Water quality microbiology

Chapter 28

- Wastewater microbiology and water purification
- Waterborne microbial diseases

Public health and water supply

- **Communicative diseases and water supply systems - John Snow and Cholera (1855)**
- **Routine monitoring of water quality using <u>indicator organisms</u>, indicating fecal contamination**

Characteristics of a useful indicator

- Useful for all water types
- Always present when pathogens are present
- Not present in the absence of the pathogen
- Correlated with degree of pollution
- More easily detectable than the pathogen
- Survive longer than the pathogen
- Not dangerous to work with

Direct tests for pathogens

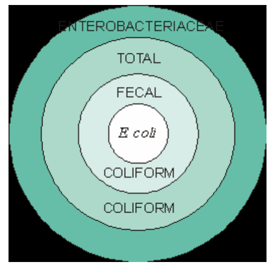
- Involve selective cultivation to large numbers
  - –Time consuming
  - –Expensive
  - –Potentially dangerous to lab personnel
- Molecular tests
  - –Require testing for each pathogen
  - –Expensive
  - –Require expertise

Bacterial-indicator organisms Common groups

- COLIFORM GROUP
  - –total coliforms
  - –fecal coliforms
  - –*Escherichia coli*
- *STREPTOCOCCI*
  - –*fecal streptococci*
  - –*enterococci*
- SPORE FORMERS
  - –*Clostridium perfringens*

Coliform Characteristics

- Total coliforms
  - –Gram negative
  - –Ferment lactose at 35 ° C
  - –Ubiquitous in the environment
- Fecal coliforms
  - –Ferment lactose at 44.5 °C
  - –May be of fecal origin
  - –Old recreational-use criteria
- *Escherichia coli*
  - –Inhabits gastrointestinal tract
  - –Specific indicator of fecal pollution

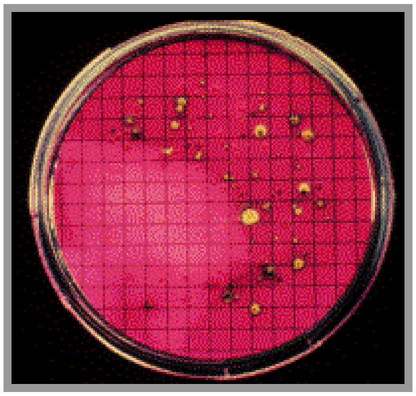


Other fecal indicators

- Fecal streptococci (enterococci)
  - –Gram-positive cocci
  - –Grows at 41 °C
- *Clostridium perfringens*
  - –Present in both human and animal wastes
  - –Produces a stress-resistant spore
  - –Indicates point sources, but poor indicator of non-point pollution sources
  - –Possible surrogate for resistant bacteria

Total coliforms by mENDO

- Basic fuchsin and sodium sulfite form a green-metallic sheen on colonies able to ferment lactose to acetaldehyde

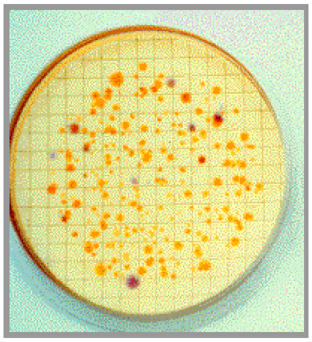


E. coli by mTEC media

- Recreational- use criteria, ambient waters, ground water, drinking water
- Sodium lauryl sulfate and sodium desoxycholate inhibit Gram + cocci and endospore-forming bacteria.
- Brom-cresol purple and brom-phenol red inhibit nontarget bacteria, color change when lactose fermentation lowers pH.
- Incubated at 35 °C (2 hr) then at 44.5 °C

E. coli by mTEC media

- Non-target colonies turn purple when breakdown of urea raises pH (*E. coli* does
  not produce urease)



