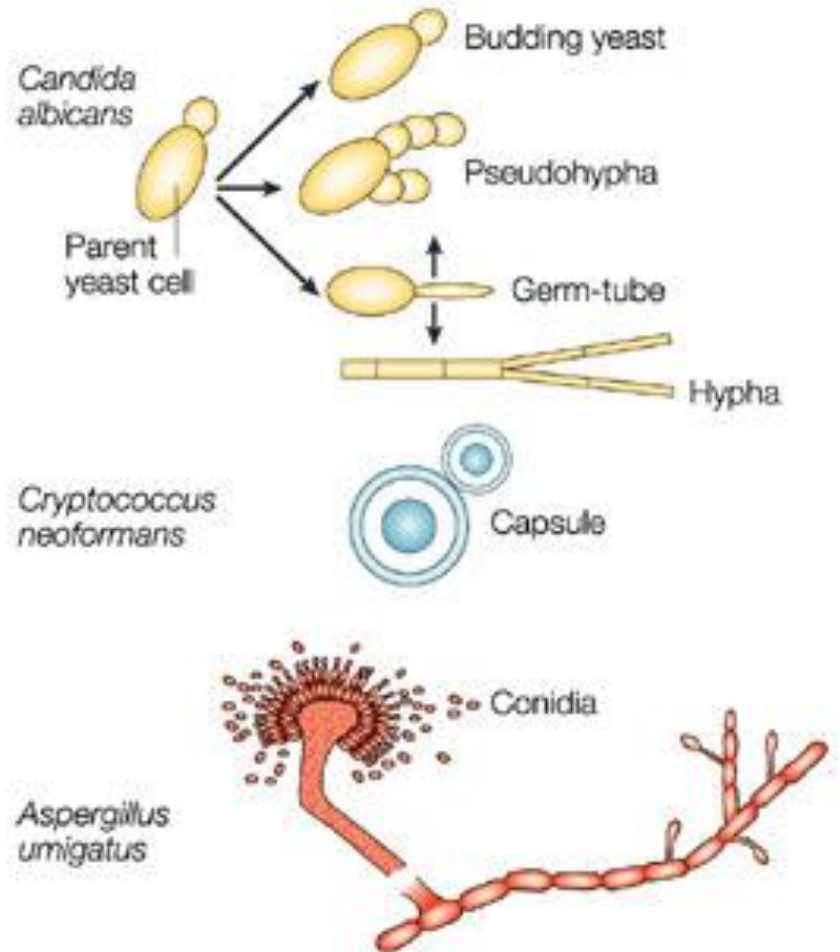
A filamentous fungus: fuzzy, powdery, woolly, velvety, or relatively smooth.



Dimorphic fungi



Opportunistic fungi



Case Report #3

22 yr old man, itchy, cracking, red feet, especially between the toes

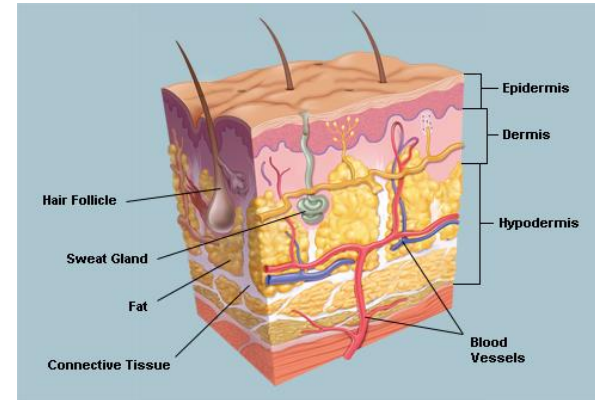
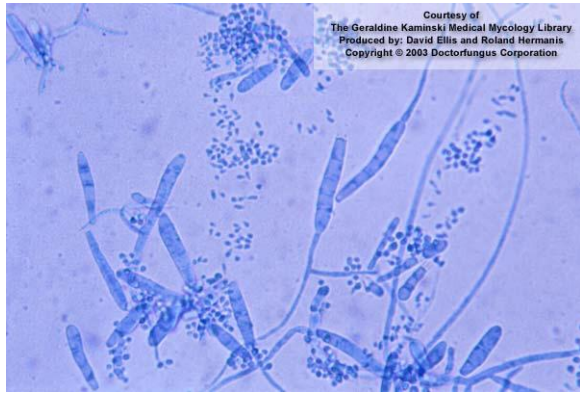
Lab cultures *Trichophyton* (Dermatophyte) from foot culture



Dermatophyte

What class of microbe is causing disease?

Where does the microbe live?



How did the patient contract the disease?



