Final Exam Review

20.106 Systems Microbiology
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Early Life, Origin of Microbes

• Early life
  – Necessities for life
  – Theories of microbial beginnings

• Timeline
  – Important events
  – Evidence

• Isotope ratios
  – Calculate
  – Examples (useful elements & how occur)
Structural Features of Bacteria

- **Capsule**
  - **Composition**
    - Capsules typically consist of a surface polysaccharide layer (‘smooth’ bacteria)
  - **Purpose**
    - Physically prevent ingestion by phagocytic cells in pathogenic bacteria

- **Peptidoglycan**
  - **Composition**
    - Cross-linked by peptides between NAM residues on adjacent chains
  - **Purpose**
    - Maintains shape of cell

- **Pili**
  - **Composition**
    - Straight projections composed of pilin protein subunits with molecule-specific proteins on pilus tip
  - **Purpose**
    - Adherence to surfaces
    - Exchange of DNA via conjugation

- **Flagella**
  - **Composition**
    - Filament (flagellin monomers), hook and motor (H⁺ driven motor)
  - **Purpose**
    - Motility

- **LPS & LTA**
  - **Composition**
    - Lipid chains that vary from bacteria to bacteria, plus polysaccharide (gram−, LPS)
    - Lipid chains, plus techoic acid (gram⁺, LTA)
  - **Purpose**
    - Stabilizes cell membrane
**Gram-negative Cell wall**

![Diagram of Gram-negative cell wall]

**Gram-positive Cell wall**

![Diagram of Gram-positive cell wall]

Figure by MIT OCW.
Motility

How at low Reynolds numbers?
• Only force at moment matters—no inertia
• Reciprocal motion useless
• Must be circular, corkscrew motion

Flagellar movement
• Random-walk pattern for environmental sampling
• Chemotaxis towards nutrients/niche

Image of a bacterium with long rotating flagella removed due to copyright restrictions.

Biased random walk: Increase concentration -> Decreased tumbling frequency

Figure by MIT OCW.
Prokaryotic Chemotaxis

Figure 2 | Schematic diagram of the chemosensory system of Escherichia coli. Two dimeric chemoreceptors—methyl-accepting chemotaxis proteins (MCPs)—are shown, one of which is interacting with a periplasmic binding protein (FEP). In addition, two chemotaxis protein (Che)W monomers and a CheA dimer are shown interacting with the highly conserved signaling domain of the MCPs in the cytoplasm. It should be noted that, given the packing within MCP clusters and the calculations of the number of chemosensory proteins, the actual arrangement will be different. One CheA monomer will probably not interact with one MCP dimer, and instead a CheA dimer might span several receptors. A decrease in attractant concentration induces trans-autophosphorylation of the CheA dimer, which phosphorylates the response regulator CheY. Phosphorylated CheY then binds to the flagellar motor to bring about a change in direction. Phosphorylated CheA also phosphorylates another response regulator—the methylase CheB. Phosphorylated CheB competes with a constitutive methyltransferase, CheR, to control the degree of methylation of specific glutamates in the MCPs. This resets the signaling state of the receptors and allows them to adapt to the present concentration of attractant and to sense subsequent changes. The dephosphorylation of phosphorylated CheY is accelerated by the phosphatase CheZ, P, phosphoryl group.
Mobile Elements and Lateral/Horizontal Gene Transfer

- Resistance plasmids
- Incompatibility groups
  - Two very similar plasmids will NOT co-exist in one bacteria
- Cloning (cloning vectors)
  - Plasmids
  - Phage
  - Cosmids
  - Bacterial Artificial Chromosomes (BAC)
Horizontal Gene Transfer: Transformation

- Release of DNA by growing or decaying cells
- Exposure to bacteria
- Stabilization
- Expression of competence
- Inactivation
- Degradation
- Expression of competence
- Uptake
- Restriction
- Recircularization
- Recombination
- Degradation
- Mutations, rearrangements
- Expression
- Selection
- Negative
- Neutral
- Positive

Figure by MIT OCW.
Horizontal Gene Transfer: Conjugation and Transduction

Chromosome

Prophage

Mobilizable plasmid

Transposon incX

incY

Integron

Conjugative plasmid

Mobile gene cassettes

Specialized Transduction

Generalized Transduction

Pilus

Chromosome

Donor cell

Recipient cell

Figure by MIT OCW.
Metabolic Diversity

**Basic needs**

- **Carbon source**
  - Organic molecules (heterotrophs)
  - Inorganic molecules (autotrophs)

