Semester - III

I8MBU304B MICROBIAL DIAGNOSIS IN HEALTH CLINIC 3H – 3C

Instruction Hours / week: L: 3 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

Acquire knowledge to identify the common infectious agents with the help of laboratory procedures and use antimicrobial sensitivity tests to select suitable antimicrobial agents.

OBJECTIVES

To provide an understanding of the natural history of infectious diseases in order to deal with the etiology, laboratory diagnosis, treatment and control of infections in the community

Unit I

Bacterial, Viral, Fungal and Protozoan Diseases of various human body systems, Disease associated clinical samples for diagnosis.

Unit II

How to collect clinical samples (oral cavity, throat swab, tissue sample, skin, Blood, CSF, urine and faeces) and precautions required. Method of transport of clinical samples to laboratory and storage.

Unit III

Examination of sample by staining – Gram stain, Ziehl-Neelson staining for tuberculosis, Giemsa-stained thin blood film for malaria. Preparation and use of culture media - Blood agar, Chocolate agar, Lowenstein-Jensen medium, MacConkey agar, distinct colony properties of various bacterial pathogens.

Unit IV

Serological Methods - Agglutination, ELISA, immune fluorescence, Nucleic acid based methods – PCR, Nucleic acid probes, Typhoid, HBV, HCV, HIV and Denque.

Unit V

Importance, Determination of resistance/sensitivity of bacteria using disc diffusion method (Kirby Bauer Method) Determination of minimal inhibitory concentration (MIC) of an antibiotic by broth dilution method (LC_{50}, LC_{90}).

SUGGESTED READINGS

1. Ananthanarayan R and Paniker CKJ (2009). Textbook of Microbiology, 8th edition, Universities Press Private Ltd.
2. Brooks G.F., Carroll K.C., Butel J.S., Morse S.A. and Mietzner, T.A. (2013). Jawetz, Melnick and Adelberg's Medical Microbiology. 26th edition. McGraw Hill Publication.
3. Randhawa, VS, Mehta G and Sharma KB (2009) Practicals and Viva in Medical Microbiology. 2nd edition, Elsevier India Pvt Ltd.
4. Tille P (2013) Bailey's and Scott's Diagnostic Microbiology, 13th edition, Mosby
5. Collee JG, Fraser, AG, Marmion, BP, Simmons A (2007) Mackie and Mccartney Practical Medical Microbiology, 14th edition, Elsevier.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore – 641 021.
(For the candidates admitted from 2017 onwards)

DEPARTMENT OF MICROBIOLOGY

SUBJECT NAME: MICROBIAL DIAGNOSIS IN HEALTH CLINIC
SEMESTER: III
SUB.CODE:18MBU304B **CLASS: II B.Sc (MB)**

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	Bacterial Diseases of various human body systems	W1
2	1	Viral Diseases of various human body systems	W1
3	1	Fungal Diseases of various human body systems	W1
4	1	Protozoan Diseases of various human body systems	W1
5	1	Disease associated clinical samples for diagnosis	W2
6	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Unit 1=06		
		UNIT-II	
1	1	How to collect clinical samples	T1: 113 - 119
2	1	oral cavity, throat swab tissue sample, skin	W3
3	1	Blood, CSF	W3
4	1	Urine and faeces - precautions required	W3
5	1	Method of transport of clinical samples to laboratory and storage	T2: 208-210
6	1	Recapitulation and discussion of question	

	Total No of Hours Planned For Unit II=06		
		UNIT-III	
1	1	Examination of sample by staining	R1: 25, 35-38
2	1	Gram stain, Ziehl-Neelson staining for tuberculosis	R1: 36 – 37, 853
3	1	Giemsa-stained thin blood film for malaria	R1:855,989 - 990
4	1	Preparation and use of culture media - Blood agar, Chocolate agar	R1: 104 – 106, 148 - 149
5	1	Chocolate agar, Lowenstein-Jensen medium, MacConkey agar	R1: 147 – 156,857
6	1	Distinct colony properties of various bacterial pathogens	R1: 108 - 110
7	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Unit III=07		
		UNIT-IV	
1	1	Serological Methods - Agglutination	T2: 101-109
2	1	ELISA, immune fluorescence	R1: 850 – 855, 866-877
3	1	Nucleic acid based methods – PCR	R1: 397 , 405 - 407
4	1	Nucleic acid probes	411- 426
5	1	Typhoid, HBV	W4
6	1	HCV, HIV and Dengue	W4
7	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Unit IV=07		
		UNIT-V	
1	1	Importance, Determination of resistance/sensitivity of bacteria using disc diffusion method (Kirby Bauer Method)	T1: 579 - 590
2	1	Importance, Determination of resistance/sensitivity of bacteria using disc diffusion method (Kirby Bauer Method)	R1: 884,W4
3	1	Importance, Determination of resistance/sensitivity of bacteria	W4

		using disc diffusion method (Kirby Bauer Method)	
4	1	Determination of minimal inhibitory concentration (MIC) of an antibiotic by broth dilution method (LC_{50}, LC_{90}).	W5
5	1	Determination of minimal inhibitory concentration (MIC) of an antibiotic by broth dilution method (LC_{50}, LC_{90}).	W5
6	1	Determination of minimal inhibitory concentration (MIC) of an antibiotic by broth dilution method (LC_{50}, LC_{90}).	W5
7	1	Recapitulation and discussion of question	
8	1	Old question paper discussion (Last Five years)	
9	1	Old question paper discussion (Last Five years)	
10	1	Old question paper discussion (Last Five years)	
	Total No of Hours Planned for unit V=10		

SUGGESTED READINGS:

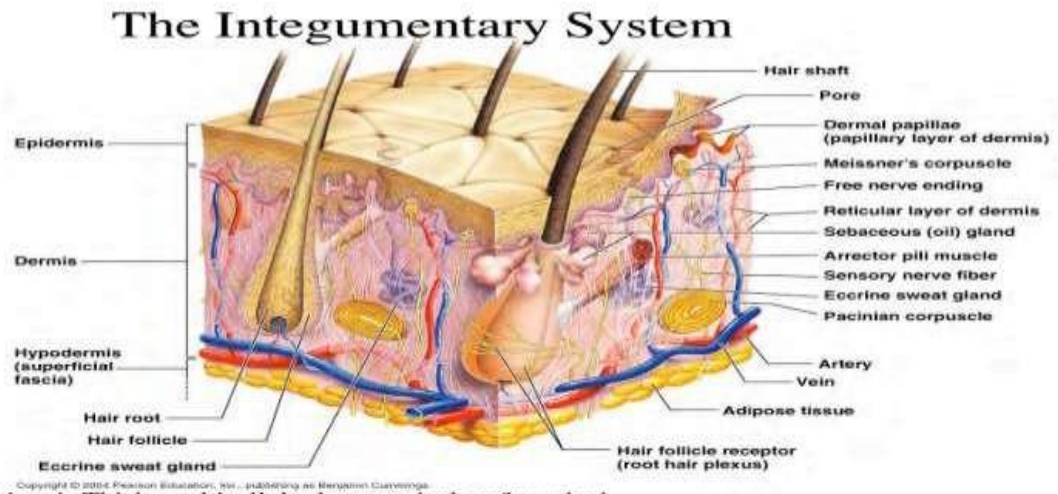
1. Rajan S and R. Selvichristy,2010. Experimental Procedure in Life Science. 1st Edition, Anjanaa Book House, Chennai
2. Paul.G.Engelkirk, 2008.Laboratory Diagnosis of Infectious Diseases: Essentials of Diagnostic 1st Edition.Lippincott Williams & wilkins.USA.
3. Kenneth J. Ryan, C.George Ray and Nafees Ahmad.2014 Sherris Medical Microbiology 6th Edition. Mc Graw Hill Medical New York.
4. Rajan, S Medical Microbiology, 2007, MJP Publishers, Chennai.
5. Ananthanarayanan, R. and C.K.J. Panicker, 2005. Text book of Microbiology. 7th Edition. Orient Longman. New Delhi.
6. Prescott, L.M., J.P. Harley and C.A. Klein, 2003. Microbiology, 5th Edition McGraw Hill Publishing Company Limited. New York.