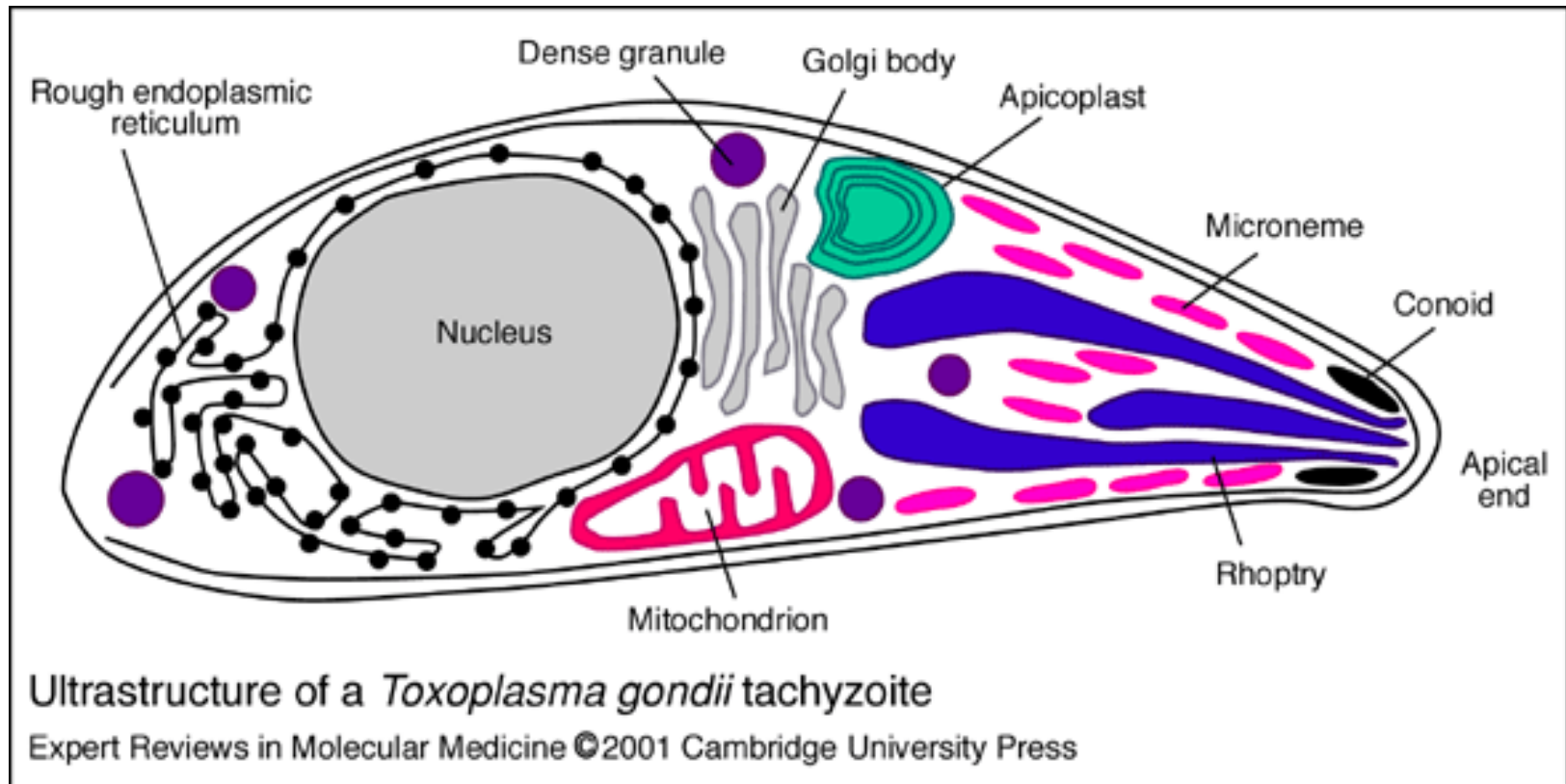
Class #4: Parasites

Eukaryotic organisms

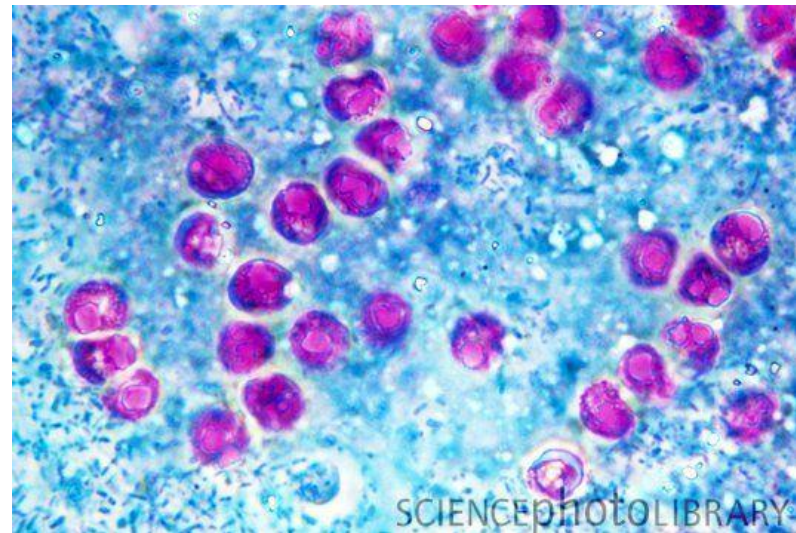
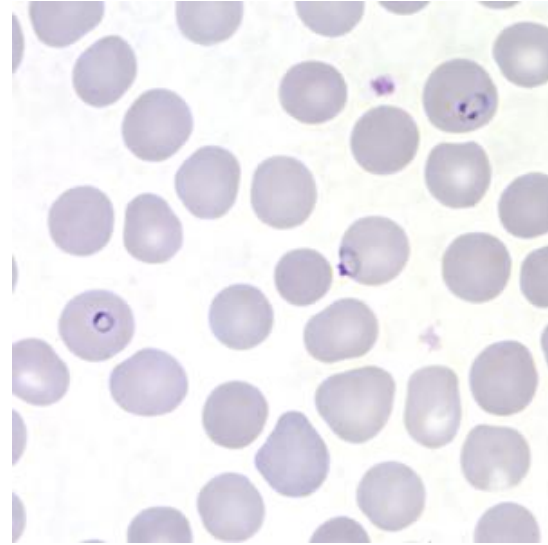
Contains a cell membrane, nucleus and membrane bound organelles

Both DNA and RNA

Has parasite specific nucleic acid, proteins, carbohydrates and lipids

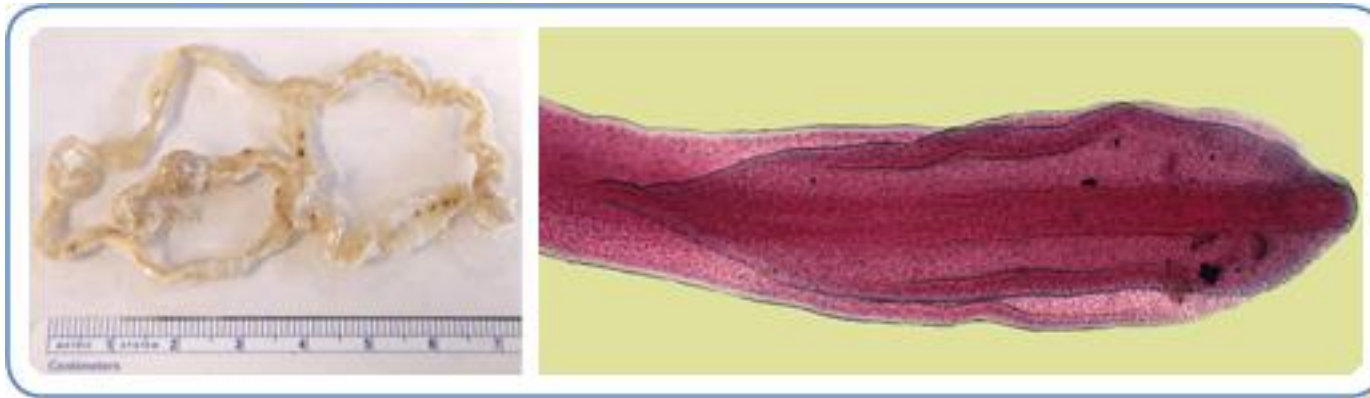


Single Celled Parasites



Large Complex Multicellular parasites

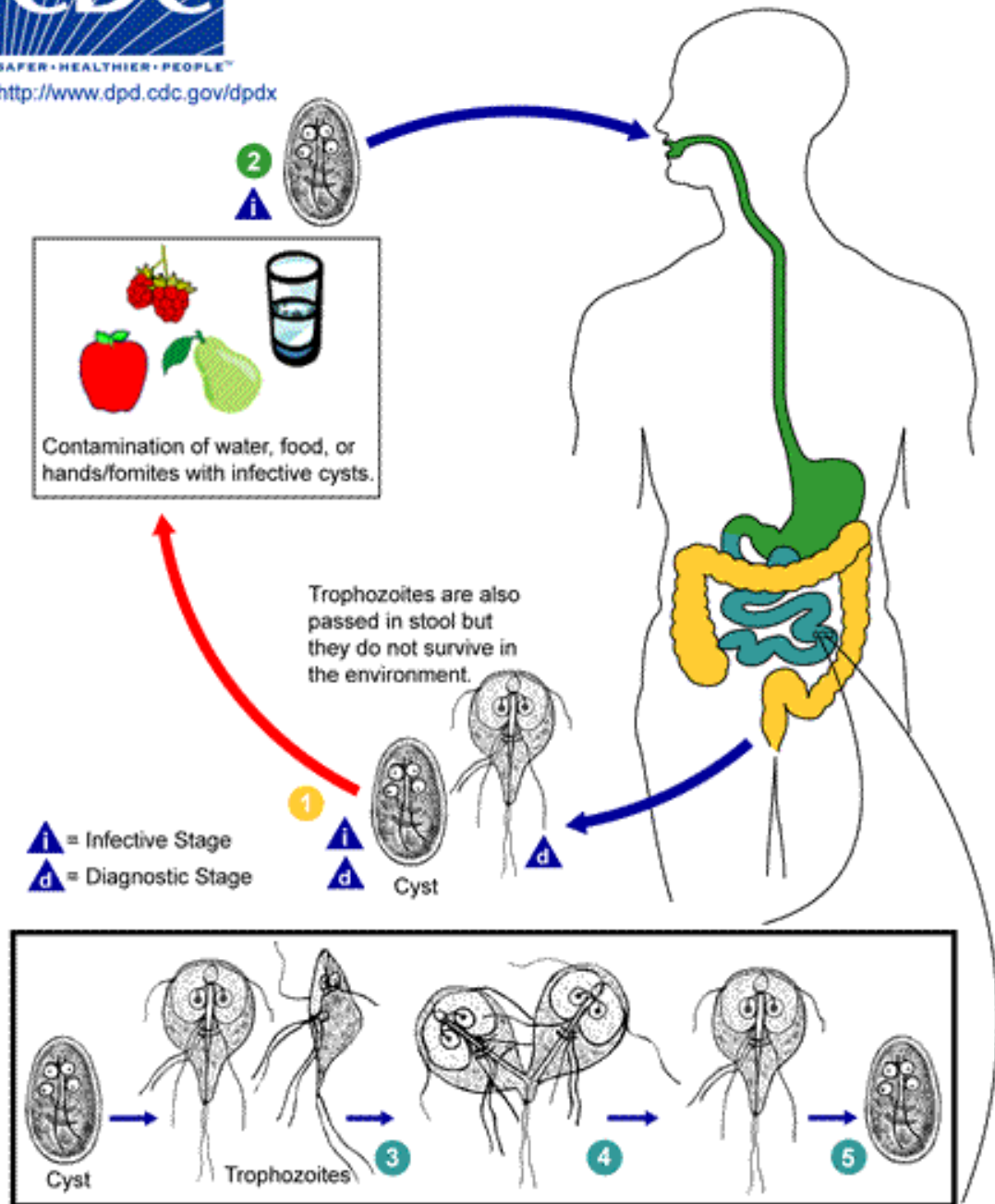
Well Defined organs and tissues (GI Tract, Genital Tract)



Parasites have complex life cycles

Infective Stage: Cyst or egg that can survive a harsh environment

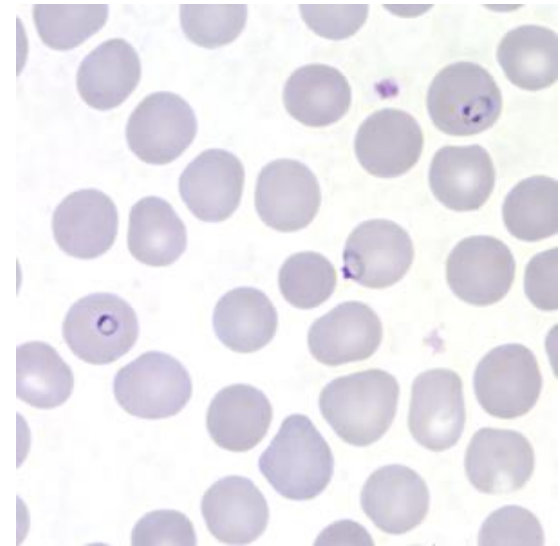
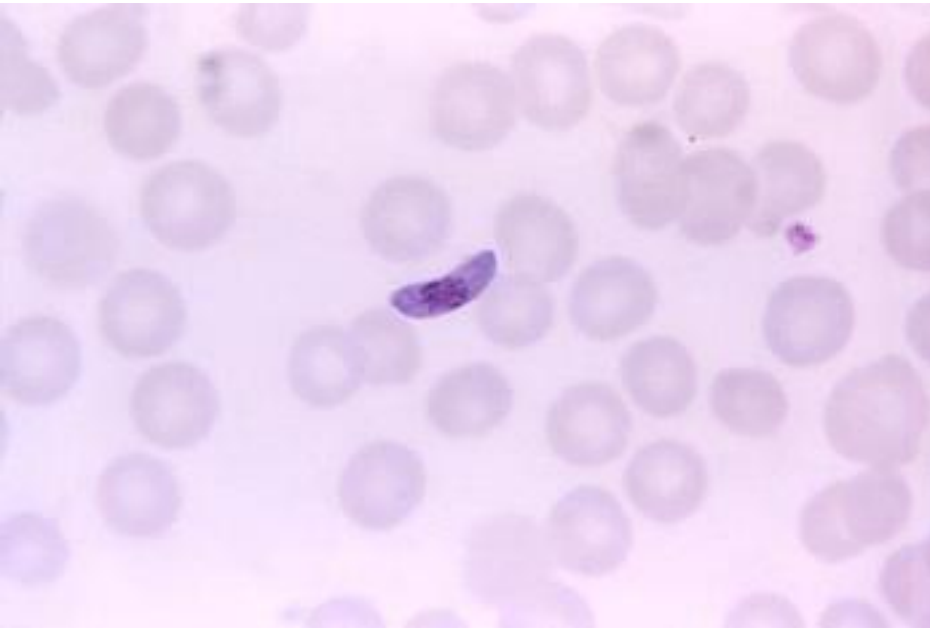
Diagnostic Stage: Trophozoite or worm that causes disease



