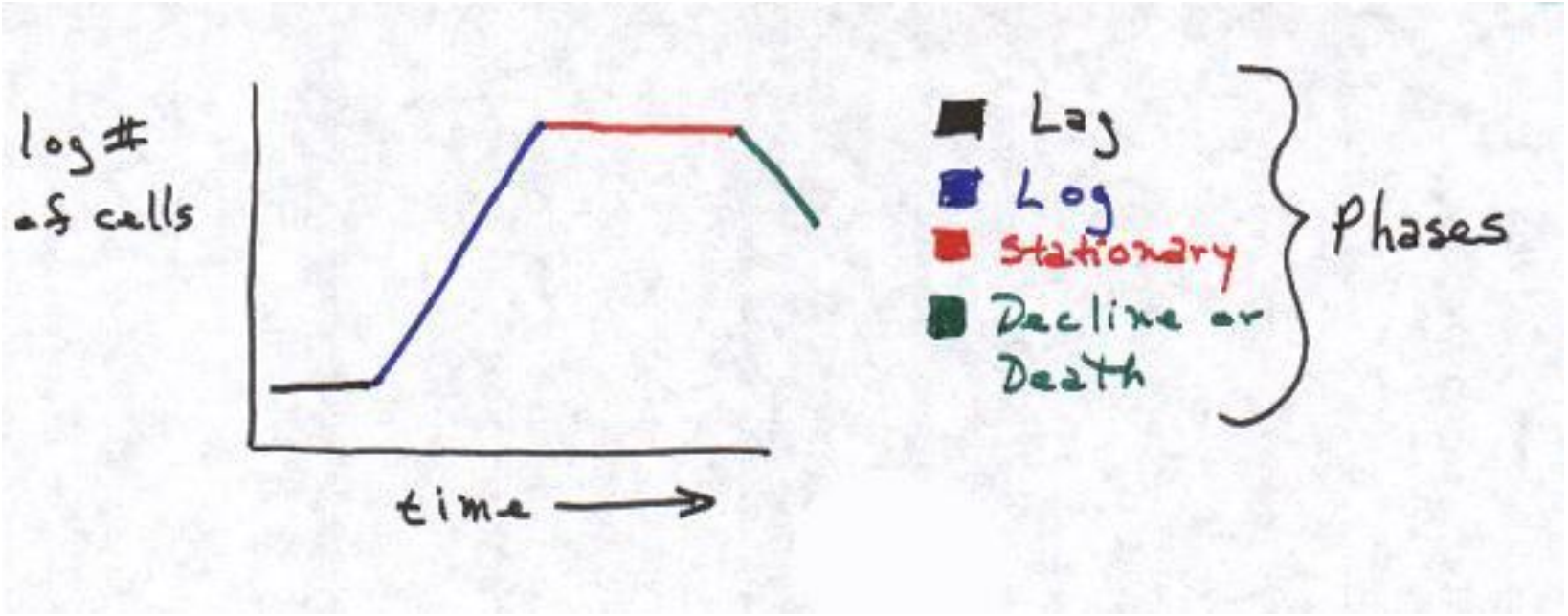
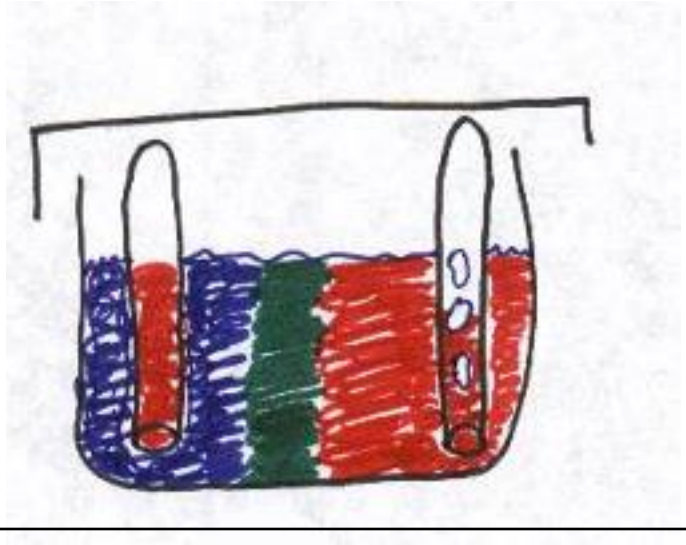
Growth and Multiplication of Bacteria

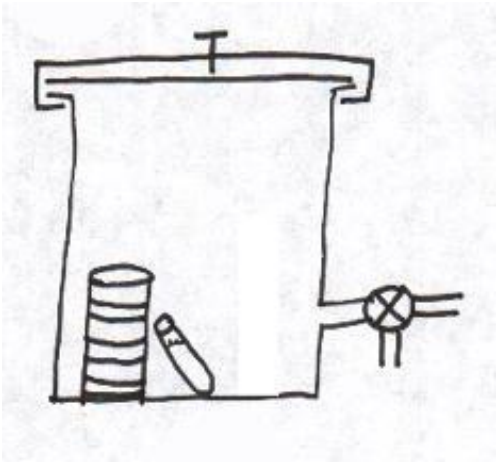
Elements of Infection

There are four phases of bacterial growth [and death]: the lag phase is in black; the log phase is in blue; the stationary phase is in red; the death or decline phase is in green.





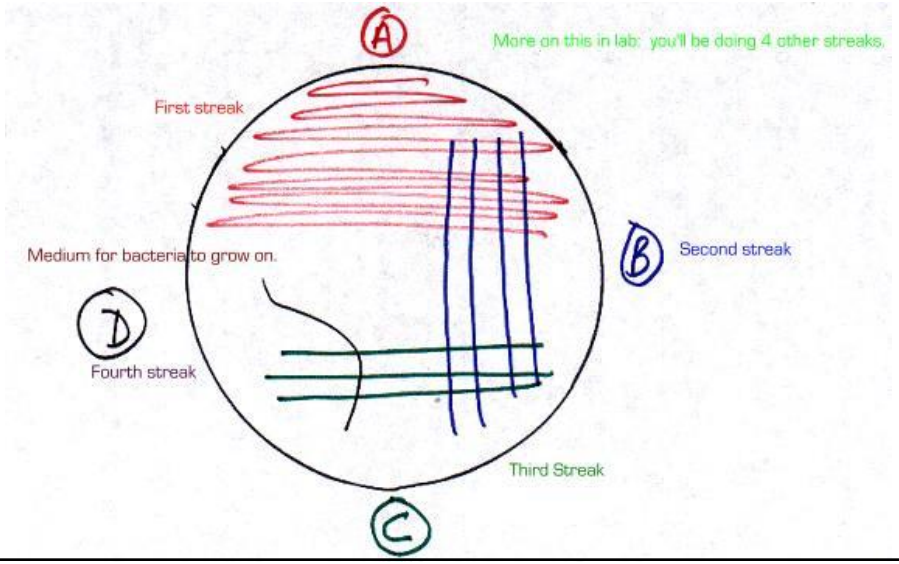
The use of Durham tubes: tiny tubes placed in solutions of peptone water with varying kinds of carbohydrates. They are placed upside down in the liquid medium. In the illustration **BLUE** is the color of the medium that it starts with. **GREEN** indicates opacity or bacterial growth in the medium. **RED** indicates that acid has been produced. The **blue squiggly circles** represent gas bubbles formed by bacteria that are fermenters. Each of these changes are used to identify different bacteria.



- An anaerobic jar: This jar is sealed after samples are placed in it with an indicator (methylene blue -- clear is reduced) or even Pseudomonas aeruginosa (if it grows, the jar is NOT anaerobic)).
- The valve (circle with "X" in it) is opened to permit a vacuum to remove air from it. Once that is complete, the valve is turned, again, and hydrogen is pumped into it, rendering the atmosphere in the jar anaerobic. The valve is turned to seal it from all gas sources, then the jar is incubated.

- An alternative to anaerobic jars: the infamous candle jar. This is handled just like the anaerobic jar EXCEPT that after samples are placed in the jar, a lit candle is, too. The lid is replaced tightly and the candle burns out, using all the oxygen in the jar, permitting enough carbon dioxide to be present to make 10-15% CO_2 in the jar. The jar is then incubated.
- Another alternative to the anaerobic and candle jars is to place your sample in a zip-loc bag with some baking soda and vinegar in separate parts of a Petri dish. Seal the bag, pressing out the air as you seal the bag. Once sealed, tilt the Petri dish so that the baking soda and vinegar may mix, releasing carbon dioxide in the bag, creating an anaerobic environment for your microbes to grow in.

- Isolation of pure cultures: pure culture = only one kind of bacteria present.
- Generally from a mixed culture (consists of more than one kind of bacteria present in culture).
- Classical method is by obtaining these colonies by plating on solid medium.



A simple manner in which bacteria can be plated out to isolate them as discrete colonies from confluently grown together plaques. A flame (or Bacti-Cinerator) sterilized loop is cooled to room temperature. A sample of bacteria is removed from another plate and streaked across a small portion of the new agar plate (**A**). The flame is then re-sterilized, cooled, then streaked across **A** into **B** as illustrated. Repeat the process from **B** to **C**. After the loop has been re-sterilized following innoculation of **C**, run a single streak from **C** into **D** and incubate after you've sterilized the loop, again.

A variation of this is to streak a swab saturated with a patient's sample across area **A**, then repeat as above with a flame sterilized loop.

- Regardless of the method, from the resulting culture, it is then possible to pick out single colonies of dissimilar bacteria and grow on other media for identification purposes.

Conditions for bacterial growth:

- Bacteria require water, inorganic salts, carbon and nitrogen sources, growth factors and a source of energy.
- Additionally, their growth depends on the redox potential, pH and temperature.
- Water: Dessication kills bacteria, although, in special cases they may survive for periods in a suspended animation state. Common method of preserving lab specimens is to lyop[hilize them and store them *in vacuo*.

- Inorganic salts: These are required for osmotic regulation and to provide trace elements necessary for certain enzyme systems. All bacteria require phosphate. Sulfate is essential if there is no other source of S. Na, K, Mg, Ca, Fe, Mn, Zn, Cu, Co, Mo are also required.

- Carbon, nitrogen, growth factors and energy: two main groups;
- Autotrophs and
- Heterotrophs.

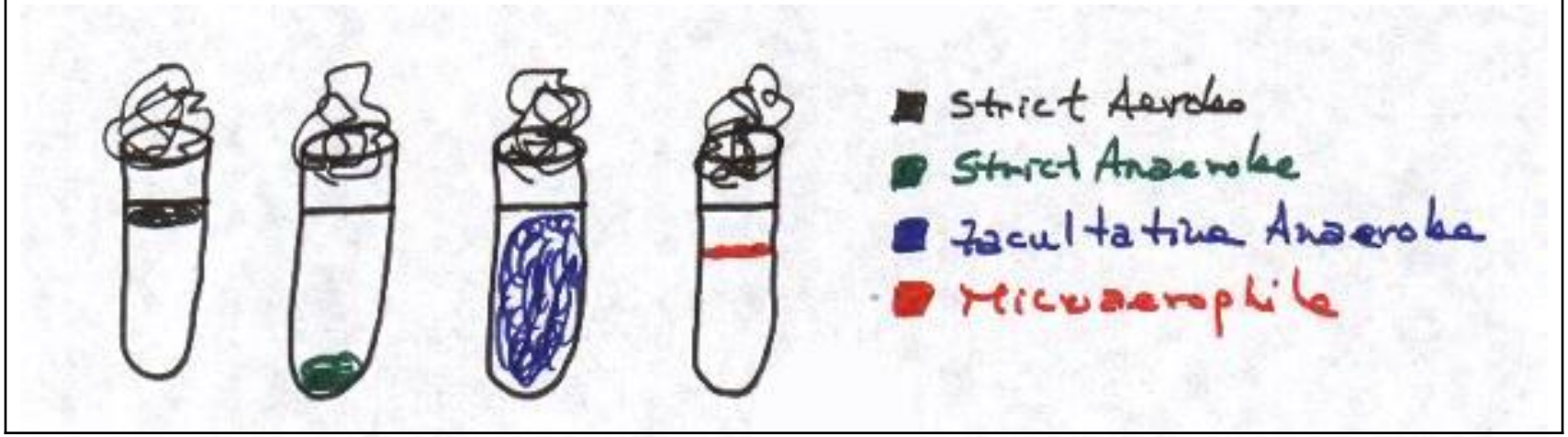
- Autotrophs are capable of living in a strictly inorganic environment. They have no direct medical importance.
- These bacteria obtain their C from CO_2 and N from NH_3, NO_2^- and NO_3^-. A few are photosynthetic and obtain their energy from light by means of chlorophyll.
- Most are chemosynthetic and obtain their energy by oxidizing substances such as NH_3, NO_2^-, Fe^{2+}, S^{2-} and H.
- They manufacture all the complicated proteins, carbohydrates, lipids, nucleic acids and enzymes needed for growth and metabolism.

- Heterotrophs require preformed organic matter for energy and synthesis.
- **All bacteria of medical importance come into this category.**
- In general, heterotrophs obtain their C, N and energy from organic compounds such as carbohydrates and amino acids.
- Many pathogenic species do not synthesize certain key substances such as vitamins, purines and pyrimidines.
- These organisms grow when they receive ready-made growth factors. E.g.,
  - most complicated: Streptococci require 17 amino acids, 9 B vitamins, adenine and guanine, cytosine, thymine and uracil and a carbohydrate for energy;
  - least complicated: E. coli obtains N from NH_3 but requires a complex source of C such as glucose.