WEBSITES

1. **W1:** www.microbeonline.com
2. W2: www.gosh.nhs.uk
3. W3: www.boundless.com
4. W4: www.en Wikipedia.org
5. W5: www.asm.org

UNIT-I
SYLLABUS

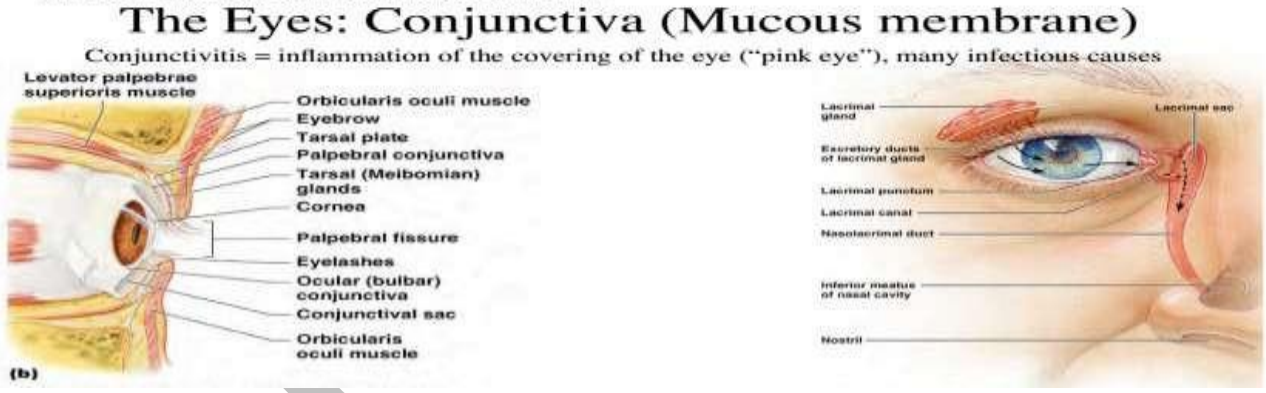
Bacterial, Viral, Fungal and Protozoan Diseases of various human body systems, Disease associated clinical samples for diagnosis



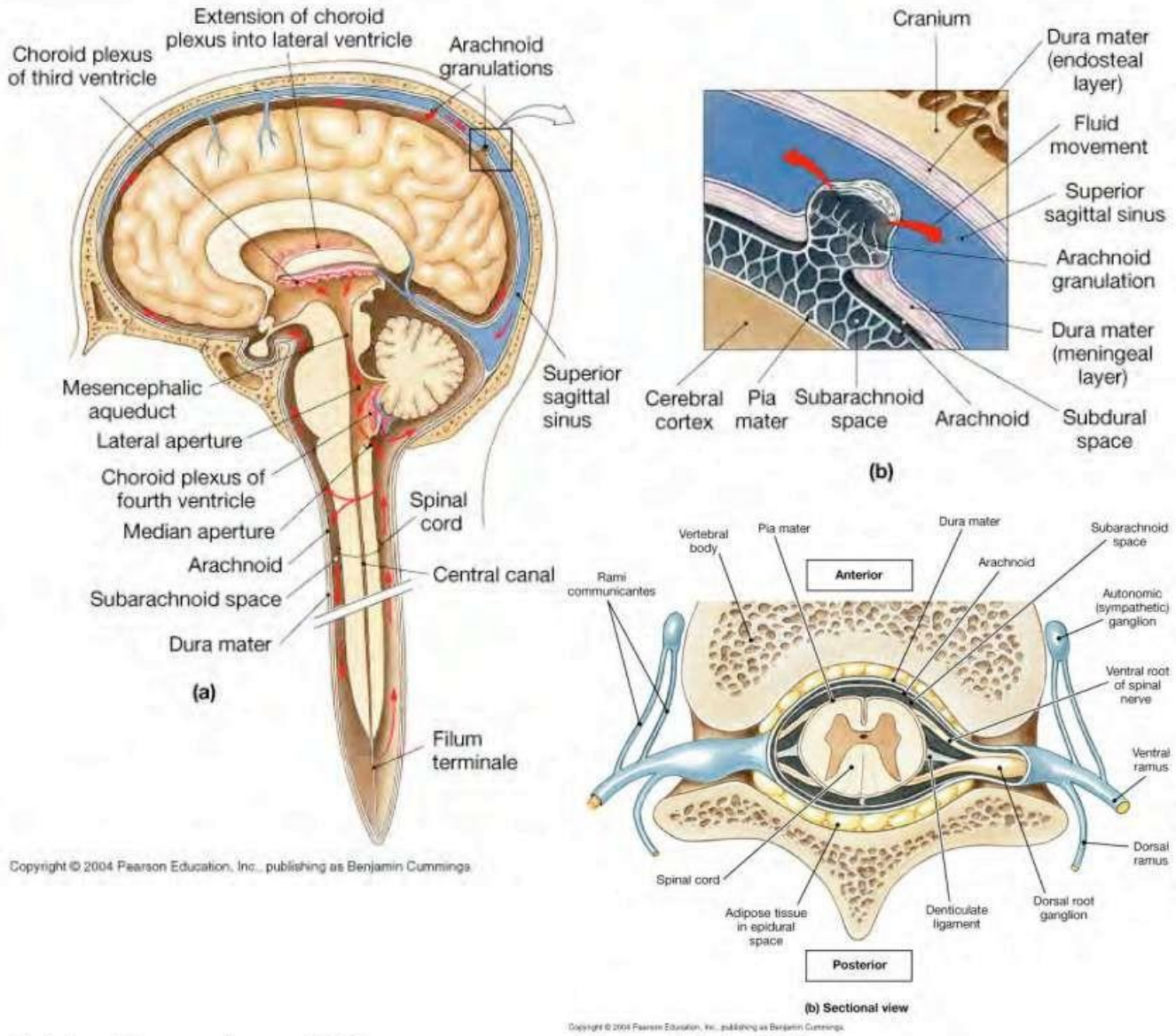
Epidermis: protective
1. Thick, multicellular layer, apical surface shed
2. Keratinized cells, impenetrable
3. Perspiration = salt & lysozyme
4. Sebum = fatty acids

Dermis: blood supply

*Normal flora/microbiota must be salt and acid resistant.
*To access to protected dermis, pathogens must penetrate epidermis through hair follicles, sweat glands, sebaceous glands, or damaged/broken epithelium



The Nervous System



Peripheral Nervous System (PNS) = nerves, nervous tissue outside the CNS

Central Nervous System (CNS) = brain & spinal cord

- -covered by meninges (connective tissue sheaths)
- -surrounded by Cerebrospinal Fluid (CSF): contains no phagocytes, complement or antibodies
- -protected by the blood brain barrier (but this also restricts access of leukocytes, complement and antibodies from the blood)