Case Report #4

23 year old man, fever (103.1) , headaches, body aches

Recent travel to the Ivory Coast for vacation



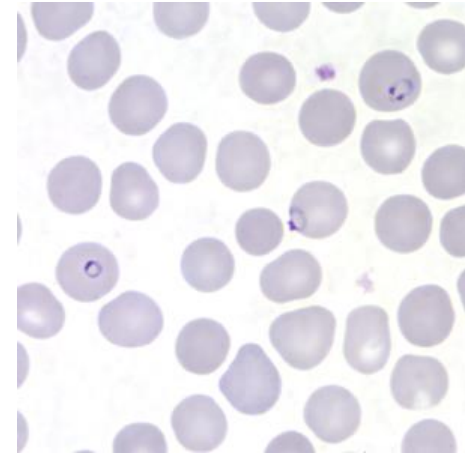
Microscopic Blood Smear analysis revealed *Plasmodium falciparum*

Malaria

What class of microbe is causing disease?



Where does the microbe live?

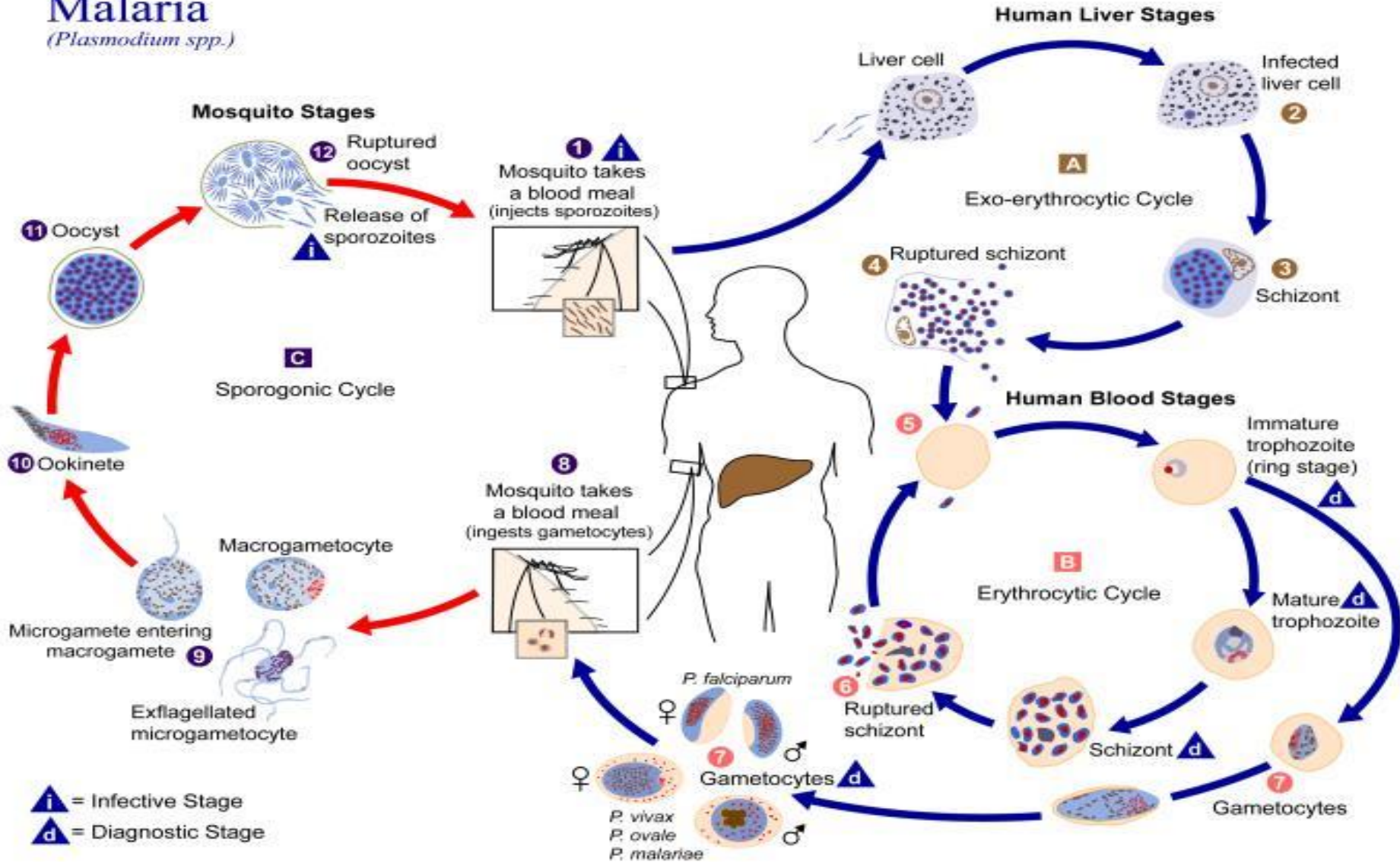


How did the patient contract the disease?



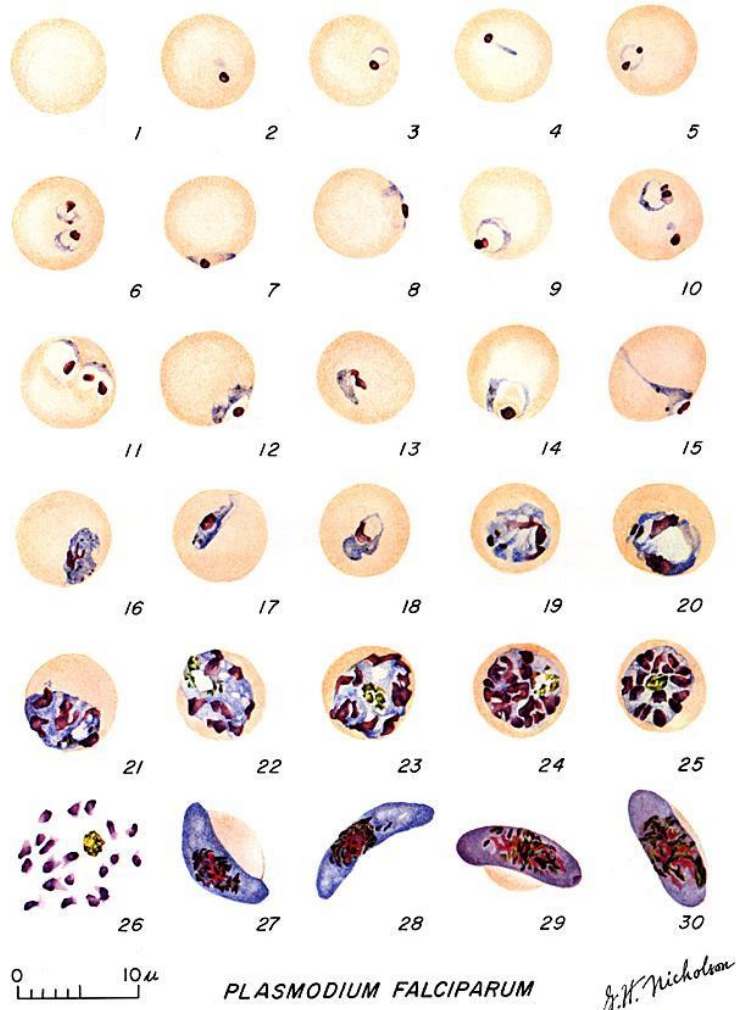
Plasmodium Life Cycle

Malaria (*Plasmodium spp.*)