- **Gaseous requirements:**
- **CO_2: all bacteria require CO_2 for metabolism. Growth of nearly all micro-organisms, particularly N. gonorrhoeae, N. meningitides, Streptococci is improved by the presenf of additional CO_2.**
- **O_2: Bacteria may be classified into four groups based upon their usage of oxygen:**

Obligate Aerobes	[Strict] Anaerobes	Facultative Anaerobes	Microaerophiles
Grow only with O_2	Grow only without O_2; killed by O_2	Will grow under either aerobic or anaerobic conditions	Grow best with lower O_2 concentrations (ca 10%)
M. tuberculosis; P. aeruginosa	Clostridium; some Strep/Staph; [Spirochaetes]	Nearly all organisms of medical importance NOT in the first 2 groups	Some Streptococci; Mycoplasma

Growth of 4 types of microorganisms: **Strict aerobes** which grow in agar shakes (heat the agar above 50° C to melt it. At 50°C, add bacteria, mix it carefully, put a plug in the top to keep dirt, etc out, then let it incubate) grow at the top of the shakes. **Strict anaerobes** grow at the bottom of the shake, where the environment is the most anaerobic. **Facultative anaerobes** grow throughout the medium as they can grow in both aerobic and anaerobic environments. **Microaerophiles** prefer to grow just below the surface of the medium where the oxygen concentration is only about 10% or so as opposed to the 21% in atmospheric air.



- Shake cultures are tubes of freshly melted agar, cooled to 50° C and inoculated.
- Anaerobes will grow under AEROBIC conditions in liquid media if they contain sufficient reducing substances: e.g., sodium thioglycollate, ascorbate, couple of dried peas or an iron nail. Cooked meat medium is the best anaerobic medium, however.

- Hyperbaric oxygen (O_2 at pressures greater than atmospheric pressure) has been used in the treatment of anaerobic infections, notably gas gangrene.
- Nitrogen: gaseous nitrogen is not required by bacteria of medical importance.
- Nitrogen fixating bacteria are important in agriculture.

- pH: most bacteria of medical importance grow best between pH 7.2 and 7.6.
- Growth is usually poor below 6 or bove 7.8.
- Growth ceases below 5 or above9.
- Exceptions are V. cholerae (8-9) and L. acidophilus (4).

Temperature: Bacteria pathogenic for man usually grow best at body temperature, 37°C. occasionally, this temperature is higher (C. jejuni – 43° C, M. avium – 40° C) or lower (Y. pestis – 30° C, M. ulcerans – 32° C). Although many species will multiply over a range of 20-43° C, some species like N. gonorrhoeae will grow only in a narrow range around 37° C.
a.Bacteria encountered in medical bacteriology are mesophilic in their temperature requirements.
a.Some psychrophilic (cold loving) species found in brine and soil will multiply at 0° C or lower.
a.Some thermophilic species found in hot springs and manure piles will multiply at temperatures as high as 55-80° C. in hydrothermal vents where water is under high pressure, certain species grow at temperatures above 100° C.

- Metabolism: the main metabolic pathways are the same as those utilized by other forms of life. Bacteria may utilize many different substrates. The end-products they produce show amazing diversity. The rate of metabolism is unusually rapid. This is due to the large surface area::volume, which facilitates the exchange of nutrients and wastes.

Carbohydrates are the major source of energy for medically important bacteria. Two types of bacteria:
1)Homofermentative: oxidize glucose by glycolysis to produce lactate as the main end product, e.g., Streptococcus and Lactobacillus.
1)Heterofermentative: produce varying amounts of other end-products.
Many bacteria carry out the intermediate reactions of the TCA (as previously discussed in BIOL 190, 223)) cycle and various reactions with pyruvate and 2 carbon fragments. These reactions serve synthetic as well as catabolic processes and act as a bridge between carbohydrate metabolism and the metabolism of proteins and lipids. Carbohydrate media may be used for biochemical tests. Sugar media: fermentative patterns are important in bacterial identification.
REMEMBER:
Fermentation: CHO $\rightarrow$ EtOH + CO_2
Oxidation: CHO $\rightarrow$ CO_2 + H_2O

Aerobic Growth

In aerobic respiration, the H^+ derived from oxidative processes are transferred to molecular oxygen by means of the cytochromes. Glucose is completely catabolized to carbon dioxide and water and maximum energy is liberated.

In fermentation, the H_2 is transferred to other hydrogen acceptors or is liberated as $H_2\uparrow$.

Depending on their use of these 2 types of oxidation, bacteria fall into 3 broad groups:

1) obligate aerobes – possess cytochromes and carry out aerobic respiration
2) Streptococcus, Lactobacillus, with rare exceptions, obligate anaerobes do not have cytochromes and carry out only fermentations
3) The great majority of organisms have cytochrome systems which they use when oxygen is available, but adapt to fermentative processes when oxygen is limited or absent.

Media Composition

- Synthetic media: chemically defined media comes from pure substances (AA's, growth factors, salts, etc). Media used in the lab usually contains a mixture of naturally occurring biological substances and their partial "breakdown", i.e., hydrolytic products. Peptone, meat extract and salt provide the basis for most media. Peptone is a complex mixture of water soluble products obtained by enzymatic digestion of meat (pepsin). Whole blood, heated blood, serum, yeast extract, glucose and glycerol may be added to increase nutritive value.
- Different components effect the way bacteria grow or don't grow. Selective media encourages or inhibits the growth of some specific bacteria. Differential media makes it possible to differentiate between organisms. Media making has already been covered in the laboratory.

Representative Media

Media	Composition	Usage
Nutrient broth	**Consists of peptone, meat extract and NaCl**	**General usage**
Nutrient agar	**Nutrient broth plus agar**	**General usage**
Blood agar	**Nutrient agar with 5-10% citrated or oxalated or defibrinated blood – may be horse or sheep – sheep more common**	**Most bacteria of medical importance will grow on this medium. Presence of intact RBC allows for detection of hemolytic properties of organisms**
Chocolate agar	**Blood agar that has been heated until it is a chocolate color. This increases the nutritive value.**	**Delicate organisms such as N. gonorrhoeae.**
Cooked meat medium	**Minced meat suspended in broth. Has excellent nutritive properties and supports the growth of a large number of organisms.**	**Great for strict anaerobes.**

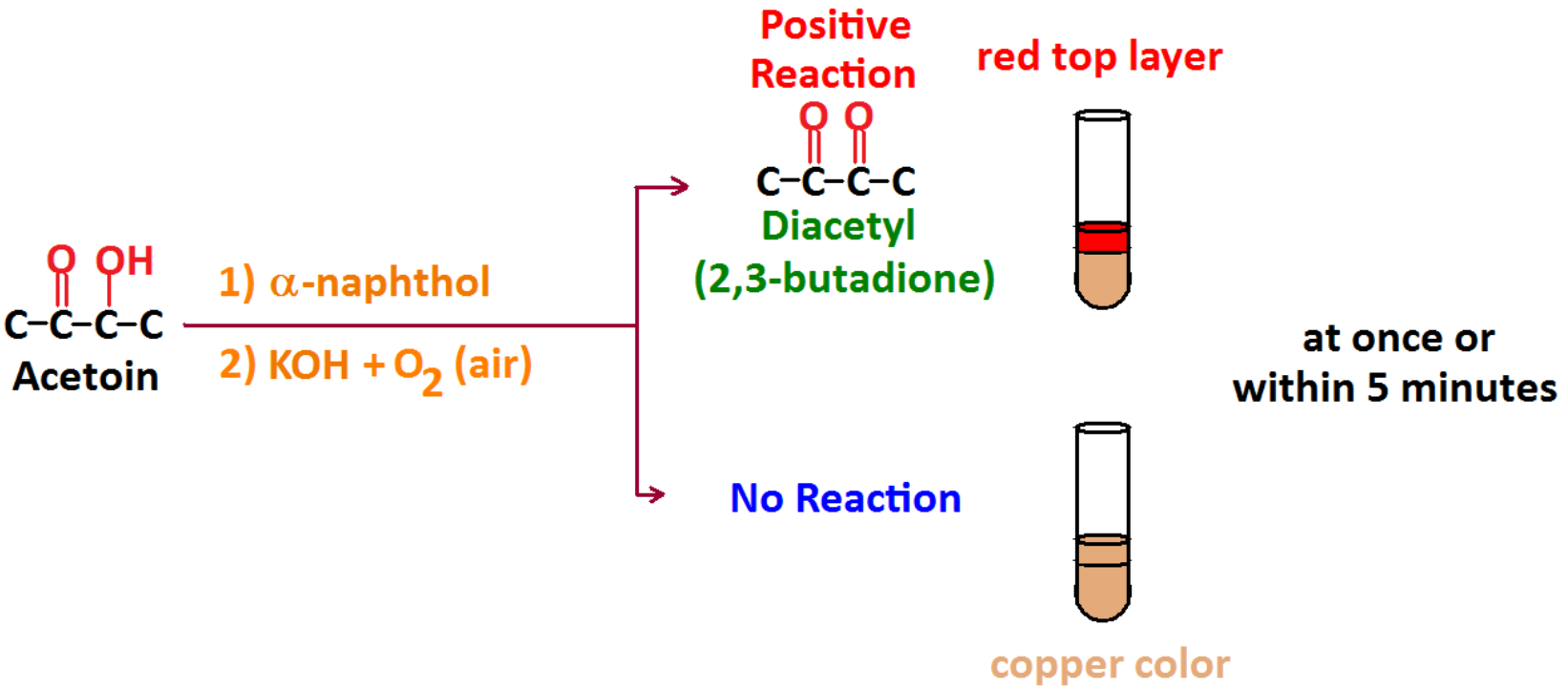
Media	Composition	Usage
Cystine-Lactose-Electrolyte-Deficient medium (CLED)	Cystine allows for growth of cys-cys dependent organisms; lactose and bromothymol blue (BTB) for differential properties; lyte deficient to prevent swarming of Proteus.	Lactose fermenters (E. coli) produce yellow colonies; non lactose fermenters (Proteus) produce blue colonies.
MacConkey agar	Contains peptone as the main source of nutriment; bile salts which have a weak suppressive effect on non-intestinal bacteria; lactose and neutral red which confer differential properties on the medium.	Most important differential medium for general purposes such as examination of urine and wound swabs. Lactose fermenters (E. coli) produce pink colonies; Salmonella and Shigella (intestinal pathogens) produce colorless colonies. S. faecalis and S. aureus (fermenters) and Proteus and P. aeruginosa (non-fermenters) grow as well. S. pyogenes is inhibited.

Media	Composition	Usage
Bacterial transport medium	**Contains salts, sodium thioglycollate to provide anaerobic conditions, methylene blue to check that these conditions are maintained (colorless0 and sufficient agar (ca. 0.3%) to render the medium semi-solid. The specimen is taken with charcoal impregnated/coated swabs which are free of inhibitory substances found in cotton wool. Swabs are inserted in the medium, snapped off and the bottle cap screwed on tightly.**	**Used to encourage survival of delicate organisms such as N. gonorrhoeae and T. vaginalis when there is delay in transporting the specimens to the lab for analysis.**

Voges-Proskauer

Metabolism and Application

Principles Behind the VP Test



α-naphthol added first as it enhances the red color sensitivity – using second may give false negative; KOH and oxygen in the air oxidize the secondary alcohol to the ketone;

α-naphthol is also known as Barritt's Reagent A

KOH is also known as Barritt's Reagent B

Application

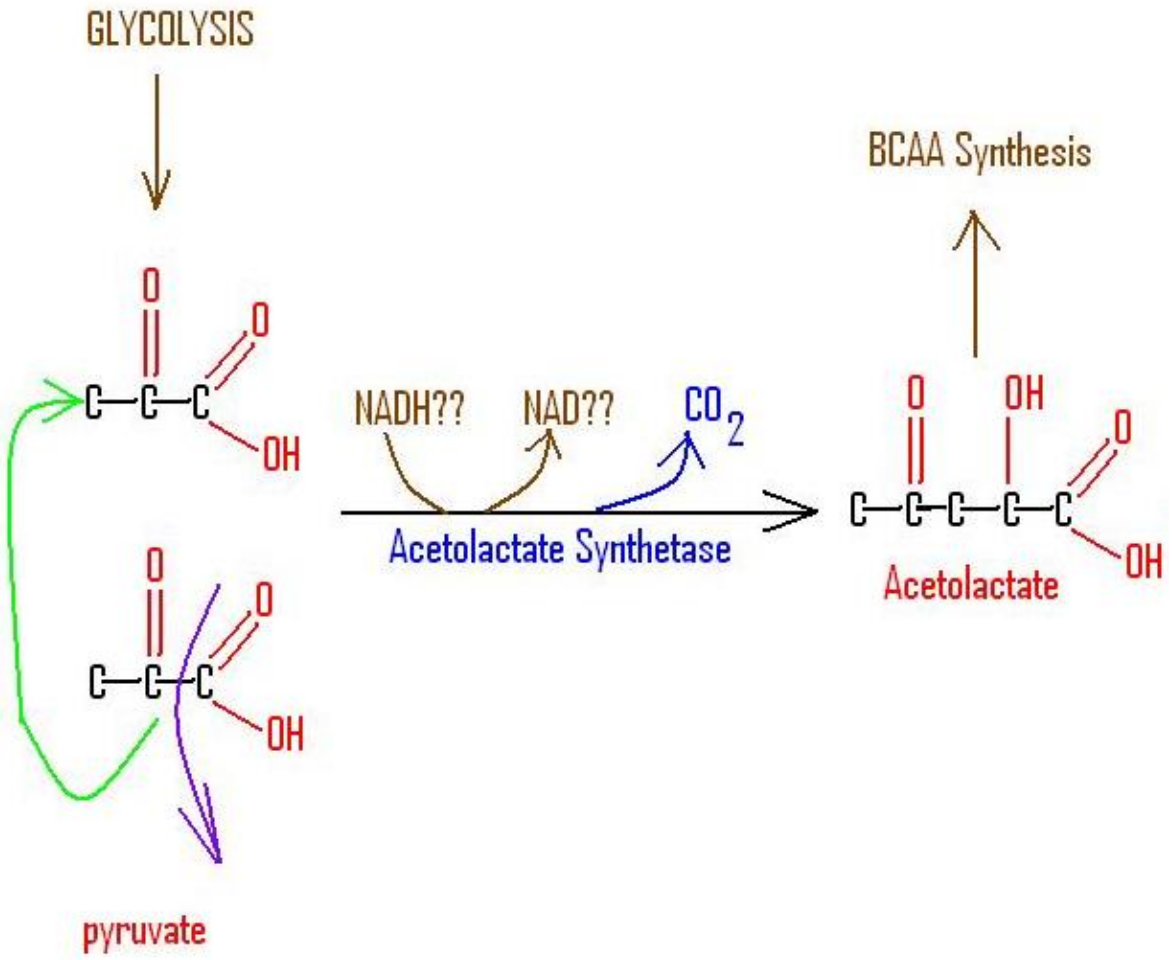
- Voges-Proskauer test is used in the identification of bacteria by identifying the presence of acetoin (3-keto-2-butanol) in the bacterial media:
- Voges-Proskauer Positive Bacteria Include:
  - E. aerogenes S. marcescens
  - K. pneumoniae
- Voges-Proskauer Negative Bacteria Include:
  - E. coli Shigella Citrobacter
  - C. freundii Yersinia
- Voges-Proskauer Inconclusive Bacteria Include:
  - Proteus

