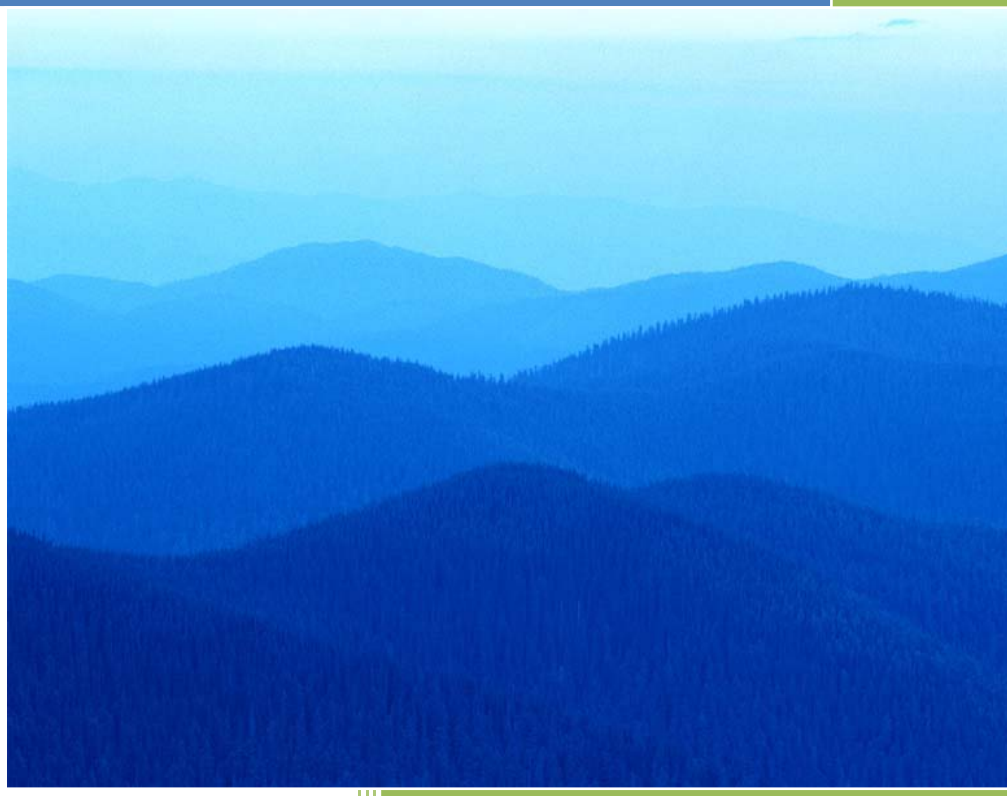
Orientation Program for Infection Control Professionals



Module 2: Microbiology

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Module 2: Microbiology

Objectives

At the completion of this module, the ICP will:

1. Describe basic elements of microbiology that are pertinent to Infection Prevention and Control
2. Provide information about specimen collection
3. Identify and interpret microbiology laboratory tests which have an impact on infection prevention and control

Number of hours

- Key Concepts - 3 hours
- Methods - 4 hours

Required readings

- Information available in Appendix A
- CHICA-Canada presentation for novice practitioners- Introduction Microbiology
- http://www.chica.org/Members/members_conf_presentations.php
- APIC Text of Infection Control & Epidemiology 2nd or 3rd Edition – Chapters 14, 15, 16, 17, 24, 25 & 63

Required text

- APIC Text of Infection Control & Epidemiology 2nd or 3rd Edition – Chapters 14, 15, 16, 17, 24, 25 & 63
- Bennett JV & Brachman PS. Bennett & Brachman's Hospital Infections. 5th ed. Ed. William R Jarvis: Philadelphia, PA, 2007 – Chapter 22

Overview

It is important to have a basic understanding of microbiological terms and organisms in order to interpret laboratory information into the infection prevention and control context.