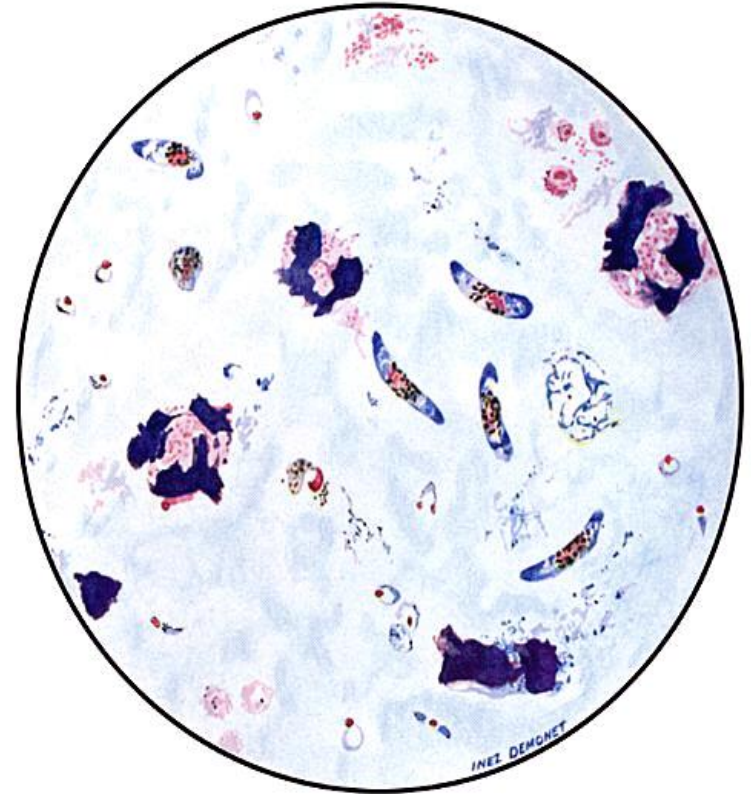


Plasmodium falciparum

Thin smear:



Thick smear:



Key characteristics on blood smear

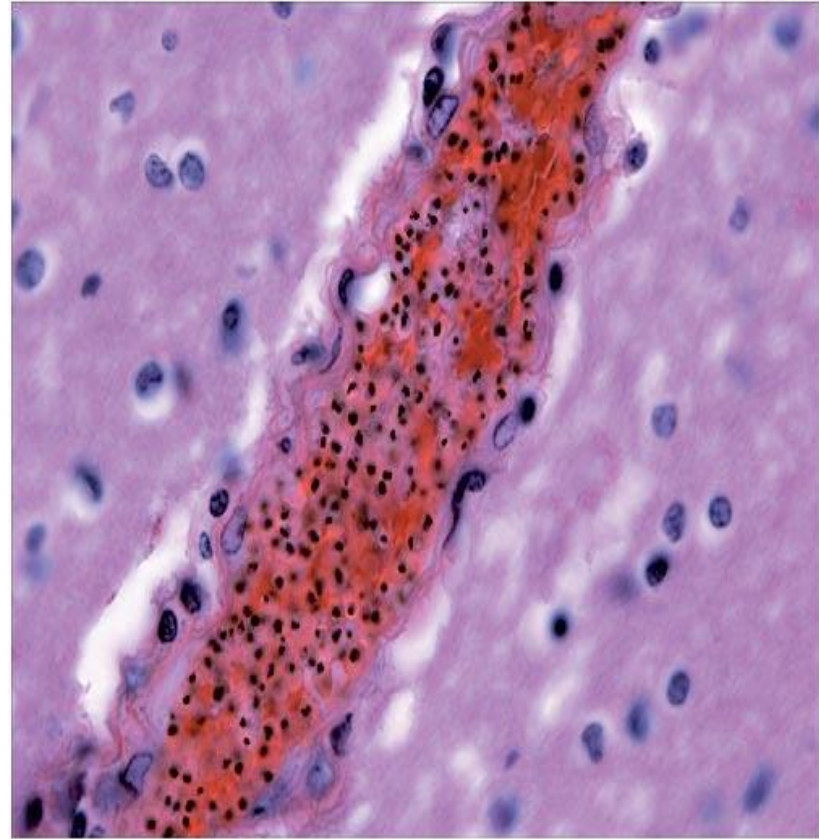
6: Multiple delicate rings per RBC

7, 8, 12: Accolé forms (on the margin of RBC)

27-30: Crescent-shaped gametocytes

Complications of *falciparum* Malaria

- Ischemia caused by the plugging of vessels in the internal organs
 - Cerebral Malaria
 - Blackwater Fever and kidney failure
 - Disseminated Intravascular Coagulation
 - Multi-organ failure



P. falciparum-parasitized blood cells plugging the capillaries in brain tissue

Relative level above detection

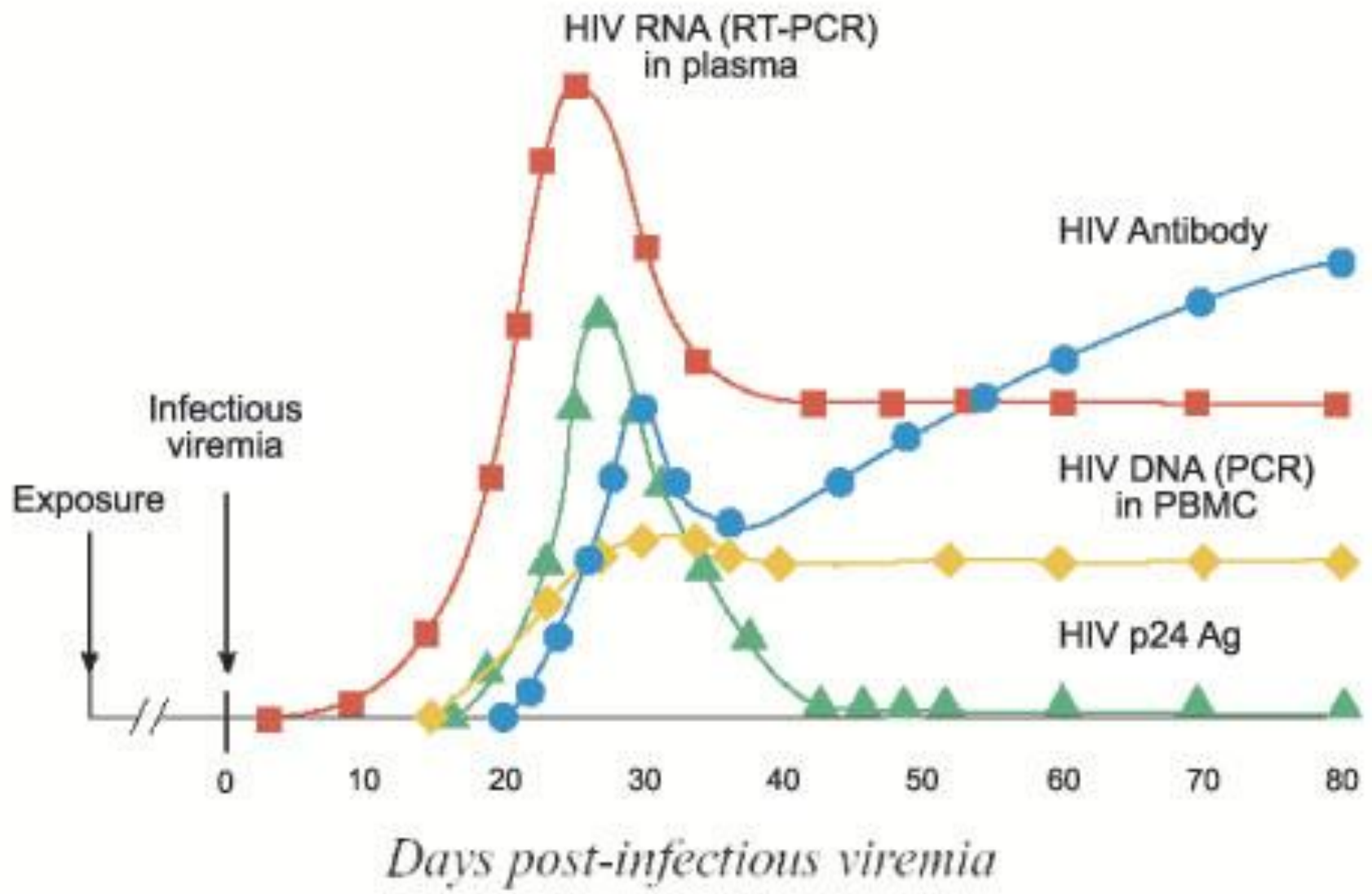
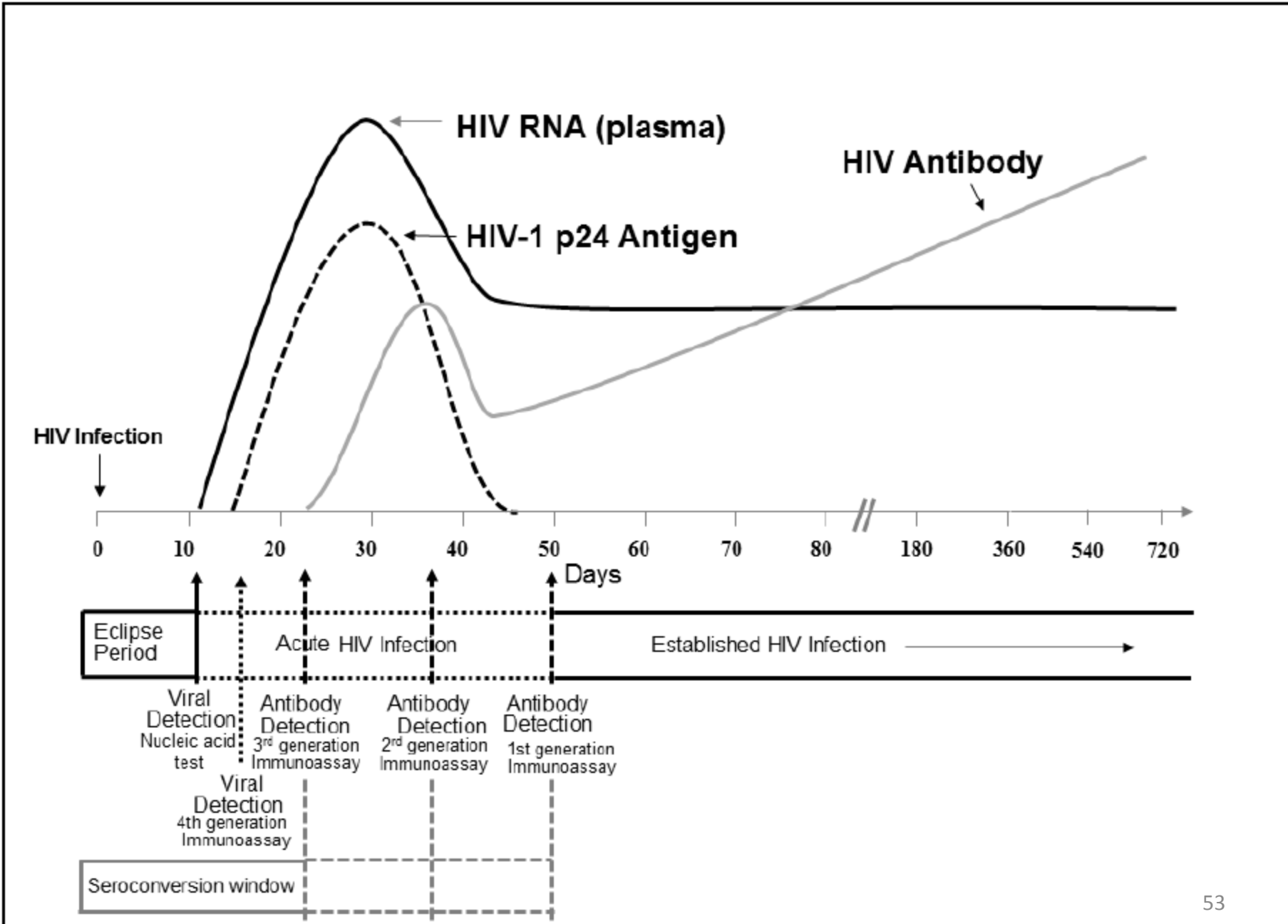