Typical water quality standards

- Drinking water
  – No coliform contamination acceptable
- Public water supply water
  – 2000 fecal coliforms per 100 mL
- Recreational water
  – 200 fecal coliforms per 100 mL
- Fish and wildlife habitat
  – 5000 fecal coliforms per 100 mL
- Shellfish
  – 14 fecal coliforms per 100 mL

Accepted criteria for the coliform test (US):

No more than:

- 1/100 ml as the means of all samples examined per month
- 4/100 ml in more than one sample if < 20 are performed per month
- 4/100 in 5% of the samples taken if > 20 are examined per month
- Reporting is to the U.S. EPA

Problems with the coliform indicator test

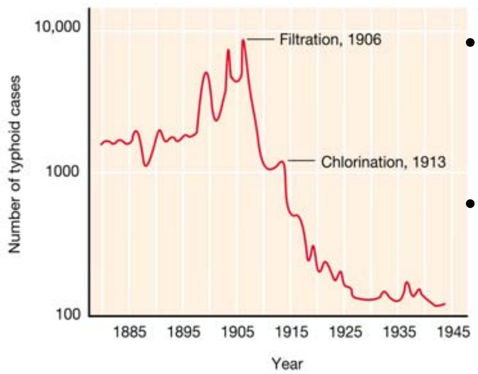
False positive:

Enterobacter aerogenes - a soil organism; use the IMViC test for distinction

False negative:

Salmonella typhi in Riverside in 1965

Public health and drinking water purification

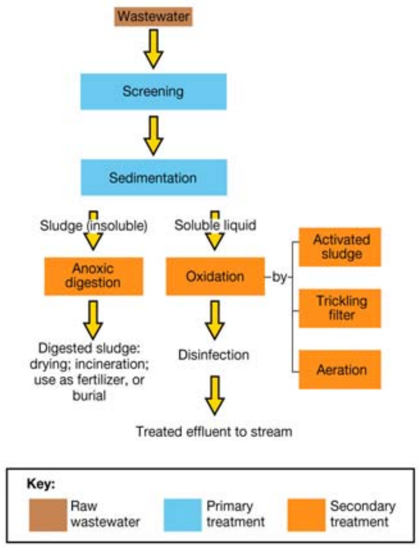


- Today in developed countries - Foodborne, rather than waterborne, diseases are the problem
- Institution of filtration and later chlorination ➲ Dramatic improvement in public health

Wastewater and sewage treatment

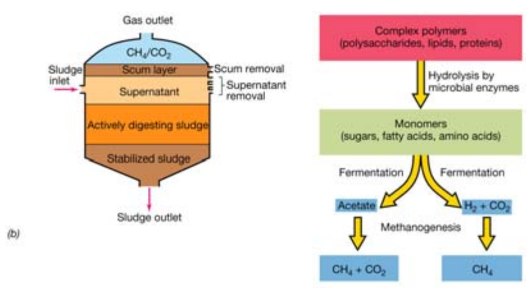
- Wastewater: domestic sewage ("gray water") and industrial wastes.
- Problems: organic compounds, harmful organic and inorganic compounds, pathogenic microbes
- Quality is defined by the Biological Oxygen Demand (BOD) - the amount of oxygen consumed during oxidation of organic matter for 5 days at 20 ^{o}C.
  - Sewage - 200 BOD units
  - Food industry as high as 1600 BOD units
  - Effluents < 5 BOD units
- In the U.S. 15,000 sewage treatment facilities treating 40 billion gallons of wastewater a day

Wastewater treatment



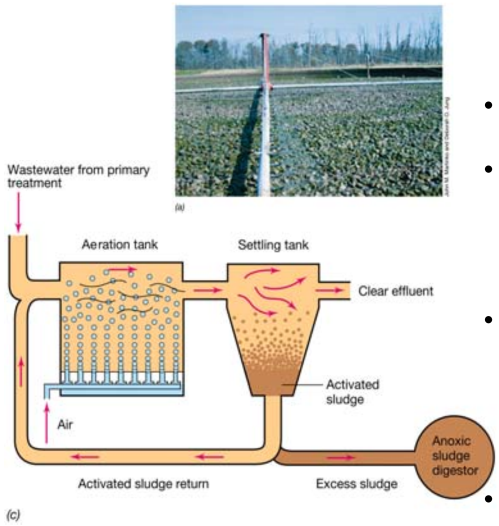
- **Primary treatment** - the removal of large objects - screening and sedimentation
- Resulting effluents have a high BOD values