Key Concepts

Key Terms

Define these key terms:

Term	Definition
normal flora	
bacteria	
virus	
colonization	
infection	
disease	
pathogenic	
non-pathogenic	
virulence	
opportunistic pathogens	
antibiogram	
aerobic organisms	
anaerobic organisms	
bacterial spores (endospores)	
endotoxins	
exotoxins	
antitoxins	
zoonosis	

The body's response to infection

Describe these processes:

Body's response	Description
Natural barriers	
Immune system (specific host mechanisms)	
Immune System (non-specific host mechanisms)	

Stages of Illness

Match the following stages of illness with the corresponding definitions :

Stage of illness	Definitions
1. Invasion	a. maximum impact of illness when pathogen is proliferating rapidly – toxic by-products of microbial metabolism and immune response produce tissue damage
2. Incubation	b. pathogen replicating, no symptoms
3. Prodromal	c. pathogen acquires entry into the body mucus membranes inhalation, self inoculation
4. Acute Illness	d. pathogen is contained and eliminated from body, damaged tissue is repaired and resolution of symptoms
5. Recovery	e. initial appearance of symptoms (usually mild and vague)

Bacteriology

Basic characteristics of bacteria

Term	Definition
Gram stain	
Gram positive	
Gram negative	
Culture & sensitivity	
Acid fast bacilli	
WBC versus epithelial cells	

Term	Definition
Aerobic	
Anaerobic	
Shape:	
• Cocci	
• Diplococci	
• Bacilli or rods	
• Spiral forms	
• Pleomorphism	
Coagulase test – positive or negative	
Motility	

Bacteria of interest

Learning objective: Identify the key characteristics and diseases most commonly associated with the following bacteria.

Bacteria	Shape (cocci, diplococci, bacilli)	Gram + or Gram -	Spore forming: Y or N	Common disease
Staphylococcus aureus				
Methicillin resistant *Staphylococcus aureus*				
Group A Streptococci (*Streptococcus pyogenes*)				
Streptococcus pneumoniae				
Clostridium difficile				
Listeria monocytogenes				
Klebsiella pneumoniae				
Neiserria meningitides				
Escherichia coli				
Klebsiella Pneumoniae				
Pseudomonas aeruginosa				
Treponema pallidum				
Mycobacterium tuberculosis				
Salmonella typhi				
Haemophilus influenzae				

Testing for bacteria

Learning objective: Give explanations for the following questions.

	Explanation
What is a colony count?	
How are antibiotic sensitivities tested?	
What is the clinical implications of resistance to antibiotics?	
Why are different growth media needed?	

Virology

Basic characteristics of viruses

Key terms

Term	Definition
Obligate intracellular parasites	
Size of viruses	
Nucleic acid	
Shapes	
Enveloped vs. non-enveloped viruses	

Describe the five stages of virus replication:

Viral Stage	Description
Attachment	
Penetration	
Replication	
Maturation	

Viruses of interest

Virus	Envelope versus non enveloped	Importance to IPC
Influenza virus		
Measles virus		
Respiratory Syncytial virus (RSV)		
Mumps virus		
HIV		
Norovirus		

Interpret the following results for hepatitis b virus testing:

Disease	Tests	Results	Interpretation
Hepatitis B	HBsAg	Negative	
	Anti-HBc	Negative	
	Anti-HBs	Negative	
Hepatitis B	HBsAg	Negative	
	Anti-HBc	Negative	
	Anti-HBs	Positive with ≥ 10 IU/ml	
Hepatitis B	HBsAg	Positive	
	Anti-HBc	Positive	
	IgM anti-HBc	Positive	
	Anti-HBs	Negative	

Differentiate between viruses and bacteria:

Characteristic	Viruses	Bacteria
Size and type of microscope to see organism		
Need a living host to multiply		
Has a cell wall and a cell membrane		
Usually tested for susceptibility to antibiotics		

Characteristic	Viruses	Bacteria
Can there be beneficial types?		
Nucleic acid type		

Other Organisms of Interest

Fungi

Fungi are organisms that derive nutrients from organic matter. Most fungi are aerobes that require a moist environment and grow best at a neutral ph. Their spores and conidia are able to survive in dry conditions for long periods of time. Some fungi are well-adapted human pathogens however most are accidental pathogens that humans acquire through contact with decaying organic matter or in airborne spores. Typically fungi are divided into two separate groups: yeasts and moulds. Common pathogenic yeasts include *Candida spp.* (vaginitis, mucositis) and *Cryptococcus neoformans* (meningitis, pneumonia in compromised individuals). Common pathogenic moulds are *Aspergillus spp.* (necrotizing pneumonia) and agents of mucormycosis (*Rhizopus and Mucor spp.*). Some fungi can grow as either a mould or yeast (dimorphic fungi). Common examples are *Pneumocyctis carinii* and *histoplasma capsulatum* which cause pulmonary infections.