Figure 1. Sequence of appearance of laboratory markers for HIV-1 infection



Evolution of HIV Immunoassay Technology

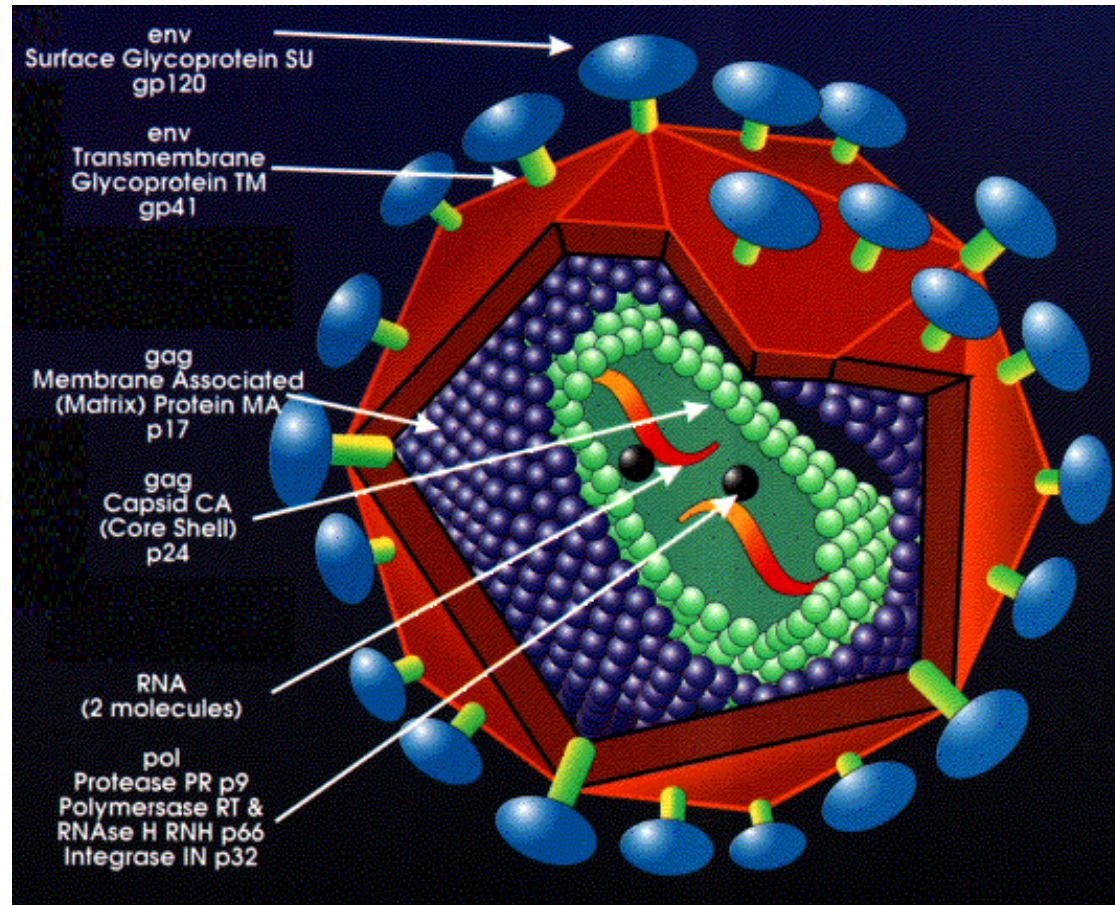
- **1st generation:** All antigens used to bind HIV antibodies are from a lysate of HIV-1 viruses grown in cell culture. Significant specimen dilution is required to overcome cross-reactivity with cellular protein contaminants. Examples: HIV-1 Western blot and HIV-1 IFA.
- **2nd generation:** Synthetic peptide or recombinant protein antigens alone or combined with viral lysates are used to bind HIV antibodies. Improves sensitivity for HIV-1 group O and HIV-2; improves specificity by eliminating cross-reactivity with cellular proteins. Examples: HIV-1 EIA and rapid HIV antibody tests.
- **3rd generation:** Synthetic peptide or recombinant protein antigens are used to bind HIV antibodies in an immunometric antigen sandwich format. Allows detection of IgM and IgG antibodies. Increase sensitivity during early seroconversion. Examples: HIV-1/HIV-2 EIA and HIV-1/HIV-2 chemiluminescent immunoassays.
- **4th generation “combo” assays:** Same as 3rd generation assays but include monoclonal antibodies to detect **p24 antigen**. Allows detection of HIV-1 infection before seroconversion. Examples: HIV-1/HIV-2 enzyme immunoassay, HIV-1/HIV-2 chemiluminescent immunoassay, and HIV-1/HIV-2 rapid test.

Evolution of HIV Immunoassay Technology

| Generation | 1st | | 2nd | 3rd | 4th | |
|-----------------------------------|--|--|---|--|--|--|
| Antigen | [Diagram: Two Y-shaped antibodies (green and blue) bound to a purple particle] | | [Diagram: One Y-shaped antibody (green and blue) bound to a purple particle] | [Diagram: One Y-shaped antibody (green and blue) bound to a purple particle] | [Diagram: Two Y-shaped antibodies (green and blue) bound to a purple particle] | |
| Sample | [Diagram: Two Y-shaped antibodies (green and blue) bound to a purple particle] | | [Diagram: One Y-shaped antibody (green and blue) bound to a purple particle] | [Diagram: One Y-shaped antibody (green and blue) bound to a purple particle] | [Diagram: Two Y-shaped antibodies (green and blue) bound to a purple particle] | |
| Conjugate | [Diagram: Two Y-shaped antibodies (green and blue) bound to a purple particle] | | [Diagram: One Y-shaped antibody (green and blue) bound to a purple particle] | [Diagram: One Y-shaped antibody (green and blue) bound to a purple particle] | [Diagram: Two Y-shaped antibodies (green and blue) bound to a purple particle] | |
| Signal | [Diagram: Two Y-shaped antibodies (green and blue) bound to a purple particle] | | [Diagram: One Y-shaped antibody (green and blue) bound to a purple particle] | [Diagram: One Y-shaped antibody (green and blue) bound to a purple particle] | [Diagram: Two Y-shaped antibodies (green and blue) bound to a purple particle] | |
| Antigen | Lysate | | | Recombinant & synthetic | | |
| Specificity | 95–98% | | >99% | >99.5% | | |
| Sensitivity | 99% | | >99.5% | >99.5% | | |
| Window period | 8–10 weeks | | 4–6 weeks | 2–3 weeks | | |
| Immunoglobulin class detection | IgG | | IgG | All | | |
| Approximate year of first release | 1985 | | 1987 | 1991 | | |
| Platforms | Plate assays Particle agglutination | | Plate assays Automated generic platforms Particle agglutination Rapid assays | Plate assays Dedicated instruments Rapid assays | | |
| | | | | Plate assays Dedicated instruments Rapid assays in development | | |

Structural genes

- Gag is p55 from which three core proteins (p15, p17 and p24) are formed
- Env gene codes for envelope proteins gp160, gp120 and gp41
- Pol codes for p66 and p51 subunits of reverse transcriptase and p31 an endonuclease