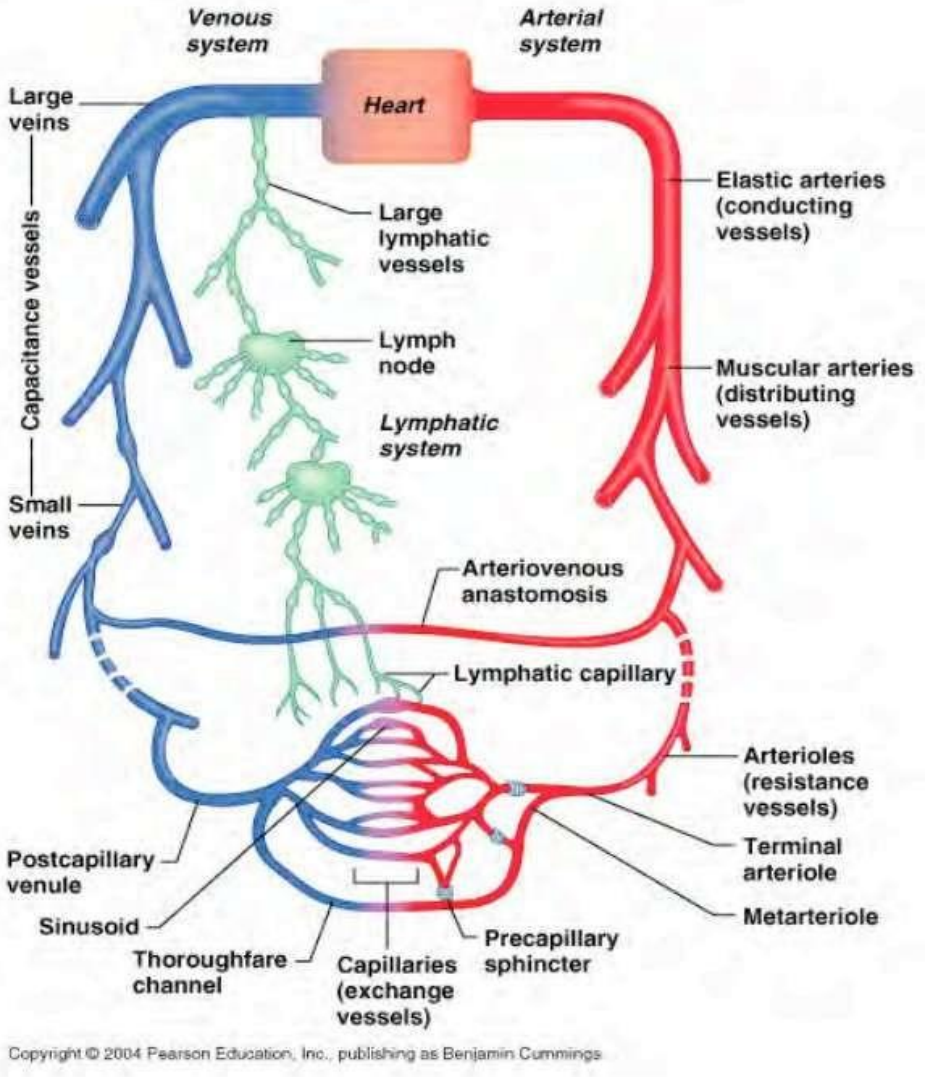
*No normal flora/microbiota

*Pathogens gain access to the CNS via trauma, travel along peripheral nerves, or by blood infection that causes inflammation to break down the blood-brain barrier

The Circulatory System: The Cardiovascular System & The Lymphatic System

Cardiovascular System:
- Heart
- Arteries
- Veins
- Capillaries
- Blood & Blood cells

Lymphatic system:
- Lymphatic capillaries
- Lymphatic vessels
- Lymphoid follicles
  - MALT (Peyer's Patches)
  - Tonsils
- Lymphoid organs
  - Lymph nodes
  - Spleen
  - Thymus
- Lymph & Lymphoid cells



*No normal flora/microbiota
*Infection in tissues easily gains access to lymphatic and blood vessels

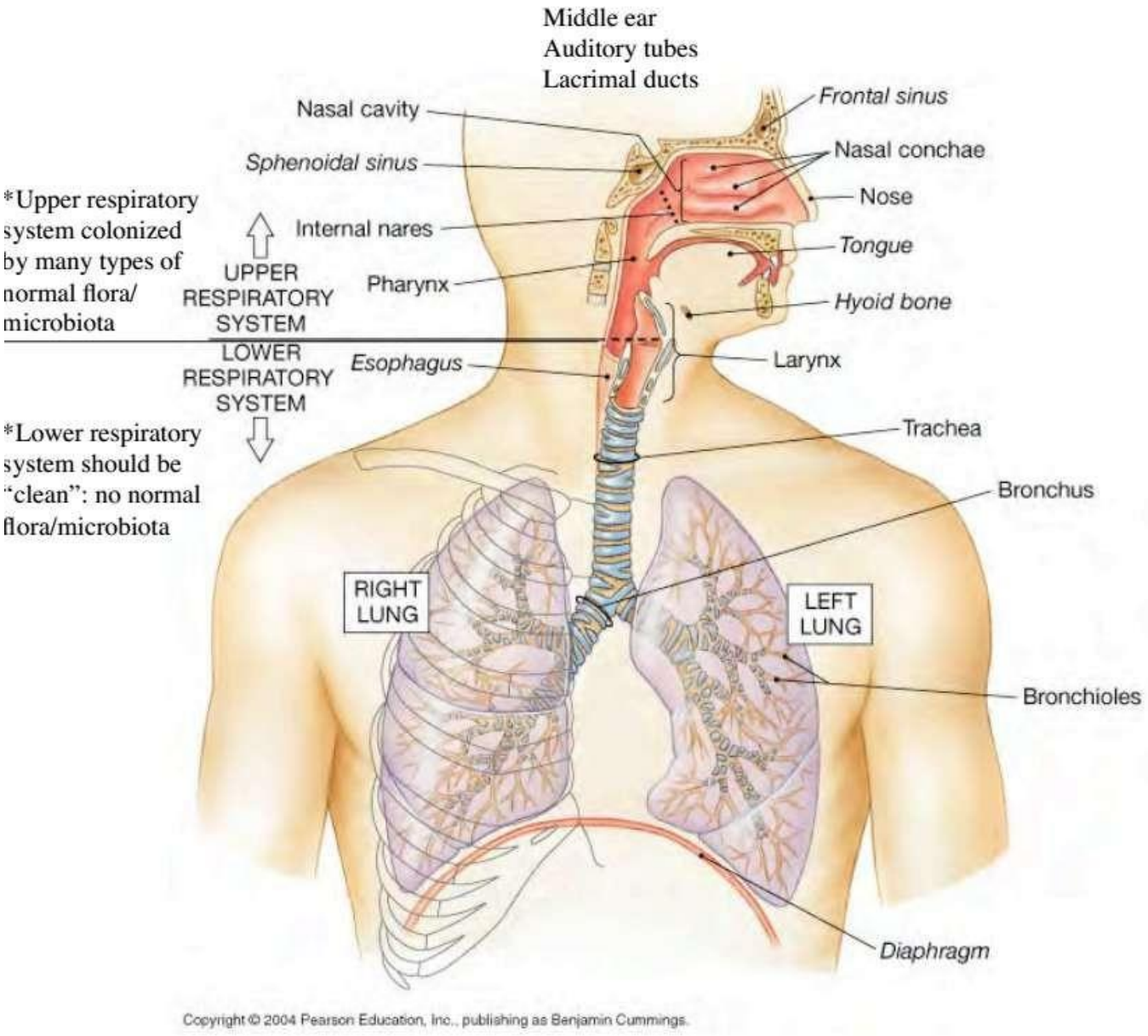
Lymphangitis = inflammation of a lymph vessel due to microbe infection or toxins

Septicemia = proliferation of microbes in the blood

Septic shock = septicemia that leads to body-wide inflammation resulting in a drop in blood pressure that could be fatal

Endotoxic shock = septic shock caused by Gram negative organisms shedding LPS into the blood stream thus triggering body-wide inflammation

The Respiratory System



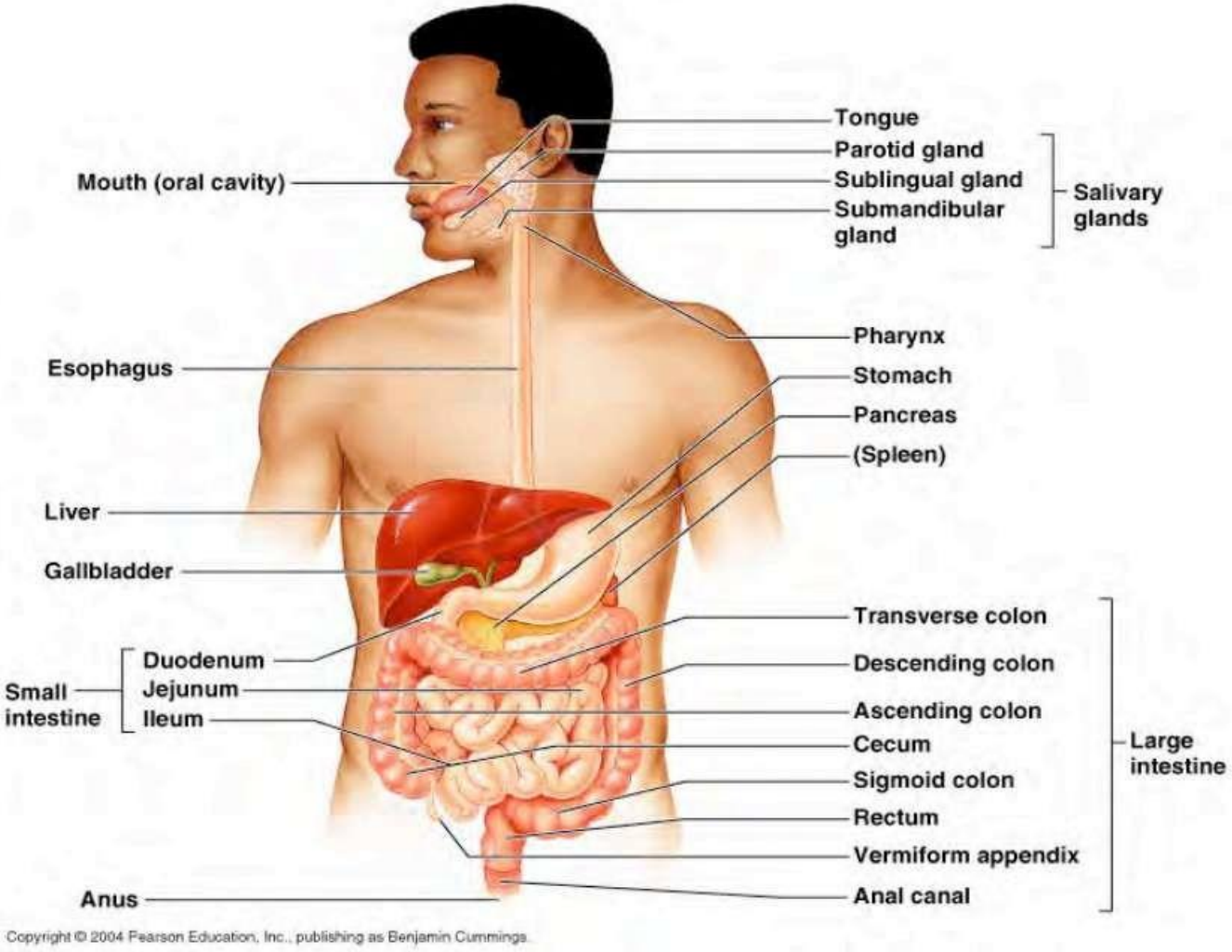
Pharyngitis = inflammation of the mucosa of the pharynx (sore throat)