Butylene glycol (2,3-butanediol) pathway

Initiation of the butylene glycol pathway, as it's also known, derives from EMP: the formation of pyruvate.

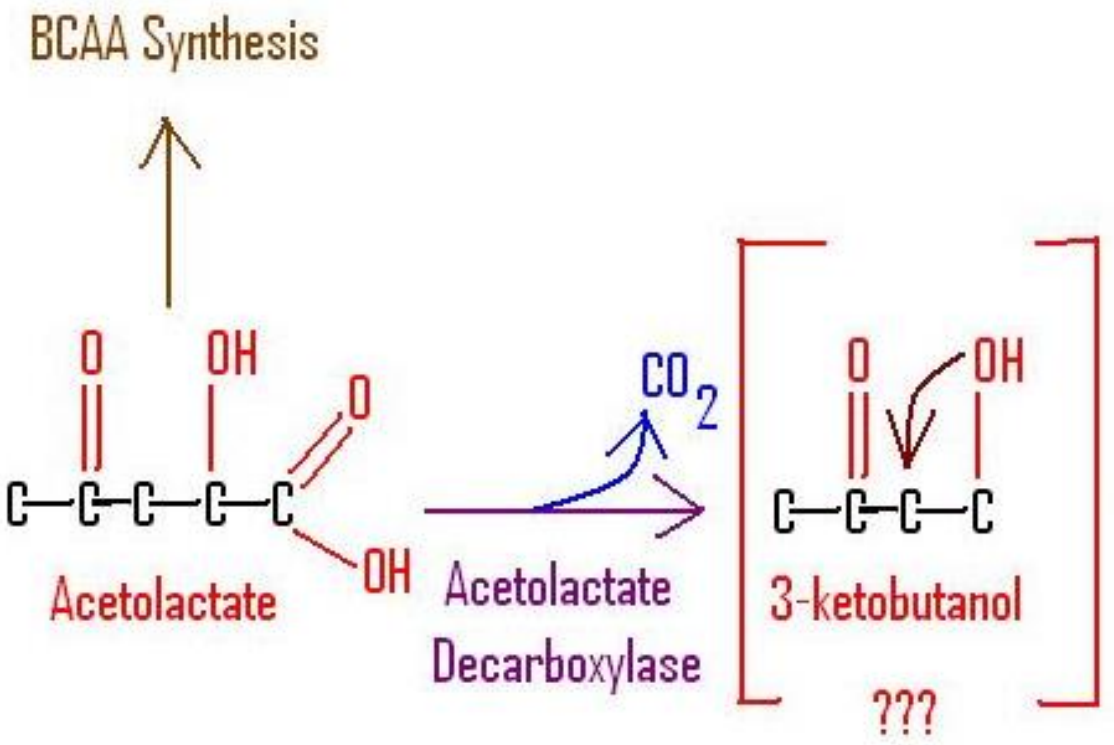
Two pyruvates are condensed with a concomitant loss of one CO_2 and the reduction of one of the ketones to an alcohol to form acetolactate.

The enzyme that catalyzes this reaction is acetolactate synthetase.



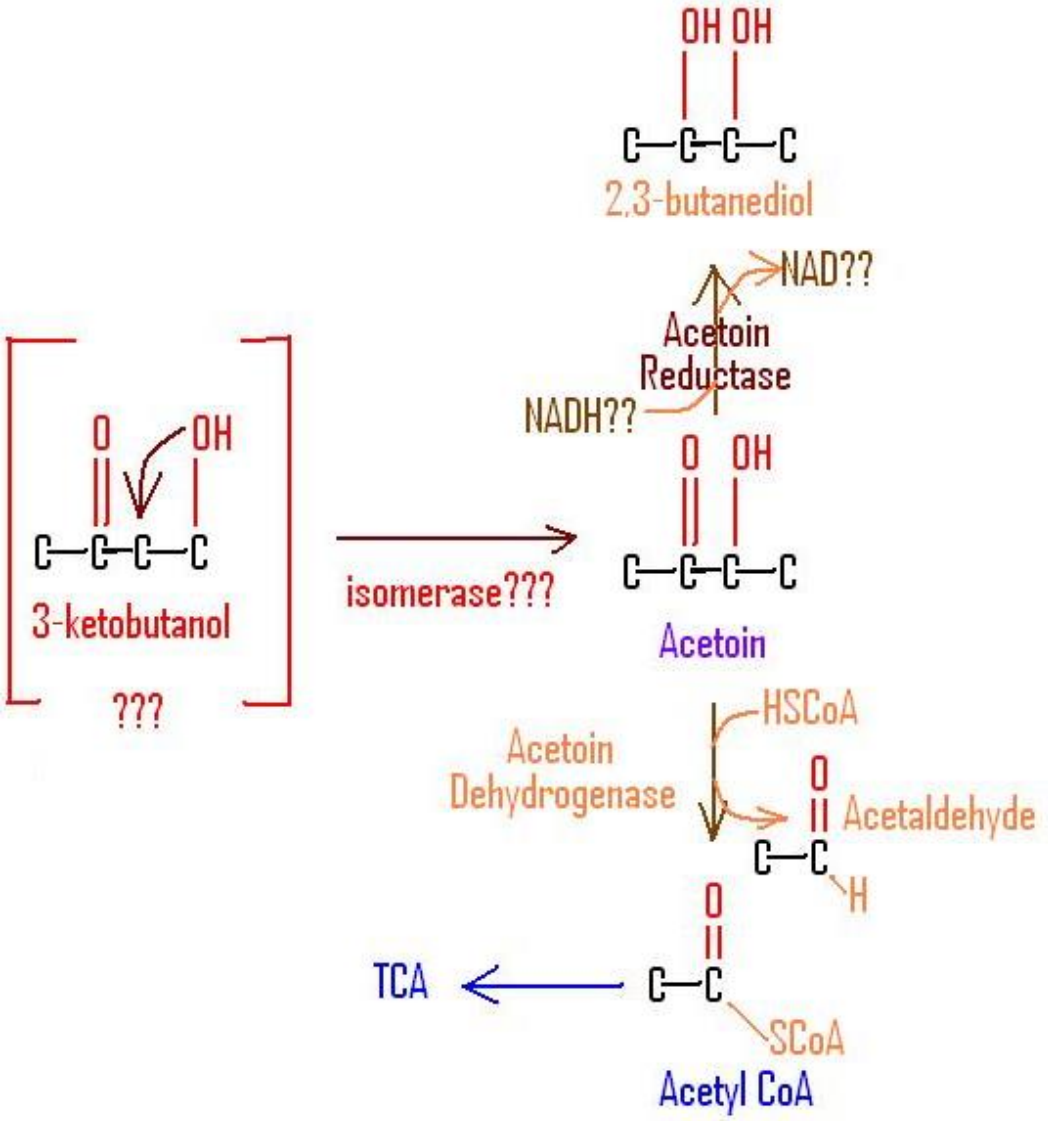
Acetolactate is commonly known as an intermediate in the synthesis of BCAA's.

If BCAA's are not needed by the prokaryote, acetolactate decarboxylase removes the carboxyl group to form [presumably] 3-ketobutanol.



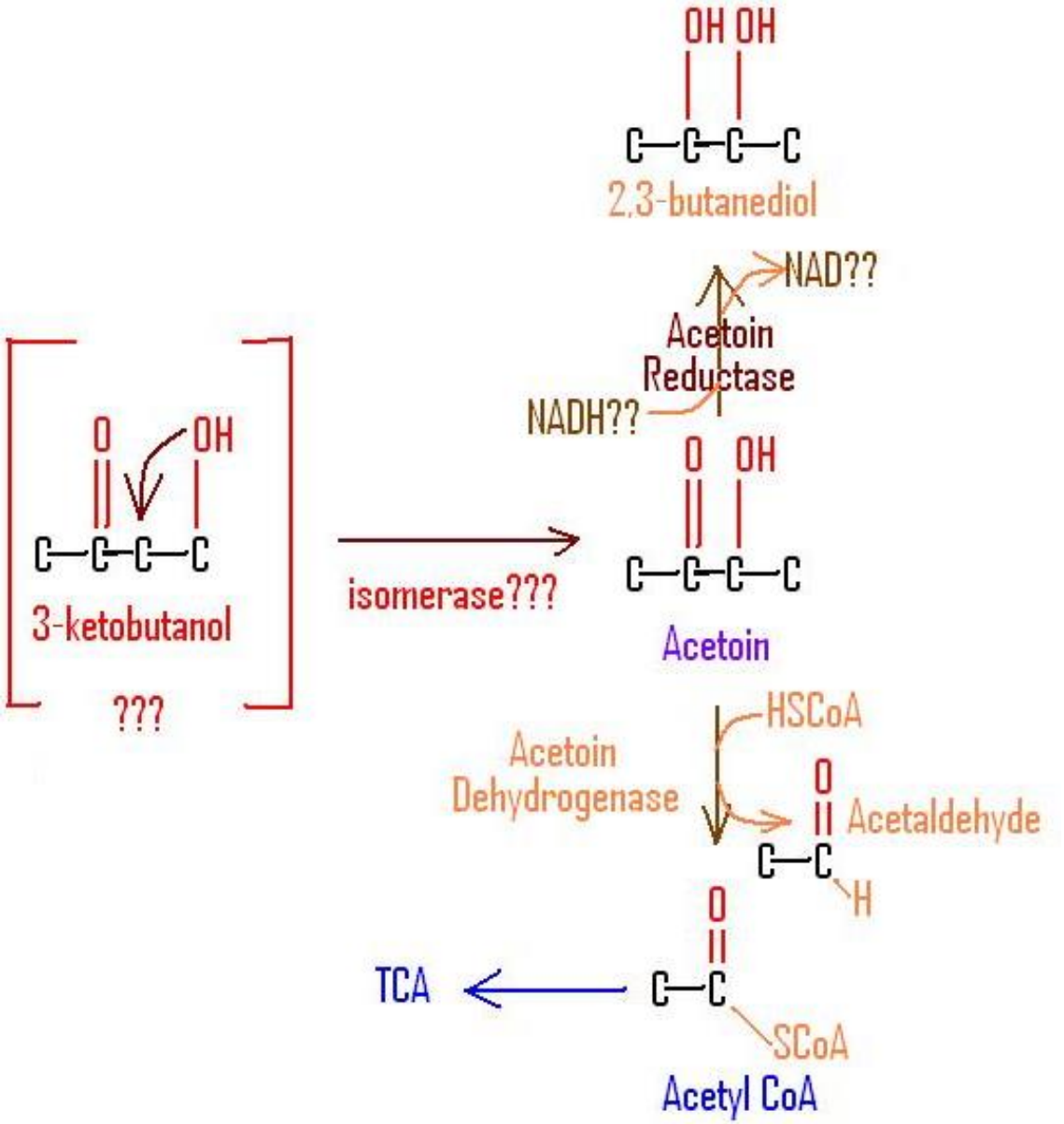
- Again, presumably,an isomerase rearranges the 1-ol to a 2-ol to form acetoin.

- It may very well be that acetoin is a branch point to regulate the bacterium or bacteria



Acetoin (top pathway) is reduced to 2,3-butanediol by, presumably, NADH.

The reaction is catalyzed by acetoin reductase.

