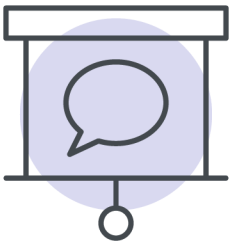
UofM

# MBIO 3000

# FINAL EXAM

## STUDY GUIDE



# Lecture Notes

MBIO 3000
Lecture 1

- Exam will be Short/Long Answer
- non-cumulative final - but need to know general content
  - assignment is 25%
  - midterm is 25%
  - final is 50%
- Stories told in class by prof are examinable
  - example: syphilis in European judges
    - changed the way they dress due to syphilis causing their hair to fall out (wigs) and wore long sleeve to cover the sores on skin from syphilis
      - this fashion in judges is still exhibited today
      - will go over more details about this topic in later lecture
  - example) 2014/2015 ebola outbreak
    - biggest ebola outbreak ever
    - ebola was common before the outbreak but had a low infection rate
    - a vaccine was made before the outbreak, but it was shelved because it was so expensive no one wanted to use it
    - by the time the new outbreak happened, the old vaccine no longer worked
    - quantified as a logarithmic infection
    - USA gave the African health care bad advice about how to handle E.bola -> 534 health care workers died while taking care of the sick because they weren't sufficiently protected themselves
    - health care workers spread the disease to family when they went home after work
    - this later spread into communities
    - around 30,000 infected, ad 15,000 died
    - only 1 E.bola virus is needed to infect the host
    - changed biosafety and how we control it
- fomite -> inanimate object transmission
- working on bacteriophage to act as antibiotics since they are failing -> antimicrobial resistance is occurring at a fast rate

Lecture 2
- Watching a documentary series called Guns, Germs and Steel - Examinable
- Part 1
  - planting/farming created a division in the world
  - in the fertile crescent, people started farming wheat - steady source of food
  - this spread into parts of Europe, Asia and Egypt.
  - places like New Guinea have a harder time growing food - they food they can grow are not sufficient in protein and take a long time to cultivate and make edible
  - since they lack protein - lack energy - need to go hunting and focus all their available time trying to make food -> no free time to evolve community and make better tools
  - domestication of animals from fertile crescent helped the wheat farmers gather a source of protein and energy - this allowed them to spend time doing something else rather than worrying about food
    - there are only 14 animals that can be domesticated - fertile crescent had 13 of them
    - animals eat the remains of wheat that humans cant eat, plus they produce fertilizer that helps the wheat grow

    - animals could be used to pull machinery in crops to lessen the workload on humans
    - no domestication of animals in New Guinea - later were introduced to the pig which they can use for meat, but the pig is not sufficient in helping with crops
    - look for animals that reproduce fast (unlike elephant) -> want a constant population to eat/help with work
  - bigger the population the more hands you had to help farm -> could make trades
    - more hands = more food = less time to work

Lecture 3
- Zoonotic organism - can be transmitted to humans from animals
  - ex) 1918 flu outbreak
  - after the war it spread to other countries with returning soldiers
  - expected that another mass flu will occur again in the future
- Question: What makes humans so advanced?
  - high supplement food sources
- Question: Do you agree with Jarod Diamond's theory?
- organisms are now becoming fully immune to all antibiotics
  - must use immune system by itself to fight off infection
  - our immune system is lower than before and will not likely save us
    - this occurred because children are not as exposed to germs -> parents don't want their children playing in the dirt -> dirt contains microbes that can help children build up their immune system
  - mycobacterium tuberculosis chimera
    - first bacterium known to be resistant to all known antibiotics
    - infecting cardiothoracic machines in hospital - 10% chance survival rate
    - no detergent or cleaning agent will kill it
- Part 2 of Guns, Germs and Steel documentary
  - Inca (America) are able to farm very efficiently -> the only domestic animal they have are the llamas -> not good at helping around the farm - will not pull machinery like horses do.
  - while Eurasia is very long east to west, America is long north to south -> Jarod Diamond believes this is the reason behind the colonization of people -> they didn't travel as far down because the climate kept changing and their farming practices would have to be changed
  - as the Spanish came to America and came into contact with the Inca people, they started to spread Smallpox
  - respiratory disease -> spreads systemically through body -> causes blisters -> burst = flu virus
  - only the europeans spread germs to the Incas, not the other way around
    - since the Europeans have been domesticating animals for a long time - they've been exposed to animal viruses that transmitted to human virus - small parts of the population died but the ones who were resistant to the infection stayed alive and passed the genes down.
    - the Incas has no immunity to flu from pigs, or measles from cows, as well as other diseases since they were never in contact with the animals before -> caused massive epidemic of disease
    - the llama does not spread any bacteria to humans, therefore the Incas could not spread any llama germs to the Spanish, or have a better immunity towards the European germs
    - 99% of the population was killed by old world disease

Lecture 4

- we will be tested on “current events” he talks about at the beginning of class
  - influenza is hitting Asian population severely
  - mumps at UofM
- Video part 3: Into the tropics
  - settlers imported crops and animals to South Africa - travelled by ship
  - temperature and climate of South Africa is almost the same as Europe
  - kicked out the original people that lived there
  - initial contact with the Europeans in South Africa caused epidemics of Smallpox and other disease
  - the settlers pushed north -> the settlers were massacred in the night by the Zoolou tribe -> it was a strong society with a powerful army
  - the settlers fought back -> decided to hide behind wagons for protection and use a hand-off system to shoot guns very fast -> killed a great deal of the zoolou army
  - invention of the machine gun helped the settlers go wherever they wanted and take whatever land they wanted.
  - they found that the land in the tropics was impossible to grow, settlers started getting sick as well as their cattle
  - they noticed the African colonies around them had no problem farming and staying safe.
  - once the settlers passed the tropic of Capricorn - their food would not grow
    - tropics have a different land set and weather system than Europe
    - tropic experience a dry season and a rain season -> due to this the wheat and barley would not grow
    - settlers and their cattle started dying from germs, while the African tribes and their cattle were not affected
    - the Europeans did not spread Smallpox and other germs to the African people because they already had their own cattle before the settlers came -> were already exposed to cattle germs for a longer period of time - even created a vaccine against it
    - Malaria was the cause of the Settlers dying, they set up their camps/villages near water where Mosquitoes were highly populated, which the African people already knew the risk and always placed their camps away from water, in the driest area they could find and kept in small populations to limit spread - over time the African people started making antibodies against Malaria
  - while the Settlers could not find land safe enough to live on in the tropics, they decided not to live here. But they found lots of resources like Diamonds and Copper.
  - Belgians went back to the African communities, and forced the people to work in the mines
  - They also forced the African people to build the railroad from South Africa to the tropics
  - because of the impact the Settlers and Belgians had on the African people, most of them live in large cities now where Malaria could spread very easily.
  - Malaria is now the #1 killer of African children under 5 years old.
  - Malaria is mutating now, there is no vaccine
  - Malaysia and Singapore are also found in the tropics, but they found a way to exterminate any possible risk of getting Malaria
- Jarod’s Hypothesis:
  - guns, germs and steel are reasons why Europe succeeded in being so powerful - because their geographic region gave them a head start on development
  - he did not acknowledge Asian societies in the video - they also had the advantages of the fertile crescent
  - never mentioned that the Europeans colonized by force

- malaria -> sickle cell anemia is the evolution of humans trying to combat the disease
- while Malaria does not occur in Canada, it can occur if people are traveling to the tropics, and coming back to Canada with Malaria.

- The prof wrote 3 exam questions from todays video

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Lecture 5

- Historical Perspective of Infectious Disease
  - read article “Workers may have been exposed in Winnipeg Lab” - Feb 2nd
  - common causes of exposure is negligence
    - different people with different skill set - not everyone knows what you know
- History of Disease
  - most diseases occur from us becoming settlers
- Before Farming
  - 4.5 min years human ancestors (hominids)
    - humans were hunter gatherers
    - small groups around 50-100 individuals
    - pathogens were unable to survive
      - had an amazing diet, compared to today
      - small populations -> did not have to worry about spread of germs and being close to waste
  - groups were never in the area long enough to pollute water sources with human and other waste
    - Canada and Russia are the only countries with non-polluted water sources
      - it could be possible that the next world war occurs not because of oil but for clean water
  - no refuse pile to attract disease carrying insects
  - they had no domestic animals as they were a mobile society
  - hunter/gatherers covered the entire earth 1.5 to 1.8 million years ago
- disease in hunter gatherers
  - were less exposed than modern man
  - principle sources
    - wild animals
      - trichinosis (sleeping sickness)
      - tularemia (rabbit fever)
      - these infections would be incidental and rare
    - if a person would fall sick they would be kicked out of the group -> prevents pop spread
    - there was a rabbit fever outbreak in Winnipeg 9 years ago
    - parasites present in prehominid ancestors continue to evolve today
      - this includes worms, lice, bacteria like Salmonella (Yaws) and Treponema (Syphilis)
- before farming
  - mobility put hunter gatherers within reach of a wide range of food
    - this helped establish the kinds and quantities of nutrients humans need today
- Scurvy
  - insufficient vitamin C
    - only a few animals, like humans cannot synthesize vitamin C by themselves
    - due to absorbic acid being well supplied in food over hundreds of thousands of years
  - didn't have to make it anymore -> evolved to not waste energy producing it if we already had enough from food
  - a shipboard disease for 300-400 years
    - 30 weeks of vitamin C deficiency will cause scurvy
    - symptoms include
      - spongy, bleeding gums

        - sunken eyes
        - old wounds reopen
        - death
    - relative rare before 15th century
        - no ships before then, people on land have a easy way to get it
    - it affected:
        - seized armies
        - prison war camps
        - irish great potato famine 1845-1846
            - as potatoes contain vitamin C and their replacement did not
    - absorbic acid is important in metabolism
- before farming
    - population crisis occurred from
        - famine -> pop.control
        - childbirth and infanticide
        - lost lives in high risk, high return endeavours associated with hunting, war and travelling
    - populations grew even with these condition
    - some groups would split in 2 if the population was too big
    - the separation of groups led people to spread all over the globe
    - peripatetic existence
    - farming became new means of getting food due to increase in population
    - after 12,000 to 10,000 years ago, ice caps melted blocking movement between continents
- farming + disease
    - farming is most important event ever engineered by humans
    - artificial selection / GMO - picked best and save it - plant it next year to get even better crops
    - wild grasses were tamed until they became domesticated varieties of wheat, barley and rice
    - dogs were probably the first animals to be domesticated - followed by cattle, sheep, goats, pigs, horses and fowl
    - population increased dramatically as constraints on the number of offspring were not longer present
    - the more people there were, the more hands there were to farm
        - over time our food is becoming less nutritious
        - ** HE WILL ASK AN EXAM Q ON DOMESTICATED ANIMALS
    - from this point people manipulated the planet
    - rearranged ecological systems, as well as the domestication of plants and animals
    - humans began undoing self-self-regulating natural environments without knowing it
    - we cannot yet fathom the effects of the agriculture evolution
    - one downside -> pesticides, land manipulation
    - by inventing agriculture humans also cultivated disease
- problems caused by Agriculture
    - irish potato famine
        - potato blight -> insect
        - destroyed almost all potato crops in Europe
        - ireland was disproportionally hit as one third of the population was dependent on potato
            - needed vitamin C in a different way -> were given wheat only which has no vitamin C
    - pathogens of domestic animals found way into humans -> adapted along with humans
    - humans share disease with:

- 65 types with dogs
- 50 types with cattle
- 46 types with sheep
- 42 types with pigs
- 35 types with horses
- 26 types with poultry

- along with sharing food with our animals, we would find their waste in our water supply
  - parasitic worms + other diseases from insects
- permanent settlements attract mice, rats, mosquitoes and other blood sucking insects
- fleas, lice on the outside, and amoeba, hookworm, and other parasites on the inside of our body
- all proliferated very easily since people lives in such close proximity with one another
  - flu is the most important
    - pig flu
    - bird flu
    - human flu
      - we do not recognize bird flu, but pigs do and they are able to transmit bird flu to us
      - bird flu genome coated in pig flu coat
        - H1N1 flu was very important - happened in 1918 and in the 2000's again - different type

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Lecture 6

Midterm is on March 14th in class
will go over question on the day before
Assignment given out on the 21st of March - due after a week?

