BIOL215: MICROBIOLOGY FOR HEALTHCARE PROFESSIONALS

Lecture notes for Exam 2

DR. PRYOR

6. Endospore stain

- allows visualization of bacterial **endospores**
- **endospores** = highly resistant to chemicals, heat, radiation, etc…
- seed-like, dormant stage of some bacteria
- ***Bacillus*** species and ***Clostridium*** species, including:

- ex. ***Bacillus anthracis*** (causes anthrax; respiratory disease -- mentioned earlier)

- ex. ***Clostridium difficile*** (causes Cdiff -- mentioned earlier)

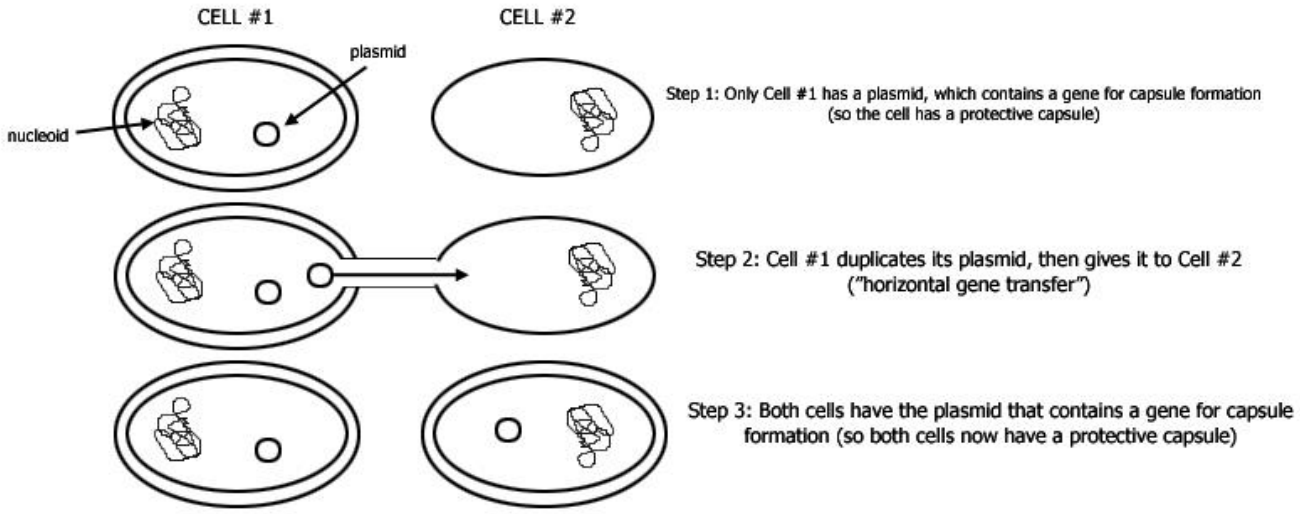
- ex. ***Clostridium botulinum*** -- causes **botulism** = muscle paralysis
  - type of food poisoning
  - can cause **Infant hypotonia** (**floppy baby syndrome**) – when babies eat *C. botulinum*- contaminated honey

- ex. ***Clostridium tetani*** – causes **tetanus** = uncontrolled muscle contraction
  - endospores enter body via puncture wound (step on rusty nail)

Prokaryotic cells (bacteria) have:

- **A nucleoid**
  - a loop of DNA
  - NOT membrane-bound, like a eukaryotic nucleus is
  - a single chromosome

- **Plasmids**
  - extra, additional loops of DNA
  - can contain functional genes
  - can be transferred between bacterial cells via **horizontal gene transfer**:



Plasmids can contain genes that cause **virulence** (ability to cause disease), such as:

1) **Capsules –** protective structures around some strains of bacteria

Ex. *Streptococcus pneumoniae*

- Frederick Griffith's experiments with *S. pneumoniae* and mice:

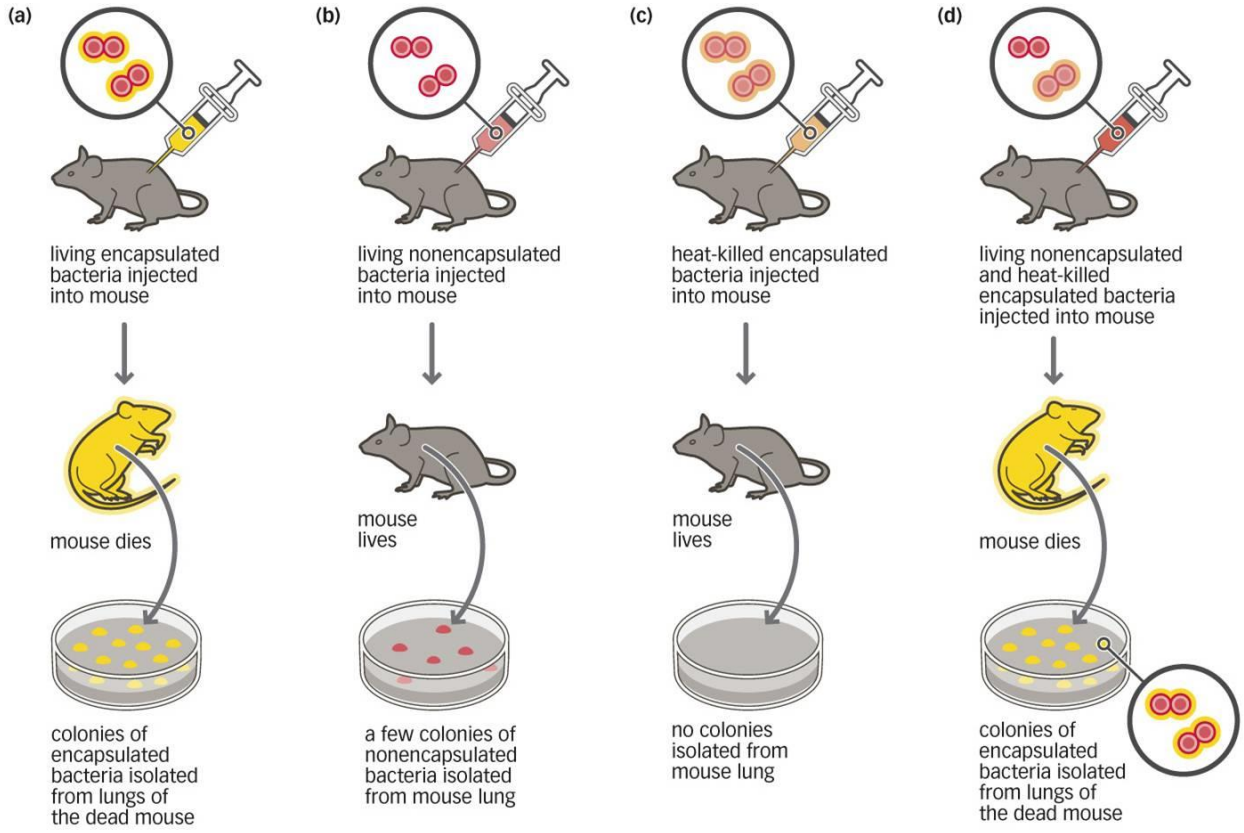


Figure 11.28 Microbiology: A Clinical Approach 2e (© Garland Science 2016)

a- **live, encapsulated bacteria** = lethal to mice; live, encapsulated bacteria recovered from dead mice
b- **live, nonencapsulated bacteria** = NOT lethal to mice; a few harmless bacteria recovered
c- **heat-killed, encapsulated bacteria** = NOT lethal to mice; no bacteria recovered
d- **heat-killed, encapsulated bacteria** mixed with **live, nonencapsulated bacteria** = lethal to mice; live, encapsulated bacteria recovered! How did that happen?

Answer: "bacterial transformation" occurred: the plasmid with the gene for capsule formation was transferred **from** dead, heat-killed bacteria **to** live, nonencapsulated bacteria, and it transformed them into live, encapsulated bacteria (**horizontal gene transfer**)

*** Remember how genes work:**

DNA (genes)
↓ (transcription)

mRNA
↓ (translation)

amino acids/proteins

First, DNA and plasmids contain genes, which are ***transcribed*** into mRNA (messenger RNA).