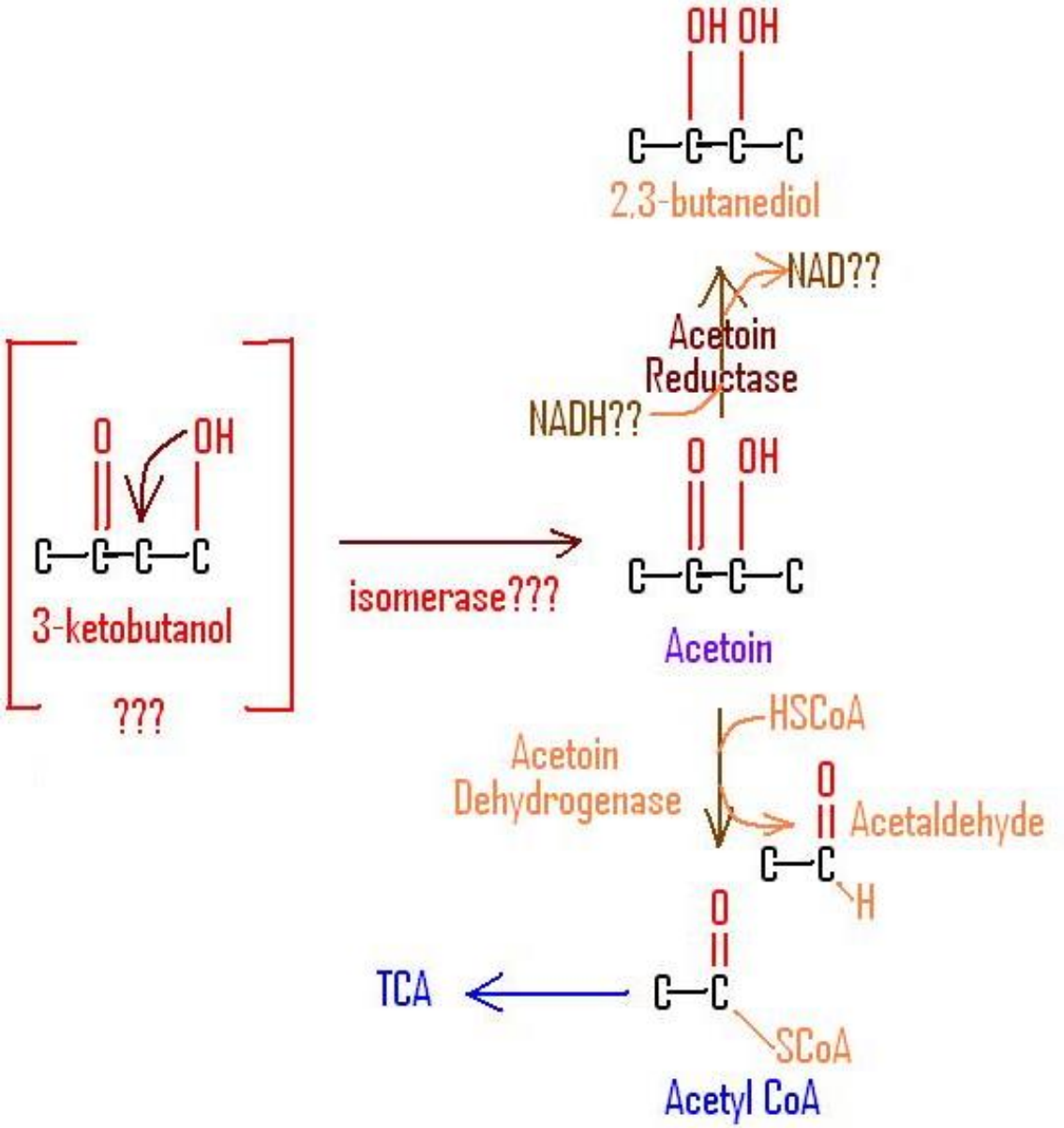


Acetoin (bottom pathway) is oxidized and thiolated to form acetyl-CoA.

The enzyme that catalyses this reaction is acetoin dehydrogenase.

An additional product formed is acetaldehyde. Its purpose is not known.

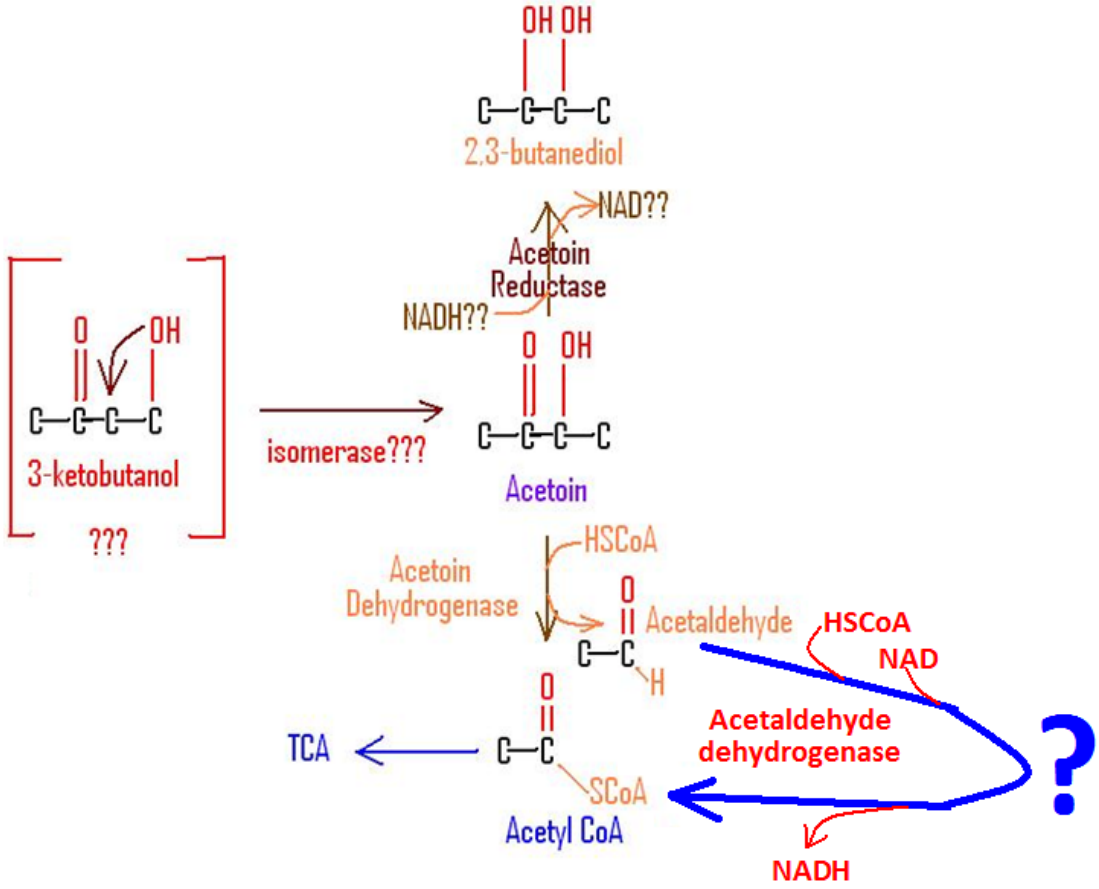
The acetyl CoA feeds into the TCA.



It seems likely that the 2,3-butanediol (butylene glycol) is probably synthesized during times when the bacteria has sources of energy and needs to regulate its population growth/decline. Top pathway.

It seems likely that the acetoin reduction to release acetaldehyde and acetyl-CoA are for when the bacterium lacks energy sources in its "diet" and uses a portion of the acetoin catabolically. Bottom pathway.

It also seems likely that the acetaldehyde that is generated is oxidized and thiolated to form more aCoA



Infection

Sources and Transmission of Infection

- There are three sources of disease-causing organisms in man:

1. Human beings (the MOST important)
2. Lower animals (much less important)
3. Inanimate nature (fomites – relatively unimportant – depending on your perspective)

1. Human Sources: Our Own Organisms

- The normal human infant is sterile at birth, but rapidly acquires a complex bacterial flora. Bacteroides, E. coli are harmless co-occupants of the bowel, but acting together following mechanical trauma to the bowel may cause peritonitis. E. coli is also the most common cause of UTI. H. influenzae, S. pneumoniae live harmlessly in the upper respiratory tract, but can cause bronchitis, bronchopneumonia, sinusitis, otitis media, if the mucous membranes are damaged by the viruses of influenza, measles and the common cold. On rare occasions, viridans streptococci may gain access to the blood, settle on a damaged heart valve and cause infective endocarditis.

Patients Incubating a Disease

- Patients who are infectious while incubating a disease are sometimes referred to as precocious carriers. This is because during the incubation period of an infectious disease the organisms multiply in the tissues but cause no clinical evidence of infection. In hepatitis A, the feces are a source of infection for about 2 weeks before the onset of jaundice. In hepatitis B, the blood is infectious for more than a month. In rubella, mumps and poliomyelitis, the upper respiratory tract is a source of infection for a few days before the onset of symptoms.

Patients with Overt Disease

- Depending on the type of disease, organisms may be present in feces, urine, droplets and discharges from the mouth, nose, ears and eyes, and in discharges of pus from internal organs, wounds, ulcers, sores and other lesions of the skin.

- In many acute diseases the organisms are rapidly killed and there may be only a short time during which the patient is infectious. Pathogens such as M. tuberculosis, T. pallidum, N. gonorrhoeae tend to produce chronic disease in which the organisms are conveyed to the exterior for much longer periods of time. Herpes simplex virus persists in the tissues in a latent form for the rest of the patient's life, occasionally awakening to produce the infectious "cold sores" of this disease.

- Not all infected patients are a source of infection for others. Sometimes the organisms attack some deep seated organ such as the meninges from which outlet to another victim is impossible. Or the pathogen may be so virulent that it rapidly kills the host and thereby exterminates itself.

Convalescent Carriers

- **Persons in contact with a patient suffering from an infectious disease may acquire the organisms and harbor them without suffering from any apparent disease.**
- **Such people are known as contact carriers or symptomless excretors.**
- **The carrier state may be temporary or chronic. The interactions between host and parasite range from very mild forms of disease to relationships which may best be regarded as commensalism (living within the body without causing disease).**
- **In outbreaks of poliomyelitis, cases of overt disease are greatly out-numbered by cases of inapparent or subclinical infection which can be recognized only because the individual produces specific antibodies. These cases are nevertheless infectious to others.**
- **The carriage of S. aureus in the nose and skin of over half the general population and an even higher proportion of hospital workers is undeniably a most important source of disease (MRSA) but is hardly a disease in itself.**
- **Other diseases in which contact carriers are common include diphtheria, strep throat, meningococcal meningitis, dysentery and hepatitis B. Since contact carriers usually go unrecognized, they constitute a special hazard for the rest of the uninfected population.**

2. Animal Sources of Zoonoses

Cattle		
Micro-organisms/Bacteria		**Cause/Disease**
M. bovis		**Tuberculosis-like**
B. abortus		**Abortions**
Coxiella burnetii		**Q-fever – influenza-like**
Campylobacter jejuni		**Gastroenteritis**
Leptospira interrogans		**Weil's disease: fever, conjunctivitis, albuminuria, hemorrhage, jaundice, renal failure**
B. anthracis		**Anthrax: inflammatory lesions, sever bronchopneumonia**
Ringworm fungi		**Ringworm**
	Microsporum	**Hair/skin NO nails**
	Trichophyton	**Skin/hair/nails**
	Epidermophyton	**Skin/nails NO hair – groin ringworm**
T. saginata		**Beef tapeworm**
Brucella melitensis		**Unspecific symptoms; undulant fever; respiratory symptoms, fever, malaise, cyanosis**

Tinea: commonly called ringworm; any fungal infection occurring on any part of the body.	
Tinea barbae	**Barber's itch; fungal infection of the bearded portions of the neck and face**
Tinea capitis	**Fungal infection of the scalp; may be due to Microsporum or Trichophyton spp.**
Tinea corporis	**Tinea of the body; starts as red, slightly raised, scaly patches that reveal minute vesicles; new patches arise from the periphery of the old; heal from inner ring out; itch like hell.**
Tinea cruris	**Jock itch; fungal disease of scrotum, crurae, anus, genitals**
Tinea nigra	**Fungal infections of the palms of the hands; deeply pigmented, non-scaly patches; Cladosporium spp.**
Tinea nodosa	**Hard, sheath-like nodular masses in beard/moustache hair from Trichosporum spp. (white) or Piedraa spp. (black). TX: shave hair off**
Tinea pedis	**Athlete's foot; fungal infection of the foot**
Tinea unguium	**Fungal disease of the nails**
Tinea versicolor	**Fungal infection that produces bran-like patches; yellow/fawn-colored; caused by Malassezia.**

Pigs/Hogs	
Streptococcus suis	**Similar to S. faecalis**
Salmonella	**Food poisoning**
B. anthracis	**Anthrax (malignant pustule)**
Erysipelothrix rhusiopathiae	**Inflammatory lesions**
Balantidium coli	**Dysentery-like illness**
Trichinella spiralis	**Trichinosis (abdominal pain, N/V, diarrhea, diplopia)**
T. solium	**Pork tapeworm**

Sheep	
B. anthracis	**Anthrax: inflammatory lesions, sever bronchopneumonia; woolsorter's disease**
Fasciola hepatica	**Jaundice**
Coxiella burnetii	**Q-fever – influenza-like**
Chlamydia psittaci	**Pulmonary infections**

Horses	
C. tetani	Tetanus

Dogs	
Rabies virus	Rabies
Pasturella multocida	Similar to Yersinia
Toxocara canis	Dog round worm

Cats	
Ringworm fungus	Ringworm
P. multocida	Similar to Yersinia
T. gondii	Toxoplasmosis; similar to infectious mononucleosis, pyrexia, lymphocytosis, lymphadenopathy