News
- Three person baby
  - already have babies produced genetically - like GATTACA
  - “science cannot be stopped, but it can be controlled”
- UK and China
  - found 1500 new viruses
  - The real virus attacking aggressive cancer cells

Farming and Disease
- Pathogens of domesticated animals now found their way into human bodies and began to adapt
- humans share disease with
  - 65 diseases with dogs
  - 50 with cattle
  - 46 with sheep
  - 42 with pigs
  - 35 with horses
  - 26 with poultry
- Along with sharing diseases domesticated animals joined humans in fouling their drinking water
- maximizing the opportunities of parasitic worms and other disease -spreading insets
- permanent settlements attracted mice, rats, mosquitoes, and other blood sucking insects
- fleas and lice would colonize the outs of the human body and amoeba, hookworm, and other parasitic worms invaded the inside
- all proliferated easily because people lived in close proximity with one another
- Russia and Canada are the only countries with pristine “clean” drinking water
- Storytime: Him and colleague: went rock climbing - colleague urinated in a pool of water in Morocco - hookworm travelled up his urethra - lodged itself in theres with spikes - cant come out. Must cut open urethra to remove hookworm
  - take into account what countries have hookworms, amoebas and parasitic worms
- 12,000 years ago permanent settlements and the cultivation of land around the domestication of animals developed many disease
- cattle contributed the poxes ******
- pigs, birds , and horses pass on flues *******
  - whales as well - they catch colds
- measles is the result of rinderpest or canine distemper form cattle to dogs ********
- small pox is from the evolution of cowpox in humans
  - humans have eradicated small pox - mass vaccinations

Small pox
- variola virus a species of Orthopoxvirus “speckled monster”
- reservoir ** REMEMBER

- human disease
- no animal or environmental reservoir
- currently the virus is maintained only in designated laboratories
- important for bioterrorism - currently still in the laboratory (BSL-4) completely eradicated from population through vaccine
  - current young population doesn't have smallpox vaccine
  - CDC and russia had small pox samples - CDC had inaccurately labelled stuff - found small pox viles in a freezer used for lunch room
    - biosafety is valuable for these types of labs

- mode of transmission
  - droplet spread
  - skin inoculation
  - conjunctivae or placenta were portals of entry
- incubation period
  - 7 to 19 days commonly 10 to 14 days to onset of illness and 2-4 days for rash onset
- period of communicability
  - earliest lesions to disappearance of all scabs (3 weeks)
  - susceptibility among the unvaccinated is universal
- an estimated 300 million people died from smallpox in the 20th century alone
- kills a third of those it infects
- known to have co-existed with humans for thousands of years
- as the world's population grew, and travel increased the virus had access to larger and larger populations
  - fear of today - governments spend millions of dollars to study viruses that could have this effect - needs to be stopped
- the earliest physical evidence of smallpox is the pustular rash on the mummified body of the Pharaoh Ramesses V of Egypt 1157 BC
  - has been in our population for decades
- traders carried the disease from Egypt to India during the 1st millennium BC
- swept into China in the 1st century AD and reached Japan in the 6th century
- returning crusaders provided a way for smallpox to spread through Europe in the 11th and 12th centuries
- Spanish inadvertently owe much of their success in conquering the Aztecs and the Incas in Mexico during the 16th century to smallpox
  - virgin population (not exposed before) **********
  - notice Indians had no immunity to the disease
- a century later north American Indians suffered a similar devastation
  - a form of bioterrorism
- in the 18th century smallpox decimated the aborigines when it reached Australia, the last corner of the world to have escaped its ravages
- Variolation
  - Edward Jenner - was the one who came up with vaccinations "in history books"
  - from Jarod's videos and other facts - vaccines came from Africa and China "not Americans or europeans"
  - variation was a process developed in the 10th century in china and India
  - taking pus from the pocks of someone suffering from smallpox and inoculating healthy people with it
    - snorted - if you survived you would be immune - 2/3 would survive
  - usually a mild case of smallpox developed, giving lifelong immunity afterwards

- about 0.5 to 2 percent of people dies after variolation, compared with 20 to 30 percent after natural smallpox
- a major disadvantage of the practice was that variolated people could pass on severe smallpox to others - they were still contagious
  - more people able to pass on to a virgin population
- lady Mary Wortley montage (1689-1762) is credited with introducing variolation to Britain in 1721 * wont ask dates but will ask questions related to the date
- severely pockmarked herself after surviving the illness, she learned about variolation in Constantinople
- she has her children inoculated and persuaded to vaccinate
- Edward Jenner
  - 1796 he inserted pus extracted from a cowpox populate from the hand of a milkmaid, into an incision on the arm of an eight-year old boy, James Phipps
  - this theory was drawn from the folklore of the countryside, that milkmaids who suffered the milk disease of cowpox never contracted smallpox
  - Jenner proved conclusively that contracting cowpox provided immunity against smallpox
  - in 1801 he wrote “it now becomes too manifest to admit of controversy, that the annihilation of the small pox, the most dreadful scourage of the human species, must be the final result of the practice”
  - James Phipps worked with Edward Jenner the rest of his life - protected Edwards work against the Germans in WWI and died for it
  - * contracting cowpox gives you immunity for smallpox*
  - it was to be well over a hundred years before Jenner’s vision finally began to be realized
  - in 1959 the World Health Assembly passed a resolution to undertake the global eradication of smallpox
    - the vaccine works by infecting a baby calf, killing it, and blending the meat and injecting it into you
    - now our vaccine is genetically made
    - national lab workers, USA soldiers and maybe Russia? otherwise you cant get the smallpox vaccine
  - disease was wiped out from developed countries in Europe and NA
    - we cannot get rid of polio like we did the smallpox
      - people are refusing the vaccine
      - CDC and non-government organizations - vaccinated people in Nigeria
        - made mistake and gave people the live polio virus - giving people polio - Nigerian people kicked the western out
        - Africans don't trust the medical advice anymore - wont vaccinate
        - still hesitancy to get vaccinated even today — E.bola outbreak
  - Nations all continued to suffer outbreaks of smallpox caused by imports from developing countries where the disease was endemic
  - number of outbreaks that demonstrated how a few smallpox causes could spark mass panic and large-scale disruption
  - in 1947 a Mexican businessman, unaware he was incubating smallpox
  - traveled by bus to New York
  - health authorities decided to act pre-emptively and mass vaccinate New Yorkers
    - side note: swine flu outbreak - a bunch of people got vaccinated - if we had a huge outbreak the ones who didn't get vaccinated would have most likely perished
    - we spent 50 million dollars - at the end we still had about 30 million worth of vaccine left
  - over 6 million people were vaccinated within a month at hundreds of vaccination stations

    - 12 people caught smallpox and two of them, including the Mexican business man died
    - six people also died form adverse reactions to the vaccine
        - look at population as a bell curve - one side doesn't need vaccine, and other side will die with or without vaccine
- one of the last major European outbreaks was in Yugoslavia in 1972
- 175 causes and 35 deaths
- a muslim pilgrim had returned from Mecca to his village in Kosovo via Iraq, where there were cases of smallpox, and spread the disease to friends and relatives. a man called L.Muzza in a nearby city also became infected
- Muzza fell ill and became of the seriousness of his condition was treated in a series of hospitals, ending up in Belgrade on 10th of March. Nurse D. Stupar was on duty that night with her colleague D. Spastic - read book GOOD FOR MEDICAL SCHOOL
- Muzza has been misdiagnosed and suffering from a bad reaction to penicillin
- contracted the most virulent and highly contagious form of smallpox - hemorrhagic
- incredibly Muzza infected 38 people, eight died
- enforced mass quarantine was instigated to stop the virus in its tracks

Farming and Disease
- the disease left the population in a diseased state
- birth rates soared with increasing farming
- more individuals living within spitting, coughing and sneezing distance of one another

The Rise of New Diseases
- as humans switch their activity from living off the land to vigorously manipulating it, they were increasingly parasitized
- humans were not totally defenceless
    - those who survived are at best protected against further infection
- humans thus began developing a complex immune system
- humans also evolved with organisms
    - war of the worlds - aliens died from our disease
    - worried of Extraterrestrial giving us disease they've lived with over their life
    - tried to make a Level 5 laboratory - vaccine lab
    - NASA has a level 5 where they look at space microbes

malaria
- believed to have originated among African primates
- genus Plasmodium, transmitted via female mosquito (anopheles)
    - P. vivax - infects young erythrocytes (red blood cells - non nucleated cells)
    - P. malariae - infects mature erythrocytes
    - P. falciparum - infects both types (most common in sub-saharan africa)
    - P. knowlesi - infects both types (most common in South East Asia
- infection gives you a characteristic fever cycle
    - 2 to 3 days you have increased ten 40 degrees temp to 42
        - fever to kill or slow down bacteria so immune sys can kill
    - the fever cycles occur with a burst of new plasmodium from erythrocytes
    - this is due to inflammation from the clean up of broken cells
        - Cloroquin - malaria drugs (must take if going to Africa)

Malaria and Immunity

- innate immunity holds down the parasite count
- acquired immunity produces antibodies but only after the person has survived several attacks
  - only work on the strain causing the infection
- natural selection (genetic evolution) in malarious regions has gradually supplemented acquired immunities by evolving innate resistances
  - creates cells that can no longer be attacked by malaria - innate
- this is known as sickle cell anemia
  - mostly found in Africa and some Asia
- individuals whose origins are in Africa may have acquired a genetic trait which protects them from Plasmodium vivax
  - 1/4 of the population have this genetic trait
  - if two people mate passion on the vesicle cell trait the resulting offspring will fall vital to Sickle Cell Anemia
  - A trait which causes the erythrocytes to turn sickle in shape
  - * must be heterozygous - must have regular cells and sickle cells to live and be immune
  - if you have homo for sickle cell you die
  - normal you're able to get malaria - if infected some of your cells turn sickle shape which the body isn't used to - disease
  - if we can genetically manipulate our bodies, can we produce a sickle cell anemia genetic version of a human without having the sickle cell dynamics

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Lecture 7

Midterm 20-25 questions
Assignment due March 30th
Correction from last lecture - treponema causes YAWS not salmonella

Malaria
- about 1.5 mil to 3 min people die of malaria every year (85% of these occur in Africa) accounting for about 4-5% of all fatalities in the world
- One child dies of malaria somewhere in Africa every 20 sec. and there is one malarial death every 12 sec somewhere in the world
- malaria kills in 1 year what AIDS killed in 15 years. In 15 years, if 5 million have died of AIDS and 50 million have died of malaria
  - Story: Guy who found a “cure” for malaria - got millions of dollars from the US government to fund his research. His “cure” only blocked a particle of malaria, did not help many people. Took the money and skipped town
  - AIDS is funded more than Malaria - due to first world having more AIDS
  - No ebola in Canada - we spend lots of money (20 million) on research for Ebola because people fear another outbreak
- malaria ranks third among the major infectious diseases in causing deaths after and tuberculosis. it is elected that by the turn of the century malaria would be the number one infectious killer disease int he world
- every year 30,000 visitors to endemic areas develop malaria and 1% of them may die
- estimated world wide expenditure is 58 mil from US, one thousandth of the us 56 billion spent globally on health research annually
- estimated world wide expenditure per malaria fatality is $65 compared to $3274 for HIV and $789 for asthma. That is to say, one HIV/AIDS death is equal to about 50 malaria deaths.
  - west nile cases in Manitoba due to different mosquito species travelling farther due to global warming - only occurred about 5 years ago.
  - This change is occurring all over the world - vectors are changing in land locations