Secondary treatment - anaerobic



- Anoxic digester: Removal of high molecular weight material; industrial wastewater
- Low production of biomass; most C is converted to CO_2 and CH_4

Secondary treatment - aerobic



- Organic carbon is converted to CO_2 and microbial biomass
- Trickling filter - a bed of rocks (10-15 cm in diameter) at a 2 m depth. The thick biofilm that forms on the rocks oxidizes organic matter
- Activated sludge - 5 -10 hours retention; flocs consisting of bacteria (*Zoogloea ramigera* - Fig. 28.7) embedded in EPS (slime) and eukaryotic microbes
- The sludge may be digested anaerobically or dried and used as a fertilizer

Tertiary treatment – Removal of inorganic nutrients

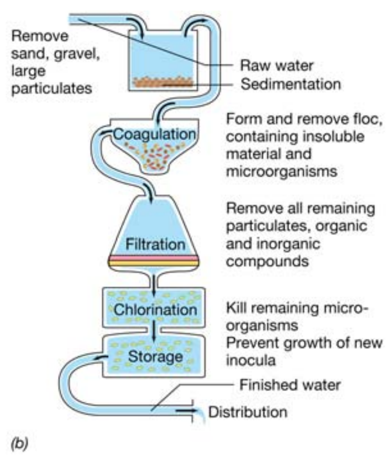
- Most treatment facilities carry out only primary and secondary treatment
- Release of nitrate and phosphate - eutrophication of receiving water bodies
- Physical chemical processes: precipitation, filtration, chlorination

Drinking water purification

- Potable water: water safe for human consumption
  - Pathogens removed
  - Turbidity (suspended particles) decrease
  - Taste improved and odors removed
  - Nuisance chemicals removed
- Achieved in water treatment plants that feed finished water to consumers through distribution systems

Water purification

- Sedimentation tank - Large particles and objects removed
- Coagulation basin (clarifier) - Raw water mixed with anionic polymers, alum, chlorine; floc formation by coagulation and flocculation; settling of large flocs
- Filtration - remaining suspended matter including microbes removed
- Disinfection - Chlorine kills most microbes in 30 min but Protozoa (e.g., *Cryptosporidium*) may survive