- **Energy source**
  - Chemical rxns (chemotrophs)
    - Light (phototrophs)

- **Electron donor**
  - Organic molecules (organotrophs)
  - Inorganic molecules (lithotrophs)

- **Electron acceptor**
  - Oxygen (aerobic)
  - \( \text{SO}_4^{\text{2-}}, \text{NO}_3^{-}, \text{Fe}^{\text{III}} \) (anaerobic)
Nitrogen Cycle

Important Reactions

✓ Nitrogen Assimilation
✓ Deamination
✓ Nitrification
✓ Denitrification
✓ N₂ Fixation
Rhizobium

- Free-living are aerobic, not $N_2$ fixers
- **When symbiotic**
  - *Rhizobium* turn on plasmid-based *Nod* genes
  - Become anaerobic $N_2$-fixing, bacteroid form
  - Legumes form nodules to control symbiotic relationship

Images of free-living *Rhizobium* and bacterioids in nodule removed due to copyright restrictions.
Symbiosis and Genome Reduction

- **Buchnera aphidicola** from two aphids *Schizaphus graminum* (Sg) and *Acyrthiosiphon pisum* (Ap)

- 70 million years
  - No chromosomal rearrangements
  - Sequence divergence (9⁻⁹ synonymous substitutions/yr
  - 1.65⁻⁹ non-synonymous substitutions/yr

- *E. coli* and *Salmonella* spp. (closest free-living relatives) 2000x more liable
Genome Dynamics in Buchnera

Obligate endosymbiont

- Substantial sequence divergence
- Prominence of pseudogenes
- Loss of DNA repair mechanisms
- Stable genome architecture HOW??
  - Gene transfer elements reduced/eliminated
    - Reduced phage
    - Reduced exchange w/other genomes
    - Fewer repeat sequences
    - Fewer transposons
  - Lack of recombination mechanisms (no recA, recF)
  - Lower frequency of recombination
**Ti plasmid & crown gall disease**

- A portion of the Ti plasmid is inserted into the plant chromosome causing the formation of the tumor or gall.
Fundamentals of Regulation

- **Substrate** → **Product**
  - Enzyme A
  - Enzyme B
  - No Enzyme

- **Translation**
  - Gene A
  - Gene B
  - Gene C
  - Gene D
  - Regulate enzyme activity
  - No mRNA

- **Transcription**
  - Regulate enzyme synthesis
  - At translation
  - At transcription
  - No mRNA

Figure by MIT OCW.
Prokaryotic Gene Regulation

1. Single-celled organisms with short doubling times must respond extremely rapidly to their environment.

2. Half-life of most mRNAs is short (on the order of a few minutes).

3. Coupled transcription and translation occur in a single cellular compartment.

Therefore, transcriptional initiation is usually the major control point.

Most prokaryotic genes are regulated in units called operons (Jacob and Monod, 1960)

Operon: a coordinated unit of gene expression consisting of one or more related genes and the operator and promoter sequences that regulate their transcription. The mRNAs thus produced are “polycistronic”—multiple genes on a single transcript.
Transcriptional Regulation

• Sigma Factors
  - Some required for binding of RNA polymerase to promoter
  - Others present under different environmental signals

• Transcription Factors
  - DNA binding proteins
  - Interact with regulated promoter to increase (activator/inducer) or decrease (repressor) transcription speed

• Transcriptional Termination
  - RNA polymerase reaches termination site, released from DNA
  - Attenuator sequence—leader peptide produced when aa is present, speeds up translation causing loop in mRNA that ends translation and transcription
Attenuation

Attenuation is mediated by the tight coupling of transcription and translation

• The ribosome translating the trp leader mRNA follows closely behind the RNA polymerase that is transcribing the DNA template.
• Alternative conformation adopted by the leader mRNA.
Translational Regulation

Ribosome binding site
Strength of ribosome binding to mRNA
“stringent” response
Shuts down translational machinery globally

Post-translational Regulation

Feedback inhibition
Covalent modifications
Affect protein activity
Cultivation, Isolation, and Identification of Microorganisms

To go from a mixed population to a pure culture...