Rats	
Y. pestis	**Plague**
Streptobacillus moniliformis	**Rate-bite fever (pyrexia, rash, polyarthritis)**
Salmonella	**Food poisoning**
T. spiralis	**Trichinosis (abdominal pain, N/V, diarrhea, diplopia)**

Mice	
Salmonella	**Food poisoning**
R. akari	**Rickettsialpox (similar to chicken pox)**

Chickens, Ducks, Turkeys	
Salmonella	**Food poisoning**
Campylobacters	**Gastroenteritis**

3. Inanimate Sources (FOMITES) of Infection

Soil	
P. aeruginosa	**Pneumonia, UTI, wound infections**
Proteus	**Ibid**
C. tetani	**Tetanus**
C. perfringens	**Type A: Human gas gangrene Type C: Necrotizing enteritis Types B, D, E: cause intestinal disease in animals**
Histoplasma capsulatum	**Histoplasmosis (respiratory disorder)**

Soil/Water	
Legionella pneumophila	**Legionnaire's disease (severe pneumonia, diarrhea, mental confusion)**

Presenting Stages of the Infective Process

Period of Infection	**Interval between the time of the infection is received and the appearance of the disease. Length of incubation depends on AT LEAST five conditions**	**1.Nature of the agent (the incubation for diphtheria is less than that of rabies)** **2.Its virulence** **3.Resistance of the host.** **4.Distance from the site of entrance to focus of action (the incubation of rabies, which effects the brain, is shorter when the inoculation site is about the face.** **5.Number of infectious agents invading the body.**

Period of Prodromal Symptoms	**Short interval (prodrome) that sometimes follows the incubation period, described by such symptoms as headache and malaise.**

Period of Invasion	**Disease reaching its full development and maximum intensity. May be rapid – a few hours as in pneumonia – or insidious – a few days as in typhoid fever. At the onset of acute infectious disease, rigors and chills often precede the temperature rise. Heat production exceeds heat loss with concomitant fever – NO SWEATING in this stage.**

Fastigium or Acme	**Disease is at its height**
Period of Defervescence or Decline	**Stage during which manifestations decline. During this stage, profuse SWEATING OCCURS. Heat loss soon exceeds heat production. Defervescence may be by crisis (within 24 hours) or by lysis (within several days, with the temp going down a little each day until it's normal). Fever that begins abruptly usually ends by crisis.**
Convalescence	**The patient regains his or her strength.**

FEVER		
Temperature measured quantitatively by one of four methods	**Orally**	**NPO for at least 30 minutes**
	Rectally	**Subtract 1° F**
	Aurally	**Infra-red in EAM – problems, sort of**
	Axillary	**Add 1° F – not used much any more, BUT, depending on the parent, may be more useful and LESS TRAUMATIC than rectal temp – communication on this with physician is important**
Measured qualitatively	**Use back of hand on nape of neck (most sensitive tissue and most heat "releasing" tissues, respectively)**	
Fever = body's response to foreign invasion/infection – is NOT the disease.		

Temperatures to 102° F do NOT need tx unless patient is uncomfortable OR unless patient is less than 6 months of age or of senior citizen age – even a slight fever in these age groups warrants calling physician since non-specific symptoms may be present for serious disease.		
High fever is defined as a temperature above 105° F.		
Diseases with high fever: UTI (gram negative); meningococcemia, typhoid fever, brucellosis, tularemia, TB, malaria, relapsing fever, leptospirosis	**Diseases with little to no fever: Diphtheria, cutaneous anthrax, sporotrichosis**	**Diseases with Tendencies to "Repeat Infections with Fever" D. mellitus, congenital hypoparathyroidism, nephrosis, SLE, leukemia, lymphomas and plasma cell disease**
CHILLS: associated with a period of rapid and widespread invasion of the host by the parasite		

When to treat fever	
1) when risk of dehydration is great	**Fluids prn**
2) to make patient comfortable	**Light clothing, bedding, cool room, vaporizer**
3) Temperature > 102 F	
4) FUO > 104 F	**Sponge no sooner than one hour after antipyrexic – if sponge sooner than 1 hour after po antipyrexic, drug doesn't have enough time to decrease fever – patient may, then, feel a chill and fever will INCREASE after sponging is complete**
	Acetaminophen: age appropriate dosage and tx. Advil: Ibid; may use acetaminophen and Advil on alternating 2 hour dosing to control high fever; ASA: Age appropriate dosage – do NOT give to children < 15 YOA – particularly when infected with/by chicken pox or influenza
5) FUO < 104 F for more than 24 hours	

Reye's Syndrome	
Acute encephalopathy; fatty infiltration of liver, pancreas, heart, spleen, lymph nodes and kidney; in children < 15 YOA after acute viral disease	**Occurs about 6 days after primary disease: N/V, disorientation, coma, seizure, hepatomegaly (> half the cases have jaundice)**
Due to ASA with primary disease: may be fatal; occurs on RECOVERY side of illness.	
Use advil or acetaminophen with children to tx fever	**Do NOT use ASA to tx fever in children < 15 YOA or senior citizens.**
Follow this PARTICULARLY in children who have chicken pox or influenza.	

Post-operative Temperatures:

- Temperature less than or equal to 34.4° C up to 12 hours post-op may be due to anesthetic or due to heat loss during surgery.
- Temperature less than or equal to 38° C up to 24-48 hours post-op may be due to inflammation due to surgery.
- Temperature greater than 38° C up to 24-48 hours post-op may be due to pulmonary congestion or atelectasis or dehydration.
- Temperature greater than 37.8° C 72 hours post-op and on may be due to an infected surgical site, UTI, LRI or phlebitis.

Ability of Bacteria to Grow in Host Tissues

Five factors are necessary to accomplish this goal:

1. Nutritional factors: the basic food stuffs needed by pathogens are available in the tissues. Paradoxically, non-pathogens require less nutritional requirements and don't grow in tissues.
2. Temperature: organisms are more likely to establish themselves as parasites if the optimal temperature for their growth is the same as the temperature of the host. It is possible to increase or decrease the susceptibility of animals to various bacterial and viral diseases by artificially altering the temperatures of the host.
3. pH: most organisms will grow at the pH of normal tissues.
4. Redox potential: a low redox potential in the host tissues is an essential requirement for the growth of strict aerobes.
5. Unknown factors: the above factors offer no satisfactory explanation for the high degree of host specificity shown by many organisms, e.g., why are leprosy, gonorrhea and measles strictly human diseases? Presumably there must be some subtle differences in the physiologicochemical properties of human tissues and tissues of other animals.

Basis of Virulence

Antiphagocytic/Antilytic Properties

Surface components: resistance to phagocytosis mainly depends on the presence of surface components which make it difficult for the phagocytes to ingest organisms. The subject has been most thoroughly studied in the case of S. pneumoniae (Griffith's transformation), but the principles involved have general validity.
Most pathogenic bacteria do not possess obvious capsules, but a similar function is served by their surface antigens such as the M proteins of S. pyogenes or the endotoxins of Gram negative bacteria. The ability of Gram negative bacteria to withstand the bactericidal action of serum is probably due to their surface antigens (endotoxins).

- Leukocidins: some organisms such as S. aureus and S. pyogenes produce complex substances which will kill WBC.
- Such substances are toxic to leukocytes and destroy WBC by lysis of the cytoplasmic granules and are partially responsible for the pathogenicity of the organisms.

- Toxins: toxins are bacterial products or constituents which have a direct harmful action on tissue cells. There are two groups:
  - Exotoxins: are produced by living bacteria and diffuse freely into the surrounding medium, i.e., they are extracellular toxins. Typical exotoxins are produced mainly by Gram positive bacteria/organisms. Organisms producing exotoxins include C. diphtheriae, C. tetani, C. botulinum, C. perfringens, S. pyogenes, S. aureus and a few Gram negative bacteria including S. dysenteriae, P. aeruginosa, V. cholerae and some trains of E. coli.
  - Endotoxins: do not diffuse into the surrounding medium, but form part of the bacterial cell from which they are liberated when the cell dies and disintegrates, i.e., they are intracellular toxins. Typical endotoxins are constituents of Gram negative organisms. Organisms producing endotoxins include Salmonella, Shigella, Brucells, Neisseria, V. cholerae, E. coli and P. aeruginosa.

- The significance of toxins is seen most clearly in the case of organisms which produce powerful exotoxins. Botulism is not an infectious disease, at all, since C. botulinum does not invade the body. The symptoms of classical botulism are entirely due to consumption of toxins produced when the organisms multiply in food stuffs. In tetanus and diphtheria, the infection remains highly localized and symptoms are due to toxins which diffuse from the infected area. The paralytic effects of botulism, the spasms and convulsions of tetanus, the hemorrhagic adrenals, degenerative changes in cardiac muscle and muscular paralysis of diphtheria can all be reproduced by injecting animals with minute doses of the appropriate cell-free culture filtrate.

- Potency of toxins: The exotoxins of C. botulimun, C. tetani and S. dysenteriae share the distinction of being the most poisonous substances known to man.
- It is estimated that 1 mg of purified botulinum or tetanus toxin would be sufficient to kill more then 1000 tons of guinea pigs.
- The toxin of S. dysenteriae is equally potent for rabbits.
- Diphtheria toxin is 300 times less toxic, but is still more than 1000 times as poisonous as strychnine.

ASIDE

- Strychinine is an extremely poisonous white crystalline alkaloid, $C_{21}H_{22}O_2N_2$, derived from nux vomica and related plants, used as a poison for rodents and other pests;
- In synapses in the central nervous system there are two functionally distinct types of receptor.
- Binding at
  - *stimulatory* receptors increases the likelihood of a signal being transmitted, while binding at
  - *inhibitory* receptors reduces it.
- Glycine receptors are one of the main types of inhibitory receptor, particularly in the lower parts of the central nervous system, and it is these that are blocked by strychnine. (Rang, 1999)

- The result is huge over-transmission of signals, resulting in reflex arcs, which would normally be suppressed by the postsynaptic action of glycine, becoming active, so that the tiniest sensory stimulus produces powerful muscular contraction.
- This leads to powerful and extremely painful convulsions, there being no impairment of cognitive or sensory function. Death occurs as a result of respiratory arrest, due to spasm and paralysis of the respiratory muscles.

- Symptoms usually begin within about 20 minutes of ingestion of the poison, but onset can be more gradual. The lethal dose is about 5mg/kg body-weight, in other words about 350mg for an adult.
- END OF ASIDE

- In contrast to exotoxins, 1 mg of a typical endotoxin constitutes a lethal dose for 1-10 mice.

- Toxoids: are toxins that have been modified so that they lose their antigenic properties.
- The difference occurs to some extent during prolonged storage but are effected more rapidly by moderate heat or by treatment with formalin.
- Formol toxoids are widely used for immunization of man and animals.

Bacterial Enzymes that Add Virulence			
Enzyme	**Source**	**Action**	**Effect**
Coagulase	**S. aureus**	**Forms fibrin clot**	**Increases resistance to phagocytosis**
Streptokinase	**S. pyogenes, Staphylococcus and Clostridia**	**Dissolves fibrin clot**	**Prevents isolation of infection; increases spread of disease**
Hyaluronidase	**S. pneumoniae, Streptococcus, S. aureus, C. perfringens and septicum, S. pyogenes, S. pneumoniae**	**Hydrolysis of hyaluronic acid; intercellular substance/cement**	**Increases bacterial spread via increasing permeability of tissue**

Bacterial Enzymes that Add Virulence			
Enzyme	**Source**	**Action**	**Effect**
Leukocidin	**Staphylococcus and Streptococcus**	**Disintegrates phagocytes**	**Decrease phagocytosis**
Hemolysins	**Clostridia and Staphylococcus**	**Lyse RBC**	**Causes anemia and decreases oxygen delivery to cells**
Collagenase	**C. perfringens and C. histolyticum**	**Hydrolyzes collagen**	**Allows bacteria to spread through tissue barriers; causes disintegration of muscle and other tissues**
Lecithinase	**C. perfringens, C. novyi**	**Hydrolyzes PC in cell membranes**	**Increases toxicity/invasiveness of organisms**

Bacterial Enzymes that Add Virulence			
Enzyme	**Source**	**Action**	**Effect**
DNA'ase	**S. pyogenes, C. prefringens, C. septicum**	**Hydrolyzes DNA (viscous part of pus and inflammatory exudates – pus from wounds)**	**Increases spread of organisms in tissues**
Enterotoxin	**S. aureus**	**Emesis**	
Exfoliatin	**S. aureus**	**Exfoliation of infant skin**	**Scalded Skin Syndrome**

Bacterial Enzymes that Add Virulence			
Enzyme	**Source**	**Action**	**Effect**
α-hemolysis	**S. aureus**	**RBC of few animal species**	**Dermonecrotizing; lethal**
β-hemolysin	**S. aureus**	**Phospholipase (sphingomyelinase C); narrow hemolytic spectrum**	**Lethal**
γ-hemolysin	**S. aureus**	**Broad hemolytic spectrum**	**Lethal**

- These and other enzymes may be taken advantage of to identify bacteria. Some of the other enzymes include:

Selected Enzymes use to Identify Bacteria	
Enzyme	**Test/Principle**
Catalase	**Add a drop of H_2O_2 on slide with bacteria – bubbles = positive test.**
Coagulase	**Citrated plasma (1:5) with an equal volume of broth culture – if clots within 1-4 hours = positive test (all coagulase positive Staph are pathogenic for man).**
ONPG (β-galactosidase)	**A colorimetric assay for the determination of β-galactosidase activity in bacteria.**

Selected Enzymes use to Identify Bacteria	
Enzyme	**Test/Principle**
Citrate utilization	**Medium begins green – as citrate is utilized from the medium, the pH goes up and the medium turns blue (bromthymol blue is the indicator – blue at or above pH 7.5; green below pH 7.5)**
Oxidase	**Produced by Neisseria (and other bacteria) – with 1% tetramethyl-p-phenylene diamine, colonies turn pink, then red, then purple, then black: oxidase positive – reaction is lethal to cells and cells must be subcultured rapidly within a few minutes.**
Gel Liquefaction	**20°C for at least 2 weeks; liquefies gelatin in a deep to a "watery – jello-y" consistency.**

- Hypersensitivity: a.k.a. allergy. Some products of bacteria, viruses and fungi are relatively harmless for normal people, but are toxic for individuals who are suffering from the relevant disease or who have had the disease in the past.
- Thus, tuberculin, a complex preparation obtained from M. tuberculosis, is about 100 times more lethal for tuberculous guinea pigs that it is for normal guinea pigs.
- Allergy develops in nearly all infectious disease, but it often does not appear until convalescence and it is by no means certain that it always favors the parasite.