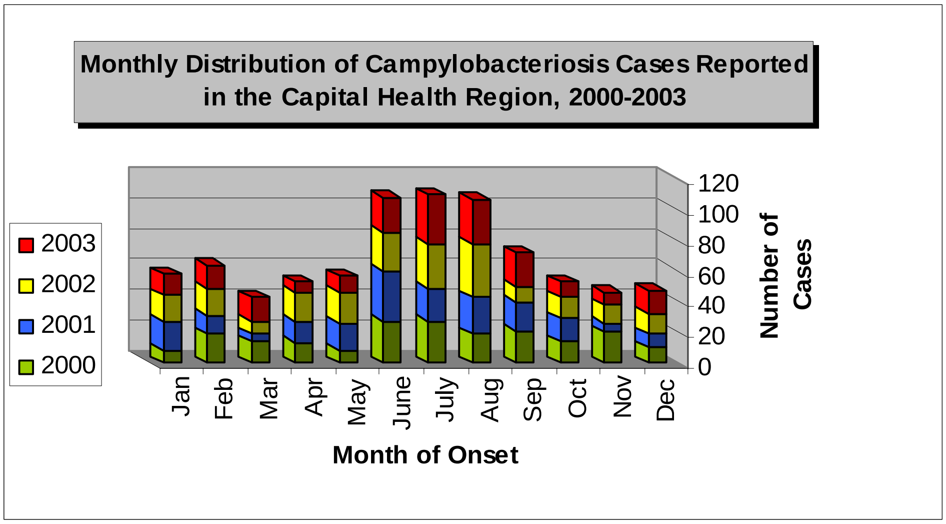


Waterborne diseases

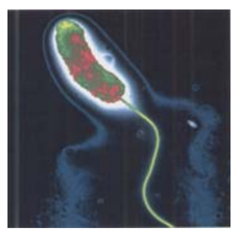
- Infection is initiated by exposure to contaminated drinking or recreational water - fecal contamination
- Recreational water (natural and public swimming pools) - regulation: monthly geometric means at $\leq$ 126/100 ml *E. coli*
- A big problem in developing countries

Waterborne infectious diseases (U.S. 1997-1998)

Disease	**Agent**	**Outbreaks**	**Cases**
Shigellosis	*Shigella sonnei*	1	183
Giardiasis	*Giardia lamblia*	4	159
Cryptoporidiosis	*Cryptosporidium parvum*	2	1432
Gastroenteritis	*E. Coli* 0157:H7	3	164
Acute gastrointestinal illness	Unknown	5	163



Vibrio cholerae



- Descriptions of epidemics on the Indian subcontinent in the 1400s but it did not spread to Europe and the Americas until 1800s
- Cholera was prevalent in the U.S. during the 1800s, but has been virtually eliminated by modern sewage and water treatment
- Currently it is common in Asia, Africa, and Latin America
- Recent outbreak in Africa (110 new cases per day)
- Over 100,000 cases with 2345 deaths in 2004

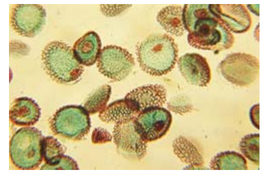
Cholera - *Vibrio cholerae*

- Transmitted through raw water and food that is contaminated by water (vegetables, shellfish)
- Since 1817 seven pandemics
  - 6 prior to 1961 caused the classic strain (Koch 1883)
  - 1961 to present, the El Tor biotype
- Endemic in Africa, Southeast Asia, and India
  - In 1999 400,000 cases with 9000 death
- Pathogenesis - enterotoxin produced in the small intestine causes diarrhea leading to dehydration and, if untreated to death (60% of cases)
  - Role of acidity in the stomach
- Diagnosis- presence of curved rod in water stool
- Treatment - hydration with electrolytes

Giardiasis and Cryptosporidiosis

- *Giardia lamblia* and *Cryptosporidium parvum* are <u>protozoans</u>
- *G. lamblia* cyst ingested and trophozoite develop, attach to intestine wall leading to diarrhea, foul smell, stomach cramps
- Transmission through water. 97% of all surface water carry cysts. Cysts are resistant to chlorine but proper water treatment removes them during flocculation and filtration
- Natural streams - transmission by wild animals
- Diagnosis: microscopic observation or antigen presence; treated with drugs