1. Establish permissive conditions for growth
2. Physically isolate the organism
3. Identify the organism

- **Microscopic examination**
  1. Presence of yeast
  2. Morphology of bacteria

- **Cultivation**
  1. Isolation (serial dilutions or streaking)
  2. Identification (Genus species)
    - Morphology
    - Metabolic characterization

- **DNA fingerprinting (strain ID)**
  *viral identification need plaque assay and serology*
Selective & Differential Media


*Escherichia coli: Enterobacter cloacae: Klebsiella pneumoniae (E. coli Green metallic sheen)*

*NON-LACTOSE FERMENTERS*

*Salmonella typhi: Shigella sonnei: Proteus vulgaris*

www.spiceisle.com/zross/Enteric%20Demo.htm

Courtesy of Dr. Z. Ross. Used with permission.
Growth Control

- **Methods**
  - Physical antimicrobial control
    - Filter
    - Radiation
    - Heat
  - Chemical control
    - Pathogenic vs non-pathogenic
    - Sterilants
    - Disinfectants

- **Antimicrobials**
  - Synthetics
  - Growth Factor analogs
  - Chemotherapeutics

- **Antibiotics**
  - Broad vs narrow spectrum
  - Different classes (macrolides, aminoglycosides, etc)

- **Resistance**
  - R plasmids
  - Other mechanisms (drug or target modification, pathway perturbations, etc)
Indigenous microbiota

- Microorganisms that inhabit body sites in which surfaces and cavities are open to the environment
- Skin, oral cavity, upper respiratory tract, gastrointestinal (GI) tract, and vagina
- Each habitat can be considered a separate ecosystem
- For every cell in human body ($10^{13}$) there are 10 viable indigenous bacteria in the GI tract
- The GI tract ($10^{14}$) harbors 100-fold more bacteria than the skin ($10^{12}$)
Defining the GI microbiota

- **Autochthonous microbiota**
  - Present during the evolution of an animal and therefore present in every member of a species

- **Normal microbiota**
  - Common and perhaps even present in every individual in a given geographic area/community, but not in every member of the species

- **True pathogens**
  - Acquired accidentally and therefore not normally present in all members of a community of an animal species

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Ecological principles

• In a stable GI ecosystem, all available habitats are occupied by indigenous microbiota.

• Transient species derived from food, water, or even another part of the GI tract or the skin will not establish (colonize).

• Habitats are physical spaces in the GI tract normally occupied by a climax community of indigenous microbiota.

• Population levels and species composition are stable and not easily disrupted.
The indigenous GI microbiota

- Does not appear spontaneously in newborn humans or animals
- Certain microbes colonize particular habitats at certain times after birth that are characteristic of a given animal species (succession)
- Fetus is normally sterile in utero
- Becomes contaminated with heterogeneous collection of microbes at birth, but within days many of these are eliminated and the process of succession begins
Succession & climax populations

- Lactic acid bacteria and coliforms predominate in infant human and animal GI tracts
- During weaning the microbiota changes drastically and obligate anaerobic bacteria become predominant
- The indigenous GI microbiota of adults consists of climax communities that are remarkably stable
- Each region of the GI tract has a characteristic population of microbes, in terms of complexity and population density
Colon microbiota as an organ

- Distinct cell lineages
- Consumes, stores, and redistributes energy
- Mediates physiologically important chemical transformations
- Maintains and repairs itself
- The “microbiome” has ≥ 100 times the genetic complement of our genome provides functional features that we have not had to evolve ourselves
- Traditionally viewed as commensal microbiota, but clearly a mutualistic relationship where both partners benefit
Continued...

Figure by MIT OCW.
Human colonic microbiota

- Highest cell densities recorded for any ecosystem
- Diversity at the division level is among the lowest
- Only 8 of the 55 known bacterial divisions have been identified in colonic bacteria to date
- 2 division dominate
  - Cytophaga-Flavobacterium-Bacteroides (CFB)
  - Firmicutes (genera Clostridium and Eubacterium)
- Proteobacteria are common, but not dominant
- Compare to many soil communities, where ≥ 20 bacterial division can be present
Immune Responses

1. Innate immunity
   - Antigen processing
   - Antigen presentation
   - T cell activation
   - Antigen-specific, antibody-mediated immunity
   - Produce antibody
   - Activate B cells

2. Antigen-specific, antibody-mediated immunity
   - Activate T cells
   - Cytokine production

3. Cell-mediated immunity
   - Antigen-presenting cell
   - MHC
   - TCR

Killing

Inflammation

Antigen destruction

Complement, opsonization

Figure by MIT OCW.
Activation of Phagocytes

**PRRs**

- Present before infection
- Evolved to recognize microbes
- PRRs interact with PAMPs shared by a variety of pathogens, activating complement and phagocyte effector mechanisms to target and destroy pathogens
- Activation of signaling cascade leads to production of chemokines and cytokines
- First discovered as the Toll receptors in *Drosophila* (the fruit fly), the evolutionarily and functionally related transmembrane proteins are called **Toll-like receptors (TLRs)** in mammals