The Rise of New Diseases
- a general rule of thumb
  - those who live in close proximity to a particular pathogen for long enough develop an ability to live with the disease
- other disease are far more difficult to live with
  - plaque, small pox, mumps, ebola
- new plagues first hit humankind around 3000 BC. when cities over 50,000 were spring up in Mesopotamia, Egypt, and Indus Valley
- the period beginning about 500BC in when pathogens had an impact on the growth of civilization in Asia and Europe
- generally these diseases would pass from human to human very quickly and need no intermediary carrier
  - fomites
    - most common fomites now are cellphones
- these new pathogens changed the course of human history
- one’s people disease became another people’s plague
- such disease outbreaks would control population growth

- which made possible the food surpluses that sped the development of cities
- relatively recently, cities were so unhealthy that population could not replace themselves
  - outlast and outcompete the speed of human reproduction - kept the population at bay
- rodent disease was responsible for mass human death, socioeconomic changes and religious turnovers
- the first documented outbreak of the plaque was in the Roman Empire
  - called the plague of Justinian
  - lost 1/4 of the population
  - devastated Constantinople in 542 AD
  - mediterranean for the two centuries
  - Story: london - big sewer systems - needed to kill rats and mice - poured arsenic into the sewer - homeless people used to live down there and would also die due to the arsenic
    - still treating for Plague in london and the rest of Europe
    - spot outbreaks occasionally
    - could buy the plague off the internet until about 5 years ago
    - “human pathogen and toxic act”
- second cycle of the plague (justinian was first)
  - greatest imprint on population
  - known historically as the black death
  - began in 1300 and ended in 1800
  - began in Asia and swept through middle east, north Africa and Europe
  - Europe alone lost over 20 million people
  - between 20% and 60% and higher dead in cities
  - often facing people to abandon their towns and villages for non infected areas (this change the socioeconomics of the cities)
    - rich people would leave, other smaller people would take over.
    - when the rich people (mayor or king) would come back, the population wouldn't let them be in charge again
    - “posies would neutralize the bad bacteria from your body” from the song about death

Plague

- Yersinia pestis
  - two types of plague infection
    - bubonic plague
    - pneumonic plague
    - ZOONOTIC DISEASE **
    - human and animal disease
- reservoir
  - arthropod reservoir
- mode of transmission
  - bitten by a rodent plea that is carrying the plague bacterium
  - handling an infected animal ‘flea transmission’
- incubation period
  - bubonic plague
    - infection of the lymph nodes
    - signs occur 2 to 6 days after being infected
    - no person to person transmission
      - if there is no surgical/ blood transmission - you will not catch the transmission by sitting beside them

- when bubonic plague is left untreated, plague bacteria invade the bloodstream
- causes septicemia
  - infection of the blood
- lives in phagocytic cells which accumulate in the lymph nodes causing swelling
  - hides from immune system
- pneumonic plague
  - infection of the lungs causes the pneumonic form of plague
  - (if lung on x-ray is shown as cloudy its infected)
  - a severe respiratory illness with, high fever, chills, cough and breathing difficulty witch expel bloody sputum
  - person to person transmission - respiratory
  - there is also swollen lymph nodes
    - if the pneumonic is untreated — you will get both after because they spread to lymph
  - without antibiotic therapy, the disease can progress rapidly to death
  - signs and symptoms
    - most common form of human plague is a swollen and very tender lymph gland accompanied by pain
    - swollen gland is called a “bubo”
    - bubonic plague should e suspected when a person develops a swollen gland, fever, chills, headache, and extreme exhaustion and has a history of possible exposure to infected rodents, rabbit or fleas

The Great Plague of London

- Began in 1665
  - the last in a long series of plague epidemics that first begin in June 1499 in London
  - the great plague killed between 75,000 and 100,000 of London's rapidly expanding population of about 460,000
  - by September 1665, the death rate reached 8000 people per week
    - if this happened now, people would stay home and cities would shut down
    - best to stay at home for at least 41 days to be safe
  - helpless municipal authorities thew their earlier caution to the wind and abandoned quarantine measures
  - tens of thousands of dogs and cats were killed to eliminate a feared source of contagion
  - well-off residents soon fled to the countryside, leaving the poor behind in impoverished and decrepit parishes
  - physicians and surgeons lanced bucoes and bled black spots in attempts to cure plague victims by releasing bad bodily humors
  - prohibited churches from keeping dead bodies on their premises during public assemblies or services
  - London's Privy Council issued a new Plague Orders in May 1666,
    - banned the burial of future plague victims in parish churches
    - enforced the use of quicklime at designated burial sites - disintegrates bodies - “bases will degrade the human body quickly”
    - strictly prohibited opening graves less than one year after interment as a safeguard against the spreads of infection
      - many people died that were grave diggers
    - carriers of the dead had to identify themselves and could not mix with the public
- plague subsides and the government reacts
  - February 1666, the great plague had nearly  run its course

- it died out during the great fire that same year and never returned
- ventral parts of london were rebuilt with wider streets to relieve crowding and better sewage systems to improve sanitation
  - before the city burned, they had terrible infrastructure and plumbing
  - after - totally reinvented the swage systems and sanitation practices

- plague today
  - mortality rate of plague
    - 14% (1 in 7) of all plague cases in the US are fatal
    - US has an average of 15 to 30 persons each year
    - globally the world health organization reports about 1,000 to 3,000 (higher now, up to 5,000)
- treatment
  - microscopic examination of lymph gland, blood and sputum samples
  - streptomycin is the antibiotic of choice
  - gentamicin si used when streptomycin is not available
  - tetracyclines and chloramphenicol are also effective
  - vaccine is not protective against aerosol in animal studies
  - can also use rodent control (protecting food)
- about 14 percent of all plague cases in the united states are fatal
- pneumonic plague is the most severe form of te disease and death occurs in approximately half of the cases
- story: fleas bite black rats (not brown), called the black death. Roofs were bad, black rats would fall into the houses, someone had to go pick them up to throw the rat outside, this is when the transmission of fleas would occur.

MBIO 3000
Lecture 8

Assignment - interesting virus going around today & mini grant proposal for grad studies
more information after midterm

Story: prominent plague scientist - smoked the pipe and had tea - walked into level 2 lab - would leave the pipe on the counter while talking to people - one day he put it down on the counter and got inoculated with the plague - systemic type of plague and died later. Biosafety is very important in preventing lives of scientists

Syphilis
- has changed our society the most - in how we act today rather than how many people it killed
  - judicial system example

Why look at social history?
- diseases cause changes in society
  - social measures are modified or abandoned in response to a disease which results from a particular societal practice
- hard science lacks societal focus
  - the scientific field is separated from the societal milieu in which research is conducted
- it is interesting to see how certain diseases have influenced or altered the spirit of the times

Syphilis
- Cause by the spirochete Treponema pallidum subsp. pallidum
- two types
  - venereal syphilis, acquired through sexual contact
  - congenital syphilis, acquired in utero from an infected mother
- enters through breaks in the skin and mucosal tissue and migrates to the lymph nodes
  - we see a lot of diseases occurring to our lymph nodes - due to trying to attack IR
- the disease is not very contagious
  - contact with a chancre during sex results in transmission in ~ one third of cases

The Organism
- Treponema
- order scpirochaetales, family spirochaetacea
- 10 species, 4 human pathogens

| Species | Subspecies | Disease |
|---|---|---|
| T. pallidum | pallidum | venereal syphilis |
| T. pallidum | endimecum | endemic syphilis (bejel) |
| T. pallidum | pertenue | yaws |
| T. carateum | N/A | pinta |

Causes of Disease
- primary stage
  - incubation period of 10 days to 3 weeks

  - first symptom is a small, red, painless ulcer - chancre
  - the chancre is filled with spirochetes
- secondary stage
  - 2 to 10 weeks after appearance of primary lesions
  - characterized by a skin rash (which is infectious)
  - also loss of hair, malaise and fever
    - loss of hair = wig, powder on face due to rash, sleeves = hiding rash spread to hands
- tertiary
  - after many years of latency, develop degenerative lesions (gummas) in skin, bone and nervous system
  - nervous system involved can result in retardation blindness and even insanity

Venereal syphilis: symptoms
- primary lesions: single, painless indurated
  - variation in morphology
  - usually clean base, though may be purulent
- usually on genitals but many be found on fingers mouth or anus
- painless** regional lymphadenpathy in 80%
  - treptonema releases enzymes that stop your neurons from working causing no pain
- may be no demarcation between primary and secondary
- secondary syphilis marked by skin rash, often hands and feet

Early Treatments for Syphilis
- mercury salves or steam baths were most common
  - mercury was mixed with pork fat, fresh butter, vinegar, myrrh, turpentine, and sulphur in a mortar
    - before people knew about microbes
  - barbers would rub these mercury salves into the sores of syphilis, which often went down to the bone -often very painful
  - salve recipes varied greatly with some physicians adding live frogs, chicken's blood, snake venom and human flesh
- the syphilitic was then wrapped in blankets and placed in a steam bath
  - the prevailing medical orthodoxy held that only salivation or sweating could expel the disease from the body
  - some doctors insisted that 4 pints of saliva was a good start, and that over the course of a month, 112 points of saliva could be collected

Treatment
- historically
  - mercury, arsenic,  bismuth
- currently
  - benzathine penicillin
- appears to clear bacteria completely
  - some controversy (is it really clear after penicillin? bell curve based - some have the genetics to not clear syphilis)
    - genital warts (most common in winnipeg - not curable)
    - chlamydia (most common in thompson)

History
- two theories of origin
  - the pre-columbian theory
  - the new world theory
- first recognized European epidemic 1400
  - confident with Naples invasion by king Charles VII (france)
  - dispersal to his army furthered the spread throughout Europe
    - king charles the well served - first theory was he was the cause of syphilis
    - Tahiti islands - Paul Gaguin - famous for paintings and passing syphilis to virgin pop
- early names: the great pox, Iues venereum (VD), morbid gallicus (french disease)
- hieronymus fracastorius
  - coined syphilis decided from mythical shepherd syphilus
- believed syphilis and gonorrhoea where same entity until 1838
  - Philippe Ricord, 2500 human inoculations by accident (google?)
- 1932: Tuskegee study by the US Public Health Service
  - government scientists believed difference in race had different disease progression
  - over 400 men enrolled and treatment was withheld
    - infected with syphilis and/or gonorrhoea - no treatment
      - regular spinal taps (more painful than now) and aspirin
      - stopped in 1970 - all individuals died

Who's who of they syphilitics
- Many many people have been associated with syphilis:
  - Al Capone, Francisco Goya, Henry VIII, Adolf Hitler, Scott Joplin, Friedrich Nietzasche, Oscar Wilde, Kaiser Wilhem II, Christopher Columbus, James Cook, Franz Schubert, Ludwig van Beethoven, Henri Toulouse - Lautrec, John Keats, Marquis de Sade, Peter the Great of Russia, Napoleon, Julius II - Pope
- the fashion of long, frilly shirts, make up and white wigs among both men and women of Baroque Europe was due tot he rash, lesions, and hair loss of tertiary syphilis
  - judges and lawyers in many jurisdictions still wear these wigs
- sequel mores were altered
  - condom use became widespread, though the catholic church condemned their use
  - sex lost much of its spontaneity
  - Victorian sexual taboos in the mid to late 19th century were a direct result of fear of syphilis transmission