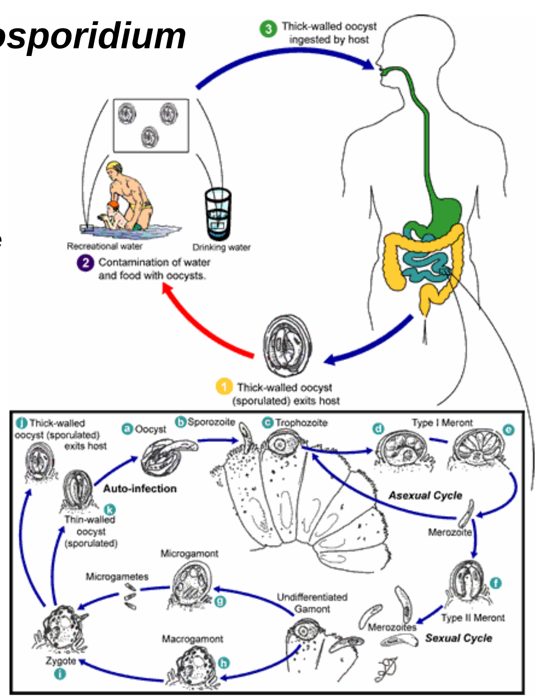
Cryptosporidiosis



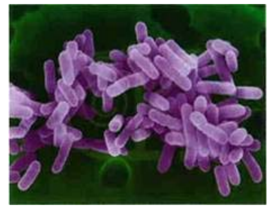
- Tiny protozoan (2-5 µm) with highly chlorine tolerant cysts; mild diarrhea but groups at risk.
- Transmission from animal carriers via water distribution systems
- 1993 an outbreak in Milwaukee (400,000 infected); breakdown of water treatment due to heavy rains

Life cycle of *Cryptosporidium*

•Transmission of *Cryptosporidium parvum* occurs mainly through contact with contaminated water
•Following ingestion (and possibly inhalation) by a suitable host, excystation occurs
•The sporozoites are released and parasitize epithelial cells of the gastrointestinal tract
•The parasites undergo asexual multiplication (schizogony or merogony) and then sexual multiplication (gametogony) producing microgamonts (male) and macrogamonts (female)
•Upon fertilization of the macrogamonts by the microgametes, oocysts develop that sporulate in the infected host.



Legionellosis (Legionnaire's disease)



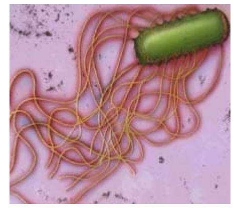
- *Legionella pneumophila*:
  - a waterborne pathogen transmitted via aerosols
  - Found in water and soil but grows to high numbers in warm waters (air conditioning cooling systems, hot pools)
- An intracellular parasite of alveolar macrophage and monocytes
  - Pontiac fever - mild headaches, sore throat, fever, that disappears after a few days
  - Pneumonia that follows flu-like symptoms in the elderly; 10% death
- Treated with erythromycin

Legionellosis (Legionnaire's disease)

Legionella pneumophila is a motile, rod-shaped, gram-negative, aerobic, bacterium. It is considered to be a "facultative parasite," which in the last twenty years has been identified as the leading cause of Legionnaire's Disease. This disease was first discovered in 1976 among a group of elderly men attending an American Legion Convention in Philadelphia, Pennsylvania (hence the name Legionnaire's Disease).

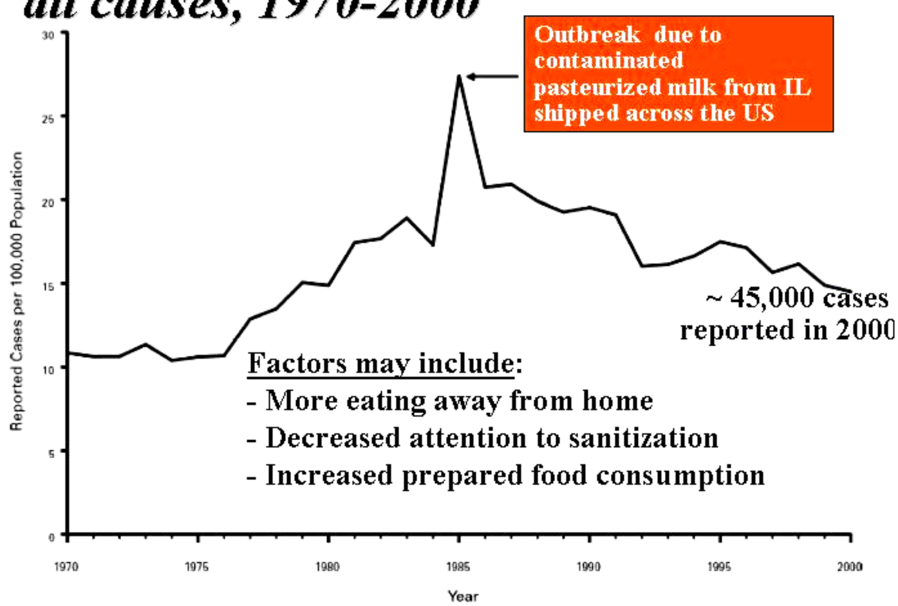
When this outbreak first occurred it shocked the nation and the world, because no one knew why all of the men at the convention were being diagnosed with with acute respiratory failure. However now, more than twenty years later, scientists have learned a great deal about Legionnaire's Disease, as well as the *Legionella pneumophila* the pathogen which causes it.