- Interference with Metabolism of Host Cells:
- In viral infections an important part of the metabolism of the host cell is diverted to the synthesis of viral material, e.g., HIV.
- In bacterial infections competition for nutrient materials may sometimes account for damage to the host cell, but at present there is little evidence that this is an important mechanism of toxicity.

- Metabolic End-Products:
- Simple end products of microbial metabolism, e.g., lactate, may reach toxic concentrations in the immediate vicinity of invading organisms.
- The contribution of such substances to microbial toxicity is obscure – for the time being, anyway.

Portals of Entry: How Micro-Organisms Get into the Body

1.

Transplacental infection: T. pallidum, CMV, HIV, Hepatitis B virus, T. gondii

2.

- Inhalation and infection through the respiratory tract: strep throat, meningococcal meningitis, whooping cough, measles, influenza, common cold.
  - Direct transfer: Kissing, use of infected eating utensils: infectious mononucleosis, HSV1 and CMV
  - Droplet Infection: in eupnea, few to no organisms; in quiet conversation, the number is small; when shouting, greater numbers are present. All of these numbers are small compared to the vast organisms released during sneezing or coughing. Nose blowing is a prolific source of nasal organisms. A vigorous cough projects about 5000 droplets; a sneeze as many as 1,000,000. Importance of direct air-borne infection is uncertain. Droplets are largely formed from the saliva at the front of the mouth and this areas is not necessarily contaminated with organisms causing disease in the tonsils, pharynx and lungs. It, however, seems reasonable to suppose that a cough or sneeze at a short range directly into the victim's face is a fairly certain way of transferring infection. N. meningitides, B. pertussis – source may be droplets, but are rapidly killed by drying.

2.

- Dust-born infections: Many respiratory tract infections are probably acquired by an indirect process involving 2 stages:
  - The donor contaminates him/herself and his/her environment, and
  - The organisms are conveyed to the recipient in the form of dust. This comes about by daily nose rubbing, pen sucking, smoking a pipe, using a handkerchief, then touching someone. NOSOCOMIAL INFECTIONS. Also by drying out of sunlight (M. tuberculosis, S. pyogenes, S. aureus, C, diphtheriae, smallpox virus) and being dispersed by a dust mop or broom.

3.

- Ingestion/Infection via GI tract: Salmonella, Shigella, E. coli, V. cholerae, C. jejuni, Y. enterocolitica, G. lamblia, round worms, tape worms – these are swallowed and produce direct effects.
- Indirectly infective are from toxins produced by S. aureus, C. botulinum, C. prefringens. Those which enter at the GI tract, but act elsewhere include Brucella, C. burnetti, Hepatitis A virus, polio, T. gondii.

4.

- Inoculation through the skin or mucous membranes:
  - Simple contact: in the absence of any damage to skin or mucous membranes, simple contact is enough to spread some diseases. Thus the STD's syphilis and gonorrhea are spread by contact of genital mucous membranes. Other STD's include granuloma venereum, NGU, HSV1, 2, 6, Genital warts, candidiasis, trichomoniasis, scabies, crab lice.
  - Close physical contact between individuals or through the intermediary of clothing, bedding, towels, utensils important in the spread of:
    - Skin infections such as scabies, impetigo, boils, warts, ring worm
    - General disease which effect the skin such as leprosy, yaws (T. pertenue), 2° syphilitic rashes and
    - Infections of the conjunctivae by H. influenzae, C. trachomatis

4.

- Wound infection: occurs when a break in continuity of skin or mucous membranes exposes the underlying tissues.
- S. aureus is one of the most frequent wound infections in hospitals.
- The contamination occurs predominantly during wound dressing, bed making and dusting.
- C. tetani is introduced at the time the wound is inflicted.

4.

- Injection:
  - Medical: these are derived from individuals who have given blood for transfusion which is positive for Hepatits A, HIV, Syphilis, CMV, HSV, Malaria, but was not screened properly. This is no longer a problem in the U.S.

4. Biting insects (subcategory of injection):

Biting Insects			
Mosquitoes	**Plasmodium**	**Vivax**	**Malaria**
		Ovale	
		Falciparum	
		Malariae	
	Arboviruses		**Yellow fever**
	Wuchereria	**bancroftii**	**Filariasis (elephantiasis)**
Fleas	**Yersinia**	**pestis**	**Bubonic plague**
Lice	**Rochalimaea**	**quintana**	**Trench fever**
Ticks	**Rickettsia**	**rickettsii**	**Rocky Mountain Spotted Fever**
	Borrelia	**burgdorferi**	**Lyme disease**
Mites	**Rickettsia**	**tsutsugamushi**	**Scrub typhus (small necrotic ulcer at site of injection)**

4.

- Transplantation: kidneys, bone marrow, corneal grafts may transmit infection from one person to another – particularly if the patient is receiving something like cyclosporine (immunosuppressive drug to decrease the chance of transplant rejection). CMV is the most common. HIV via grafts has been mentioned from time to time in the literature. Semen for AI and in vitro fertilization may convey HIV and Hepatitis B if the donor has not been properly screened. This is not a problem in the U.S.
    - Multiple portals: M. tuberculosis via inhalation, ingestion, inoculation.

- Usually infectious if only through a particular portal, e.g.
    - Salmonella: gain access via bowel, but doesn't infect wounds
    - S. aureus: readily infects wounds, but is unlikely to be infectious by ingestion
    - Exceptions, of course

Bacterial Virulence

- Saphrophytism: mode of free living organisms which obtain their nourishment from soil and water.
- Do not require a living host and only in extremely rare circumstances do any of the establish residence as a parasite.

- Parasitism: adaptation to life on or in the bodies of higher organisms. The association may take one of three forms:
  - Symbiosis: is the ability to live in the tissues of the host with mutual benefit. This does not occur in man.
  - Commensalism: literally "eating at the same table" – is the ability to live on the external or internal surface of the body without causing disease. Common harmless commensals are sometimes referred to, erroneously, as saprophytes. Pathogenic organisms may occasionally be carried as temporary commensals.
  - Pathogenicity: the capability of the organisms to produce disease. All pathogens are endowed with **virulence**, a measure of their degree of pathogenicity. Virulence depends on **invasiveness**, the ability to penetrate the tissues, overcome host defenses, multiply and disseminate widely. Virulence depends on **toxicity**, the capability of the micro-organism to damage the tissues.

- Virulence of the Parasite: all the functions of the parasite that favor its survival, growth, multiplication and ability to produce pathological changes in the host tissues.
- Resistance of the Host: All the inherent and adaptive mechanisms of the host that enable it to withstand the deleterious effects of the parasite.
- Virulence of the parasite is always dependent upon the "balancing" of resistance of the host with the virulence of the parasite.
- Opportunist: normally harmless organisms which take the opportunity afforded by lowered host resistance to act as pathogens. Very common nowadays, e.g., immunosuppressive therapy, AIDS, protective effects of normal flora removed by antibiotics (PCN's and candidal infections).

Sterilization

Sterilization Definitions

Sterilization	The process of destroying all forms of microbial life. A sterile object is free of living microorganisms.
Disinfectant	An agent, usually chemical, that kills the growing forms but not necessarily the spore forms of disease producing microorganisms. Applied to substances used on inanimate objects (fomites).

Properties of a good disinfectant	1. Attack all types of microorganisms	2. Be rapid in its action
	3. Not destroy body tissues or act as a poison if taken internally	4. Not be retarded in its action by organic matter
	5. Penetrate material being disinfected	6. Dissolve easily in or mix with water to form a stable solution
	7. Not decompose when exposed to heat, light rays or unfavorable weather conditions	8. Not damage materials being disinfected such as instruments or fabric
	9. Not have an unpleasant odor or discolor the material being disinfected	10. Be easily obtained at a comparatively low cost and readily transportable
	11. MOST IMPORTANT: The ability to form lethal combinations with microbial cells	

Antiseptic	**A substance that prevents the growth or action of microorganisms either by destroying them or by inhibiting their growth and activity. Applied to the body.**
Sanitizer	**An agent that reduces the microbial population to safe levels as judged by public health requirements. Commonly applies to inanimate objects and are used in dairies and food plants and in restaurants.**
Germicide (Microbicide)	**In practice almost the same thing as a disinfectant, but is more commonly used for all kinds of germs for any application.**
Bactericide	**An agent that kills bacteria.**
Bacteriostasis	**A condition in which the growth of bacteria is prevented. The action caused by these agents is called bacteriostatic action.**
Antimicrobial agent	**One that interferes with the growth and activity of microbes. When used to treat infections are called therapeutic agents.**

Hand Washing

1. Wash hands and arms well with water.
2. With soap and a sterile brush, scrub nails and knuckles of both hands.
3. Scrub the hands, beginning with thumbs and in succession scrubbing inner and outer surfaces of thumbs and fingers, giving several strokes to each area.
4. Scrub palms and backs of the hands and then forearms to the arms 3 inches above the elbows for three (3) minutes; rinse well with running water.
5. With a sterile orange stick, or plastic stick for the purpose, clean under each nail thoroughly.
6. With a second sterile brush, scrub hands and forearms for 7 minutes in the same manner as with the first brush.
7. Thoroughly rinse off all the soap with water and then rinse hands in 70% alcohol.
8. With one end of a sterile towel, dry one hand and, with a circular motion, dry forearm to arm above elbow. With the other end of the towel, dry the other hand and forearm in the same manner.

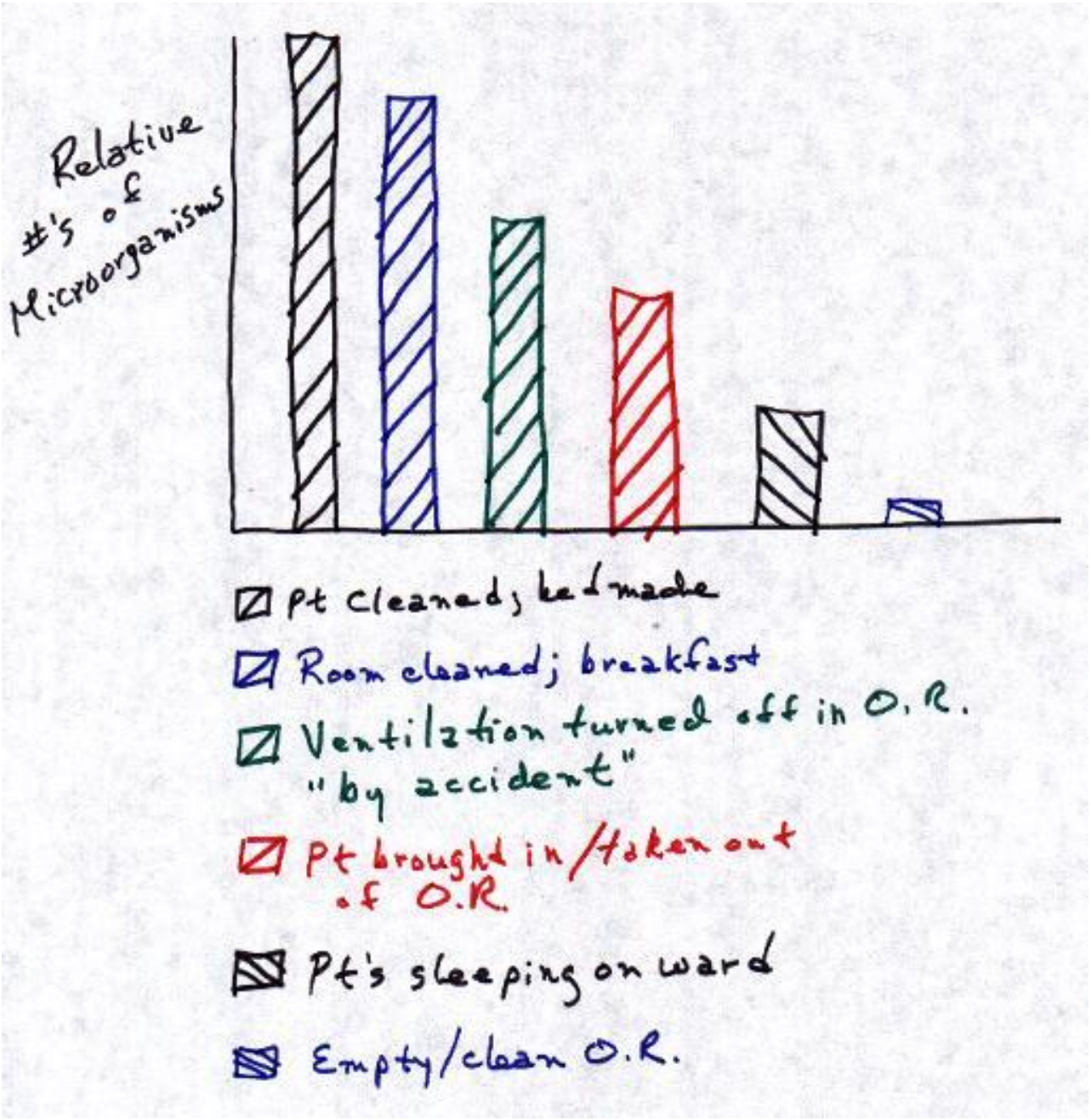
Control of Hospital/Community Infection

General Principles
1. Removing the source of the infection
2. Interrupting the route of transmission
3. Increasing the resistance of the individual

Routine Surveillance
Infection Control Officer **Infection Control Nurse**
Both are the individuals who are responsible for maintaining up to date records of all infections occurring in the hospital. Two sources of information for them:
1. Lab records of the more important organisms isolated.
2. Information acquired from members of the staff and visiting family members.