Medical Milestones in Syphilis
- the venereal transmission of syphilis was not shown conclusively until 18th century
- philippe record (1838) recognized the different stage of sypohilis
-

Where is syphilis today
- early stage can be easily treated with penicillin
- later stage syphilis, particularly neurosyphilis, is much more difficult to treat
- immunity to syphilis is not complete, so patients can suffer relapse
- curiously, syphilis appress to have attenuated in virulence, though no one knows why
  - loss of virulence gene
  - or adapt over time, through evolution to be less virulent

New World Plagues
- africa also passed deadly diseases westward
- 1700 yellow fever found its way up the eastern coast
- ports in the caribbean, central america and mexico
- in the north, it hit New York in 1668, Philedephia in 1690 and boston in 1691
- now mostly in central america and top of south america
  - our health care system in north america has outcompeted yellow fever

Yellow Fever
- Flavivirus, family Flaviviridae
- 2 transmission cycles
  - Jungle cycle 'Aedes' or "Haemagogus"
    - restricted to tropical regions
  - urban cycle "aedes"
- reservoir
  - humans, monkeys, and marsupials
- incubation period
  - 3 to 5 days
- communicability
  - before fever onset and the first 3 to 5 days of illness
- susceptibility
  - after recovery from illness life long protection
  - vaccine
  - insect control
- decisive in Caribbean military campaigns
  - 80,000 men died in the west indies 1793to 1796
  - most of the 40000 french who trained to regain San Dominique in Haiti
  - slaughtered most spinach troops during Cuba's rebellion of 1868 to 1878
- hit numerous cities in Europe
  - 1821 in Barcelona
  - 1857 in Lisbon

Typhus
- Turned the 1812 expedition of Napoleon to Russia into a catastrophe
- spread through Ireland in 1816-1819
- in 1848 revolution sin Europe triggered epidemics
- WWI killed a reported 2 to 3 million soldiers and civilians
- poor hygiene caused the spread of most outbreaks

The Birth of Rickettsiales
- Howard Ricketts
  - contracted typhus and died at the ago of 39
  - Stanuslaus von Prowazek also died due to Typhus
- gram negative bacilli
- single-circular chromosomes
- vector-borne
- two types
  - Louse-borne (epidemic) typhus (R. prowazekii)

- human louse ticks
- flying squirrels, crowded and natural disaster areas and un laundered clothing
- Eastern USA and wold wide
- 1-20% fatality

- Endemic flea borne (murine) typhus (R. typhi)
  - Oriental/tropical rat flea, human flea to —>
  - rats, mice, dogs, and cats
  - south america and ethiopia wold wide
  - 1-20% fatality

MBIO 3000
Lecture 9

Outbreak in the US: Hantavirus outbreak
NML - more ebola research (more from the government)

Midterm covers the movies - history classes and disinfection section (4 classes) after spring break

Symptoms and treatments
- Fever, rash, headache, chills, achiness, myalgia, and prostration
- CNS involvement
  - respiratory, gastro intestinal, and multi organ dysfunction/hemorrhages
- treatment
  - antibiotics
  - doxycycline (7 to 10 days)
  - except pregnant woman (other tetracycline antibiotics)
    - from (https://www.drugs.com/pregnancy/doxycycline.html) animal studied have revealed evidence of embryo- and fetotoxicity, including toxic effects on skeletal formation. animal studies indicate doxycycline crosses the placenta ad is found in fetal tissues. There are not controlled data in human pregnancy.
- prevention
  - limit contract with rats and other high risk areas
  - topical ointments
  - in Manitoba we are worried about lyme disease though ticks - deer ticks (smaller) - if you see a bullseye rash ask for the lyme disease test “very expensive - people weren't being tested”

Fleas and Lice
- requires blood meals - around 50 eggs/day
- eggs (shed) -> larvae -> pupae -> adult flea
- nits -> 3 larval stages -> adult louse
- requires blood meals
- wont be asked on exams

Vector Transmission
- vector borne diseases: transfer of an infectious agent from host to host via hematophagous arthropod including bacteria, viruses and parasites
- spreading attracted to: travel and shipping, climate and environment changes
- fleas and lice: must look at cleanliness - can grow in unclean areas

Diagnostic assays for Rickettsia spp.
- Shell vial technique
- cell staining assays
- immunofluorescent assays
- PCR based assays - not as accurate as staining and immunofluorescence

Bioterrorism
- similar symptoms, very general

- diagnostics are time consuming and require containment
- highly infectious!
- louse-borne: < 10 rickettsia prowazekii particles
- flea-borne: R. type is not listed

Summary
- typhus groups are vector borne diseases
  - similar symptoms and death rates
  - rats and poor sanitary conditions
- bioterrorism threat/ select agent
  - very infectious
  - BSL - 3
- sometimes.. less is more (genome reduction)
  - high virulence
  - want to infect you, and will continue to cause problems inside your body

GI Tract Infections: Salmonella typhi
- Typhoid fever (salmoella and rickettsia are different ** exam)
  - from food and sewage water or fecal contamination
  - 2 week incubation period
  - fever headache from the endotoxins
    - followed around 2 weeks later by diarrhea (cytotoxin & enterotoxin)
    - it is invasive, likes phagocytic cells which when infected can result in a systemic infection
    - recovering patients can become carriers
      - bacteria live in gall bladder & is shed in the feces
        - example; typhoid mary - cook in new york area: ring pattern of infection: mary tested positive for salmonella typhi - did not wash her hands - she did not listen, banned from cooking - she was medically arrested and removed her gallbladder - also received either prison time or asylum
        - prof used to be a paramedic in thunder bay - husband and wife come in, wife has a rash around her face - it was chlamydia - she said the only thing they did differently was they ate at a restaurant and ate Caesar salad and had soup - employee was masturbating in the salad and urinating in the soup - he was arrested
      - KNOW: gram neg produce endotoxins, why?
        - enterotoxins: found in intestines
- Treatment
  - fluid replacement
  - antibiotics are essential
    - broad range that effect DNA replication or protein synthesis

Cholera
- the biggest epidemic of the nineteenth century
- confined to India until early 1800
- 1817 increasing frequency outside India
- first appearing in Europe and North America beginning in 1831 - 1832
- seven pandemics over 150 years killing many thousands
- during most of this time doctors had no recourse for this disease
- bonfires were lit to disinfect the air
  - recently in Haiti - contaminated UN worker - don't know if we can get rid of it

- first pandemic 1817
  - began though southeast asia to china
  - Persia to Egypt
- second pandemic 1824
  - covered same ground as the first but penetrated into russia
  - firs time we had a disinfection team - go in to check if we are cholera free “biosafety in the 1800’s”
  - spread to north america in 1832
  - latin america later
- third pandemic
  - began when cholera accompanied British troops to Afghanistan and china
  - covered most areas, central asia, atlantic north and south america
  - 1854 the old and new world was awash in disease
- fourth - seventh pandemic
  - covered most counties over many decades
  - european and american physicians believed cholera was a locally produced miasmatic disease
    - an illness brought about by direct exposure to the products of filth and decay
- causative agent of cholara
  - Vibrio cholerae
  - first isolated by Robert Koch in 1883
  - bacterial enteric disease
  - causing profuse and violent cramps , vommiting and watery diarrhea
  - dehydration so rapid and severe the blood thickens and the skin becomes deathlike and blue
    - blood isn't moving fast enough to deoxygenate your system
  - cholera victims can die in a  matter of hours
  - asymptotic
    - EXAM Q: koch’s postulates: Dead animal - isolate organism (one clonal population) - insert into new animal - same diseases and same symptoms - found culprit
    - “what are two organisms that cant follow Koch’s postulates”
      - HIV cant infect humans **
      - H.pylori - cant culture it in the lab  **
      - possibly treponema
- Tranmission
  - fecal contamination **
  - ingestion of an infective dose on contained food and water
- reservoir
  - humans
  - environmental observation of zooplankton in brackish water
- incubation period
  - few hours to 5 days (usually 2 o 3 days)
- period of communicability
  - as long as stool samples are positive, a few days after recovery
  - carrier state can last a few months
  - infective for live during febrile stages
- susceptibility variable
  - breasted infants are protected
  - susceptibility to O blood group

- http://www.bloodjournal.org/content/115/23/4635?sso-checked=true
- treatment
  - has not changed much over the century
  - antibiotic treatment is effective if you use the right one
    - resistant to tetracycline, chloramphenicol
    - only works with nalidixic acid
  - patients with family were cared for at home
  - water replacement and rest will resolve the infection
  - physicians, would use such characteristics treatment as bleeding or opium
    - bleeding will only dehydrate you faster
  - homeopathic methods are popular

- by the end of the 19th century cholera epidemics no longer appear in Europe and NA
- why?
  - standards of living had risen
  - many communities had made major changes in sanitation practices
  - establishment of permanent boards of health
    - native populations have sanitation pipes extremely close to water where people drink and clean the water

Classifying Infectious Disease : Side notes
Disease: noticeable impairment of body function
- symptoms - what the patient feels
  - pain, malaise
  - this is subjective & can change
- sign - objective and concrete
- communicable - spread to one individual from another ex. gonorrhoea
  - this occurs through either contact direct or indirect
- contagious: easily spread ex chicken pox
- non-communicable: tetanus - spores
- endemic: low and constant levels of the disease in a given population
- epidemic: high numbers of infected people in a given area
  - short duration chicken pox in school
- pandemic - world wide epidemic
- acute: rapidly developing with short duration
- chronic - slow developing with continual direction - tuberculosis
- latent - inactive for certain periods of time
  - needs to be triggered for reactivation - stress , cold ore hides in nerve ganglion at the back of the neck
  - HIV - chicken pox, cold sores, shingles

Emerging infectious disease
- newly identified disease or organism & is noticeable in a number of cases
  - ex. lyme disease increase because we are not helping population
  - hantavirus (level 4 organism)
  - KFD - Kyasanur forest disease virus - if it ever finds a good vector in NA it can cause a lot of problems in the population

MBIO 3000
Lecture 10

Disinfection & biological waste
News: Plasmid driven super bugs - uprising
Why Disinfect?
- to get rid of unwanted pathogenic microorganisms
  - to eliminate or at least reduce - exposure risk
    - medical waste treatment
    - spill cleanup
    - minimization of nosocomial infections
    - routine surface decontamination
    - bio-terrorism attack
  - to eliminate contamination risk
    - preparation of microbial media & supplies
    - preparation of pharmaceuticals production supples and equipment
      - oxycontin needs to be made in a very sterile environment
      - ex) recall on dog food -> components were contaminated with heavy metals -> would stop their kidneys from working
      - these pharmaceuticals can be contaminated with many ingredients - could be dangerous to us
    - preparation of food (surface sanitization)
    - preparation of work area for cleanliness-critical tasks
      - contamination of self and other people

Spread of Pathogens
- Vertical transmission
  - susceptible hosts - maternal or germ line
- Horizontal transmission
  - direct contact
  - indirect contact - aides - tangible items
    - food, fingers, flies, feces, fomites) water air vehicles transplanted blood and tissue
      - remember the 5 Fs (will be on exam)