Typhoid fever and other waterborne diseases

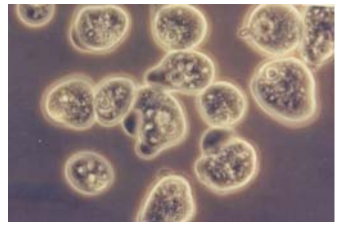


- Typhoid fever - *Salmonella typhi*
  - Diarrhea
  - Transmitted by contaminated food, direct contact, and drinking water
- Viruses - polio and hepatitis A
  - Maintaining of 0.6 µg/ml residual chlorine assures elimination
- Both diseases are transmitted from infected individuals and in the developed world are associated with the breakdown of water treatment practices

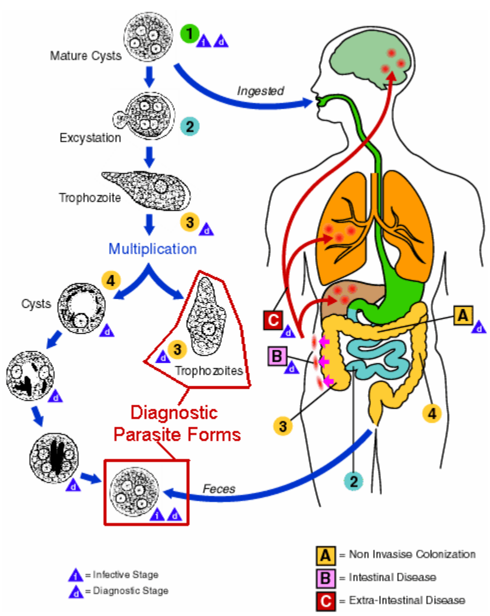
Salmonellosis, all causes, 1970-2000



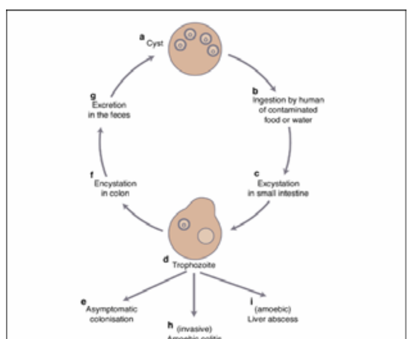
Amebiasis



- Protozoa
- *Entamoeba hystolytica* - dysentery
  - Anaerobic (no mitochondria)
  - Cysts in water and food
  - Germination in the intestine and growth of trophozoites in mucus cells - many individuals are asymptomatic
  - Invasion into epithelial cells lead to diarrhea, inflammation, and fever
  - Severe cases, further penetration into blood, liver and brain
  - Drug treatment available - dehydroemetine, diloxanide furoate (for asymptomatic cases)
- Worldwide: 100,000 deaths a year, 50M infected (5M asymptomatic)



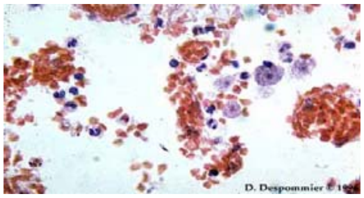
Amebiasis



Life cycle of *Entamoeba histolytica* and the clinical manifestations of infection in humans

Expert Reviews in Molecular Medicine © 1999 and 2005 Cambridge University Press

Meningoencephalitis



- *Naegleria fowleri* - a free-living ameba
- Transmission from recreational waters (warm soil contaminated hot springs, rivers and ponds)
- Through nose and subsequent passage to the brain where propagation occurs
- Hemorrhage and brain damage and death
- In the U.S. sporadic (a few cases a year) but highly lethal