Epidemiological Survey
More detailed information is needed when an outbreak occurs or is threatened. This information will assist in identifying the source of the infection and accelerate its clean-up:
1. Name, age, sex of the patient and disease
2. Name of surgeon, ass't surgeons, nurses and anesthesiologist
3. Nature of procedures, date and staff involved
4. Type of sepsis (superficial or deep) and date of onset
5. Pathogens isolated, antibiotic sensitivities and results of typing procedures

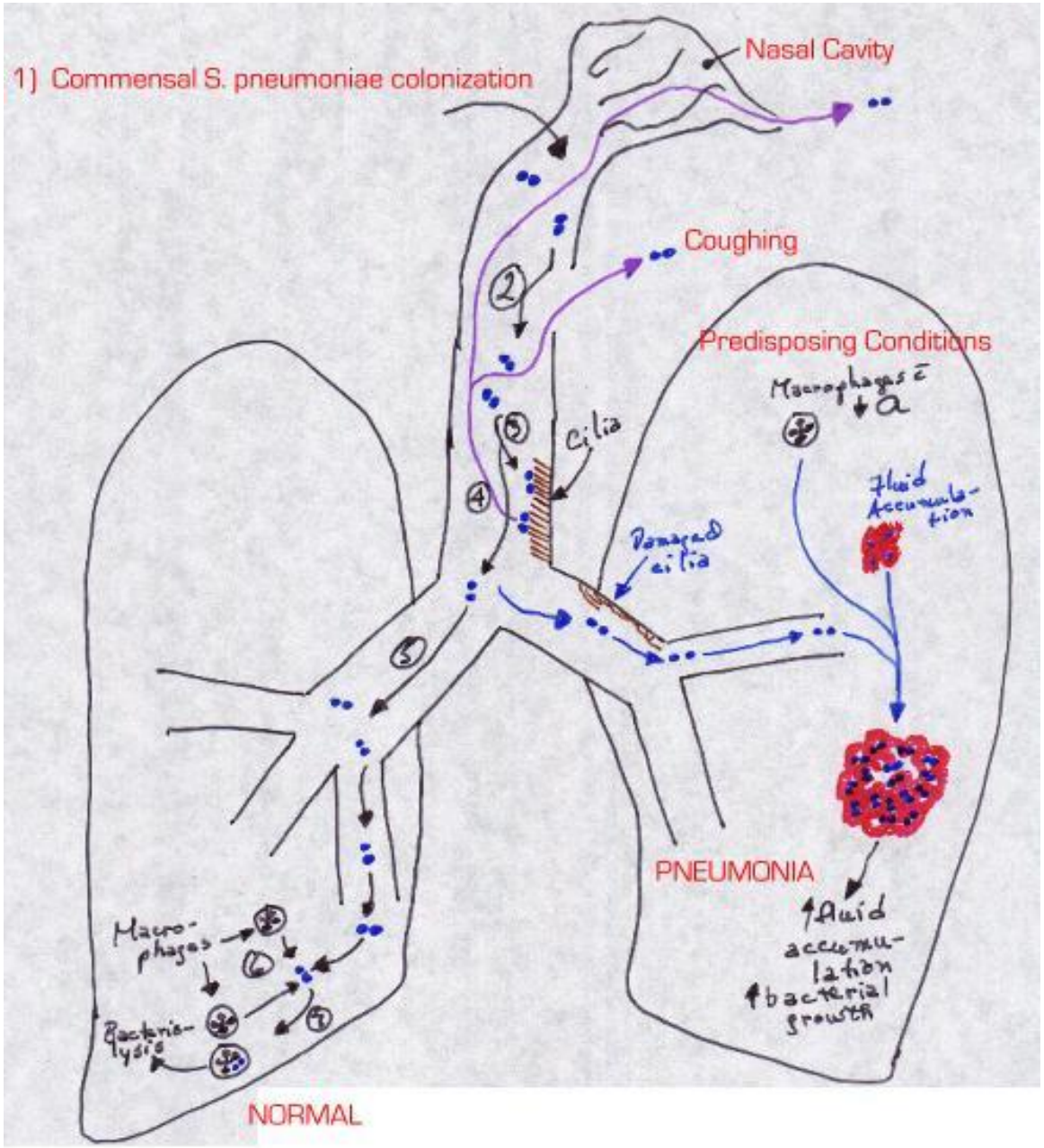
Bacteriological Studies of the Environment	
Air	Simplest way is to expose open agar plates to the air and allow to grow
Blankets, sheets, clothes	An open plate of medium is moved to and fro across the surface of the fabric (a.k.a. sweep plate method). Press plate method: smooth fabrics are pressed against the nutrient medium with an object
Ledges, equipment, dust	A swab moistened with broth is usually adequate. This is then streaked directly onto the plate
Disinfectants, lubricants, irrigation fluids	Large samples are usually required. Disinfectant my of necessity need to be diluted or neutralized to be cultured.


Relative #'s of Microorganisms
Pt cleaned; bed made
Room cleaned; breakfast
Ventilation turned off in O.R. "by accident"
Pt brought in/taken out of O.R.
Pt's sleeping on ward
Empty/clean O.R.

- The important features to get from the previous page are as follow:
- 1) LOTS of organisms are in patient's rooms. When making their beds, do NOT snap the sheets around -- rather, carefully move the sheets and blankets around so you don't spread some nice little obnoxious organism around the hospital (can you say nosocomial?).
- 2) When cleaning rooms, wet mop, and wet dust -- do everything you can to retain microbes on your tools of the job, and not to put them onto your patient.
- 3) Note that when no one is bustling around, there are few microbes that can be detected in the air. Keep airflows to a minimum except to filter the air to remove the microbes from the environment.

Biochemical Effects in Disease Pathogenicity
1. Interfere with mechanical blocks to the spread of infection set up in the body.
2. Slow up or stop the ingestion of microbes by the phagocytic WBC.
3. Destroy body tissues (hemolysins, necrotizing exotoxins).
4. Cause generalized unfavorable reactions in the host, resulting in fever, discomfort and aching (endotoxins).
5. Collectively called <u>TOXICITY</u>

- The development of Streptococcal pneumonia from "normal“ – next slide.
- In short, S. pneumoniae colonizes under commensal conditions. It gets into our airways and is typically removed by healthy cilia, coughing and/or nose-blowing. Any bacteria that are not expectorated will be dealt with in the lungs by macrophages and lysed. This is what's SUPPOSED to happen.
- When the immune system is down, though, or the cilia are damaged (e.g., by SMOKING!), S. pneumoniae gets past the damaged cilia and past macrophages that have reduced activities (predisposing conditions). With any fluid accumulation, the bacteria go nuts. With increased fluid accumulation comes increased dark, dank, warm, anaerobic regions in the lungs that are wonderful growing conditions for S. pneumoniae: pneumonia. One of the characteristic features of S. pneumoniae pneumonia is that you will cough up a rust-colored/flecked sputum.


1) Commensal S. pneumoniae colonization
Nasal Cavity
Coughing
Predisposing Conditions
Macrophages ↓
Cilia
Fluid Accumulation
Damaged cilia
PNEUMONIA
↑fluid accumulation
↑bacterial growth
Macrophages
Bacteria-lysis
NORMAL

How Disease-Producing Agents Leave the Body	
Feces	Bacteria of salmonellosis, bacillary dysentery, cholera; protozoa of dysentery; poliomyelitis and infectious hepatitis viruses
Urine	Bacteria of typhoid fever, tuberculosis (when affecting the GU tract) and undulant fever (caused by Brucella abortus, melitensis, suis -- sx: insidious weight loss, increased irritability, headache/chills/diaphoresis, aches/pains -- enlarged reticuloendothelial system; contagious abortions -- recovery: within 2-6 months -- tx: prolonged streptomycin and tetracycline therapy -- prevention: pasteurization of milk
Discharges from the Mouth, Nose and Respiratory Passages	Bacteria of tuberculosis, whooping cough, pneumonia, scarlet fever (Group A, β-hemolytic streptococci -- a.k.a. scarlatina) and epidemic meningitis; measles, smallpox, mumps, poliomyelitis, influenza and epidemic encephalitis viruses.
Saliva	Viruses of rabies
Blood (Removed by Biting Insects)	Malarial protozoa; tularemic bacteria (Francisella tularensis -- plague-like disease, produces a local ulcerative lesion and often a generalized infection

So … How Does One Keep a "Sterile" Environment?

Sterilization Techniques			
Technique	Temperature (°C)	D/A[1]	Time (minutes; minimum)
Pressurized steam	140		0; Instant
Sat'd steam	121		12; (spores)
Std Technique	121		15; (everything)
EtO (12% E to 88% Freon; humidity, explosive)	40	D; 3.5[2]	150
Hot, dry air	160		120+; (spores)
Boiling water	100		120+; (spores, bacteria)
Flame	"red hot"		0.17; (all micro-organisms)
Pasteurization	63		30; (dairy pathogens)
UV light	RT		varies; (not useful in liquids)
Ionizing radiation	RT		varies; (heat sensitive materials)
Ultrasound	RT		varies; (very few practical uses)
Glutaraldehyde (aq)	RT	D; 3	varies; (spores)
Hydrogen peroxide with phosphoric acid	RT	D; 3	varies; (spores, toxic to skin)
Formaldehyde with EtOH	RT	D; 3	varies; (spores, toxic fumes)
Formaldehyde (aq)	RT	D; 1.5	varies; (spores, toxic fumes)
EtOH (aq)	RT	A; 3	varies; (spores, toxic fumes)
Iodine and EtOH	RT	A; 4	varies; (corrosive; stains)
Silver nitrate	RT	A; 4	varies; (burns, may be irritating)
Chlorine	RT	D; 3-4	varies; (viruses; bad taste)
Phenol	RT	D; 2-3	varies; (toxic, gram + bacteria)
Benzoic and undecylenic acids	RT/Body Temp	A; 3-4	varies; (fungi)

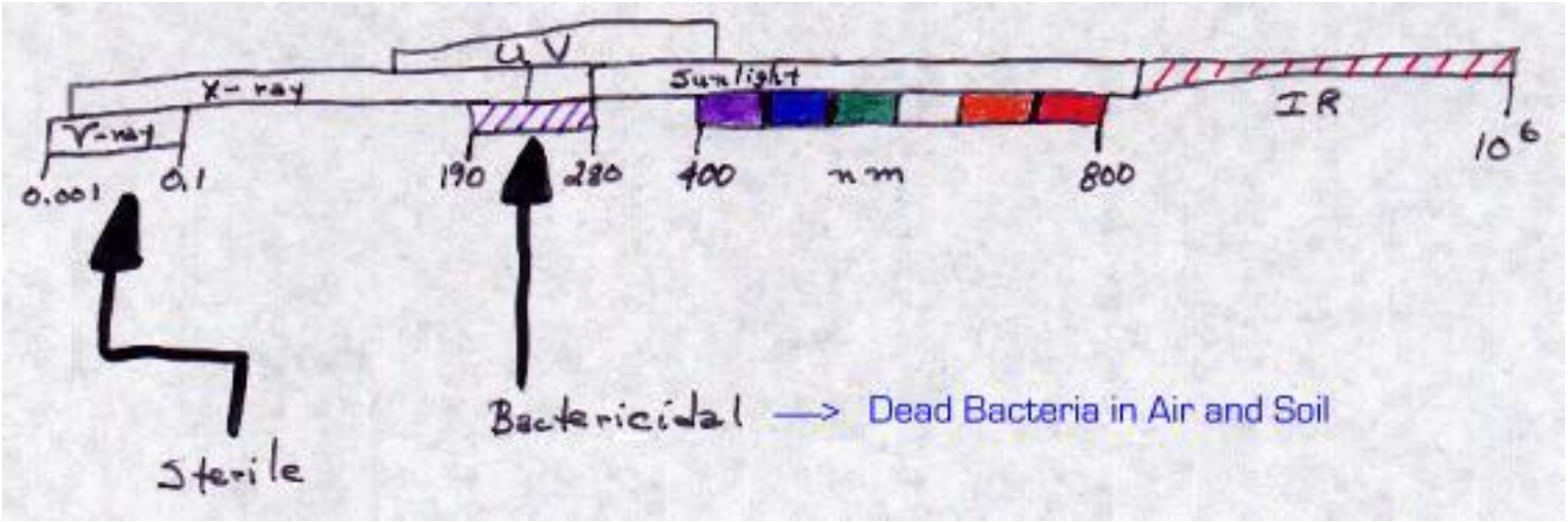
[1]D = disinfectant; A = antiseptic; [2]the greater the number, the better it is

Comments

- Sat'd steam: at 12 minutes generally is NOT enough time to destroy (spores).
- Std technique: (everything) is either destroyed, killed or inactivated by standard autoclave techniques.
- General rule: if it's not contaminated, run for 15 minutes. If it's contaminated or has obvious growth on it, double the sterilizing time to CYA.