Disinfection Terms
- Sterilization - act of process, physical or chemical that destroys or eliminates all forms of life, especially microorganisms (including spores)
  - also harms us - ex) gamma radiation
    - don't want to be in contact
- Disinfectant - an agent, usually chemical that inactivates viruses and kills vegetative microbes but not necessarily resistant forms, such as spores
- Antiseptic - a substance that prevents or arrests the growth or action of microbes, either by inhibiting their activity or by destroying them (living tissue use)
  - must be used on humans without hurting us - ex. bleach will not work
- Decontamination - disinfection or sterilization of contaminated articles to make them suitable for use
- Sanitizer - an agent that reduces the numbers of vegetative bacteria only
  - most of the time only contains ethanol

Disinfection Suffices
- cide: kills
  - bactericide - (=germicide) destoryes vegentatice bacteria only
  - sporicide - destroys bacterial spores
  - fungicide - destroys fungal spores
  - tuberculocide - destroys M Tuberculosis
  - virucide - destroys specific viruses
- static (prohibits growth but may not kill)
  - bacteriostatic - prevents growth of veg bacteria
  - tuberculostatic - prohibits growth of mycobacterium tuberculosis

Resistance to Disinfectants
MOST RESISTANT
- Prions
- Bacterial spores
  - most bacterial resistant substance
- Coccidia (cryptsporidium)
- Mycobacterium
- Nonlipid viruses (ex Hep A, polio)
- Fungi
- Rickettsiae, chlamydiae
- Vegetative bacteria
- Lipid-containing viruses (ex. HIV, Ebola)
  - easy to break down a lipid membrane compared to non-lipid viruses

LEAST RESISTANT
- least resistant only requires low level disinfectant - from lipid viruses to vegetative bacteria
- intermediate level - to fungi
- high level disinfectant - to coccidia
- cold sterilant - to bacterial spores

Classes of Disinfectants
- Choline
  - Available as liquid, powder or tablet form
  - household bleach usually 5-6% Na Hypochlorite
  - in use dilutions depend on application and amount of organic material present
    - bleach is inactivated by organic material - would need a higher concentration of bleach or multiple washes
    - general disinfection
    - 5,000 ppm available Cl (10% bleach)
    - works well against organic material
      - only if there is more bleach than organic material
    - 10,000 ppm available Cl (20% bleach)
    - effective vs vegetative bacteria, mycobacteria, viruses, and fungal spores and has some sporicidal activity
      - only works against some spores
    - working solutions should be prepared fresh (within 24 hours) and kept in light-protected containers
      - if not, then sitting bleach will go through a reaction to kick off the Cl ion which makes the solution inactive and will not work

    - stock liquids lose Cl concentration over time
    - corrosive; neutralized by organic material
    - $ClO_2$ gas used in mail centre against anthrax
- Iodine
    - available as aqueous solution, tincture (solution in alcohol), and iodophor (iodine complexed with a carrier molecule to increase solubility and provide sustained release of the halogen) ex. povidone-iodine
        - also neutralized by organic material
    - effective against enveloped (lipid containing ) viruses, vegetative bacteria and fungi, exhibits variable activity against mycobacteria, non-enveloped viruses and bacterial spores
    - disadvantage include staining of treated items, corrosiveness and neutralization by organic material
    - wescodyne, providone-iodine (product names)
- Alcohol
    - typically
        - ethyl alcohol (ethanol, EtOH)
        - isopropyl alcohol (isopropanol, IPA)
    - 70% in water is most effective concentration; 100% alcohol is not nearly as effective
        - causes a dehydration reaction, without water in the ethanol, we cannot make a dehydration reaction, which makes it ineffective
    - effective against
        - enveloped viruses and
        - vegetative bacteria
    - longer contact times (10 minutes) required for any activity against fungi and mycobacteria
    - variable activity against non-enveloped viruses
    - no activity against bacterial spores
    - longer contact times difficult to achieve due to loss by evaporation
    - flammable
- Phenolics
    - wide variety of phenolics available: generally used in combination with detergents
    - effective against
        - enveloped viruses &
        - vegetative bacteria
    - variable activity against fungi and mycobacterium, depending on production
    - limited activity against non-enveloped viruses
    - no activity against bacterial spores
        - outside shell of spore is impermeable to phenols
    - disadvantages:
        - bad for our skin - itchy
        - toxicity
        - neutralization by hard water
            - hard water - lots of mineral containing water
        - pungent, unpleasant smell
    - advantages include:
        - not easily neutralized by organic material
        - if you mix it with detergents it is non-corrosive
    - vesphene, LpH are examples

- Quaternary Ammonium Compounds (QACs)
  - quats have innate determent action but may be inactivated in the presence of some soaps or soap residues
  - odourless, colourless, non-irritating and deodorizing
  - effective vs gram + bacteria
  - limited activity vs enveloped viruses, fungi and gram - bacteria
  - effectiveness reduces by organic material
  - roccal, germex, zephiran, coverage plus
- Glutaraldehyde
  - 204% aqueous solution, often with activator
  - broad spectrum antimicrobial activity, including
    - non enveloped viruses and mycobacteria (20 min)
    - bacterial spores (3 hours)
  - very effective cold sterilant commonly used to disinfect endoscopes
    - issues with disinfection in hospitals
  - use requires adequate ventilation
  - activated product has limited shelf-life
  - controversial product because of adverse health effects, indulging mucous membrane irritation, contact dermatitis, occupational asthma
    - contact dermatitis - skin rash
    - occupational asthma - damage to lungs if inhaled
      - cidex taken off the UK market May 2002
- Formaldehyde

MBIO 3000
Lecture 11

Mumps outbreak in Winnipeg
Mumps in Manitoba - WILL HAVE EXAM Q ON THIS
- 176 cases since September
- normally cases range form 4-8 a year
- most cases were initially from university students 18-29 years old
- some individuals were vaccinated but still got infected (vaccines is 85% effective)
- sports teams are to blame and are at risk
- this out break has already spread to the NHL, university hockey and football team

What is Mumps?
- Mumps virus - genus Rubulavirus ( -ssRNA virus)
- portal of entry
  - mucous membranes of the upper RT
  - generally infects children between 2 and 12
- Signs and symptoms
  - parotitis (swelling of the salivary glands) face pain, headache and sore throat
    - gland is beside the ear, to the side of the chin
    - may cause deafness in ear
  - some infections may be asymptomatic * easily spread
- incubation period
  - twelve to twenty four days
  - infects via the RT enters blood and many organs (mainly the salivary glands)
- susceptibility
  - unimmunized individuals are at risk
  - fomite transmission or person to person
- treatment
  - comfort care only… soft fluids, warm water gargles
  - will self resolve over time - if child
  - if adult - sterility problems if the infection gets bad
- prevention
  - MMR vaccine
- virus infect the saliva glands
- was once among the more common of childhood diseases
- nearly nonexistent in developed nations due to effective childhood immunizations
- infections in late winter and early spring still occur in countries that lack an effective immunization programs
- patients suffer from inflammation of the testes, meninges, or pancreas, and rarely deafness in one ear

MMR vaccine
- measles mumps rubella
- no live virus - component vaccine (piece of the pathogen in order to make antibodies)
- some speculations (bad science) like thimerosal in vaccines in autism
  - Jenny McCarthy made this popular - UK scientist published a paper that was entirely fraudulent, that paper was published - after it was retracted and shown to be false.
  - becoming very problematic - people aren't vaccinating children - not doing background work

Classes of Disinfections
- Formaldehyde
  - supplied as solid paraformaldehyde (flakes or tablets), or as liquid formalin (37% formaldehyde in water with 100 ml/l of methanol stabilizer)
  - disinfectant use is typically 4%
  - broad spectrum of activity against all classes of microorganisms
  - less susceptible to inactivation by organic material
  - corrosive, caustic, irritant, carcinogen
  - can be neutralized with ammonium bicarbonate
    - in the lab: use formaldehyde gas - leaves a white film
    - go in again and pressure wash the room with ammonium bicarbonate
    - now we use vaporous hydrogen peroxide
- hydrogen peroxide
  - available as concentrated 37% solution in water (6% in use solution)
  - effective against
    - vegetative bacteria
    - mycobacteria
    - fungi
    - viruses and spores
  - vapour phase hydrogen peroxide (VHP) is good volume disinfectant (35 to 37% solutions in converted to vapour phase by heat)
  - leaves no residue (breaks down to H2O and O2)
  - incompatible with some metals, aluminum copper zinc brass natural rubber and some plastics
  - accepta 8101 is new biocide based on stable combination of H2O2 and silver ions - product literature indicates high levels of activity against nearly all microorganisms
    - ie. does not kill all microorganism - not as good as formaldehyde
- chlorhexidine
  - available as a 2-4% solution of chlorhexidine gluconate (a biguanide) in a detergent base (used undiluted) and as
  - concentrated alcohol- based solutions requiring dilution prior to use
  - alcoholic solutions shows superior activity to aqueous solutions
  - effective against enveloped viruses and vegetative bacteria: limited activity against fungi, mycobacteria, non-enveloped viruses; no sporicidal activity
  - generally used as an antiseptic (skin disinfectant) and hand wash

Graphs
- sodium hypochlorite
  - does not kill spores
  - mycobacteria - acid fast cell wall - more resistance to disinfectant
  - phenols are able to break down acid fast walls

Comparing anti-microbials
- the phenol coefficient = highest dilution of the test solution divieed by the highest dilution of phenol that gives “no growth” of a bact. culture under standard condition
- example:
  - at 10 minutes a disinfectant exhibited no bacterial growth at a dilution of 1 to 500
  - phenol exhibited no bacterial growth at 10 minutes at 1 to 95
  - phenol coefficient of the disinfectant tis 500/95 = 5.36 or 5.2 x created than phenol

Factors Influencing the Efficacy
- Surface
  - topography
- Temperature
  - maple leaf outbreak - disinfectant worked great at room temperature but not at 4 degrees which is what their temp was
- Relative humidity
  - important for decontamination agents that are gaseous
- Water Hardness
- organic load
- concentration
- contact time

Some Factors
- uneven cradled or pitted surfaces, especially wooden surfaces, can hide microorganisms and are difficult to disinfect
- elevated temperatures may enhance germicidal action but also evaporation rate
- many disinfectants have an optimal relative humidity range for maximum effectiveness
  - formaldehyde gas works best at RH(relative humidity)> 70% while VHP is best at 50-70%
- some disinfectants may be less effective when diluted in hard water; distilled water is used in many manufacturer's testing protocols

Organic Load
- blood, sputum, milk, bedding, feed, manure
- proteins physically protect and stabilize many microorganisms
- adverse effect on action of many disinfectants

MBIO 3000
Lecture 12

Organic Load
- Blood, sputum, milk, bedding, feed, manure
- proteins et al physically protect and stabilize many microorganisms
- adverse effect on action of many disinfectants
- organic load interferes with many disinfectants

Concentration
- in most cases, the higher the concentration, the more rapid the kill
  - a major exception is 70% ethanol
- consider potential damage to surfaces or tissues (if concentration is high enough)
  - reducing concentration to avoid damage will require additional contact time
  - ultimately, disinfectant will no longer be active enough to be useful, if we decrease the concentration and increase contact time we have evaporation occurring

Contact Time
- disinfectants should be effective with a short CT
- manufacturer's recommended contact time may be unrealistic in-use conditions
- CT may depend on the method of application:
  - surface disinfection: 1 min CT is practical
  - instrument soak: 20 min CT is practical
- for surface application, loss by evaporation may require frequent applications to achieve CT
  - if it evaporated we need to reapply
- extending contact time may increase effectiveness, but not in all cases
  - if it can make spores we may induce spore formation which could be very dangerous
    - C. difficile
- evaluate disinfectants on case by case basis according to need