Laryngitis = inflammation of the laryngeal folds (vocal cords)

Sinusitis = inflammation of the mucosa of the sinuses

Pneumonia = inflammation of and fluid accumulation in the lungs

The Digestive System



Accessory structures: teeth, tongue, salivary glands, liver, gallbladder, pancreas

Gastrointestinal Tract (G.I.): Mouth to anus

*Heavily colonized by normal flora/microbiota, most are located in the colon

Diseases:

1. Infection = colonization and growth of the microbe in the G.I. causes signs and symptoms of disease
2. Intoxication = ingestion of preformed bacterial toxin which causes signs and symptoms of disease (organism need not be present in the G.I.)

Both cause cramps, nausea, diarrhea and vomiting

Dysentery = diarrhea + blood + mucus

Gastroenteritis = inflammation of the mucosa of the stomach and intestine, fluid results in diarrhea

Bacterial Infections of the Skin

Staphylococci (*Staphylococcus aureus*): Gram + cocci

- coagulase: clot fibrin
- leukocidin: kill leukocytes

Scalded skin syndrome

- exfoliative toxin: peeling skin
- bacteremia → septicemia

Toxic shock syndrome

- bacteremia → septicemia

Streptococci (Group A β-hemolytic e.g. *Streptococcus pyogenes*): Gram + cocci

- >80 types classified by M-protein: antiphagocytic, aids adherence to mucosa
- hemolysins: RBC lysis
- streptokinases: fibrinolysis
- hyaluronidase: dissolve hyaluronic acid
- deoxyribonucleases: hydrolyze DNA
- proteases: hydrolyze proteins

Skin infections:
- folliculitis
- sty
- abscess

Menigitis

Pneumonia

Pharyngitis

Heart infections

Dental cavities

Ear aches

Impetigo

Necrotizing fasciitis

- exotoxin A: superantigen

Pseudomonas species: Gram – bacilli

Skin infections

Rash

Ear infections

Propionibacterium acnes: Gram + bacilli

Acne: in sebaceous glands, sebum → free fatty acids, elicit neutrophil attack

Viral Infections of the Skin

Warts – Papilloma virus (HPV)

infects & integrates in skin cells → proliferation

Small pox – Variola virus

Variola major: 20% mortality

Variola minor: < 1% mortality

respiratory route transmission → organ infection, lesions

no animal reservoir

vaccination: last case 1977

Chicken pox – Varicella-Zoster Herpes Virus

respiratory route transmission → skin, pus-filled vesicles

low mortality

latent in nerve ganglia

reactivation = Shingles

blindness, paralysis

Herpes

Herpes Simplex Virus 1 (HSV1): oral lesions (cold sore, fever blister)

oral & respiratory route transmission

trigeminal nerve ganglia latency

Herpes Simplex Virus 2 (HSV2): genital lesions

sexual transmission

sacral nerve ganglia latency

Measles – Rubeola virus

respiratory route transmission → respiratory infection → rash & Kopliks spots

can be fatal in infants & elderly

no animal reservoir

vaccination

Fungal Infections of the Skin

Candidiasis – *Candida* species, *C. albicans*: dimorphic fungi

Oral thrush

Vaginitis

(commensal of GI → opportunistic systemic mycosis in immunocompromised host)

Ring worm – Various fungi genera/species

Mycosis of hair follicles, nails, epidermis

Bacterial Infections of the Eyes

Neonatal gonorrheal opthalmia – *Neisseria gonorrhoeae*: Gram – cocci

corneal scarring → blindness

Trachoma – *Chlamydia trachomatis:* Gram – pleomorphic cocci

corneal scarring → blindness

greatest cause in infectious blindness worldwide

Viral Infections of the Eyes

Herpetic keratitis – HSV1

corneal ulcers → blindness

Protozoan Infections of the Eyes

Acanthamoeba keratitis – Amoebozoa protozoan

corneal digestion → blindness

Bacterial Infections of the Nervous System

Bacterial Meningitis

Streptococcus pneumoniae: Gram + diplococci

Haemophilus influenzae: Gram – bacilli

Neisseria meningitidis: Gram – cocci

transmitted via nasal secretions

nasal & pharyngeal mucosa → throat infection → bacteremia → menigitis → shock

40% mortality

Tetanus – *Clostridium tetani*: Gram + bacilli, obligate anaerobe, endospore forming

soil → puncture wound → anaerobic infection → toxemia

tetanospasmin: neurotoxic to motor neurons = spastic paralysis

100% mortality untreated, treatment with tetanus immunoglobulin 25% mortality

toxoid vaccine

Botulism – *Clostridium botulinum*: Gram + bacilli, obligate anaerobe, endospore forming

soil & water → growth in anaerobic food → ingestion → toxemia

botulinum toxin: blocks Acetylcholine release at neuromuscular junction = flaccid paralysis

70% mortality

Botox

Leprocy – *Mycobacterium leprae*: acid-fast bacilli

generation time 12 d, optimal temp 30°C

inhalation → neuron infection, peripheral regions →

Tuberculoid leprocy: numbness and nodules on skin patches, spontaneous recovery or

Lepromatous leprocy: infection & necrosis of skin cells (poor cell mediated immunity)

Viral Infections of the Nervous System

Poliomyelitis – Poliovirus

replicates in GI, transmission via oral fecal route → tonsil infection → viremia → CNS motor nerve infection → necrosis → paralysis

no animal reservoir

vaccines

Rabies – Rabies virus

transmission via bite or saliva → muscle & CT (30-50 d)→ nerves → CNS: fatal encephalitis: paralysis & death

encephalitis causes agitation

vaccination effective after exposure

Fungal Infections of the Nervous System

Cryptococcosis – *Cryptococcus neoformans*: thick capsule yeast

inhalation of pigeon droppings → lung infection → blood → CNS: chronic meningitis

30% mortality

Other Infections of the Nervous System

Transmissible spongiform encephalopathies – prion

prion = infectious protein, misfolded, directs misfolding of other proteins

transmitted by ingestion or direct contact → CNS: progressive spongiform degeneration of neurons

Creutzfeldt-Jakob disease: source? genetic?