- Hot, dry air: (spores) are destroyed at 160° C for 1 hour.
- Boiling water: most (spores) of pathogenic bacteria can be destroyed by boiling for 30 minutes.
  - This is not always reliable, though.
  - For every 1000-ft of elevation above sea level, increase the boiling time by 5 minutes.
  - Thermophiles, some (bacteria), will survive boiling.
- Flame: Kills everything.
- Pasteurization: kills non-sporulating pathogens and non-pathogens at 63° C for 30 minutes without destroying the food value.

- UV light: aqueous based liquids absorb UV light, making it not very useful with solutions.
- Ionizing radiation: excellent for use with heat sensitive agents.
- Is also being used experimentally to sterilize meat so it won't require refrigeration and will have a longer shelf life.
- The meat does NOT retain the radiation, so you won't "eat" it if you eat food of this treatment.

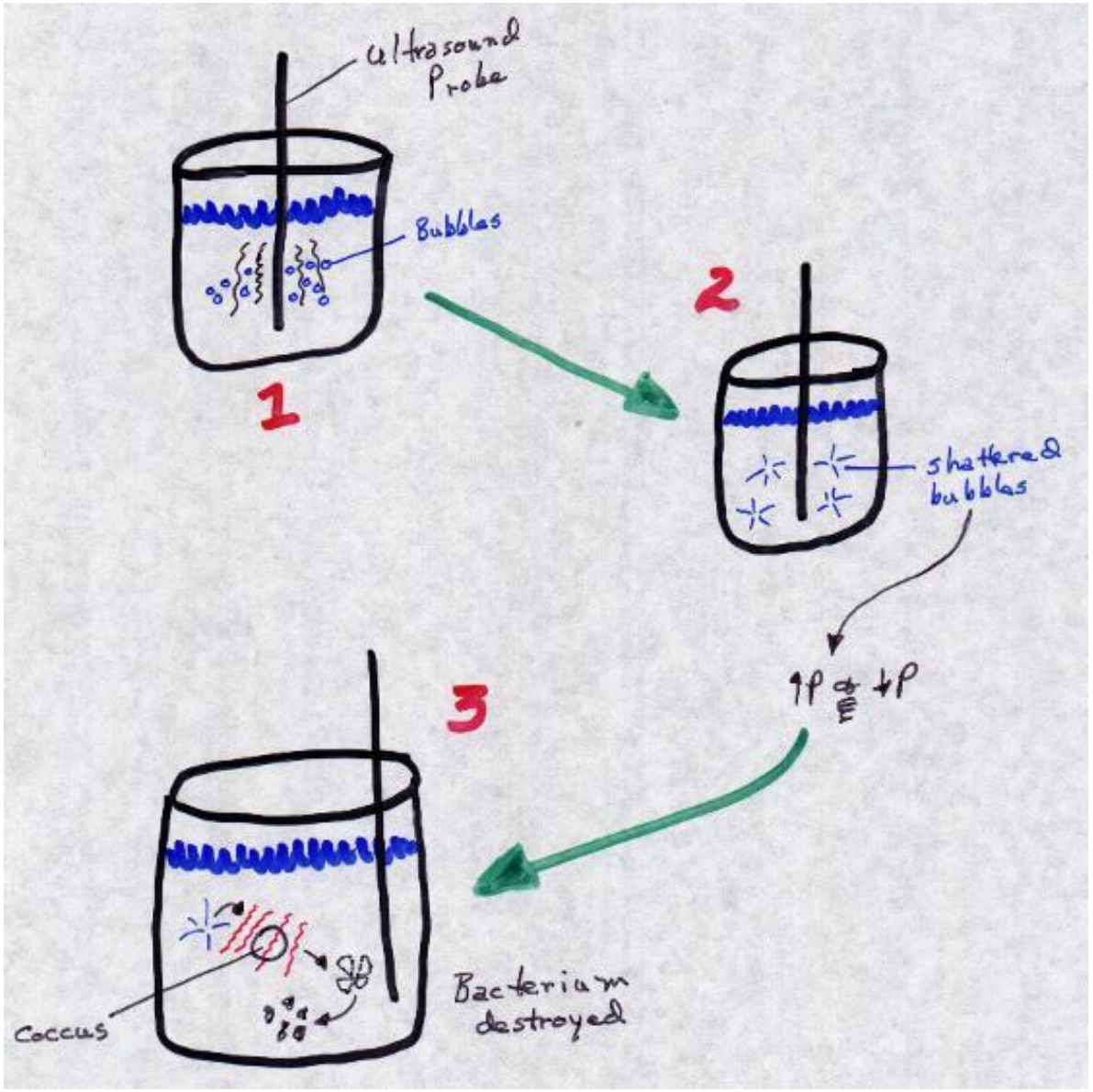

UV
X-ray
Sunlight
IR
γ-ray
0.001
0.1
190
280
400
nm
800
10^6
Sterile
Bactericidal → Dead Bacteria in Air and Soil

The visible part of the spectrum (the part we can see) is between about 400 and 800 nm.

The region of the spectrum that kills bacteria in air and soil is in the ultraviolet (UV) region between 190 and 280 nm.

The region of the spectrum that renders thing sterile is in the gamma (γ) region between 0.001 and 0.1 nm.

- Ultrasound: has no practical use in health care for sterilization.
- Is used routinely in respiratory therapy in many breathing types of treatments.


Ultrasound Probe
Bubbles
1
2
shattered bubbles
3
Coccus
Bacterium destroyed

- What happens when an ultrasound probe is inserted into an ultrasound generator, then into a solution?
- The sound waves vibrate the solution creating bubbles (cold boiling). The bubbles shatter or explode. When they explode, they make regions of high and low pressure, literally blowing up the bacteria. This renders the solution sterile. As mentioned, above, there seems to be no practical use of this technique for sterilization at this point.

- Glutaraldehyde: when sold as activated glutaraldehyde, it's one of the best disinfectants around. It is virucidal, bactericidal and sporicidal.
- Hydrogen peroxide with phosphoric acid: the phosphoric acid is to stabilize the hydrogen peroxide. The hydrogen peroxide is toxic to the skin and doesn't inactivate spores. Frankly, hydrogen peroxide is one of the most over-used and over-rated antiseptics in health care. You can get the same results from cleaning cuts and abrasions with soap and water.
- The reason most people think it's working is because it "fizzes". Of COURSE it fizzes! There is blood with iron (II) and Staph with catalase in/on the wound: both lyse hydrogen peroxide to water and oxygen (fizzing).

- Formaldehyde with EtOH: doesn't get (spores) and has toxic fumes. Not recommended.
- Formaldehyde (aq): See entry immediately above.
- EtOH (aq): Does not inactivate spores. May be toxic to those on Antabuse. Is tuberculocidal. Otherwise, it just "rearranges the dirt" on skin when used in short term applications.
- Iodine and ethanol (EtOH): is (corrosive) and makes (stains) -- forms inclusion compounds with the starch in cotton.

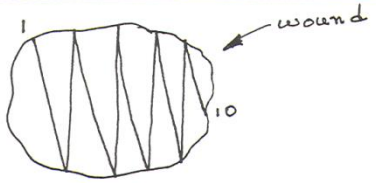
- Silver nitrate: it chemically burns skin; effectiveness varies; may be irritating to the skin. This is no longer used in the eyes of newborn infants to prevent against delivery-transmitted gonorrhea -- antibiotic ointments are now used.
- Chlorine: is inconsistent. Organic material blunts its effectiveness. May destroy some viruses, but not others. Also has a bad taste.

- Phenol: used to be the gold standard for disinfection. The phenol coefficient was used to compare the effectiveness of one compound against that of phenol for killing bacteria. A large number meant that it was better than phenol; a number less than 1 meant it was worse than phenol. Phenols are very good at killing gram positive bacteria. Lysol is a form of a phenolic disinfectant that is useful against gram positives.
- Benzoic and undecylenic acids: for fungi. Desenex contains the latter. When using Desenex, throw out your old shoes, pour in enough powder into new shoes that breathe that when you put your foot in, a small white cloud forms. That will take care of your athlete's foot -- as long as you do it for at least 2 weeks.

Sterilization/Disinfection Techniques of Commonly Used Clinical Items			
Item	Autoclave Method of Choice	Chemical Disinfection, Choice Indicated	Other techniques
Surgical Instruments and Supplies, mechanically clean:			
Noncutting instruments	Y	Activated glutaraldehyde, 10 minutes to 10 hours Ethylene oxide gas, 2-12 hours	Boil completely submerged for 30 minutes
Sharp Instruments	Y, in certain instances	" " " " " "	Boil 30 minutes -- cutting edges wrapped with cotton; instrument kept from tossing about; water boiling before instruments placed in it[+]
Surgical Needles	Yes, in pkgs -- remember disposable now available	Isopropyl alcohol 70-90%, 20 minutes EtO gas 2-12 hours	Hot air at 160°C, 2 hours
Hypodermic syringes and needles	Yes -- remember disposable now available	Cold sterilization INADEQUATE to remove hepatitis viruses	Dry heat, 170°C, 2 hours Boil 30 minutes
Endoscopes and instruments with optical lenses (cystoscopes)		EtO gas, 2-12 hours (MOC) Activated glutaraldehyde (2% aq) 10 hours -- @ least 15 minutes to eliminate tubercle bacilli and enteroviruses	
Thermometers		Activated glutaraldehyde, 10 hours EtO gas, 2-12 hours, cold cycle only	
Surgical Instruments and Supplies, contaminated	Yes, wherever possible -- as a general rule, double exposure time, other conditions the same	EtO gas, 2-12 hours	
[+]$NaNO_2$ added to alcohols, formalin, formaldehyde-alcohol, quaternary ammonium compounds and iodophor compounds prevents rusting. $NaHCO_3$ added to phenolic solutions prevents corrosion. Anti-rust tablets are available commercially			

Culturing Wounds

- In terms of culturing wounds, here are some "Do's and Don'ts":
- 1) Rinse the wound with sterile saline before swabbing.
- 2) Leave pus alone; refrain from swabbing pus.
- 3) Swabbing over eschar will not result in culturable organisms.
- 4) Use Ca alginate or charcoal impregnated wool swabs. Cotton has certain fatty acids in it that will kill off some bacteria before you get it streaked.
- 5) When swabbing the wound, rotate the swab 360° one way, then 360° the other way.
- 6) With a hard eschar, swab the edges of the wound and the exposed wound tissue.
- 7) With a soft or open wound, swab the wound in a zigzag motion (10-point contact) and the edges of the wound, itself.

Pressure Ulcer Treatment: Outdated *vs* Preferred				
Outdated Treatment	Rationale	Culturing Do's and Don't's	Alternative Treatment	Comments
Enzyme preparations	Inactivated by soap; other products with hexachlorophene; are expensive; won't penetrate thick eschar	1) Rinse wound with sterile saline before swabbing 2) Leave pus alone; refrain from swabbing pus	Moist coarse gauze in wound; remove after 4 hours	Helps loosen dead tissue
Hydrogen peroxide	Disturbs new capillaries in granulation tissue; toxic to fibroblasts; can cause subcutaneous gas which may lead embolism (a plug in a blood vessel, clot or air)	3) Swabbing over eschar will not result in culturable organisms 4) Use Ca alginate or charcoal impregnated wool swabs; cotton has certain fatty acids in it that will kill off some bacteria before you get it cultured	Saline solution; Lactated Ringer's solution	Saline is physiologically neutral; lactated Ringer's solution contains electrolytes which help tissue grow
Povidone-iodine	Retards collagen synthesis; when left in wound to soak, it may be absorbed into the blood and possibly affect renal function	5) When swabbing wound, rotate swab 360° one way, then 360° the other way		
Dakin's solution	(0.45-0.5% NaOCl and 0.4% boric acid) Toxic to granulation tissue and fibroblasts; retards collagen synthesis; delays epithelialization; inhibits 90% of neutrophils migrating to the wound -- shoots down body's defense, here			
Acetic acid	Eliminates P. aeruginosa in wound bed; ineffective against G(+) and (-) that replace it; toxic to fibroblasts and slows epithelialization	6) With hard eschar, swab edges of wound and exposed wound tissue 7) With soft/open wound, swab the wound in a zig-zag motion (10 point contact) and the edges of the wound, itself		
Hair dryers/Heat lamps	Dries the wound, delaying healing			
Open-air Healing	Inappropriate			Cover and moisten wound

- The difference between the healing of a moist wound bed vs. that of a dry wound bed.
- The moist wound bed heals better and with less scarring than does the dried bed.
- Even Band-Aid knows that ☺

Clues to Anaerobic Infections			
1) BAD smell			
2) Next mucous membranes; bite originated			
3) Gas gangrene			
4) Fluoresces red to UV (black exudates with blood; B. melaninogenicus)			
5) See 'em on Gram stain, but not in routine cultures			
Clinically Important Anaerobes: A Smattering			
Rods			
	Gram Negative	Bacteroides	Mouth, bowel
	Gram Positive	Lactobacillus	Vagina
		Propionibacterium	Skin
		Clostridium	Bowel, soil
Cocci			
	Gram Positive	Peptostreptococcus	Bowel
	Gram Negative	Veillonella	Mouth, bowel