Fungi	
Name	Describe a disease caused by this fungi and any infection control precautions recommended.
Candida albicans	

Parasites

A parasite is an organism that lives in or on and takes it's nourishment from another organism. A parasite cannot live independently. Parasitic diseases include infections caused by protozoa, helminths, and arthropods:

- Protozoa – Malaria is caused by plasmodium protozoa, a single-cell organism that can only divide within its host organism.
- Helminths – Schistosomiasis, another very important parasitic disease, is caused by helminths (worms) in the Schistoma family.
- Arthropods – The arthropods include insects and arachnids (spiders, etc.), a number of which can act as vectors (carriers) of parasitic diseases.

Parasites	
Name	Describe disease caused by this parasite and identify any infection control precautions recommended.
Giardia lamblia	

Methods

As a critical component of this module, you will be allocated time to be spent with a preceptor in the microbiology laboratory. Your mentor for the ICP orientation will arrange for this clinical experience. In preparation for you time in the laboratory here are some exercises which you should do. If you need further clarification on the exercises you can bring them to your preceptor in the laboratory.

Contact Information

Local laboratory

Key contacts	
Name:	
Location:	
Phone:	
Email address:	
Required contacts:	
Are there different labs for different tests – microbiology, serology etc? If yes, contact numbers for them.	

Public Health Laboratory (PHL)

Key contacts	
Name:	
Location:	
Phone:	
Email address:	
Required contacts:	
Contact for Regional Medical Health Officer	

Microbiology

Specimen collection and transportation

Learning objective: Describe the appropriate method for the collection, storage and transportation of specimens to the bacteriology lab.

Specimen collection and transport to the lab is an essential part of the culture process. In general, all specimens should be collected aseptically and placed in a sterile container; in some cases specimens may be placed directly into culture media (e.g., blood cultures, genital cultures). Special handling techniques may be necessary for some specimens such as those for anaerobic culture. Prompt delivery to the laboratory is essential to prevent the death of pathogenic organisms or the overgrowth of commensal organisms. If transport is delayed, some specimens may be refrigerated (e.g., urine, stool, sputum) while others should be maintained at room temperature (e.g., genital, eye, or spinal fluid).

Specific procedures for specimen collection and transport are institution dependent. Please refer to your institution's laboratory manual for specific procedures and protocols.

Test	Usual transport medium	Important points on collection of the specimen	Common problems with specimen collection and transportation to lab	Usual test result time
Blood culture				
Wound Culture				
Urine culture				
Stool for C&S				
Stool for *C diff*				
MRSA screen				
VRE screen				
Throat culture				
Eye culture				
Sputum culture				
AFB smear/culture				

ii. Interpretation of Microbiology laboratory results

Review 2 or 3 microbiology requisitions and determine the laboratory significance:

Criteria	Laboratory significance
Demographics	
Date collected	
Time collected	
Diagnosis	
Gender	
Person ordering the test	
Date received in lab	
Time received in lab	
Date reported	
Gram stain	
Mixed count	
Amount of growth	
Specimen number	
Cell count	
Organism	
Sensitivity	
Intermediate sensitivity	
Beta lactamase positive	
Resistance	
Thymidine dependent strain (TFG)	
Source of the specimen Leg, vagina, etc. –	
Type of test required; i.e. not viral studies but HSV	

Why is full work-up on stool not sufficient to guide the lab staff? Is it for *C. diff, salmonella*, ova and parasites, etc.?

Common Microbiology Requisition Problems

Discuss with your preceptor if there are requisition problems commonly experienced in the microbiology and how they affect the testing methods and possibly the results.

Problems	**Suggestions for improvement**
Information not filled in correctly	

Virology

Specimen collection and transport

Test	Usual transport medium	Important points on collection of the specimen	Common problems with specimen collection and transportation to lab	Usual test result time
Stool for parasites				
CSF for viral studies				
Nasopharyngeal swab for RSV				
Nasopharyngeal swab for influenza				
Varicella zoster swab from vesicle				
Herpes simplex 1 & 2				
Buccal swab for mumps				
Stool for norovirus				
Stool for rotavirus				

Testing for viruses

Direct examination methods for antigen detection:

Unlike most bacteria, viruses are not complete cells that can function on their own. They cannot convert carbohydrates to energy, the way that bacteria and other living cells do. Viruses depend on other organisms for energy. And viruses cannot reproduce unless they get inside a living cell.