Kuru: cannibals of New Guinea

Bacterial Infections of the Cardiovascular System

Puerperal sepsis/Childbirth fever – *Streptococcus pyogenes*: Gram + cocci

uterus → abdominal cavity → blood → septicemia

Bacterial endocarditis – α-hemolytic Streptococci, *Staphylococcus aureus*: Gram + cocci

mouth (tooth extraction) → blood → heart valves: destruction

Rheumatic fever – *Streptococcus pyogenes*: Gram + cocci

pharyngitis →

autoimmune disorder: reaction to M protein → cross react with CT (joints & heart valves): progressive destruction

Tularemia – *Francisella tularensis*: Gram – pleomorphic bacilli

rabbits & squirrels contact, or deerflies, ticks, lice bites → ulcer → lymph nodes → septicemia → body-wide abscesses & organ infection

highly transmittable: 10 bacteria = full infection

survives in phagocytes

Brucellosis – *Brucella* species: Gram – bacilli

mucous membrane → uterus → chills, fever, malaise, abortion

grows on fetal carbohydrate

transmitted in milk

reproduces in macrophages

Anthrax – *Bacillus anthracis*: Gram + bacilli, endospore forming

soil → spore inhalation → multiply in macrophages → fatal septicemic shock

capsule does not elicit protective immune response

high mortality unless very early treatment

Gangrene – *Clostridium* species: Gram + bacilli, anaerobic, endospore forming

ischemic wound → necrotic tissue → anaerobic infection = gangrene

toxins promote necrosis of live tissue

enzymes digest into deeper tissues

Clostridium perfringes: soil & GI

treatment: amputation, hyperbaric chambers

Bubonic plague – *Yersinia pestis*: Gram – bacilli

mild infection in rodents

flea bite → blood & lymph → proliferates in phagocytic cells → buboes → fever &

hemorrhages →

septicemic plaque → septic shock

pnuemonic plague → death, 100% mortality in 3 days

(542-767 AD 25% of pop. of Europe died of plague)

Lyme disease – *Borrelia burgdorferi*: spirochaete

mild infection in mice

deer tick bite → rash → flu-like → body wide organ & tissue infections →

heart damage, facial paralysis, arthritis

Typhus – *Rickettsia* species

obligate intracellular parasite

endothelial cells → vessel hemorrhaging → "spotted" fever

Epidemic Typhus – *Rickettsia prowazekii*: Gram – pleomorphic bacilli

human body lice feces → fever & hemorrhaging

high mortality

Endemic murine typhus – *Rickettsia typhi*: Gram – pleomorphic bacilli

rat flea → mild disease

Rocky Mountain Spotted Fever – *Rickettsia rickettsii*: Gram – pleomorphic bacilli

tick (parasite of tick) → rash, headache, fever → kidney & heart failure

Viral Infections of the Cardiovascular System

Infectious Mononucleosis – Epstein Bar Virus (EBV)

saliva →incubation 4-6 w → B cells → lymphocyte proliferation → fever, sore throat,

swollen lymph nodes, weakness → latent B cell infection

Burkitts Lymphoma – EBV

EBV + malaria concurrent → jaw tumor

Viral Hemorrhagic Fevers

zoonotic diseases: animal host no illness

transmission to human → internal organ hemorrhage

vector borne: (mosquito)

Yellow fever: monkey

Dengue fever: unknown

body fluid transmission:

Ebola: unknown

Lassa fever: rodents

Hantavirus pulmonary syndrome: rodents

Protozoan Infections of the Cardiovascular System

Toxoplasmosis – *Toxoplasma gondii:* Apicomplexa protozoan

rodent & cat feces → blood cells → brain damage or death to fetus (& immunocompromised)

Malaria – *Plasmodium* species: Apicomplexa protozoan

Anopheles mosquito → RBC lysis → capillary blockage: kidney & liver failure, brain damage

sexual cycle in mosquito, proliferation in human

Bacterial Infections of the Respiratory System

Streptococcal Pharyngitis (Strep throat) – *Streptococcus pyogenes*: Gram + cocci

streptokinases: fibrinolysis

streptolysins: host cell lysis

resistant to phagocytosis

Scarlet fever – *Streptococcus pyogenes*: Gram + cocci

erythrogenic toxin → pink skin rash

high fever

Diptheria – *Cornebacterium diptheriae*: Gram + pleomorphic bacilli

phage lysogeny = toxin production → inhibit protein synthesis

airborne transmission → throat infection: sore throat, fever, neck swelling: gray membrane of fibrin, necrotic tissue & bacteria → suffocation

toxoid vaccine

Pertussis/Whooping Cough – *Bordetella pertussis*: Gram – cocobacilli

capsule inhibits phagocytosis

throat infection → tracheal cytotoxin produced → ciliated cells destroyed: violent coughing, suffocation

Tuberculosis – *Mycobacterium tuberculosis*: acid-fast bacilli

generation time 20 h

replicates in macrophages

high resistance

inhalation → lung tubercles → Ghon complexes → septicemia

weigh loss, coughing, with blood in sputum, fatigue

3 million die annually

Bacterial Pneumonia

Streptococcus pneumoniae: Gram + cocci

Haemophilus influenzae: Gram – bacilli

Mycoplasma pneumoniae: tiny, no cell wall

lung inflammation → suffocation

Legionnaires disease – *Legionella pneumophila*: Gram – bacilli

fresh water amoeba → inhalation → macrophages → pneumonia → death

no person to person transmission

Viral Infections of the Respiratory System

Common Cold – Rhinovirus

respiratory mucosa → sneezing, coughing, nasal secretion, congestion

self limiting, non fatal

Influenza – Influenza virus, enveloped with spikes

chills, fever, headache, malaise, cold-like symptoms → secondary infections → death

antigenic shift of spikes

recombination between animal and human flu results in deadly strains

Fungal Infections of the Respiratory System

Histoplasmosis – *Histoplasma capsulatum*: dimorphic fungi

inhalation of spores: bat feces → lung infection → blood & lymph → body-wide lesions

Mississippi & Ohio River valleys

Coccidiomycosis – *Coccidioides immitis*: dimorphic fungi

inhalation of spores: dust storm → tuberculosis like respiratory illness

California & Arizona

Bacterial Infections of the Digestive System

Staphylococcal Food Poisoning – *Staphylococcus aureus*: Gram + cocci

resistant: heat, drying, salt, radiation, osmotic pressure

grows in food at room temp, produces enterotoxin (stable through 30 min boiling)

nose → food: enterotoxin (super antigen) → vomiting & diarrhea

quick onset, no mortality

Shigellosis – *Shigella* species: Gram – bacilli

survives stomach, replicates inside intestinal epithelial cells

shiga toxin: inhibits protein synthesis, causes necrosis of cells

human/primate feces → intestinal epithelium → shiga toxin → dysentery

some mortality

infectious with low dose exposure

Salmonellosis – *Salmonella enterica, S. typhimurium*: Gram – bacilli

pathogen of animals and humans

transmitted on contaminated eggs, meat, contact with reptiles

animal feces → intestinal mucosa, multiplies in cells → diarrhea → blood & lymph → or
infections → septic shock,

requires high dose to be infectious

mortality 1% due to shock

Typhoid fever – *Salmonella typhi*: Gram – bacilli

oral fecal route of transmission

carriers: organism in gallbladder, shed to intestine, no symptoms
Typhoid Mary

human feces → ulceration of intestine → bacteremia → death
fever, headache, diarrhea

20% mortality

vaccine poorly effective

Cholera – *Vibrio cholerae*: Gram – vibrio, polar flagellum

oral fecal route of transmission from fecal contaminated water

phage conversion → cholera toxin: fluid & electrolyte loss from human cell

water → small intestine → cholera toxin → electrolyte & water loss → shock → death

vaccine poorly effective, many strains

Escherichia coli Gastroenteritis: Gram – bacilli

usually common harmless flora

pathogenic stains: altered fimbrae & toxin production

Traveler's diarrhea strains: adhesive fimbrae & toxins: self limiting gastroenteritis

Enterohemorrhagic strains: adhesive fimbrae & shiga toxin → colon hemorrhage → bloo

organ infection → death

Cows & deer unaffected by shiga toxin

Peptic ulcer disease – *Helicobacter pylori*: spirilium

urease: urea → ammonia (buffer) for stomach colonization → ulcer, self destruction via a

environmental source unknown

Viral Infections of the Digestive System

Mumps – Mumps virus

transmitted in saliva and respiratory secretions

saliva → respiratory tract → lymph nodes → viremia → salivary glands

can also infect testes & ovaries, cause meningitis, pancreatitis

vaccine effective

Hepatitis = viral infection and inflammation of the liver

Hepatitis A – Hepatitis A virus (HAV)

fecal/oral → intestinal epithelium → viremia → liver, kidney, spleen

anorexia, nausea, diarrhea, fever, chills, jaundice

self limiting

effective vaccine

Hepatitis B – Hepatitis B virus (HBV)

blood & body fluids → liver: chronic = cirrhosis → cancer

anorexia, nausea, diarrhea, fever, chills, joint pain, jaundice

some mortality

effective vaccine

Hepatitis C – Hepatitis C virus (HCV)

blood & sexual contact → liver: asymptomatic 20 years + → cirrhosis → cancer → death

high mortality

no vaccine

Hepatitis D – Hepatitis D virus (HDV)

coinfect with HBV → chronic liver infection → progressive damage → death

(requires HBV envelope)