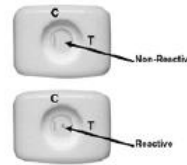


Old HIV Diagnostic Algorithm

1. Screen

immunoassay (EIA/CIA)

rapid tests

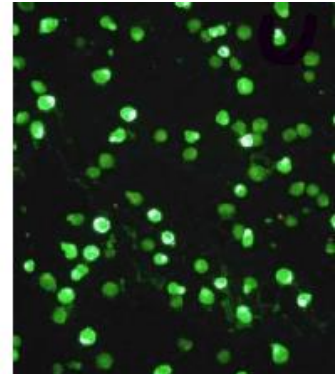
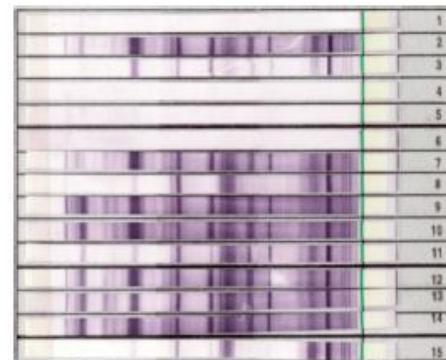


2. Confirm

Western Blot (98%)

IFA

APTIMA qualitative NAAT



Laboratory diagnosis of HIV infection

ELISA Testing

- first serological test developed to detect HIV infection
- antibodies detected include those directed against p24, gp120, gp160 and gp41, detected first in infection and appear in most individuals
- used for screening only, false positives do occur (recent acute illness, allergies)
- highly sensitive, not specific

Laboratory diagnosis of HIV infection

Western Blot Testing

- most popular confirmatory test
- antibodies to p24 and p55 appear earliest but decrease or become undetectable
- antibodies to gp31, gp41, gp120, and gp160 appear later but are present throughout all stages of the disease

Western Blot Testing = interpretation of result

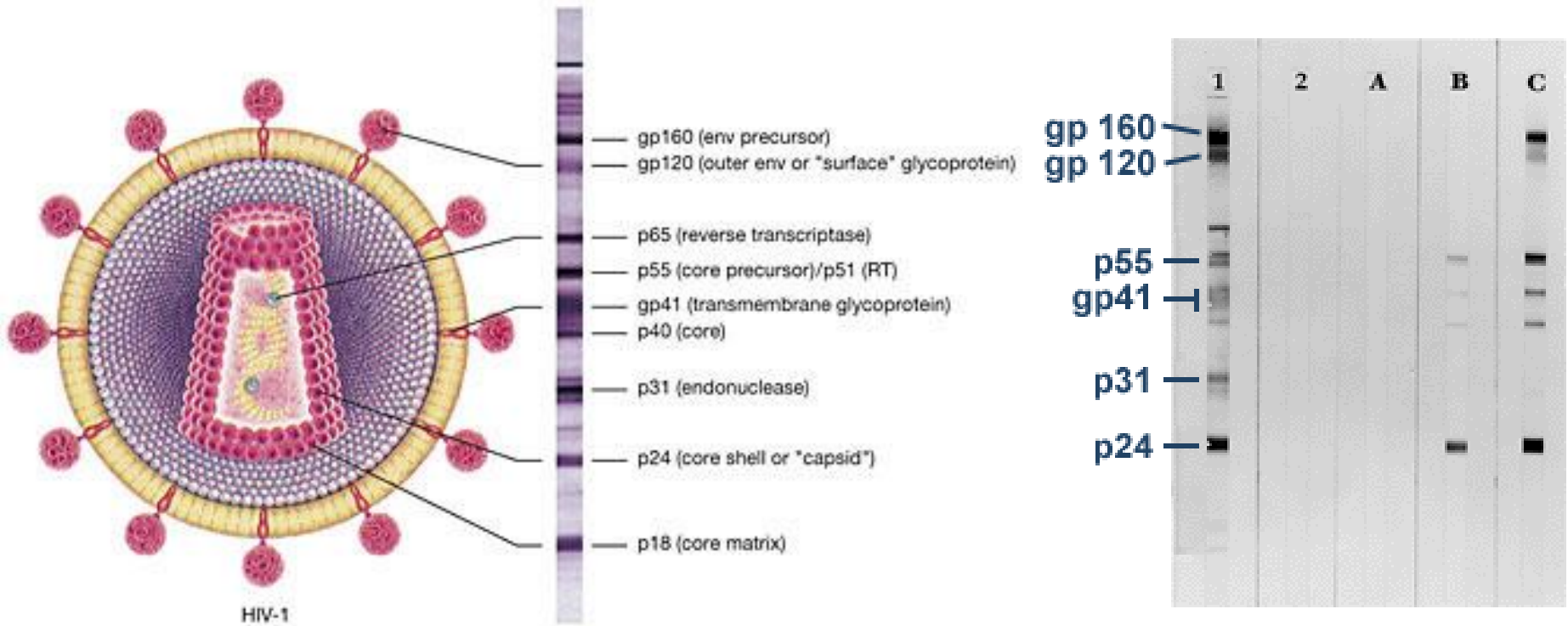
- no bands, negative
- in order to be interpreted as positive a minimum of 3 bands directed against the following antigens must be present : p24, p31, gp41 or gp120/160
- CDC criteria require 2 bands of the following : p24, gp41 or gp120/160

Laboratory diagnosis of HIV infection

Western Blot Testing = interpretation of result

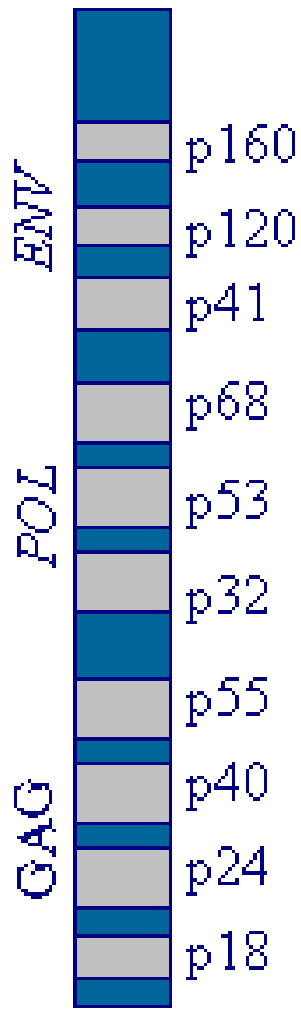
- indeterminate results are those samples that produce bands but not enough to be positive, may be due to the following:
 1. prior blood transfusions, even with non-HIV-1 infected blood
 2. prior or current infection with syphilis
 3. prior or current infection with malaria
 4. autoimmune diseases
 5. infection with other human retroviruses
 6. second or subsequent pregnancies in women

*** run an alternate HIV confirmatory assay



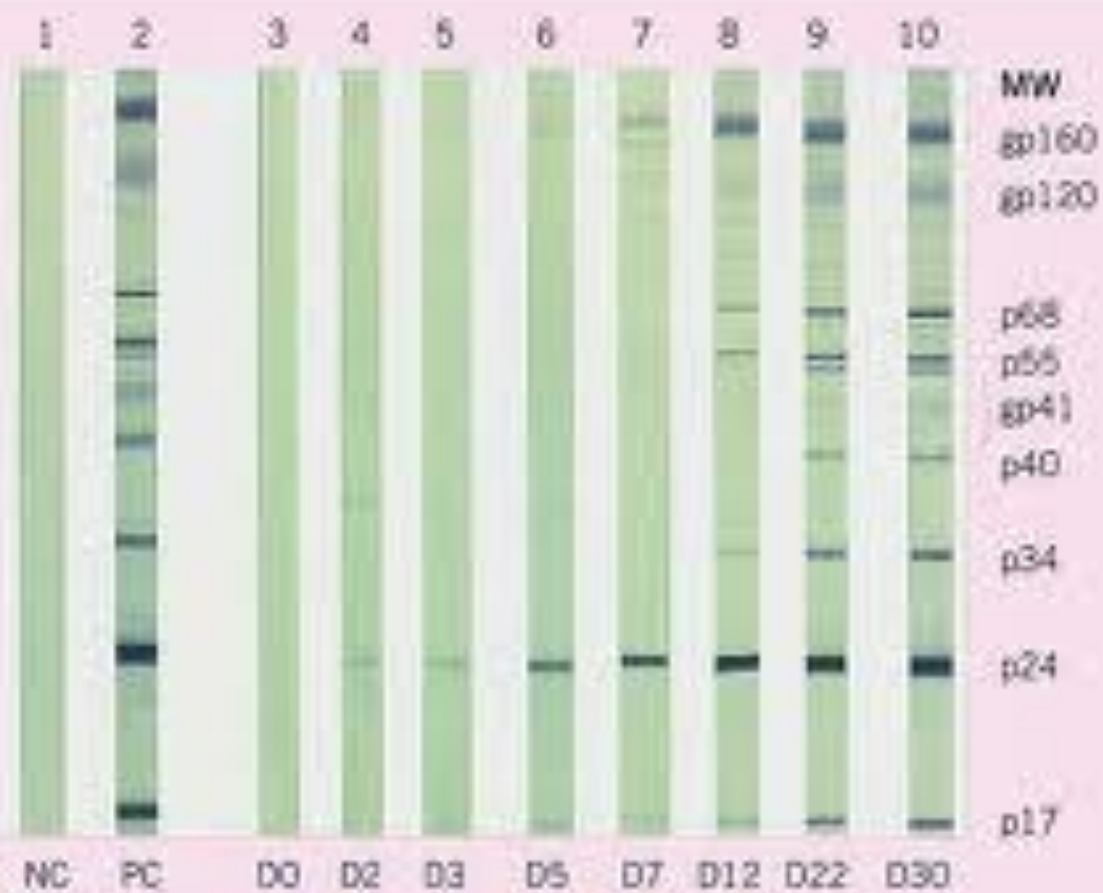
CDC criteria require 2 bands of the following : p24, gp41 or gp120/160