Learning objective: Describe methods for identifying viruses in the lab.

There are three categories of diagnostic tests for viruses: i) Direct examination of the specimen ii) virus isolation (cell culture) and serology.

Test method	Give examples of two diseases where this testing is used	Type of sample required (urine, blood, nasopharyngeal etc.)
Antigen detection immunofluorescence		

Test method	Give examples of two diseases where this testing is used	Type of sample required (urine, blood, nasopharyngeal etc.)
Molecular techniques for the direct detection of viral genomes		
Electron Microscopy		

Virus isolation method:

Cell culture can take a long time; thus it is not used often.

Test method	Give examples of two diseases where this testing is used	Type of sample required
Cell culture		

Serology methods for antibody detection:

Serology forms the mainstay of viral diagnosis. Following exposure, the first antibody to appear is IgM, which is followed by a much higher titre of IgG. Detection of rising titres of antibody between acute and convalescent stages of infection, or the detection of IgM in primary infection are often used for diagnosis of viral infections.

Test method	Give examples of two diseases where this testing is used	Type of sample required
Enzyme-linked immunosorbent assay (ELISA)		
Particle agglutination		
Western Blot		

Interpretation of virology laboratory results

Review 2 or 3 virology requisitions and determine the laboratory significance:

	Significance on report
Date reported	
PCR report	
IgM	
IgG	

Common Requisition Problems

Discuss with your preceptor if there are requisition problems commonly experienced in the virology laboratory and how they affect the testing methods and possibly the results.

Problems	Suggestions for improvement
Information not filled in correctly	

Public Health Laboratory

Find answers to the following questions:	
What tests are referred to BCCDC?	
Is there a different protocol for sending samples to BCCDC on week-days versus week-ends?	
Is there a specific protocol for sending samples to BCCDC during an outbreak?	
Is there a requirement for specific collection methods for samples which must be transported to BCCDC?	
How long does it take to get a report from BCCDC?	
Does BCCDC do a panel of virus on some respiratory samples? Is there a criterion around this procedure? E.g. is it done only on patients less than 5 years and over 75 years?	
Are samples for MRSA, VRSA, VRE, ESBLs, carbapenem resistance sent to the BCCDC routinely?	
Are any samples referred to the National Microbiology Laboratory?	
Discuss with your ICP mentor whether you need to have a tour of the BCCDC	

Clinical Microbiology Laboratory Experience

Follow a specimen from the time it is received in the laboratory until the report is finalized and sent to the ordering professionals.

Discussion with mentor

Item	Notes
Get an understanding of how lab work is divided	
How long different tests take and why	
The differences in the type of media for different tests	
How the media are selected	
How contamination of the specimens is avoided	
Tests for identifying organisms	
Review antibiotic sensitivity testing	
How is a Gram stain done	
How are reports generated	

Observation of procedures

Observe the following procedures:	
Procedures	**Notes**
Gram stain	
Sensitivity method	
Blood culture	
Specimen for AFB	
Urine culture	
Wound culture	

Viewing of specific organisms on slides/plates

View the following slides/plates:	Comments
Staphylococcus aureus	
Streptococcus pneumoniae	
Mycobacterium tuberculosis	
Neisseria meningitidis	
Bordetella pertussis	
Streptococcus pyogenes (Group A strep)	
Fungi	

Documentation and Reporting

Laboratory reporting mechanism to IPC

Criteria	Description
Determine the lab reports which are sent to IPC on a daily basis	
How are routine reports sent to IPC?	
Is there a process for stat reports to IPC for TB, GAS, MRSA, VRE, ESBL, Carbapenem resistance?	
How long does the lab keep specific samples such as MRSA, VRSA, VRE, ESBLs?	

Responsibility of IPC for laboratory reports:

- Is there a designated surveillance program for certain microorganisms such as MRSA?
- How are the reports stored? i.e., database
- Who is responsible for entering the data?
- Who is responsible for analyzing the laboratory data collected?
- Are there reports generated from the data and to whom are these reports sent?

Other Issues

Ethics

Discuss with your ICP mentor the steps which have been taken at your facility to ensure the confidentiality of reports.