Some other factors
- dried spills (from media, buffers) can protect microorganisms form contact with the disinfectant
  - must wet it first then disinfect
- dirt, grease and oils - all can protect the organisms
  - grease and oils will repel water based disinfects
- types of microbes present
  - spores, vegetative cells, viruses
- pH
- age of the product/solution
- method of application
  - spray vs wipe
- rate of application
- storage conditions

Prion Disinfection
- Decontaminate surfaces with 1N NaOH
  - alternatives include bleach solution (>2% free chlorine), 4M guanidine hydrochloride
- autoclave solids or liquids at 132 degrees C for 4.5 hours - best in presence of 1M NaOH

- incineration
  - UCSF holds liquids 24 hrs in 1M NaOH before sending for incineration
  - Winnipeg doesn't have an incinerator - we have to go to north Dakota
- formaldehyde has little effect on prion infectivity; how fixed tissues in 96-100% formic acid/1hr then 10% formalin/48 hrs
- wherever possible, use disposable plasticware and incinerate

Properties of Ideal Disinfectants ***** EXAM Q
- broad spectrum (kills almost everything)
- high efficiency (kills rapidly)
- unaffected by organic matter, soaps & detergents, water hardness, pH
- nontoxic, noncorrosive, nonflammable
- odourless
- cheap
- stable
- environmentally friendly

US, EU, Canada Disinfectants where to get ore Information
- In the US
  - disinfectants are regaled as EPA Pesticides
    - pesticide EPA Law 1972
- In the EU
  - Disinfectants are regulated by multiple organizations
- In Canada *** EXAM Q ON THIS “what are the two laws”
  - therapeutic products program (TPP) for disinfectant drugs
  - the pest management regulatory agency (PMRA) for antimicrobial products for purposes other than for treatment of human or animal disease
    - example: greenhouse disinfectant

Autoclaves
- heat kills
- moist heat kills more effectively because of the mass transfer of heat as steam condenses its very efficient
  - surface sterilization requires 15 min at 121 decrees C with steam and 60 mins at 170 degrees C dry heat
- steam must be in contact with the material being sterilized to be effective **
  - sealed containers in autoclaves (would blow up) material in the box would not be sterilized
- most autoclaves are “gravity displacement” - higher density of the steam drives air from the chamber
- pre-vacuum or hi-vac autoclaves use a pump to remove air during conditioning and to remove steam during exhaust cycle, shortening the total cycle time
- the autoclave works by increasing he pressure on steam
- not by increasing the temperature
- pressure
  - 15 psi ->>> 121 degrees C
  - 30 psi ->>> 132 degrees C
- must have adequate pressure to achieve the temperature

Autoclave Personal Protective Equipment (PPE)
- Required
  - high heat resistance gloves
    - longer than the reach inside
- Recommended
  - lab coat /clothing protect
  - slip-resistant shoes for wet autoclave room floors
  - non-fogging eye protection

Autoclave Quality Assurance (QA)
= sterilizing conditions achieved
  - monthly “spore test” with geobacillus stearothermophilis
  - thermosensitivie indicators such as tape and red bag labels
    - autoclave tape (clear and goes to brown if it worked)
  - hi-lo thermometer
- mechanical systems should undergo periodic system safety checks
  - seals and drains should be checked daily
- formal QA program may be required by regulation
  - now we have to due to the HPTA (human pathogens and toxins act) regulation (EXAM Q)
  - increasing costs by a lot
  - LOOK UP MANDATE FOR HPTA (EXAM Q?)

Volume Decontamination
- Formaldehyde
- ethylene oxide
- ozone
  - hurts eukaryotic organisms just as much as prokaryotes
  - ozonate blood to get rid of pathogens in Russia
- vapor phase hydrogen peroxide (VHP)
  - formaldehyde is better than VHP (look up from old notes)
- gas H2O2 plasma
- new technologies:
  - propylene oxide
  - beta propiolactone
  - chlorine dioxide (ClO2)

Formaldehyde
- generated by heating parafomaldehyde flakes to generate folamdehyde gas
- advantages
  - low temperatures
  - highly effective disinfectant
- disadvantages
  - toxic / carcinogenic
  - explosive 7-73% by volume in air (roper use at 0.3 g/cu ft will yield concentration well below explosive level)
  - RH and temp control important to success
- standard protocol for biosafety cabinet decon

Ethylene Oxide
- USEPA registered anti-microbial
- used in gas sterilizers
  - used to sterilize instruments
  - used in fumigation process for mail (2001)
- human carcinogen and monitoring is necessary during its use

VHP
- VHP generator by heating concentrated (35 to 37%) $H_2O_2$
- advantages
  - no harmful emission
  - no resides
  - highly effective
- Disadvantages
  - incompatible with some metals and plastics
  - does not go through organic load * ?
  - poor penetration through solid load; requires surface cleaning
- 5000 ft3 volume limit

Ozone
- usually made by passing oxygen through electrodes or UV light
  - utilizes the very unstable free radical
  - strong oxidizing agent
- used in water supply purification
  - first used in 1880's
  - effective against bacteria, algae, viruses
  - oxidizes metals and cell walls/ filtration required
  - expensive and byproducts could be toxic
  - common industrial applications
- has been used in Medicine (ozone therapy)

Physical Disinfection Approaches
- Radiation
  - microwave - destroys protein structure; alt medical waste treatment; expensive
  - ultra-violet (UV) - required direct exposure; effect decreases rapidly with distance; dangerous
  - Gamma irradiation - good penetration; effective; expensive (machine doesn't last long)
- Incineration
  - destroys matrix; not socially acceptable; expensive; exhaust concerns; regulated
- Filtration
  - no good on solids
  - HEPAs are very effective for air; wide range of effective choices for liquids; matrix preserved

Choosing a Decon Method **** (EXAM Q)
1. Identify the contaminants
2. Determine need to preserve matrix (wont destroy the things in the room)
3. Identify effective disinfectants and processes
4. Perform risk assessment with attention to

1. hazard and risk **mitigation**
2. regulatory issues
3. manufacturer's claims vs reality
   - can say it takes 3 minutes (in vegetative form) but it will actually take 9 hours for spores

5. compare choices; select method which ensures adequate rests (within budget and space constraints) with maximum safety

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Lecture 13

New in the World
- Small out breaks of measles and pertussis
- Frontier school division national microbiology lab - google for video of lab

Hand decontamination
- hand washing is the single most important procedure for preventing
  - lots of bacteria and virus on hour hands, we are always on our phone and transmitting bacteria (fomite)

Basic Hand Hygiene
- 3 levels - social hand wash, hygienic hand wash and surgical scrub
- when to wash hands
  - whenever contaminated
  - before and after a patient contract
  - before and after gloving
  - before leaving laboratory
- what to use
  - soap, plain or anti-microbial
    - do not use anti-microbial soap - using antibiotics in not a prescribed way (causing resistance)
  - alcohol-based rub (liquid, gel, foam)

Disinfection Summary
- there is a resistance hierarchy
- there are many classes of chemical disinfectants and they come in many forms
  - susceptibility to disinfection varies within biological species
- environmental and other factors can influence efficacy
- choose the disinfectant wisely
- EPA (US) and other countries may regulate disinfectants
- there are other alternatives to chemical disinfection
  - autoclaving common (non chemical) disinfecting procedure
- hand washing - simple, inexpensive and critical

Medical waste
- lots of ways of storage - a lot of the time they are used incorrectly
- proper ways is very important

Who Defines Medical Waste?
- International Associations (ICAO, IATA)
- countries
  - laws
  - regulations
  - guidance
- states
  - laws
  - regulations

- guidance
- institution
  - policies

Common Defining Elements
- USEPA:
  - medical infectious waste means any waste generated in the diagnosis, treatment or immunization of human beings or animals, in research pertaining thereto, or in the production of testing of biologicals that is listen in.. this definition
    - cultures and stocks of infectious agents and associated biologicals
    - human pathological waste
    - human blood and blood products
    - sharps that have been used on animals or humans
    - contaminated animal waste from research
    - contaminated soil waste (gloves, plates, tubes, pipettes, dishes, ect)
    - isolation waste
    - unused sharps

Notes on Waste Definition
- the most applicable definition of medical waste often depends on both geography and regulatory context (air emissions (incinerations) solid waste disposal, occupational health, shipping
- the best way to ensure compliance is to ask the regulatory agency with jurisdiction over your area and activity
- need to ensure no one else gets infected with your medical waste

Types of Biological Waste
- solid, non-sharp waste from laboratory or clinical settings
  - plastic lab ware (flasks, tubes, plates, bottles, vials, oct)
  - lab waste (stocks, specimens, cultures, swabs, vaccines) (must be decontaminated so no cross contamination)
  - tissue or carcass waste (animal waste)
  - gloves, apparel, wipes
  - pipettes (could also be sharps)
- sharps
  - anything with a pint of edge capable of piercing or cutting human skin
  - glass lab ware (sometimes also broken plastic)
  - needles, scalpels, blood tubes, Pasteur pipettes
  - syringes (with and sometimes without needles)
- liquids
  - aspirates, culture fluids, rinses, washes, ect
  - sera, body fluids
  - spill clean up waste
  - put it in a container and sterilize in autoclave (non explosive container)
    - AMOEBA with parts of HIV genes - from putting genetic info down the drain
- management alternatives
  - drain disposal without pretreatment
  - at-source pretreatment
  - centralized pretreatment

Special Cases
- Pharmaceutical & chemotherapy waste
  - most states have pharm & chemo MW categories
  - differences between bulk chemicals and medical waste items contaminated with chemo agents
- research waste
  - regulated by some states
    - usually includes mammalian cell cultures, rDNA, ect
  - check your country of states regulations
- mixed chem/rad/bio waste
  - biowaste is always at the bottom of the hierarchy

MW program elements
- A medical waste management program should have systems or procedures in place to properly execute these elements:
  - Cradle to grave**
  - written medical waste management plan
  - waste stream segregation at te point of generation
  - controlled storage/holding for treatment
  - transport on-site and off-site
  - training in MW requirement and procedures

Medwaste Management
- Regulatory compliance
  - know the agencies laws and regulations that apply
  - permits?
  - tracking
  - inspections
- cost
  - tracking system (UPS)
  - recovery
  - internal bulling
  - overhead

MW treatment options
- cradle to grave responsibility applies to MW
- do it yourself
  - standards practice for liquids
  - autoclaves and other solid waste treatment devieces generally require permitting, usually by local entity
- have someone else do it
  - very common, requires due diligence
  - transporter may be delivery onto or full TSDF
    - should generate manifest of types and amounts of MW transported
    - treatment facility should provide certificate of destruction

Intermediate vs Final treatment
- intermediate treatment
  - usually performed for worker protection

  - autoclaving most common method
  - standard microbiology lab practice
  - permed before transport to final treatment
- final treatment
  - on site treatment by facility staff
  - off site treatment by disposal contractor
    - usually very costly
  - much more effort involved
    - permitting
    - monitoring
    - record keeping
    - reporting
    - risk management

Treatment Technology
- autoclave (variety of capacities)
- microwave
- incineration
- chemical treatment
- alternative technologies
  - irradiation
  - high-temp pyrolysis (vitrification)
  - macrowave

Autoclave Validation or QA
- Autoclave tape
  - temperature-sensitive tape
  - color change when heated
  - does not indicate decontamination
- biological indicators
- position in load is critical
- frequency?