Viral sources of waterborne disease

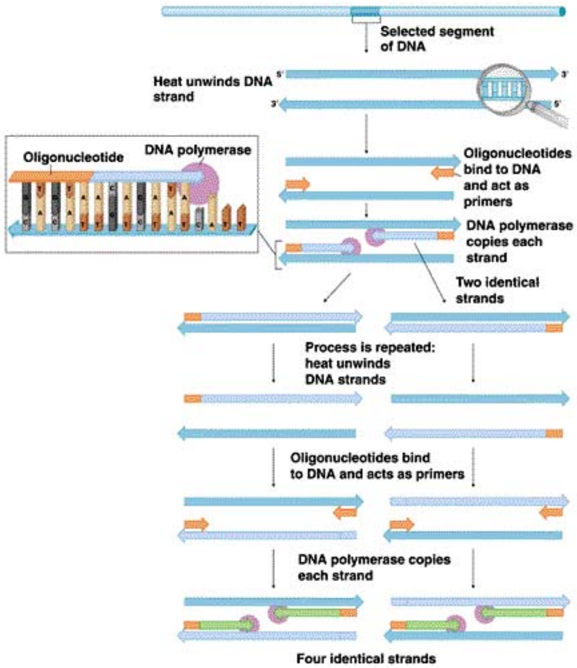
- Hepatitis A: readily transmitted via water; causes inflammation and necrosis of liver
- Norwalk-type viruses: cause acute gastroenteritis
- Rotavirus: causes acute gastroenteritis, especially in children
- Enteroviruses: many types can infect both the intestines and the upper respiratory tract
- Reoviruses: infect the intestines and upper respiratory tract (usually without disease symptoms)

Methods used to detect viruses

- Electron microscopy
- Immunoassays
- Cell culture
- Reverse transcriptase-polymerase chain reaction (RT-PCR)

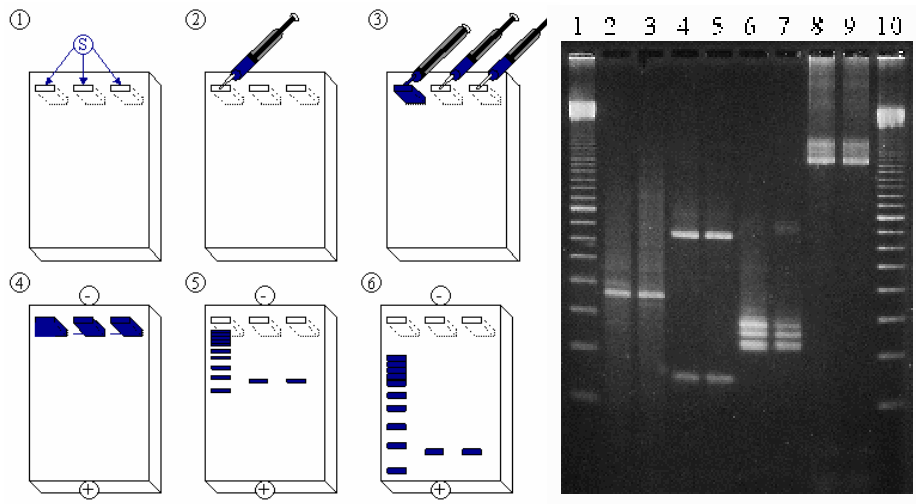
RT-PCR

- Reverse transcriptase (RT) uses RNA as a template to make a DNA copy
- Polymerase chain reaction (PCR) enzymatically amplifies specific DNA sequences
- Uses short oligonucleotide primers that are complementary to the DNA sequence of interest



Gel electrophoresis

- Size separation of PCR products
  –charged DNA molecules migrate through a gel in an electric field
  –small molecules migrate more rapidly than larger molecules
- The gels are stained and observed under UV
- PCR products from each target virus have a known size and can be compared to a ladder of standard molecular weights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