Appendix A

Terminology

colonization – multiplication of an organism in or on a body surface without causing tissue invasion or cellular injury or immune response. The person is “asymptomatic”.

infection – multiplication of an organism in a host causing tissue invasion or cellular injury accompanied by an immune response – occurs with (e.g. pneumonia) or without clinical illness (e.g. HCV infection)

disease – a pathological condition of the body that presents a group of symptoms particular to it and that sets the condition apart as an abnormal entity differing from other normal or pathological body states (e.g. CDI)

pathogenic – microorganisms that can cause disease and illness

non-pathogenic – microorganisms that do not cause illness

virulence – invasiveness, toxin production, ability to survive within the cell and cause illness

opportunistic pathogens – microorganisms that do not usually cause infection except when a person’s immune system has been compromised

antibiogram – antibiotic sensitivity patterns of the organisms being tested

aerobic organisms – grows in the presence of oxygen

anaerobic organisms – will not grow in the presence of oxygen

facultative organisms – will grow with or without oxygen

bacterial spores (endospores) – produced by some Gram-positive bacilli –difficult to kill (used for sterilization testing)

endotoxins –harmful substances released when bacterium dies which are toxic to host – primarily associated with gram negative bacilli

exotoxins – harmful substances released into environment by living bacterium (i.e.) botulism, tetanus, diphtheria, some forms of food poisoning; exotoxin may be released from a small infected area into the bloodstream or absorbed from the gut

antitoxins – chemicals produced to bind to the exotoxins to inactivate them

zoonosis – from animals or animal products

Key Information from Reading

Normal flora

Microorganisms are found everywhere in nature and are also naturally present in and on humans. The term used for those microorganisms that can establish populations in a host, such as the human body, without causing disease is “normal flora”. The normal flora that establish permanent populations are called “resident flora” and the microorganisms with temporary or semi-permanent populations are called “transient flora”.

The body's response to infection

Natural Barriers

- Skin and mucous membranes provide mechanical barriers
- Cilia of respiratory tract entrap organisms and cough mechanism expels them
- Gastric acid of stomach helps destroy some ingested pathogens, peristaltic waves prevent them from attaching and multiplying
- Mechanical flushing protects urinary tract
- Tears flush the eyes

Immune System

- Specific host defence mechanisms
  - Humoral (produces an antibody for each antigen recognized)
  - Cell mediated (macrophages and lymphocytes)
  - B lymphocytes and T-lymphocytes (4 types)
    - Regulatory, killer and suppressor and memory
- Non-specific host defence mechanisms
  - Can distinguish between self and non-self but do not differentiate between antigens
  - Complement system: destroys pathogens by enabling the body to produce inflammation and facilitate localization of the infectious agent
  - Cytokines: influence other inflammatory cells, including macrophages, neutrophils and lymphocytes
  - Phagocytosis: injured cells and foreign substances (including microorganisms) are ingested by phagocytic cells (e.g. neutrophils, monocytes)
  - Fever is produced to augment the immune system, inhibit microbial growth, increase the rate of chemical reactions, raise the temperature above the organism's optimal growth temperature and decrease the individual's activity.

Stages of illness

- Invasion – pathogen acquires entry into the body
  - mucous membranes, inhalation, self inoculation
- Incubation – pathogen replicating, no symptoms
- Prodromal – initial appearance of symptoms (usually mild and vague)
- Acute Illness – maximum impact of illness when pathogen is proliferating rapidly – toxic by-products of microbial metabolism and immune response produce tissue damage
- Recovery – pathogen is contained and eliminated from body, damaged tissue is repaired and resolution of symptoms

Common Normal Flora

Body Site	Common organisms
Mouth	*Staphylococci, S. viridans, Enterococci, S. pneumoniae, Neisseriae, Corynebacteria, Haemophilus, Enterobacteriaceae, Actinomyces, Lactobacilli, Bifidobacteria, Fusobacteria, anaerobic* Gram neg. *cocci,* anaerobic Gram neg. cocci
Upper Respiratory Tract	*Staphylococci, S. viridans, S. pneumoniae, Corynebacteria, Haemophilus, Propionibacteria, Actinomyces, Bacteroides, Fusobacteria,* anaerobic Gram neg. *cocci,* anaerobic Gram neg. *cocci*
Skin	*Staphylococci, Corynebacteria, Propionibacteria*, anaerobic Gram neg. *cocci*
Conjunctiva	*Staphylococci, Corynebacteria*, anaerobic Gram neg. *cocci*
Lover Intestine	*S. viridans*, *Enterococci, Corynebacteria, Enterobacteriaceae, Clostridia, Lactobacilli, Bifidobacteria, Fusobacteria*, anaerobic Gram neg. *cocci*
External Genitalia	*Staphylococci, S. viridans, Enterococci, Corynebacteria, Enterobacteriaceae, Bacteroides, Fusobacteria,* anaerobic Gram neg. *cocci*
Anterior Urethra	*Staphylococci, Enterococci, Neisseriae, Corynebacteria, Bacteroides, Fusobacteria,* anaerobic Gram neg. *cocci*
Vagina	*Staphylococci, S. viridans, Enterococci, Neisseriae, Corynebacteria, Lactobacilli, Bifidobacteria, Bacteroides,* anaerobic Gram neg. *cocci*