Figure by MIT OCW.
Phagocytosis stimulates respiratory burst

- NADPH or phagocyte oxidase (Phox)

- PMNs produce myeloperoxidase that converts \( \text{H}_2\text{O}_2 \) to HOCl

Efficient killing

Figure by MIT OCW.
Leukocyte Extravasation

1. Margination, rolling, adhesion
   - E-selectin, P-selectin, and L-selectin
   - ICAM-1, VCAM-1, and integrins LFA-1, MAC-1, $\alpha_4\beta_1$, and $\alpha_4\beta_7$

2. Transmigration across the endothelium (diapedesis)

3. Migration in interstitial tissues towards a chemotactic stimulus

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**Figure by MIT OCW.**
Antigens are molecules recognized by antibodies or T-cell receptors (TCRs).

Antibodies recognize conformational determinants.

TCRs recognize linear peptide determinants.

Antibodies and TCRs interact with a distinct portion of the antigen called an antigenic determinant or epitope.

Immunoglobin Superfamily

Immunoglobin (Ig) gene superfamily encodes proteins that are evolutionarily, structurally, and functionally related to Igs (antibodies).

Images removed due to copyright restrictions.

MHC-antigen Processing and Presentation

Image removed due to copyright restrictions.
T cell Selection and Tolerance

Image removed due to copyright restrictions.
T cell Activation

Image removed due to copyright restrictions.

Requires two signals
- Binding of TCR to MHC-antigen complex
- Binding of CD28 on T cell to B7 receptor on APC
Cytotoxic T cells ($T_c$)

- CD8 co-receptor to TCR, binds MHC-I protein during TCR-MHC-antigen interactions
- Recognize antigens mainly on virus-infected or tumor cells
- Antigen recognition triggers killing via release of perforins and granzymes

Image removed due to copyright restrictions.
\[ \text{T}_{\text{H}1} \text{ T cells} \]

- CD4 co-receptor to TCR, binds MHC-II protein during TCR-MHC-antigen interactions
- Recognize antigens mainly from intracellular as well as extracellular bacteria
- Antigen recognition triggers release of proinflammatory cytokines that further enhance phagocytosis

Image removed due to copyright restrictions.
$T_{H2}$ T cells

- CD4 co-receptor to TCR, binds MHC-II protein during TCR-MHC-antigen interactions
- Typically interacts with antigen presented via MHC-II on a B cell
- Activated $T_{H2}$ cells secrete cytokines to stimulate production and secretion of soluble antibodies by the B cell

Antibody Production/B cell Clonal Selection

1. Antigen is carried to the nearest lymph node

2. After initial antigen exposure, stimulated B cells multiply and differentiate into both antibody-secreting plasma cells and memory B cells
   - Plasma cells mainly produce IgM and last less than 1 week
   - More specific antibodies appear after a time lag

3. Upon second exposure to antigen, memory B cells immediately produce specific IgG
   - No requirement for T cell help
   - IgG is main class of antibody produced (over IgM)

Image removed due to copyright restrictions
Antibodies

Purpose: bind to virus, toxins, pathogen surface markers to inactivate and mark for phagocytosis and destruction by other immune cells

- Immunoglobulins (Ig) collectively most abundant protein component in blood (~20%)
- Produced by B-cells (naïve or memory) once activated by BOTH antigen and helper T-cells
  - Surface bound (IgD, IgM) not very specific
  - Soluble (IgG, IgA, IgE) specific to peptide
Classes of antibodies

- **IgM-μ heavy chain**, first Ig produced, mainly surface bound, secreted upon activation in pentameric form (early infection)
- **IgD-δ heavy chain**, same antigen binding site as IgM, surface bound, only on mature naïve B-cells
- **IgG-γ heavy chain**, many isotypes, monomer, major class in blood, Fc regions bind Fc receptors on macrophages and neutrophils, only Ig able to breach placental barrier (Fc regions)
- **IgE-ε heavy chain**, monomer, very high affinity ($K_A \sim 1 \times 10^{10} \text{L/mole}$) Fc receptor on mast cells (tissue) and basophils (blood), also binds Fc receptors on eosinophils
- **IgA/sIgA-α heavy chain**, main Ig in secretory fluids, monomer in blood and dimer in secretions, Fc region binds Fc receptors on epithelial cells allowing for trans-membrane transport (inefficient transport of IgM, but occurs)
Roles of antibodies during infection