Hepatitis E – Hepatitis E virus (HEV)

fecal/oral → (see Hep A) → mortality in pregnant females

Viral Gastroenteritis – Rotavirus

fecal/oral transmission → fever, diarrhea, vomiting

low mortality

Protozoan Infections of the Digestive System

Giardiasis – *Giardia lamblia*: Archaezoa protozoan

fecal contaminated water, fecal/oral route

water: cysts → intestine colonization → malaise, nausea, flatulence, weight loss, diarrhea

interferes with nutrient absorption

low mortality

Cryptosporidiosis – *Cryptosporidium parvum*: Apicomplexa protozoan

fecal contaminated water, fecal/oral route

water: oocysts → intestinal epithelium → diarrhea

some mortality

Amoebic dysentery – *Entamoeba histolytica*: Amoebozoa protozoan

fecal contaminated water, fecal/oral route

water: cysts → trophozoites digest intestinal epithelium → dysentery

Bacterial Infections of the Urinary and Reproductive System

Gonorrhea – *Neisseria gonorrhoeae*: Gram – diplococci

fimbrae for attachment

infect between mucosal epithelial cells

STD → mucosa infection → inflammation → scarring & infertility

pus in males

initially asymptomatic in female → pelvic inflammatory disease

transmission to eyes of new born → blindness

does not survive outside the body: human to human transmission only

Nongonococcal Urethritis (NGU) – *Chlamydia trachomatis* and others: Gram – pleomorphic cocci

STD → mucosa infection → inflammation → scarring & infertility

(see Gonorrhea)

C. trachomatis is most common STD

Syphilis – *Treponema pallidium*: spirochete, obligate pathogen

Stages:

Primary: chancre → blood

Secondary: rashes & lesions (contagious), hair loss, malaise, fever → latent →

transmission to fetus → brain damage or still birth

Tertiary: immune reaction to latent infection → lesions in organs

paralysis, blindness, seizures, heart problems

Viral Infections of the Urinary and Reproductive System

Acquired Immune Deficiency Syndrome (AIDS) – Human Immunodeficiency Virus (HIV)

mutated monkey virus, entered human population in Africa ~1930

transmitted in blood or sexual body fluids

STD & blood → infects T Helper cells (CD4 cells) → integrates (reverse transcription) → latent or replicating, death of CD4 cells → immunocompromised → death by secondary infections

rapid antigenic variation: high mutation rate

single patient can have > 100 million variants

stages (10 year progression):

Category A: asymptomatic, swollen lymph nodes

Category B: Candidiasis of mouth, throat, vagina
Shingles, diarrhea, fever, cancers

Category C: AIDS (CD4 cells <200)
severely immunocompromised
Candidiasis of bronchi & lungs
Tuberculosis, Pneumonia,
Toxoplasmosis of the brain,
Kaposi's sarcoma (HSV8): cancer of CT

Concepts related to diagnosis

Sub-types of diagnoses include:

Clinical diagnosis

A diagnosis made on the basis of medical signs and patient-reported symptoms, rather than diagnostic tests

Laboratory diagnosis

A diagnosis based significantly on laboratory reports or test results, rather than the physical examination of the patient. For instance, a proper diagnosis of infectious diseases usually requires both an examination of signs and symptoms, as well as laboratory characteristics of the pathogen
involved.

Radiology diagnosis

A diagnosis based primarily on the results from medical imaging studies. Greenstick fractures are common radiological diagnoses.

Principal diagnosis

The single medical diagnosis that is most relevant to the patient's chief complaint or need for treatment. Many patients have additional diagnoses.

Admitting diagnosis

The diagnosis given as the reason why the patient was admitted to the hospital; it may differ from the actual problem or from the *discharge diagnoses*, which are the diagnoses recorded when the patient is discharged from the hospital.

Differential diagnosis

A process of identifying all of the possible diagnoses that could be connected to the signs, symptoms, and lab findings, and then ruling out diagnoses until a final determination can be made.

Diagnostic criteria

Designates the combination of signs, symptoms, and test results that the clinician uses to attempt to determine the correct diagnosis. They are standards, normally published by international committees, and they are designed to offer the best sensitivity and specificity possible, respect the presence of a condition, with the state-of-the-art technology.

Prenatal diagnosis

Diagnosis work done before birth

Diagnosis of exclusion

A medical condition whose presence cannot be established with complete confidence from history, examination or testing. Diagnosis is therefore by elimination of all other reasonable possibilities.

Dual diagnosis

The diagnosis of two related, but separate, medical conditions or co-morbidities; the term almost always refers to a diagnosis of a serious mental illness and a substance addiction.

Self-diagnosis

The diagnosis or identification of a medical conditions in oneself. Self-diagnosis is very common.

Remote diagnosis

A type of telemedicine that diagnoses a patient without being physically in the same room as the patient.

Nursing diagnosis

Rather than focusing on biological processes, a nursing diagnosis identifies people's responses to situations in their lives, such as a readiness to change or a willingness to accept assistance.

Computer-aided diagnosis

Providing symptoms allows the computer to identify the problem and diagnose the user to the best of its ability. Health screening begins by identifying the part of the body where the symptoms are

located; the computer cross-references a database for the corresponding disease and presents a diagnosis.

Overdiagnosis

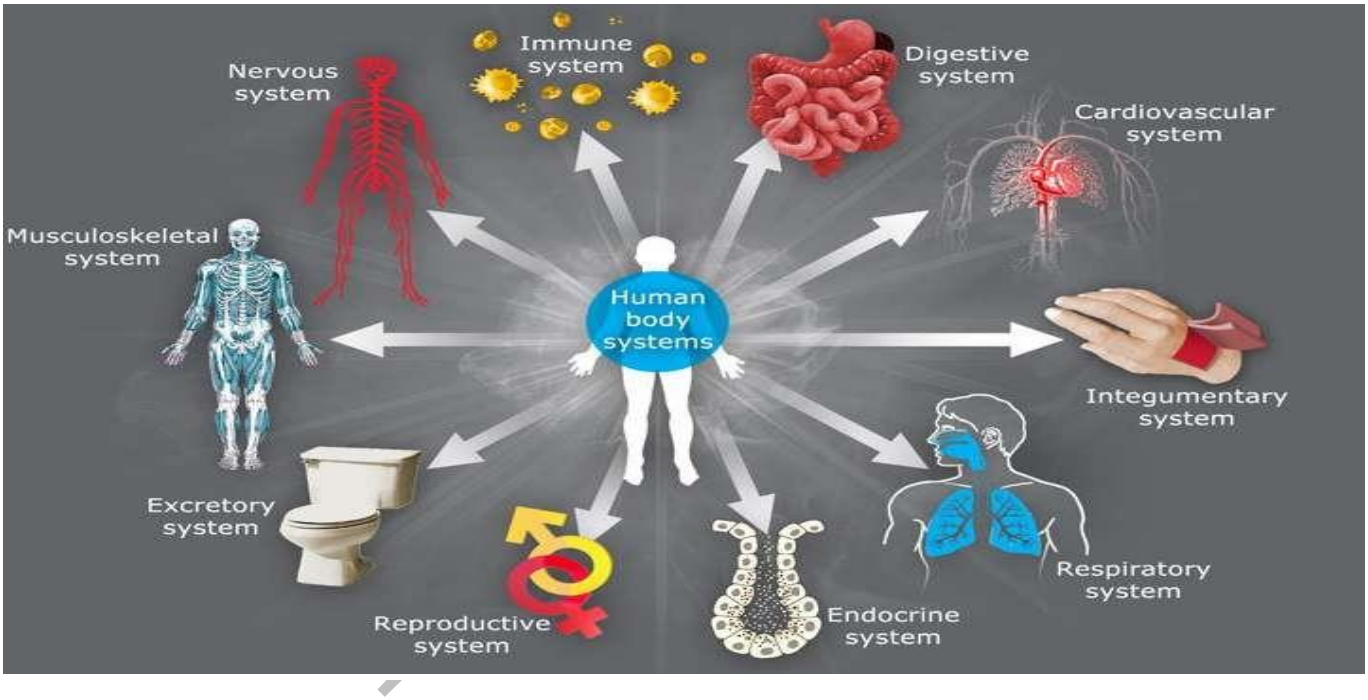
The diagnosis of "disease" that will never cause symptoms, distress, or death during a patient's lifetime

Wastebasket diagnosis

A vague, or even completely fake, medical or psychiatric label given to the patient or to the medical records department for essentially non-medical reasons, such as to reassure the patient by providing an official-sounding label, to make the provider look effective, or to obtain approval for treatment. This term is also used as a derogatory label for disputed, poorly described, overused, or questionably classified diagnoses, such as pouchitis and senility, or to dismiss diagnoses that amount to overmedicalization, such as the labeling of normal responses to physical hunger as reactive hypoglycemia.