Next, mRNA is ***translated*** into amino acids, which combine to form proteins.

Proteins can include the building blocks of capsules, biofilms, enzymes, and other cell features that make bacteria **virulent** (able to cause disease). Virulent bacterial biofilms and enzymes are discussed next.

Plasmids can also contain genes that are responsible for:

2) **Biofilms –** sticky assemblages of bacteria on surfaces, such as teeth, catheters, etc…

Ex. *Streptococcus mutans*

- causes dental cavities (caries)
- they are **cariogenic** bacteria (cavity-causing)
- how do these bacteria cause cavities?
- *S. mutans* bacteria digest sucrose (sugar), release acid, and the **acid erodes dental enamel**

How to reduce dental cavities?

- **fluoride** inhibits bacterial enzymes (little to no acid produced), resulting in fewer cavities
- experiments with fluoride and *S. mutans* and **gnotobiotic** (sterile, germ-free) rats:

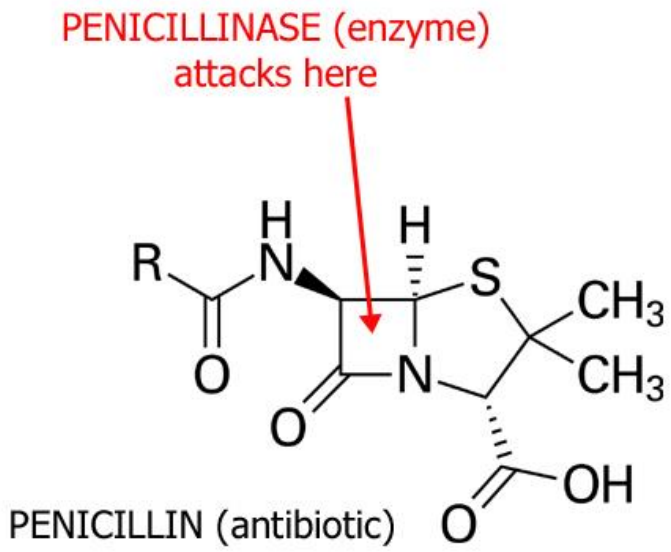
Group 1 Rats	Group 2 Rats
- rats given *S. mutans* in mouth - sucrose in diet - no fluoride Results: low pH (high acidity) in mouth *this would cause cavities	- rats given *S. mutans* in mouth - sucrose in diet - **fluoride** Results: neutral pH (low acidity) in mouth *this would cause fewer cavities

- the fluoride added to toothpaste, some mouthwashes, and drinking water helps prevent cavities

Plasmids can also contain genes that are responsible for:

3) **Enzymes that provide antibiotic resistance –** some bacteria produce enzymes that break down antibiotics

Ex. *penicillinase*
- enzyme that breaks down the antibiotic **penicillin**
- the gene for the enzyme penicillinase is on a plasmid, can be transferred between bacteria
- bacteria that produce penicillinase are <u>not</u> killed by penicillin!



PENICILLIN (antibiotic)

Some bacteria also have **cell walls:**

Gram-positive bacteria cell wall

- outer cell wall layer is made of **peptidoglycan**
- peptidoglycan is destroyed by the antibiotic **penicillin**
- thus, Gram-positive bacteria are often vulnerable to penicillin

Gram-negative bacteria cell wall

- outer cell wall layer is made of **LPS (lipopolysaccharide)**
- therefore, they are <u>less likely</u> to be killed by penicillin than Gram-positives , because there is no peptidoglycan in the outer cell wall
- alternative antibiotics must be prescribed for Gram-negative bacterial infections

- the LPS layer also can slough off live or dead cells, and act as a toxin (**endotoxin**)
- the toxins cause:

    - shock (low blood pressure, or hypotension)
    - fever
    - death

- more specifically, the LPS layer is made of:

    - **Lipid A** (which acts as the toxin)
    - 1 picogram of Lipid A/mL of blood is toxic! 1 pg = 0.000000000000001g

- the LPS layer is also made of:

    - **O polysaccharide** (which can be used in diagnostics)
    - the O polysaccharide can be used to identify deadly strains of bacteria
    - for example, there are hundreds of strains of *E. coli*
    - some strains are harmless, like the ones in your intestines right now
    - other strains are pathogenic, such as:

EHEC
- enterohemorrhagic *E. coli*
- severe food poisoning; causes intestinal bleeding, vomiting, diarrhea
- can cause **HUS** = hemolytic-uremic syndrome (hemolytic = blood; uremic = kidney)
- antidiarrheal drugs NOT recommended ex. **Loperamide** (“Imodium AD”)
- diarrhea is beneficial in this case, because it evacuates the deadly bacteria

- virulent strains of **EHEC** include ***E. coli* O157:H7** and ***E. coli* O104:H4**
- O refers to O **polysaccharide** (there are over 160 different O polysaccharides in strains of *E. coli*)
- **H** refers to flagella **proteins** (there are dozens of different flagella proteins)
- scientists analyze the bacteria and classify them according to the **O** and **H**
- then, the source of the bacteria can be determined

- for example, in 2011 there was an outbreak of EHEC food poisoning in Germany that affected almost 4000 people and killed dozens of patients (mostly from **HUS**, described above). The source of the bacteria was initially thought to be either contaminated cucumbers or sprouted seeds that were eaten by the patients before they became sick. The specific strain of bacteria, ***E. coli* O104:H4**, was identified from the patients, and it matched the bacteria found on the sprouted seeds.

How to test for EHEC toxins:

LAL – Limulus amoebocyte lysate test kit for endotoxins, primarily for pharmaceutical testing

- this test uses a reagent that will clump in the presence of endotoxins
- it is named after ***Limulus*** = genus name of the horseshoe crab
- these crabs have special blood cells called **amoebocytes**
- when the amoebocytes burst, the **lysate** (cell contents) clumps together with endotoxins

- for example, before it is packaged for sale, insulin is tested with the LAL to make sure it does not contain endotoxins that could make diabetic patients sick or die

Some bacteria have **mycolic acid** in cell wall (*Mycobacterium* spp.)

- mycolic is a waxy lipid
- it makes ***Mycobacterium*** resistant to antibiotics and immune system attack
- *Mycobacterium* can cause tuberculosis (***M. tuberculosis***) and leprosy (***M. leprae***)

Some bacteria do not have a cell wall

- ex. ***Mycoplasma pneumoniae*** has no cell wall
- it causes "walking pneumonia," a mild form of a respiratory disease
- these bacteria are not killed by penicillin because they do not have peptidoglycan
- therefore, the patient will not respond to penicillin and keep "walking around"
- alternative antibiotics must be prescribed

Some bacteria have **M protein** in cell wall

-M protein is **anti-phagocytic** (phagocytic immune system cells cannot ingest these bacteria)
- this protein prevents the immune system cells from "eating" the bacteria (**phagocytosis**)
- bacteria with M proteins resist attack from the immune system

- ex. *Streptococcus pyogenes*, which causes

    - strep throat (**streptococcal pharyngitis**)
    - **necrotizing fasciitis** ("flesh-eating bacteria" disease)
    - **scarlet fever** (fever, redness on skin)

* note: sometimes, a single species of bacteria can cause multiple diseases, as described above

Fever is a common sign of many bacterial infections. It's actually helpful, to "burn off" the bacteria. It would be like putting the bacteria in an incubator that's too hot. However, too high of a body temperature can be deadly to the patient.

* The importance of fever (called **pyrexia**)
  - fever is the body's defense against pathogenic microbes
  - normal body temp = **98.6F**
  - fever up to **102F** is OK for brief periods of time
  - fever above **104F** is DANGEROUS!