Bacteria

Bacteria are very small, relatively simple, single celled organisms. They contain a single long circular molecule of double strand DNA. This “bacterial chromosome” is not surrounded by a nuclear envelope and is attached to the plasma membrane.

The cell wall of bacteria is a rigid structure that maintains the shape of the cell and prevents bursting of the cell from the high osmotic pressure inside it. There are several different types of cell wall structures in bacteria, which have traditionally been categorized according to their staining characteristics. The 2 major types of cell walls are gram positive and gram-negative. In addition, some mycobacteria have an acid fast wall (e.g. *M. tuberculosis*) and mycoplasms have no cell wall.

A Gram positive cell wall is composed of a very thick protective peptidoglycan layer. Because this layer is the principle component of the Gram positive cell wall, many antibiotics effective against Gram positive organisms act by preventing synthesis of peptidoglycan.

The cell wall of the Gram negative microbe is composed of two layers. The inner peptidoglycan layer is much thinner than in gram positive cell walls. Outside this peptidoglycan layer is

another outer membrane that is unique to the Gram negative cell wall. The outer membrane contains proteins, phospholipids and lipopolysaccharide. This outer membrane

- Acts as a barrier to hydrophobic compounds and harmful substances
- Acts as a sieve, allowing water-soluble molecules to enter through protein-lined channels called porins
- Provides attachment sites that enhance attachment to host cells

Because of these cell wall structure differences, gram negative bacteria are less affected by antibiotics.

Shapes of bacteria (morphology)

Bacteria vary in size from 0.4-2 um. They occur in four basic shapes:

Cocci (spherical) – usually round but may sometimes be irregularly shaped. Cocci that remain in pairs are after dividing are called diplococci and those that remain attached in a chain are called streptococci while those that remain attached in clusters or broad sheets are called staphylococci.

Bacilli (rod shaped) – most appear as single rods and are fairly uniform in shape although some are oval and look so much like cocci that they are called coccobacilli

Spirochetes (spiral shaped) – vary in length and in number of turns

Pleomorphic lack a distinct shape (like jello)

Mycobacteria

Are weakly Gram positive but stain better with an acid-fast stain. This group includes organisms that cause tuberculosis and leprosy.

Mycoplasma

Mycoplasmas are extremely small bacteria that lack cell walls and are surrounded only by an outer plasma membrane. Because they lack a rigid cell wall they are resistant to cell wall-active antibiotics (penicillins). Mycoplasms associated with human infections are *mycoplasma pneumoniae* (atypical pneumonia), *ureaplasma urealyticum* (UTIs) and *mycoplasma hominis* (urogenital infections).

Other cell attributes

Surface polymers: some pathogenic bacteria produce a covering called a “capsule” which acts as virulence factors in helping the pathogen evade phagocytosis. Slime layers are similar to capsules but are more diffuse layers surrounding the cell. They also serve to inhibit phagocytosis or in some cases to aid in adherence to host tissue or synthetic implants.

Cell Appendages: flagellum is an organ of locomotion. They are exterior protein filaments that rotate and cause bacteria to be motile. Flagella that extend from one end of the bacterium are called “polar”. Flagella that occur on all sides of the bacterium are called peritirichous. Pili (also known as fimbriae) are hair like protein structures that aid in attachment to surfaces. Some (known as sex pili) are involved in bacterial conjugation and gene exchange. Proteins exist within the pili that aid in attachment and are called adhesins.

Endospores are formed by 2 genera of bacteria *Bacillus* and *Clostridium.* Endospores are dormant forms of bacteria that are resistant to heat, cold, drying and chemical agents. Spores form when there is a shortage of needed nutrients and can lie dormant for years. When the spore is exposed to a favourable nutrient rich environment, it becomes active again.