Retrospective diagnosis

The labeling of an illness in a historical figure or specific historical event using modern knowledge, methods and disease classifications.



Possible Questions

Part B

1. Define microbial diagnosis.
2. Say about bacteria
3. What is disease and infection?
4. What is lab diagnosis?
5. What are MIC and MBC?

Part C

1. give a detailed note on diagnosis and its types
2. Write about the transport media and its importance.
3. Discuss about the investigation by culturing.
4. Describe various sources and methods of infection.
5. Comment on the ELISA
6. Give the laboratory diagnosis of Acid fast bacilli.
7. State the pathogenic role and diagnosis of GI tract pathogenesis.
8. Discuss the properties and UTI caused by *E.Coli*.
9. Explain in detail about Kirby bauer technique of susceptibility testing.
10. Comment on respiratory tract infection and its symptoms.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB **COURSE NAME: MICROBIAL DIAGNOSIS IN HEALTH CLINIC**

COURSE CODE: 18MBU304B **UNIT: I** **BATCH-2018-2021**

Unit I Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
In Greek 'pathos means_____.	Suffering	Violence	Disease	Infection	Suffering
The lodgement and multiplication of a parasite in host constitute _____	Disease	Infection	Immunity	Parasitism	Infection
Initial infection with a parasite in a host is termed _____	Primary	Secondary	Re-Infection	Nosocomial	Primary
Subsequent infection by the same parasite in the host is termed _____infection.	Primary	Secondary	Re-Infection	Iatrogenic	Re-Infection
When a new parasite sets up an infection in a host whose resistance is lowered by a preexisting infectious disease, this is termed _____infection.	Primary	Secondary	Re-Infection	Iatrogenic	Secondary
Infection or sepsis at localized sites is called_____infection	Primary	Secondary	Focal	Iatrogenic	Focal
In a patient already suffering from a disease, a new infection is set up from another host or external source it is termed_____infection.	Cross	Focal	Re-Infection	Nosocomial	Cross
Cross infection occurring in hospitals are called____infection	Cross	Focal	. Re-Infection	Nosocomial	Nosocomial
Physicians induced infections are termed_____infection	Iatrogenic	Focal	Nosocomial	Re-Infection	Iatrogenic
When clinical effects are not apparent it is called _____ infection.	Atypical	In Apparent	Subclinical	Clinical	In Apparent
_____Infection is the one in which the typical or characteristic clinical manifestation of the particular infectious disease are not present.	Endogenous	Exogenous	Inapparent	Clinical	Inapparent

Some parasites, following infection may remain in the tissues in a hidden form proliferating and producing clinical disease termed _____ infection.	Endogenous	Exogenous	Latent	Atypical	Latent
A person who harbors the pathogenic microorganism without suffering from any ill effects because of it is called_____.	Patient	Carrier	Healthy Person	Immunodeficient Person	Carrier
A person one who harbours the pathogen but has never suffered from the disease caused by the pathogen is called_____.	Healthy Carrier	Convalescent Carrier	Contact Carrier	Paradoxical Carrier	Healthy Carrier
A person who has recovered from the disease and continuous to harbour the pathogen inside the body is called_____carrier.	Healthy	Convalescent	Contact	Paradoxical	Convalescent
The_____carrier state lasts than six months	Acute	Temporary	Chronic	Healthy	Temporary
The term_____carrier is applied to a person who acquires the pathogen from a patient.	Acute	Temporary	Contact	Paradoxical	Contact
The_____carrier state lasts for several years.	Acute Or Chronic	Temporary	Contact	Paradoxical	Acute Or Chronic
The term_____carrier refers to a person who acquires the pathogen from another carrier.	Healthy	Convalescent	Contact	Paradoxical	Paradoxical
Infectious diseases transmitted from animals to human being are called _____.	Zoonosis	Anoosis	Xanthosis	Phytosis	Zoonosis
When the pathogen multiplies in the body of the vector and then transmits the infection to humans are called	Mechanical	Biological Vector	Healthy	Contact	Biological Vector

_____vector.					
Some pathogens are able to cross the placental carrier and infect the fetus in uterus. This is known as _____transmission.	Congenital	Intracelaine	Vertical	Horizontal	Vertical
_____is generally employed to refer to the ability of a microbial species to produce diseases.	Infection	Immunity	Pathogenicity	Virulence	Pathogenicity
_____is applied to the same property in a strain of microorganism to produce disease.	Infection	Immunity	Pathogenicity	Virulence	Virulence
_____is the science that evaluates the occurrence, determinants, distribution and control of health and disease in a defined human population.	Epidemiology	Oncology	Infection	Physiology	Epidemiology
An individual who practices epidemiology is a_____.	Epidemiologist	Scientist	Investigator	Environmentalist	Epidemiologist
A_____is an impairment of the normal state of an organism or any of its components that hinders the performance of vital components.	Health	Disease	Infection	Immunity	Disease
_____is the condition in which the organism performs its vital functions normally.	Health	Disease	Infection	Immunity	Infection
When a disease occurs occasionally and at irregular intervals in a human population it is a_____disease.	Epidemic	Endemic	Sporadic	Pandemic	Endemic
When the disease occurs at a steady low-level frequency at a moderately regular interval it is an_____disease.	Epidemic	Endemic	Sporadic	Pandemic	Endemic
_____disease gradually increase in the occurrence frequency	Hyperendemic	Epidemic	Endemic	Pandemic	Hyperendemic

beyond the endemic level but do not to the epidemic level.					
An_____is a sudden increase in the occurrence of a disease above the expected level.	Epidemic	Endemic	Sporadic	Pandemic	Pandemic
The first case in an epidemic is called_____	Index Case	Infection	Disease	Outbreak	Index Case
A sudden unexpected occurrence of a disease in a limited segment of population is called_____	Index Case	Infection	Disease	Outbreak	Outbreak
A_____is an increase in disease occurrence within a large population over a very wide region.	Epidemic	Endemic	Pandemic	Sporadic	Pandemic
Food poisoning is caused by	Clostridum Tetani	Clostridum Welchi	Diptheria	Clostridium Botulinum	Clostridium Botulinum
Koplic's spots will develop in	Hiv	Measles	Mumps	Rubella	Measles
Which of the following is an example of live vaccine?	Pertusis	Mumps	Cholera	Rabies	Cholera
Triple toxoid vaccine gives protection against	Diphtheria, Tetanus And Rabies	Tetanus, Whooping Cough, Tuberculosis	Whooping Cough, Tetanus And Diphtheria	Whooping Cough, Cancer And T.B	Whooping Cough, Tetanus And Diphtheria
AIDS is caused by	Retrovirus	Prion	Rhabdovirus	Retroprison	Retrovirus
Listeriosis was_____disease	Food Borne	Water Borne	Milk Borne	Air Borne	Food Borne
Pus-forming forms are called as	Pyoderm	Pyogenic	Pyrogen	Python	Pyogenic
In Elisa technique, the antibodies are labeled by	Acridine Orange	Alkaline Phosphate	Neutral Red	Bromothymol Blue	Alkaline Phosphate
_____is a genetic disease characterized by a total or partial inability to synthesize globulins.	Apitososis	Agamma Globulinemia	Gammaglobulinemma	Sickle-Cell Anemia	Agamma Globulinemia
ELISA test is used for the identification of	Jaundice	AIDS	Cancer	Diabetis	AIDS
Incubation period for infective	45 – 80 Days	15 – 35 Days	35 – 50 Days	5 – 15 Days	5 – 15 Days