Treated Medical Waste
- in most states, potentially infectious medical waste that has been treated by an acceptable method is considered non-contaminated and non-hazardous; the liquid an be sewer-disposed and the solids put in sanitary landfills

Medical Waste Summary
- vocabulary varies from state to state and country to country
- USA-usually defined and regulated by states
- medical waste can be solid lab/clinic waste, pathological waste, sharps, or liquid
- a medical waste program should address:
  - policy, training, and education
  - packaging, transportations and storage
  - monitoring and record keeping
  - treatment and disposal
- there are two treatment options
  - do it yourself  or contract

- there are several treatment technologies available for each type of medical waste

COURSE REVIEW:
Nomenclature
- correct name of bacterial names
  - underline bacterial names
  - genus capitalized, species are not
  - abbreviation: must first give full name then do E.coli
- viruses
  - can follow the binomial system - no underline
  - subtypes are put in brackets

Questions:
- what infectious agent is also known as the speckled monster? what are the reservoirs, mode of transmission, and period of communicability? (4 marks)
  - small pox (variola virus) , human reservoir, droplet transmission, skin inoculation, 3 weeks.
- describe lady mary motagu's contribution to infectious disease control in 1721 how did this process differ rom that of edwards in 1796?
  - lady montague brought variolation to europe from india. variolation = inoculation of pus from pocks of person suffering from smallpox disease into healthy individual to provide protection, may result in smallpox disease
  - edward jenner inoculated pus form cowpox pustules into healthy individuals to provide protection against smallpox, safer than variolation
- List the three subspecies found in the genus Treponema pallidum discussed in class and the disease associated with them?
  - pallidum - vinereal syphilis
  - endemicum = endemic syphilis - bejel
  - pertenue, disease = Yaws
- A small number of Spanish took over the entire inca nation, how many of the 165 Spanish died during the inca battle?
  - zero

- HPTA
  - go on canadian website (read what its about) very specific - what does it actually do? what is its mandate? (freebie question on the exam

MBIO 3000
Lecture 14

Assignment - 10 page paper on three topics to pick from
- base grant on a topic through class
- two other topics to pick from

- final is cumulative
  - wont ask specific questions on details
  - still need to know general concepts

Section two
Principles of Containment Laboratories

Biosafety Levels and Risk Groups
- BSL -1 through BSL 4, RG-1 thorough RG-4
- CL-1 (containment level) through CL-4 in Canada
- Risk Groups are agent-specific (specific organisms)
- Biosafety Levels assignment based on :
  - Agent characteristics
  - lab function or activity
  - quantity and state of agents
  - operation performed
  - others

Biosafety Levels/Contaiment Levels
- Four biosafety levels provide increasing degrees of protection
- combination of:
  - laboratory practices and techniques
  - administrative controls
  - engineering controls
    - safe equipment (primary barriers)
    - facilities design and construction (secondary barriers)
- Applications
  - Labs (BSL-1, 2, 3, 4 )
  - (small) animal containment (ABSL -1, 2, 3, 4)
  - Large animal containment (BSL - 3 AG)
  - Plant Containment (BSL -1P, 2P, 3P, 4P )

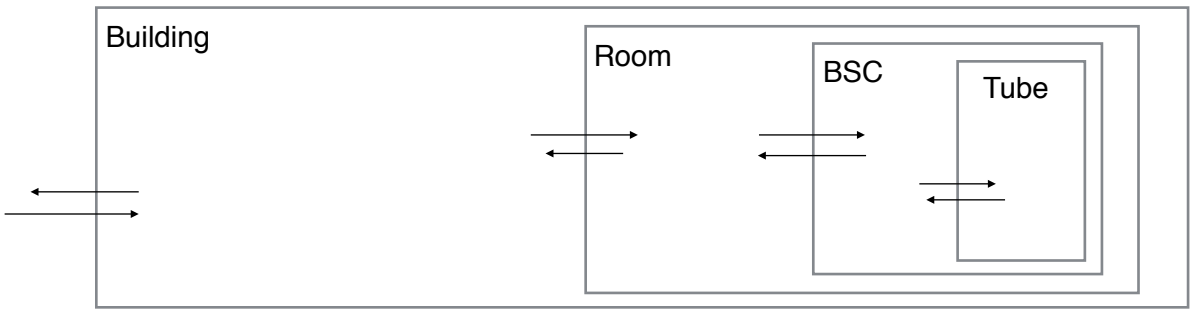
Biosafety Levels
- BSL-1
  - defined organisms,
  - not known to cause disease in healthy adults
    - ex) ecoli
- BSL - 2
  - moderate risk agents present int he community
  - disease of varying severity
  - ex. salmonella
- BSL - 3

- indigenous or exotic agents, aerosol transmission
- serious and potential y lethal infections
  - HIV, Typhus
- BSL 4
  - dangerous or exotic, high risk agents
  - life threatening disease
    - E.bola
- Hierarchy
  - last two are containment labs

Guidance on Biosafety Levels
- Some guidelines take a performance approach generally defining what rest is intended, leaving how to achieve the result up to the user
- other guidelines are more prescriptive, They outline specific requirement that must be done.

Engineering Controls
- The fist and best strategy in bio containment is to control the hazard at the source ***
- work environment and the job itself should be designed to be eliminate hazards or reduce risk
- If feasible, design the facility equipment, o process to remove the hazard or substitute something that is not hazardous
- if removal is not feasible, enclose the hazard to prevent exposure in normal operations
- where complete enclosure is not feasible, establish barriers and/or localized controls to reduce exposure to the hazard in normal operations
- apply before work practices and personal protective equipment are used
- engineering and other types of controls (including PPE) are NOT mutually exclusive
- employers may need to use multiple types of controls to prevent employee exposure
- degree to which engineering controls are required depends on the applicable regulatory framework (incl. guidelines) and a site-specific risk assessment

Containment Barriers
- Barriers protect by: ( FIVE PRINCIPLES OF BARRIER PROTECTION) *
  - physical separation
  - directional air flow (differential pressure)
  - practices and procedures
  - decontamination
  - filtration
- barriers are penetrated by:
  - personnel
  - fluids
    - air, gases, water, drainage
  - materials
  - products
- continents laboratories are layers of open and closed systems;



Primary and Secondary Barriers
- Primary barriers:
  - engineering controls (equipment)
    - biological safety cabinets and other ventilation equipment
    - animal caging
    - specialized lab equipment (centrifuges, fermenters)
  - personal protective equipment (PPE)
- primary barriers contain the agent at the source
- Secondary barriers (engineering controls)
  - building and room construction
  - HVAC issues:
    - directional air flow
    - exhaust filtration
  - other engineering controls:
    - solid waste treatment
    - wastewater treatment
- secondary barriers contain the agent within the room or facility in case an agent escapes form the primary barriers

Primary Barriers & Protection ******
- Personel protection
  - any aerosols generated within the cabinet is contained and kept away from the researcher
- Product protection
  - air within the work space of the cabinet has been filtered so that it is virtually free of airborne particles and organisms, thus protecting the work from outside contamination
- Environmental protection
  - aerosols generated within the unit are removed from the air before the air is discharged

Primary Barriers - Ventilation Equipment
- Picture

| | Personnel | Product | Environetm |
|---|---|---|---|
| Chemical Fume Hoods | X | | |
| Laminal Flow Clean Benches | | X | |
| Class I Biological Safety Cabinet | X | | X |
| Class II Biological Safety Cabinet | X | X | X |
| Class III Biological Safety Cabinet | X | X | X |
| Isolators | X | X | X |

  - laminar flow - protect the product only

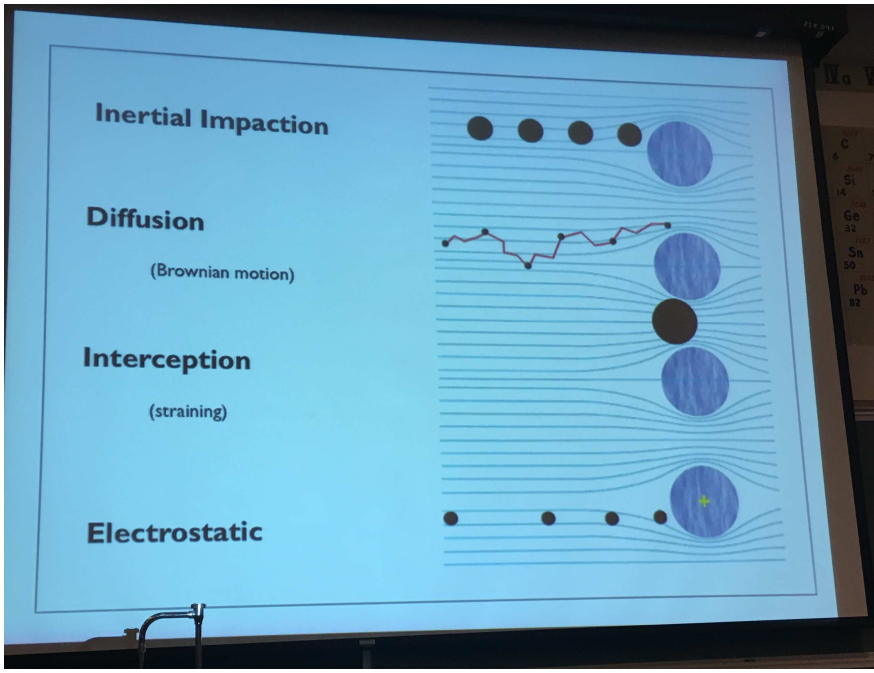
- chemical fume hoods (doesn't care about product or environment, just personnel
  - ie. if chemical spill its piped right outside
- class 3 - wouldn't be at uni, just at NML - ensures we protect the environment
  - cheaper to function than level 3 laboratory

Fumehoods, Biosafety Cabinets, and Laminar low cabinets
- fumehoods
  - work with volatile chemicals and radioactive materials
- biosafety cabinets
  - recirculates aerosol particulates through HEPA away fro user
  - no vocative chemicals
- Laminar Flow
  - protects samples, airflow towards user

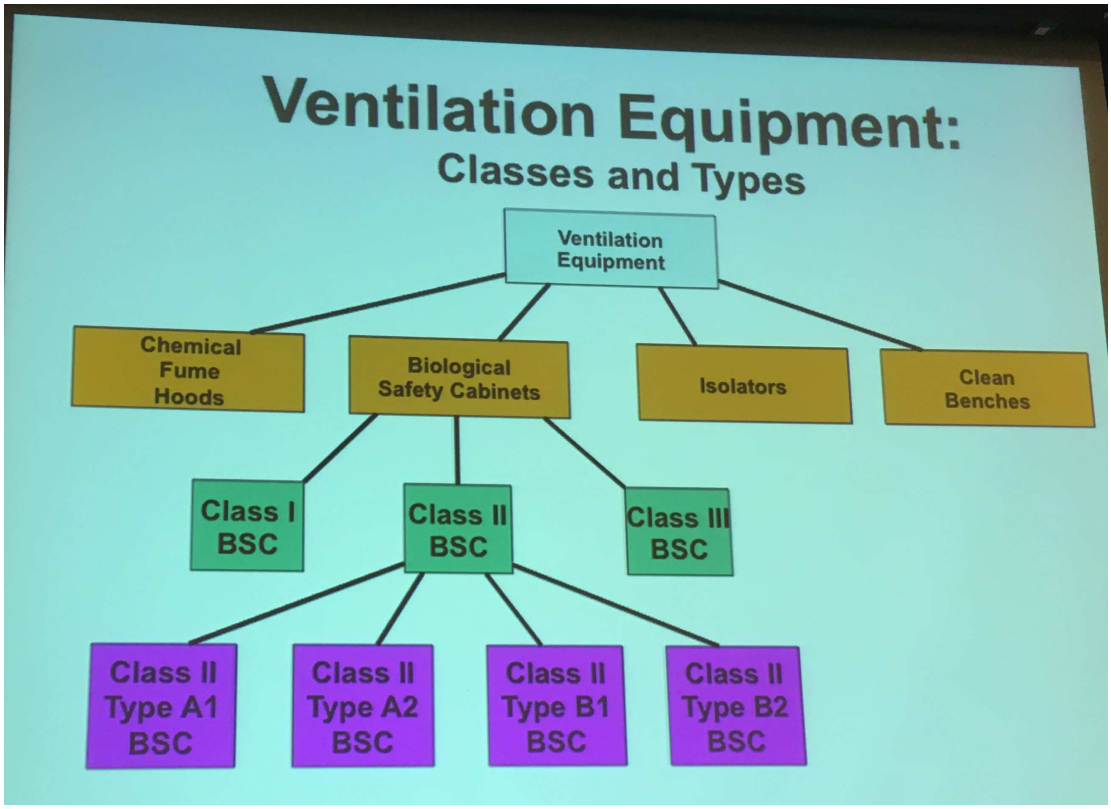
HEPA filters
- High Efficiency Particulate Air
  - Components:
    - Filter media
      - folded cellulose/borosilicate media
      - very large surface area
    - Sealants
      - epoxy, poluurethane
    - Frames
      - metal
      - wood
    - Edge Sealant
      - gasket with clamp
      - gel seal with knife edge
- minimum efficiency of 99.97% removal of 0.3 micron particles