**Opsonization**
- Antibodies bind to antigen and Fc region to Fc receptors on phagocytic cells
- Antibody-dependent cell-mediated cytotoxicity (ADCC)
  - antibodies bind viral proteins on surface of host cells or large microbes
  - cells killed by secreted toxic compounds from phagolysosomes

**Neutralization**
- Antibodies bind toxins or viruses
- Blocks entry into cells via receptors

**Activate complement cascade**
- Cascade activated by microbial molecules or antibodies on microbes' surface

**Prevent breach of epithelial barrier**
- sIgA in mucin binds antigens and Fc region sticks to mucin components
- Microbes prevented from reaching epithelium
# Four Types of Hypersensitivity

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
<th>Immune Mechanism</th>
<th>Time of Latency</th>
<th>Examples</th>
</tr>
</thead>
</table>
| Type I         | Immediate   | IgE sensitization of mast cells | Minutes          | Reaction to bee venom (sting)  
Hay fever                        |
| Type II        | Cytotoxic*  | IgG interaction with cell surface antigen | Hours           | Drug reactions (penicillin)       |
| Type III       | Immune complex | IgG interaction with soluble or circulating antigen | Hours           | Systemic lupus erythematosis (SLE) |
| Type IV        | Delayed type | $T_H1$ inflammatory cells | Days            | Poison ivy Tuberculin test        |

*Autoimmune diseases may be caused by Type II, Type III, or Type IV reactions.

Figure by MIT OCW.
Immediate Type I Hypersensitivity (Allergies)

Image removed due to copyright restrictions.
Type IV Hypersensitivity--Delayed-Type Hypersensitivity (DTH)

- cell-mediated hypersensitivity
- characterized by tissue damage due to inflammatory responses ($T_{H1}$)

Typical antigens
- certain microorganisms
- a few self antigens
- several chemicals that bind covalently to the skin, creating new antigens.

Image removed due to copyright restrictions.
**Immunologic Memory**

- **Initial Immune Response**
- **Protective Immunity**
- **Immunological Memory**

![Graph showing the timeline of immune responses over time](image)

- **First Infection**: Antibody and Effector T-cells levels rise rapidly.
- **Inapparent Reinfection**: Lower levels of antibody and Effector T-cells, no need to activate DCs and naïve lymphocytes.
- **Mild or Inapparent Reinfection**: Increased levels of antibody and Effector T-cells, faster clearance than primary infection.

**GOAL of IMMUNIZATIONS**

Induce pathogen-specific **humoral** and **cell-mediated** immune responses and immunologic memory to **prevent or limit effects of re-infection**.

- Effector T-cells and antibody levels decline after primary infection.
- Second exposure activates memory T-cells and B-cells to expand (faster clearance than primary), no need to activate DCs and naïve lymphocytes.
The Ideal Vaccine

- Effective at birth
- Single dose
- Oral or non-invasive administration
- Safe and efficacious when administered with other vaccines
- Temperature stability
- Low cost
- Global availability and accepted

- Cells required from immunization
  - Memory cytotoxic T-cells
  - Memory helper T-cells
  - Memory B-cells

- Downfall: not eliciting robust response and lack of appropriate cellular or humoral response
  - Multiple immunizations, sometimes with different administrations
  - Booster shots
Adjuvants

Substances that enhance immune response to an antigen typically by providing stimulation (second signal) to DCs

- **Current adjuvants**
  - Aluminum (widely used)
  - Ribi (monophosphoryl lipid A w/mycobacterial cell walls)
  - MF59 (oil-surfactant emulsion)
  - polymers

- **New ideas**
  - Cytokines
  - Delivery systems (liposomes, microcapsules)
  - Bacterial toxins (*E. coli* heat-labile toxin, cholera toxin)
Types of Immunizing Agents

- **Attenuated/related organism**
  - Infection with weaker or related organism or lower inoculum

- **Viral/bacterial vector**
  - Carriers to deliver antigens from pathogens that are unsafe as attenuated

- **Subunit vaccines**
  - Purified components or known peptide motifs of antigen, toxoid vaccines

- **Conjugate vaccines**
  - Protein carrier/conjugate to present polysaccharide as antigen