  <u>Stages of Fever:</u>

1. **Prodromal** stage	- normal body temp
2. **Chill** Stage	- shivering (generates heat) - put more clothes, put blankets on - **vasoconstriction** of blood vessels in skin (keeps heat in body)
3. **Flush** stage	- high body temp
4. **Diaphoresis** stage	- sweating - throw off blankets, clothes - **vasodilation** (blood vessels relax, release heat from skin to air)

These stages often repeat themselves over and over when you are very sick. If your fever persists or gets too high, the following drugs are generally safe for reducing fever in adults:

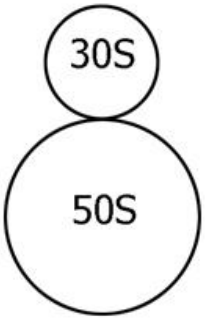
Acetaminophen ("Tylenol")
Ibuprofen ("Advil")
Naproxen ("Aleve")
Aspirin

All bacteria have ribosomes:

Ribosomes: sites of protein synthesis

Prokaryote ribosomes:
- measured in **Svedberg units** (larger "S" number is bigger molecule)
- prokaryote ribosomes have two subunits:

50S subunit
30S subunit

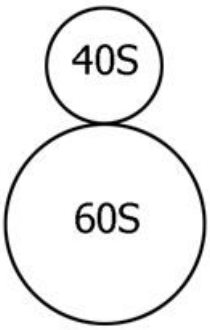


- the 50S is the target of **erythromycin**, an antibiotic derived from bacteria, ***Streptomyces erythreus***
- the 30S is the target of **streptomycin**, an antibiotic derived from bacteria, ***Streptomyces griseus***

Why aren't people or other eukaryotes affected by those antibiotics, considering that we have ribosomes, as well?

Eukaryote ribosomes:

60S subunit (not affected by those antibiotics)
40S subunit (not affected by those antibiotics)



Some **Gram-negative** bacteria have **pili** – short hair-like structures on cell wall

- help to attach the bacteria to host cells
- also assist in **conjugation** (transfer DNA from one cell to another cell; horizontal gene transfer)

Some bacteria have **axial filaments** – found only on spiral bacteria

- these bacteria rotate like a corkscrew and burrow into tissues, cells

- ex. *Treponema pallidum* – causes **syphilis** (STD)
    - bacteria that are sexually transmitted from host to host
    - fever
    - pus discharge from urethra
    - **lesions** (open sores) on genitals
    - rarely fatal, but can cause long-term disease, ex. **neurosyphilis** (brain infection)
    - can be treated with antibiotics

- ex. *Borrelia burgdorferi* – causes **Lyme disease**
    - bacteria that are transmitted by tick bite
    - fever
    - muscle and joint pain
    - **erythema migrans** ("bull's eye rash" that spreads)
    - rarely fatal, but can cause long-term disease, ex. **arthritis** (joint inflammation)
    - can be treated with antibiotics

Both syphilis and Lyme disease are caused by **spiral bacteria with axial filaments**, so they look almost identical under the microscope. How can microbiologists and doctors tell them apart?

1. **Symptoms and signs**
- both present with fever, but
- each disease has unique signs (**syphilis** = lesions on genitals; **Lyme disease** = spreading rash on skin)

2. **Patient history**
- sexual activity is related to **syphilis**
- outdoor activity, such as hiking or hunting, is related to **Lyme disease**

3. **Immunologic tests** (explained below)

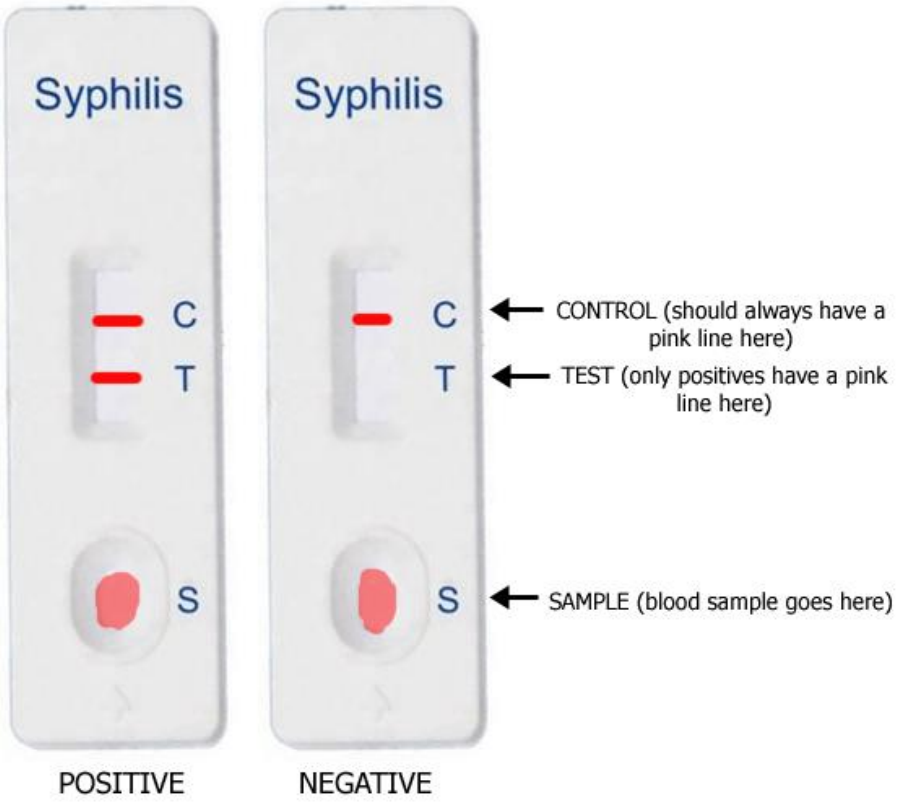
Immunologic tests are based on:

Antigens - molecules that trigger an immune response
- ex. proteins on surface of a microbe

Antibodies - proteins produced by the immune system that bind to antigens
- extremely specific!
- each type of antigen only binds to one type of antigen

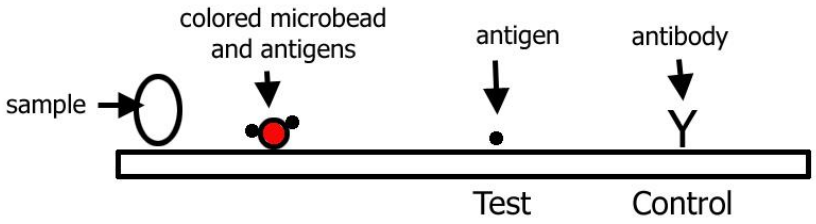
For example:

Syphilis and many other infectious diseases can be diagnosed with a "test strip" similar to a pregnancy test strip (below). These are based on antigens and antibodies.

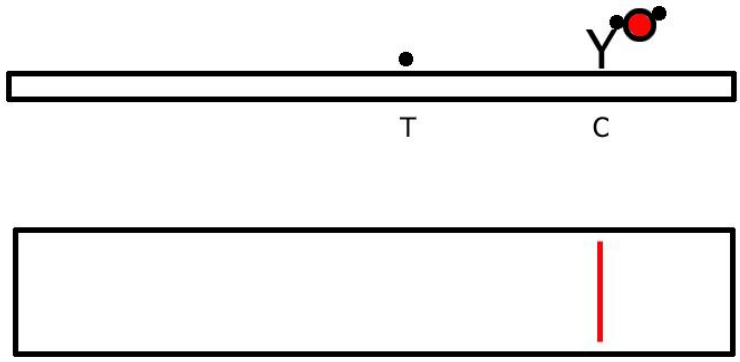


Technically speaking, these test strips are called **Lateral Flow Immunochromatographic Assays**. The patient's sample (typically, a small amount of blood) is added to the **S**. After several minutes, the **C** and **T** locations are examined for pink lines. If both have a pink line, the patient has syphilis (**positive**). If ONLY the C has a pink line, the patient does not have syphilis (**negative**). But how does it work?

Let's look at the side view of the test strip, used on a NEGATIVE patient. The sample does NOT contain antibodies specific to syphilis. The sample moves from left to right, picking up colored microbeads (very tiny beads, shown below as a red dot) with syphilis antigens (the black dots) attached to them.