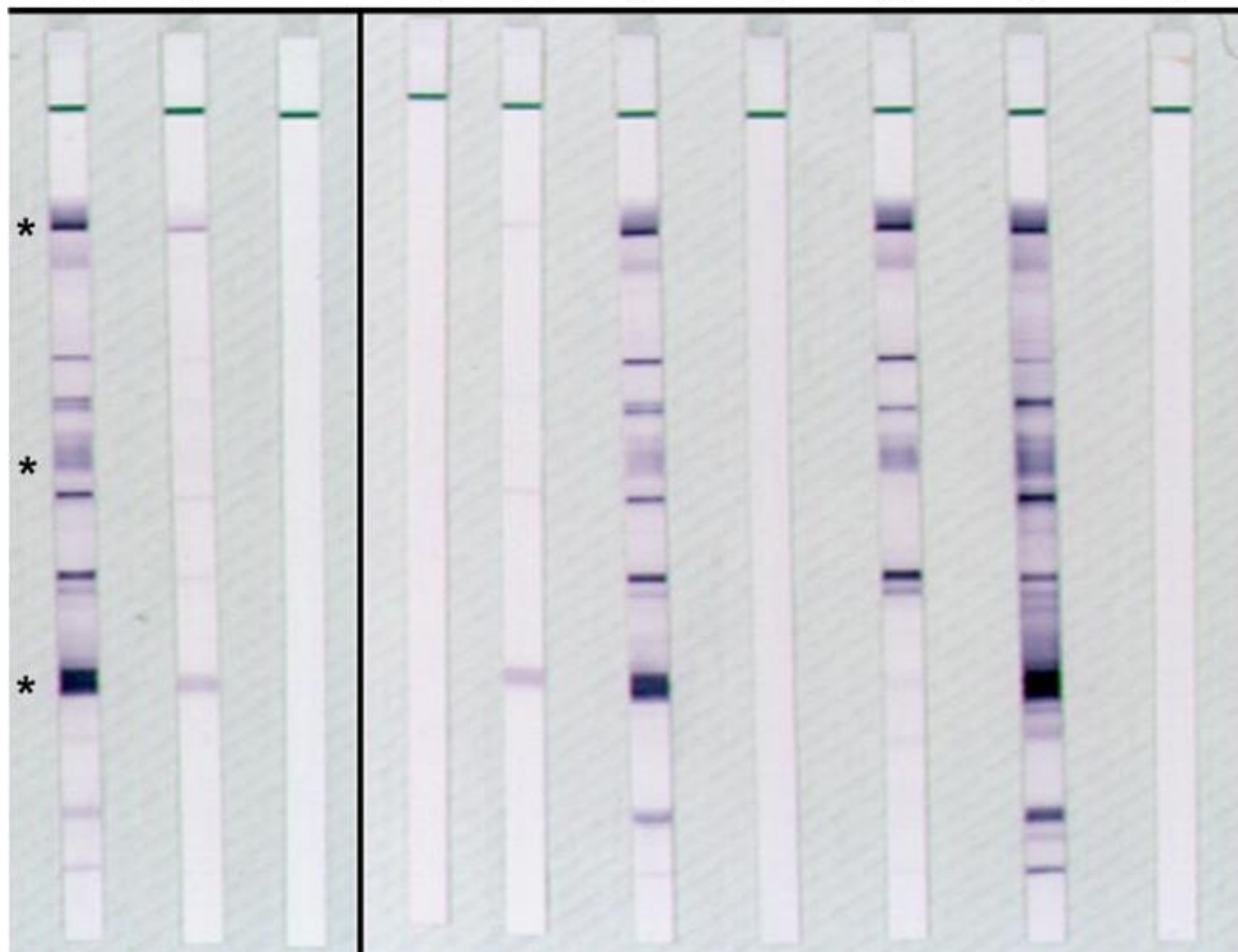
HIV
WESTERN
BLOT STRIP



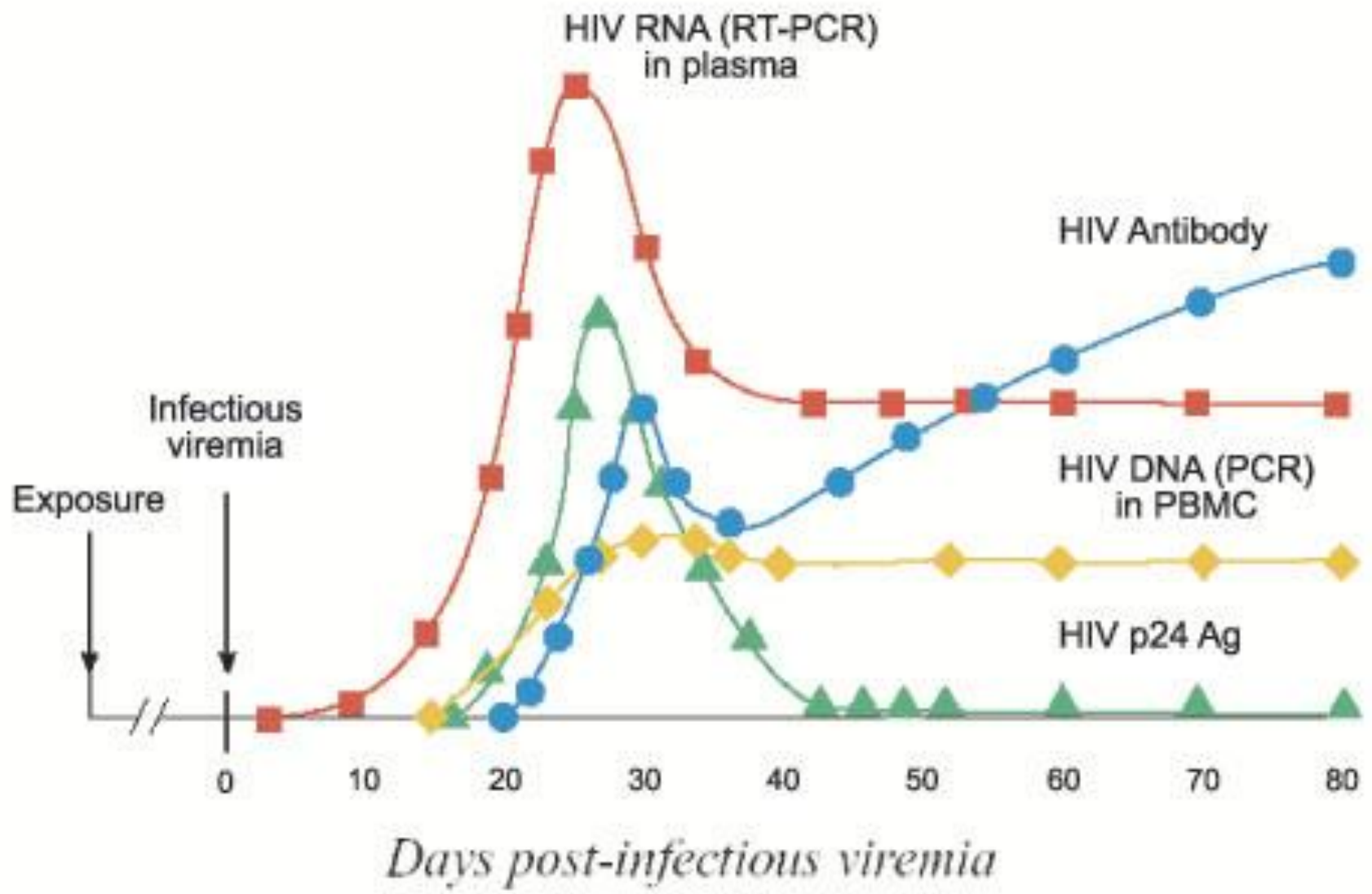
| AFR | AUS | FDA | RCX | CDC 1 | CDC 2 | CON | GER | UK | FRA | MAC |
|----------|------------------|-------------------|-----------------------------|-----------------------------|----------------------------|----------------------------|------------------|-------------------|----------------------------|---------------------------------|
| ANY 2 | ANY 1 | ANY 1 | ANY 1 | p160/ p120 AND p41 | p160/ p120 OR p41 | p160/ p120 OR p41 | ANY 1 | ANY 1 | ALL 3 | 3 WEAK BANDS OR ANY STRONG BAND |
| | ANY 3 GAG OR POL | p32 AND p24 | ANY 1 AND ANY 1 | | AND p24 | p32 OR p24 | ANY 1 GAG OR POL | p32 AND p24 | ANY 1 OR ANY 1 | |