Environmental factors influencing growth

Three factors influence the growth rate of bacteria: pH, temperature and gaseous composition of the atmosphere.

- Most bacteria of concern grow best at a neutral pH
- Bacteria that have adapted to humans grow best near body temperature
- Some require oxygen (obligate aerobes), some cannot grow in the presence of oxygen (obligate anaerobes) and some can grow either with or without oxygen (facultative anaerobes).

They also need:

- A source of carbon
- A source of nitrogen
- A source of energy (ATP)

Smaller amounts of elements such as phosphates and a variety of metals and ions must also be present.

All bacteria that inhabit the body are heterotrophic: require more complex substances for growth such as an organic source of carbon and they obtain energy by oxidizing or fermenting organic substances. Often the same substance (e.g. glucose) is used as both a carbon source and energy source.

Fungi

Fungi are organisms that derive nutrients from organic matter. Most fungi are aerobes that require a moist environment and grow best at a neutral pH. Their spores and conidia are able to survive in dry conditions for long periods of time. Some fungi are well-adapted human pathogens however most are accidental pathogens that humans acquire through contact with decaying organic matter or in airborne spores. Typically fungi are divided into two separate groups: yeasts and moulds. Common pathogenic yeasts include *Candida spp.* (vaginitis, mucositis) and *Cryptococcus neoformans* (meningitis, pneumonia in compromised individuals). Common pathogenic moulds are *Aspergillus spp.* (necrotizing pneumonia) and agents of

mucormycosis (*Rhizopus* and *Mucor spp.*). Some fungi can grow as either a mould or yeast (dimorphic fungi). Common pathogenic ones are *Pneumocyctis carinii* and *Histoplasma capsulatum* both which cause pulmonary infections.

Viruses

Viruses were originally classified according to the diseases they caused or where they were found. Now they are classified by the type and structure of their nucleic acids, chemical and physical characteristics, size, type of replication and host. They are ultramicroscopic particles that contain nucleic acid (either RNA or DNA) surrounded by protein and in some cases a membrane-like envelope.

Viruses that contain only the viron are called “naked” or “non-enveloped” viruses and are relatively stable to temperature, pH and chemicals. Viruses that wrapped in a membrane are called enveloped viruses and are more fragile because anything that disrupts their envelope inactivates them.

Outside the host cell the virus is known as a viron. A viron is metabolically inert and does not grow or multiply. All viruses replicate in a similar fashion: (APEC)

1. **Attachment:** the viron attaches to a receptor site on the host cell.
2. **Penetration:** the viron enters the host cell
3. **Replication:** viral DNA or RNA directs the host cell to begin synthesis of viral components. Replication uses host cell energy sources and amino acids to produce these components.
4. **Maturation:** the viral components spontaneously assemble into a viral particle: new virons are formed
5. **Release:** the host cell breaks open or the virus buds through the cell wall and new virons are released. Some viruses lie dormant in the host cell for months or years; after this latent period new virons form and cause damage to host cells.

Common Infections and the Usual Organisms That Cause Them

Infection/site	Common Organisms
Bronchitis	*S. pneumoniae*, *H. influenzae*, respiratory viruses
Device-related	Coagulase-negative *staphylococci*, *Corynebacteria sp.*
Endocarditis	*S. viridans*, *S. aureus*, *Enterococci*
Gastroenteritis	*Salmonella sp.*, *Shigella sp.*, *Campylobacter sp.*, *E. coli 0157:H7*, viruses
Meningitis	*H. influenzae*, *N. meningitides*, *S. pneumoniae*
Pelvic Inflammatory Infection	*C. trachomatis*, *N. gonorrhoeae*, *Bacteroides sp.* *Enterobacteriaceae*
Pharyngitis	*S. pyogenes*, respiratory viruses
Pneumonia (community)	*S. pneumoniae*, *H. influenzae*, *M. pneumoniae*, *C. pneumoniae*, *M. tuberculosis*
Pneumonia (healthcare)	*Pseudomonas sp. S. aureus*, *Enterobacteriaceae*
Septicemia	*S. aureus*, *S. pneumoniae*, *E. coli*, *Klebsiella sp.*, *Salmonella sp.*

Infection/site	Common Organisms
Sinusitis	*S. pneumoniae*, *H. influenzae*, *S. pyogenes*, *S. aureus*
Skin	*S. aureus*, *S. pyogenes*, *Candida sp.*, dermatophytes
Urinary Tract	*E. coli, Enterococci, Candida sp., Klebsiella sp., Proteus sp.*