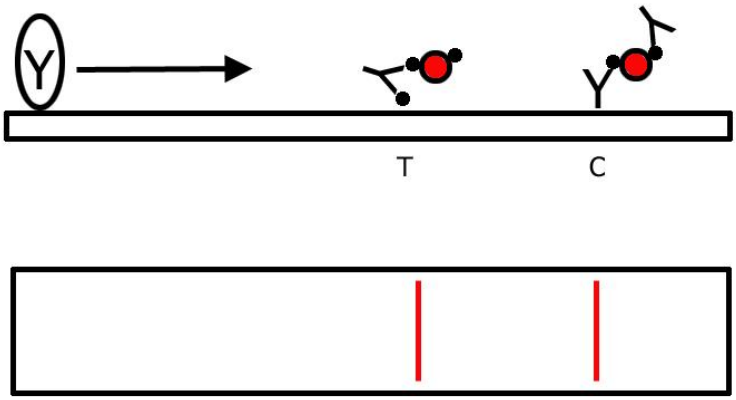
Next, the sample passes by the T (TEST) location. The T location has syphilis **antigens** attached to it (the black dot). But the sample will not stick because there is no antigen-antibody reaction. The sample moves to the C (CONTROL) location. The C location has syphilis **antibodies** attached to it (the Y-shaped structure). The sample will stick because there is an antigen-antibody reaction. The result is one pink line.



Negative (above) – no antibodies in blood for syphilis

Now let's look at the side view of the test strip, used on a POSITIVE patient. The sample contains antibodies specific to syphilis, because the patient is producing antibodies in response to the syphilis antigens. The sample moves from left to right, picking up colored microbeads (very tiny beads, shown below as a red dot) with syphilis antigens (the black dots) attached to them.

Next, the sample passes by the T (TEST) location. The T location has syphilis **antigens** attached to it (the black dot). Some of the sample will stick because there is an antigen-antibody reaction. Some of the sample then moves to the C (CONTROL) location. The C location has syphilis **antibodies** attached to it (the Y-shaped structure). The sample will stick because there is an antigen-antibody reaction. The result is two pink lines.



Positive (above) – antibodies in blood for syphilis

Eukaryotic cells have (relevant to healthcare):

- a **nucleus**
- membrane-bound organelle containing DNA
- paired chromosomes (humans have 23 pairs, or a total of 46 chromosomes)

- **no plasmids** in human cells

Some eukaryotes have **cell walls**

-**no cell wall** in **human** cells, BUT:

- a **chitin-based cell wall** in **fungi**:

    - fungi are **not** affected by penicillin or other **antibiotics**

        - antifungal drugs target **ergosterol** (found only in fungi cell wall)
        - human cells have no ergosterol (not affected by antifungal drugs)
        - bacterial cells have no ergosterol (not affected by antifungal drugs)

- antifungal drugs include:

- ex. **Clotrimazole ("Lotrimin")** which is used for **cutaneous** (skin) infections, such as:

Medical name of disease	**Location of rash on body**
tinea cruris	groin (also called "jock itch")
tinea pedis	feet (also called "athlete's foot"
tinea capitis	scalp
tinea barbae	face
tinea corporis	ring-shaped rash anywhere on body (also called "ringworm")

    - **dermatophytes** – fungi that cause skin infections
    - fungi that are dermatophytes include:
    Epidermophyton species
    Trichophyton species
    Microsporum species

- ex. **Miconazole ("Monistat")** which is used for vaginal yeast infections (yeast are fungi)

Some eukaryotes can exhibit **phagocytosis:**

- ingestion of particles, including bacteria
- ex. many types of **leukocytes** (white blood cells)
- attracted to the site of infection by chemical signals (phenomenon called **chemotaxis**)
- once inside leukocyte, bacteria move to **lysosome** (organelle containing enzymes), digested

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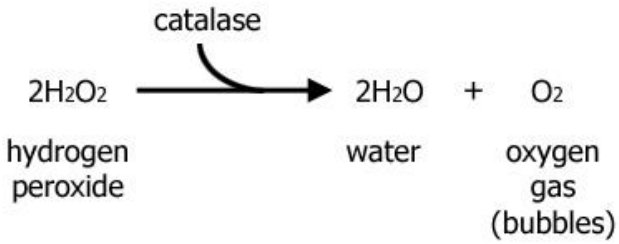
Some eukaryotes have **peroxisomes:**

- organelles containing **catalase** (enzyme that breaks down **peroxides**)



- bacteria that do not have the enzyme catalase are killed by hydrogen peroxide
- therefore, peroxide does not harm human cells but kills some bacteria
- peroxide is a first aid product, applied to an open wound or cut, to prevent infection

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