WESTERN BLOT REACTIVITY IN ONE HIV-1 SEROCONVERTER

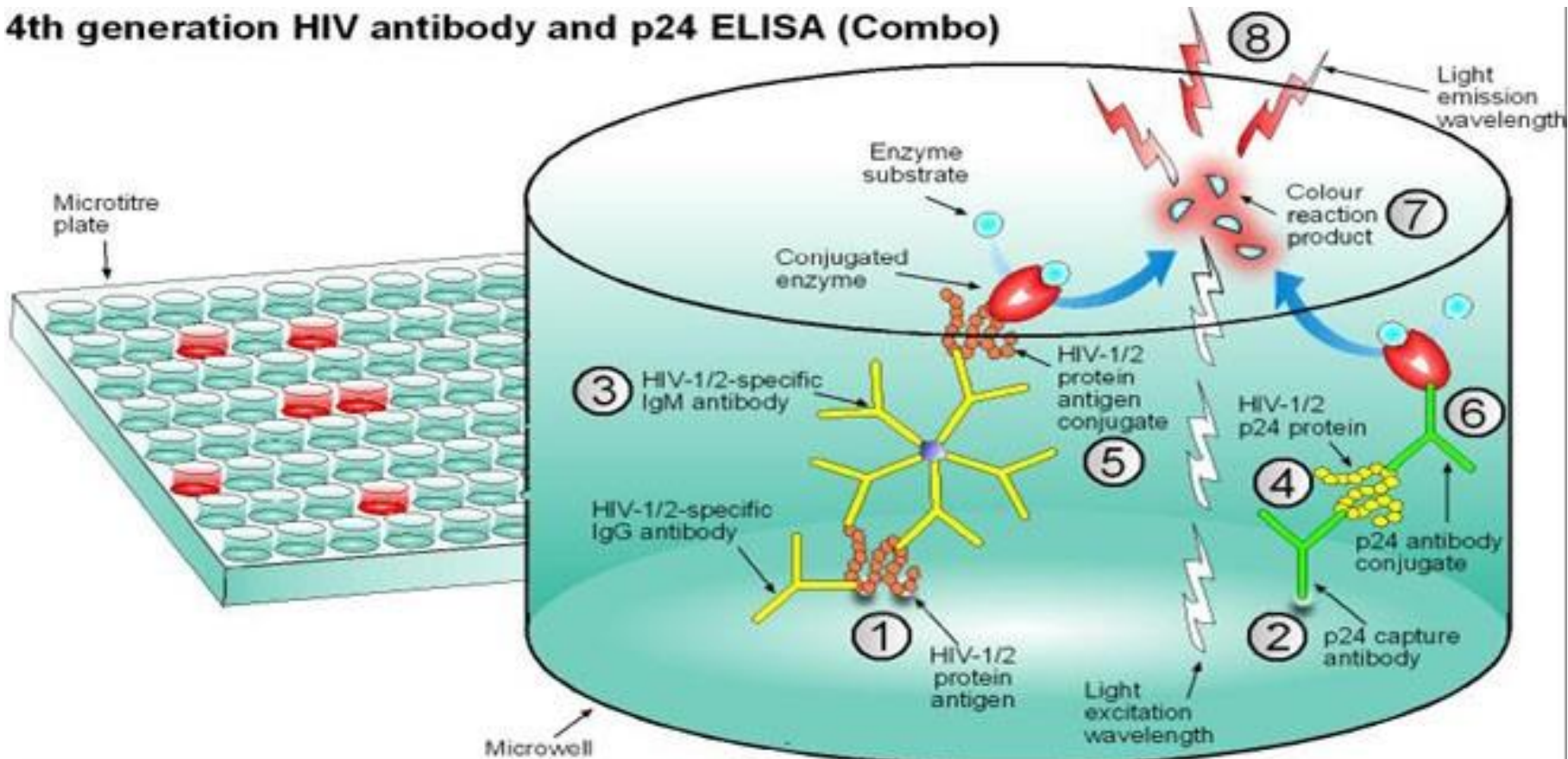


++**+****-****1****2****3****4****5****6****7**

Relative level above detection



4th generation HIV antibody and p24 ELISA (Combo)



The 4th generation HIV antibody and p24 ELISA (Combo) tests enhanced the sensitivity of the assay to detect early or acute HIV infection by including detection of p24 antigen in plasma samples. This was achieved by (1) immobilising HIV-1 and HIV-2 proteins on the surface of wells in a microtitre plate as well as (2) an anti-p24 capture antibody, (3) capture of HIV-1 or HIV-2-specific antibodies from plasma and (4) capture of p24 antigen, (5) detection of bound IgM and IgG antibodies by formation of immune complexes using HIV-1 and HIV-2 antigen-enzyme conjugates and (6) detection of bound p24 using an anti-p24 antibody-enzyme conjugate, (7) addition of enzyme substrate and (8) a spectrophotometric measurement of the completed colour reaction as an indicator of the amount of bound IgM, IgG and p24.



HIV-1 / HIV-2 Ag/Ab Immunoassay

HIV-1/2 antigen/antibody combination immunoassay

(+)

(-)

Negative for HIV-1 and HIV-2
antibodies and p24 Ag

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+)

HIV-1 (-)

HIV-1 (+)

HIV-1 (-) or indeterminate

HIV-2 (-)

HIV-2 (+)

HIV-2 (+)

HIV-2 (-)

HIV-1 antibodies
detected

HIV-2 antibodies
detected

HIV antibodies
detected

HIV-1 NAT

(+) indicates reactive test result

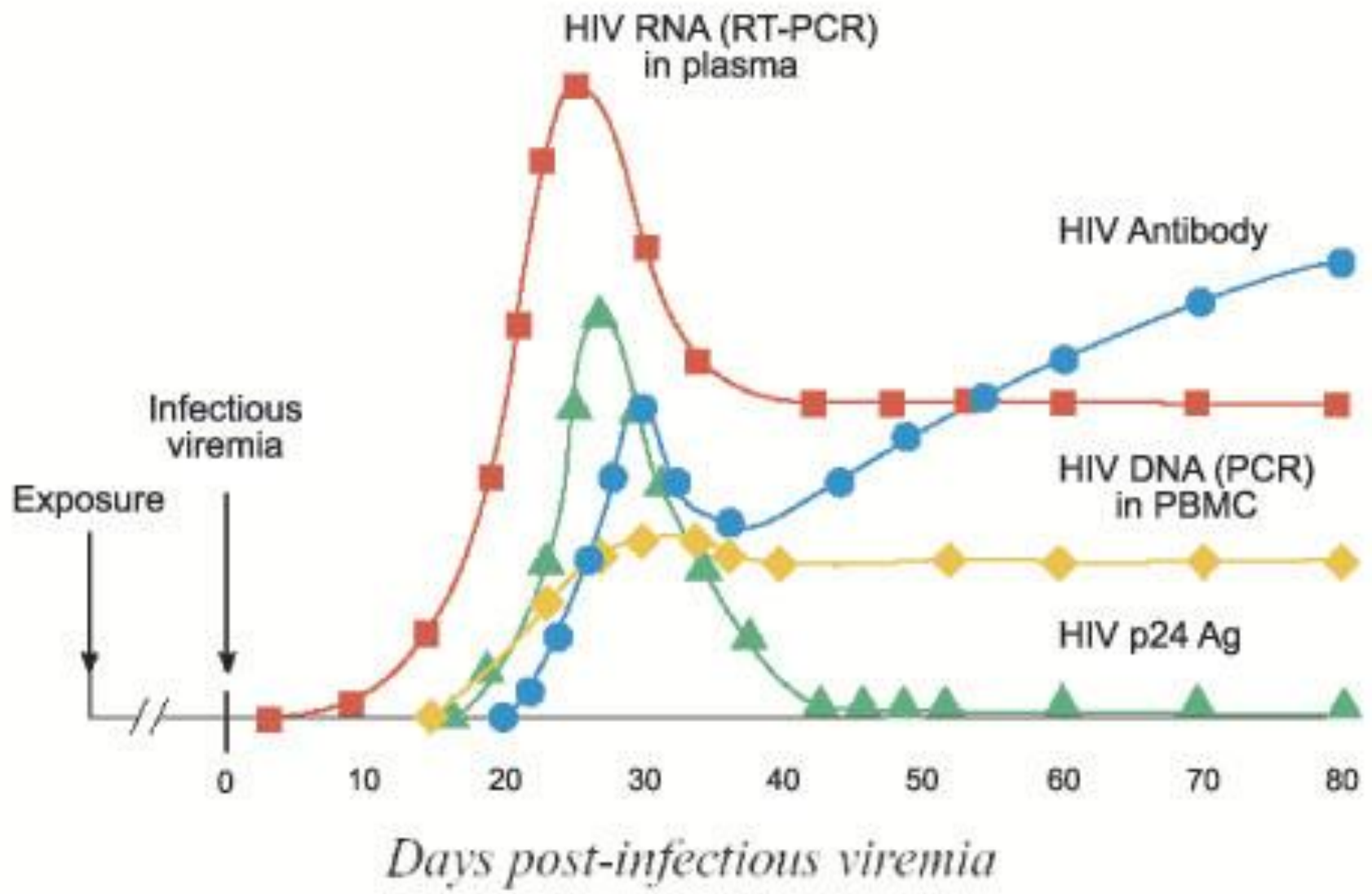
(-) indicates nonreactive test result

NAT: nucleic acid test

HIV-1 NAT (+)
Acute HIV-1 infection

HIV-1 NAT (-)
Negative for HIV-1

Relative level above detection



Laboratory diagnosis of HIV infection

Viral Load Tests

- viral load or viral burden is the quantity of HIV-RNA that is in the blood
- measures the amount of HIV-RNA in one milliliter of blood



HIV RNA Test

- The COBAS[®] AmpliPrep/COBAS[®] TaqMan[®] HIV-1 Test (Roche) uses reverse transcription and PCR amplification primers that define sequences within the highly conserved regions of the HIV-1 *gag* gene and of the HIV-1 LTR region.
- Reportable range: 20–10,000,000 copies/ml; LOD = 20