Reviewing and Interpreting Culture Results

Specimens and culture results

1. Gather as much information as possible!!
2. Know what "normal flora" is and what potential "pathogens" are
3. Some specimen types such as sputum and feces will always contain organisms as "normal flora" and potential pathogens must be separated from them (i.e.) coughing up sputum will always be contaminated with saliva and potentially non-pathogenic organisms
4. Other specimens such as blood and CSF are normally sterile so any growth needs to be evaluated
5. Is it clinically significant? (is the person sick with symptoms)
6. Is it a contaminant? (skin contamination with blood collection)
7. Is it a transient loss of sterility? (transient bacteremia after brushing teeth)
8. Quantitative values – the quantity of organisms is expressed as colony forming units per litre (CFU/L) helps in identifying contamination from infection – used for urine testing (i.e.) counts > 100,000 usually considered a potential UTI
9. Number of positive cultures important (i.e.) the same organism isolated from blood and another site suggests bacteraemia arising from infection at that site
10. Clinical findings important in interpreting cultures (i.e.) signs and symptoms of dysuria and frequency of urination as important as urine culture in diagnosing UTI
11. Person's history important (i.e.) the presence of a prosthetic heart valve increases the likelihood of coagulase negative staphylococcus (CNS) in a blood culture representing endocarditis than when the person has no history of heart surgery.
12. Keep in mind that some heavily colonized wounds will heal spontaneously, and conversely, some organisms are able to cause serious infection at much lower levels of colonization. Infection depends on the pathogenicity of the organism, the type of wound, and the patient's response
13. Person's who are immunosuppressed, on steroids or neutropenic have a greater chance of infection with "opportunistic pathogens" (i.e.) *aspergillus* in the sputum of a neutropenic person has more serious implications than in a normal host

Wound culture

- If necessary, remove debris from the wound base
- Cleanse the wound with sterile normal saline or sterile water prior to culture collection.
- **NOTE: Do not swab superficial eschar, or other necrotic tissue**
- Use appropriate sterile swab and culture medium – usually a sterile C&S swab.
- If wound is dry, moisten swab tip with sterile normal saline without preservative.
- Use sufficient pressure to cause tissue fluid to be expressed.
- For small wounds, using the side of the swab tip; roll it for one full rotation over the granulation tissue that has the most obvious signs of infection (avoid slough and surface purulent discharge).
- For larger wounds rotate swab over wound surface using a 10 point zigzag pattern.
- Place swab into culture medium.

Blood cultures

- Preparation of the site will decrease the potential for a contaminated specimen. Tincture of iodine, isopropyl alcohol, chlorhexidine, or povidone-iodine combined with ethyl alcohol rather than povidone-iodine alone should be used for skin antisepsis prior to venipuncture for blood cultures, recognizing that studies have shown significantly reduced rates of contamination with use of these agents.
- 2 cultures taken from 2 separate sites, one of which is drawn from a peripheral vein by percutaneous venipuncture.
- At least 20 ml (preferably 30 ml) is required (each specimen containing 10-15 ml, inoculated into aerobic and anaerobic media).
- Up to 30% of blood cultures positive for coagulase-negative *staphylococcus* (CNS) represent true infection, however, the majority of single positive cultures represent contamination, a finding that should reemphasize the need to obtain cultures from two separate sites whenever BSI is suspected.

Urine cultures

Clean-catch midstream specimens

- clean perineal area with skin antiseptic
- expose urethra with clean fingers
- void a small amount of urine before collecting to clear urethra of skin contaminants
- collect specimen from urine stream

Sterile specimens from an indwelling catheter or ileal conduit

- use sterile technique
- sample from diaphragm of catheter tubing
- Catheters that have been in place for an extended period of time may not reflect the microbiological status of the patient's urinary tract.
- urine should be obtained after catheter replacement for more reliable results

Transport urine specimens to lab as soon as possible. Culture within 2 hours of collection or refrigerated with no preservative.

Sputum cultures

- best collected early morning
- mouth should be rinsed and teeth or dentures cleaned
- sputum may need to be induced or suction used
- special precautions (airborne) should be taken when TB is suspected
- if results show predominantly oral flora, the test is non-diagnostic
- transport promptly to the lab

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PICNet welcomes your comments and feedback on these modules.
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