How a HEPA filter works?
- Intertial Impaction
  - particle is so large it is unable to adjust to abrupt changes in streamline direction near a filter, due to its inertia, will continue along its original path and hit the filter
- Interception
  - straining
- Diffusion
  - movement of molecules from an area of higher concentration to an area of lower concentration
- Electrostatic forces
  - the forces between particles that are caused by their electric charges
- HEPA filters do not filter out gases and vapours, the only filter out particles

- remember the four concepts



Filter Replacements
- filters are replaced when the resistance makes it impossible to deliver the required airflow
- as resistance increases, efficiency increases
- filters are replaced when repairs exceed the size allowable by standards




Chemical Fumehood
- 100 fpm face velocity
- offer only personnel protection
- always exhaust air to the outside
- do not offer protection to the produce or environment, as there is no flotation of intake and exhaust air (sometimes air cleaning testament is added to the exhaust)
- do draw contaminants in the laboratory air directly over the product being worked on
- **used for work with chemical hazards**

Isolators
- designed to produce products in a clean environment
- air is HEPA filtered as it goes into and our of the unit
- isolators provide protection specific to each design
- applications
  - pharmaceutical quality control testing
  - super clean manufacturing without creating clean room
  - pharmaceutical manufacturing of potent compounds

Clean Bench
- produce product protection only
- product protection is provided by creating a unidirectional airflow generated through a HEPA filters
- discharge air goes directly into workroom
- applications
  - any application where the product is not hazardous but must be kept contaminant free
  - preparation of non-hazardous intravenous mixtures and media
  - particulate free assembly of sterile equipment and electronic devices

Biological Safety Cabinets
- designed to contain biological hazards
- inward airflow for personnel protection
- HEPA filtered exhaust air or environment protection
- Supply air HEPA filter for product protection (except Class I)
- Separated into Classes and Types
  - Class I
  - Class II
    - Type A1, A2
    - Type B1, B2
  - Class III
- Microbiological studies, cell cultures, pharmaceutical research and procedures..

Class I Cabinet
- 75 fpm face velocity
- provides personnel and environmental protection
- no product protection
- requires an exhaust blower to pull the air through - usually to the outdoors
- applications
  - housing centrifuges, fermentors
  - cage dumping in an animal lab
  - aerating cultures

MBIO 3000
Lecture 15

Assignment due March 30th
Do not email him the assignment - give him a hardcopy

Class I Cabinet
- 75 fpm face velocity
- provides personnel and environmental protection
- no product protection
- requires an exhaust blower to pull the air through - usually to the outdoors
- applications
  - housing centrifuges, fermentors
  - cage dumping in an animal lab
  - aerating cultures

Class II Cabinet
- ventilated cabinet
- **provides personnel, product and environmental protection**
- open front with inward airflow for personnel protection
- downward HEPA filtered laminar airflow for produce protection
- HEPA filtered exhaust air for environment protection

Class II A1 Cabinet
- 75 fpm face velocity
- **70% recirculated air, 30% exhausted (thru HEPA)**
- Exhaust to room or thimble connected to external exhaust dust
- Potentially contaminated ducts and plenums under positive pressure to the room
- **Not suitable for work with volatile toxic chemical and volatile radionuclides**

Class II A2 Cabinet
- 100 fpm face velocity
- **70% recirculated air, 30% exhausted (thru HEPA)**
- Exhaust to room or thimble connected to eternal exhaust duct
- potentially contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums
- **May be use for work with minute quantities of volatile toxic chemicals and tracer amounts of radionuclides if they are exhausted through properly functioning exhaust canopies**

Class II B1 Cabinet
- 100 fpm face velocity
- **30% recirculated air, 70% exhausted (through HEPA)**
- Air in the back of the cabinet is exhausted to the outdoors through a dedicated exhaust plenum and the air in the front is recirculated
- **must be hard ducted to the outside for the cabinet to function**
- all biologically contaminated ducts and plenum under negative pressure or surrounded by negative pressure ducts and plenums

- **minute quantities of volatile toxic chemicals and tracer amounts of radionuclide permitted** if work is done in the direct exhausted portion of the cabinet

Class II B2 Cabinet
- 100 fpm face velocity
- what we use in level 4 contaminent laboratories
- **exhaust 100% of the air to the outside** after filtration through a HEPA filter
- must be hard ducted to the outside
- sometimes called **total exhaust**
- all contaminated ducts and plenums under negative pressure, or surrounded by (direcly exhausted non-recirculating through the work area) negative pressure ducts and plenums
- **may be used for work with volatile toxic chemicals and radionuclides** required as an adjunct to microbiological studies

- will most likely be tested on bolded

Biosafety Cabinet Technique
- proper technique is critical to maintaining personnel and product protection
- elements
  - decontamination
  - setup
  - work flow
  - locations of supplies and waste containers
  - movement in and out
  - others
  - want to have everything in the cabinet before working - don't want to be walking in and out

BSC Pitfalls
- overloading
- blocking airflow elements
- BSC location in lab
- Bunsen burners
- UV lamps
  - the majority of biosafety cabinets have a UV bulb in it, sterilization techniques
  - put the hood down, UV is supposed to sterilize for you to work in the morning - not totally effective
  - you wont notice if the UV light is on while you're working - could be damaging to eyes
  - before starting work - make sure UV switch is off

Class III Cabinet
(level 3 lab on legs)
- **Gas-tight absolute containment enclosure**
- product, personnel and environmental protection
- minimum air intake velocity through a glove port is 100fpm, if the glove is cut or removed
- air must be exhausted to the outdoors
- integral equipment allows the safe introduction and removal of materials
- exhausted air is treated by double HEPA filtration or HEPA filtration and incineration
- supply air is HEPA filtered
- operations in cabinet performed through rubber gloves

- operates under negative pressure of at least 0.5” w.g. (124 Pa)

- Japan is not allows to work with organisms that can be used for bioterrorism - due to treaty after the war
  - now they can start doing some infectious work

Class III Cabinet Line

| **Advantages** | **Disadvantages** |
|---|---|
| Relative ease of movement | Heavy-duty rubber gloves |
| No direct contact with agents | Susceptibility to sharps |
| Maximizes personal safety | difficult working conditions |
| Particulate -free air within cabinet | turbulent airflow within cabinet |
| self-sufficient:<br>- refrigerators, incubators,<br>- special equipment (aerosol apparatus), animal cages | maximum working areas |
| | Dexterity minimized |

Animal Equipment
- small animal cages
  - made to protect the researcher and animals
  - every cage is HEPA filtered on its own to prevent cross-contamination

Other (equipment) Engineering Controls
- Gasketed blenders, homogenizers
- cotton plugs, fliters for flasks in shakers
- filtered pipette tips
- HEPA and hydrophobic vacuum line filters
- plasticware substituted for glassware
- gas burners with shield, micro incinerators
  - gas burners not allowed in the NML anymore
- centrifuges
  - interlock, solid cover, safety buckets, O-rings

Filtered Pipette Tips
- prevents cross contamination
- prevents aerosols

Micro-incinerators/Sterilizers
- alternative to bunsen burner
- safe to use in a BSC

Safer Sharps Devices
- use one needle for a large number of small animals
- use

Biosafety Level 1
ie. Kitchen
- basic laboratory - like the ones we have at university

Biosafety Level 2
- BSL 2 is similar to BSL 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment

BSL-2 Facilities
- lockable doors (restricted agents)
- sink for hand washing
- easily cleaned, bench tops are impervious
- biological safety cabinet as needed
- eyewash available
- inward airflow without recirculation to non-lab spaces
- windows fitted with fly screens

Animal BSL-2
- containment caging should be used as appropriate for the species
- cages must only be opened in biosafety cabinets

Biosafety Level 3
- BSL 3 is applicable to clinical, diagnostic, teaching, research or production facilities in which work is down with indigenous or exotic agents which many cause serious or potentially lethal disease as a result of exposure by the inhalation route…

Biosafety Level 3
- start with BSL-1 and 2 facilities plus:
  - separate building or isolated zone within a building
  - directional inward airflow
  - single-pass air
  - double door entry
  - room penetrations sealed (or capable of being sealed)
  - enclosures for aerosol generation
  - walls, floors and ceilings are water-resistant for easy cleaning
- BSCs mandatory
- Additional requirements depending on work and agents:
  - HEPA filtration of the exhaust
  - Effluent decontamination
  - Personnel showers (for decontamination)
- follow the guidelines, regulations and site-specific risk assessment for your unique situation!

ABSL - 3
- physical separation from access corridors
- animals in containment cages
- cubicles sometimes used for segregating caging units

Biosafety level 3
- Laboratory facilities secondary barriers
  - room penetrations sealed
  - directional inward airflow
  - pressure monitoring devices at entry
  - smoke test (smoke pencil (cigarette if none available))
  - HVAC controlled to prevent sustained positive pressurization
  - interlock exhaust/supply
  - alarms for HVAC failure (inside and outside the facility)
    - story: negative pressure went positive
  - exhaust air HEPA filtered (sometimes necessary)
  - sealed ductwork
  - back-flow prevention on supply air (damper, HEPA)

Assignment
- generate information package for your research director. This package must provide historical (ie. how pathogen was first discovered, past outbreaks ect.) and scientific information (ie. morphology, transmission, incubation, period, reservoirs, containment level, treatments, ect.) regarding your specific pathogen. Additional information to provide may include: interesting historical facts, recent outbreaks, decontamination methods, current global relevance, ancient emergence of disease ect.

- Lassa Fever - the next E.bola
  - arthropod vector (can be found in Canada)
- Methicillin-resistant staph aureus
  - antibiotic resistance
  - can change to a different antibiotic resistant
- West Nile Virus
  - starting to come into Manitoba
  - emerging exotic organism
- Research Grant
  - look at CRTI (granting group)
  - follow their dicuscssion on how to do objectives and hypothesis
  - using in Canada is easier to get funded
  - email him for template if you're really interested
  -
- doesn't matter how its referenced
  - should be like a science journal
  - need references from good source
  - introduction or abstract
  - okay with tables and figures, they do not account for the ten pages
  - double spaced, Times new Roman, 12 point font
  - okay with going over the page limit, but don't go crazy (ie. don't go over 15 pages)