- **Nucleic Acid (DNA) vaccines**
  - Bacterial plasmids encoding antigens

- **Edible vaccines**
  - Transgenic plants, produce antigenic proteins

- **Mucosal vaccines**
  - Nasal or oral delivery of antigens
Measuring Immune Responses

- **Humoral Response**
  - ELISPOT
    - Number of antibody secreting cells (B-cells) during culture with antigen
    - Measure different classes and isotypes (IgG)
  - ELISA
    - Amount of antibody in serum or mucosal secretions
    - Measure different classes and isotypes (IgG)

- **Cell-mediated Response**
  - Lymphocyte proliferation *ex-vivo*
    - $^3$H-thymidine incorporation of immune cells upon culture with antigen
  - ELISPOT
    - Cytokines secreted by T-cells (CD8+, CD4+, total) or ‘immune cells’
Toxins & Monoclonal Ig

- Enterotoxins
- Exotoxins
- Cytolytic Toxins
- Superantigens

Production of Monoclonal Antibodies

Image removed due to copyright restrictions.
Epidemiology

- **Direct Host-host transmission** occurs when infected host transmits to susceptible host.

- **Indirect Host-host transmission** occurs when pathogens are spread from infected host to susceptible host via a vector (arthropods or vertebrates), fomites (inanimate objects) or vehicle (food or water).

**Key Terms**

- Acute
- Chronic
- Carrier
- Reservoir
- Morbidity
- Mortality
Classification of Disease Incidence

Outbreak: number of cases are observed in short period of time in area previously only having sporadic cases

- **Common source epidemic**
  - Infection of a large number of people from contaminated common source

- **Host-to-host epidemic**
  - May be started by one individual
  - Numbers of reported cases gradually, and continually rise

(a) Endemic Disease  
(b) Epidemic Disease  
(c) Pandemic Disease

Figure by MIT OCW.
Eradication & Elimination

**Control** -- reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as a result of deliberate efforts; continued intervention measures are required to maintain the reduction. i.e., diarrheal diseases

**Elimination of disease** -- reduction to zero of the incidence of a specified disease in a defined geographical area as a result of deliberate efforts; continued intervention measures are required. i.e., neonatal tetanus

**Elimination of infection** -- reduction to zero of the incidence of infection caused by a specific agent in a defined geographical area as a result of deliberate efforts; continued measures to prevent reestablishment of transmission are required. i.e., measles, poliomyelitis

**Eradication** -- permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts; intervention measures are no longer needed. i.e., smallpox

**Extinction** -- specific infectious agent no longer exists in nature or in the laboratory. i.e., nothing
Control Measures

- **Against reservoir**
  - Eliminate infection in domestic animals
  - No control over wild animals
  - Prevent contact or eliminate insect vectors

- **Against transmission**
  - Prevent contamination of vehicle (water, milk)

- **Immunization**

- **Quarantine**
  - Restrict movement and contact of infected individuals with general population
  - Time limit is longest period of communicability of the disease
  - International required quarantine for smallpox, cholera, plague, yellow fever, typhoid fever and relapsing fever

- **Surveillance**
  - Observation, recognition, and reporting of diseases as they occur
  - Typically pathogens with potential for epidemic
Resistance of a group to infection due to immunity of a high enough proportion of the members of the group.

Typically >70% of population must have protective immunity

Highly infectious agents require up to 95% protection

**Protective immunity, not solely immunization**
Emergence Factors

1. Demographics
2. Technology and industry
3. Economic development and land use
4. International travel and commerce
5. Microbial adaptation and change
6. Breakdown of public health measures
7. Abnormal natural occurrences
Questions?
ELISA and ELISPOT

BD ELISPOT Assay Procedure

1. Capture Antibody
   - For Sets and pairs: Coat microwells with anti-cytokine capture antibody.
   - For Kits: Go to step 3; Steps 1 and 2 not necessary.

2. Blocking
   - Block unoccupied sites with protein

3. Add Cells
   - Incubate cells in well with Ag/stimulus etc.

4. Wash
   - Cells are washed off

5. Detection Antibody
   - Add Biotinylated anti-cytokine detection antibody

6. Enzyme-Avidin
   - Add Avidin-HRP

7. Develop With Substrate
   - Add substrate and monitor formation of colored spots

Capture Ab or antigen of interest

Sample (cells, plasma, culture media, etc)
Lymphocyte Proliferation Assay

Measures cell-mediated immune response to antigen of interest

- Pulse culture with $^3$H Thymidine
- Harvest cells at various time points
- Measure incorporation in scintillation counter