Hepatitis disease					
Antibiotic produced from streptomyces orientalis is	Streptomycin	Penicillin	Vancomycin	Amikacin	Vancomycin
The drug of choice for dermal, oral and vaginal candidiasis is	Griseofulvin	Amphoterein B	Gentian Violet	Nystatin	Nystatin
Botulism means	Food Adultration	Food Poisioning By Streptococcus Bacteria	Chemical Contamination Of Food	Food Processing	Chemical Contamination Of Food
Chloramphenicol is obtained from	Streptomyces Griseus	Streptomyces Venezuelae	Streptomyces Pyrogenes	Streptomyces Aureus	Streptomyces Venezuelae
Streptomycin is obtained from	Streptococcus Species	Streptomyces Griseus	Straphylococcus Aureus	Straphylococcus Citrus	Streptomyces Griseus
Septicaemia is	Bacteria In Blood	Toxin In Blood	Pus In Blood	Multiplication Of Bacteria And Toxins In Blood	Multiplication Of Bacteria And Toxins In Blood
In AIDS, Kaposis sarcoma may respond to	Interleukin – 2 Infusion	Azathioprine	Alpha Interferon	Beta Interferon	Alpha Interferon
Ciprofloxacin acts by inhibiting	Cellwall Synthesis	RNA Synthesis	Folate Synthesis	DNA Gyrase	DNA Gyrase
Lyme disease is caused by	Bacteria	Fungi	Spirochaete	Virus	Spirochaete
Toxic shock syndrome is caused by	Staph. Albus	Staph. Aureus	Strep. Viridana	Staph Citrus	Staph. Aureus
Black water fever is caused by	P. Vivax	P. Falciparum	P. Ovale	P. Xyle	P. Falciparum
Mantoux test detects	M. Tuberculosis	Cynaobacteria	Clostridia	Staphylococcus	M. Tuberculosis
Kinetosomes are observed in	Algae	Fungi	Protozoa	Viruses	Protozoa

UNIT-II
SYLLABUS

How to collect clinical samples (oral cavity, throat swab, tissue sample, skin, Blood, CSF, urine and faeces) and precautions required. Method of transport of clinical samples to laboratory and storage.

How to collect clinical samples

Manifestations of Infection

The clinical presentation of an infectious disease reflects the interaction between the host and the microorganism. This interaction is affected by the host immune status and microbial virulence factors. Signs and symptoms vary according to the site and severity of infection. Diagnosis requires a composite of information, including history, physical examination, radiographic findings, and laboratory data.

Microbial Causes of Infection

Infections may be caused by bacteria, viruses, fungi, and parasites. The pathogen may be exogenous (acquired from environmental or animal sources or from other persons) or endogenous (from the normal flora).

Specimen Selection, Collection, and Processing

Specimens are selected on the basis of signs and symptoms, should be representative of the disease process, and should be collected before administration of antimicrobial agents. The specimen amount and the rapidity of transport to the laboratory influence the test results.

ORAL CAVITY

The oral cavity includes the lips, the inside lining of the lips and cheeks (*buccal mucosa*), the teeth, the gums, the front two-thirds of the tongue, the floor of the mouth below the tongue, and the bony roof of the mouth (hard palate). The area behind the wisdom teeth (called the *retromolar trigone*) can be included as a part of the oral cavity, but it's often thought of as part of the oropharynx.The oropharynx is the part of the throat just behind the mouth. It starts where the oral cavity stops. It includes the base of the tongue (the back third of the tongue), the soft palate (the back part of the roof of the mouth), the tonsils, and the side and back walls of the throat.The oral cavity and oropharynx help you breathe, talk, eat, chew, and swallow. Minor salivary glands throughout the oral cavity and oropharynx make saliva that keeps your mouth and throat moist and helps you digest food.

Tumors and growths in the oral cavity and oropharynx

Many types of tumors (abnormal growths of cells) can develop in the oral cavity and oropharynx. They fit into 3 general categories:

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- **Benign growths** are not cancer. They do not invade other tissues and do not spread to other parts of the body.
- **Pre-cancerous conditions** are harmless growths that can turn into cancer over time.
- **Cancer** tumors are growths that can grow into nearby tissues and spread to other parts of the body.
- Keratoacanthoma
- Leiomyoma
- Osteochondroma

- Lipoma
- Schwannoma
- Neurofibroma
- Papilloma
- Condyloma acuminatum
- Verruciform xanthoma
- Pyogenic granuloma
- Rhabdomyoma
- Odontogenic tumors (tumors that start in tooth-forming tissues)

These non-cancerous tumors start from different kinds of cells and have a variety of causes. Some of them may cause problems, but they're not likely to be life-threatening. The usual treatment for these types of tumors is surgery to remove them completely since they are unlikely to recur (come back).

Leukoplakia and erythroplakia (possible pre-cancerous conditions)

Leukoplakia and erythroplakia are terms used to describe certain types of tissue changes that can be seen in the mouth or throat:

- Leukoplakia is a white or gray patch.
- Erythroplakia is a flat or slightly raised, red area that often bleeds easily if it's scraped.
- Erythroleukoplakia is a patch with both red and white areas.

Your dentist or dental hygienist may be the first person to find these white or red patches. They may be cancer, they may be a pre-cancerous condition called *dysplasia*, or they could be a relatively harmless change.

Dysplasia is graded as mild, moderate, or severe, based on how abnormal the tissue looks under the microscope. Knowing the degree of dysplasia helps predict how likely it is to progress to cancer or go away on its own or after treatment. For example, severe dysplasia is more likely to become a cancer, while mild dysplasia is more likely to go away completely.

The most common causes of leukoplakia and erythroplakia are smoking and chewing tobacco. Poorly fitting dentures that rub against the tongue or the inside of the cheeks can also cause these changes. But sometimes, there's no clear cause. Dysplasia will often go away if the cause is removed.

A biopsy is the only way to know for certain if an area of leukoplakia or erythroplakia contains dysplastic (pre-cancerous) cells or cancer cells.But other tests may be used first to help determine if they might be cancers (and will need a biopsy) or to choose the best area to sample for a biopsy.

Most cases of leukoplakia do not turn into cancer. But some leukoplakias are either cancer when first found or have pre-cancerous changes that can progress to cancer if not properly treated.

Erythroplakia and erythroleukoplakia are less common, but are usually more serious. Most of these red lesions turn out to be cancer when they are biopsied or will develop into cancer later. Still, it's important to note that most oral cancers do not develop from pre-existing lesions (either leukoplakia or erythroplakia).

Oral cavity and oropharyngeal cancers

The different parts of the oral cavity and oropharynx are made up of many types of cells. Different cancers can start in each type of cell. These differences are important, because they can impact a person‟s treatment options and prognosis (outlook).

Squamous cell carcinomas

Almost all (more than 90%) of the cancers in the oral cavity and oropharynx are squamous cell carcinomas, also called *squamous cell cancers*. These cancers start in early forms of squamous cells, which are flat, scale-like cells that form the lining of the mouth and throat.The earliest form of squamous cell cancer is called *carcinoma in situ*. This means that the cancer cells are only in the layer of cells

called the *epithelium*. This is different from invasive squamous cell carcinoma, where the cancer cells have grown into deeper layers of the oral cavity or oropharynx.

Verrucous carcinoma is a type of squamous cell carcinoma that makes up less than 5% of all oral cancers. It's a low-grade (slow growing) cancer that rarely spreads to other parts of the body, but it can grow deeply into nearby tissue.If not treated, areas of ordinary squamous cell cancer may develop inside some verrucous carcinomas. And some verrucous carcinomas may already have areas of ordinary squamous cell cancer in them that aren't seen in the biopsy sample. Cells from these areas of squamous cell carcinoma may then spread to other parts of the body.For all of these reasons, verrucous carcinomas should be removed right away, along with a wide margin (edge) of surrounding normal tissue.

Other types of cancer in the oral cavity and oropharynx

Minor salivary gland carcinomas: these cancers can start in the glands in the lining of the mouth and throat. There are many types of minor salivary gland cancers, including adenoid cystic carcinoma, mucoepidermoid carcinoma, and polymorphous low-grade adenocarcinoma. To learn more about these cancers, as well as benign salivary gland tumors.

Lymphomas: the tonsils and base of the tongue contain immune system (lymphoid) tissue, where cancers called *lymphomas* can start. For more information about these cancers.

Cancers in other parts of the throat

Cancers can also start in other parts of the throat, but these cancers aren"t covered here:

- Cancers of the nasopharynx (the part of the throat behind the nose and above the oropharynx) are covered in Nasopharyngeal Cancer.
- Cancers that start in the larynx (voice box) or the hypopharynx (the part of the throat below the oropharynx) are covered in Laryngeal & Hypopharyngeal Cancer.

Throat swabs

- Specify on the laboratory form if this is a routine admission screen for MRSA or a swab for the investigation of a suspected infection.
- For the collection of throat swabs as part of screening for MRSA refer to the policy for the control of Methicillin-resistant Staphylococcus aureus (MRSA).
- Place the child in a position with a good light source. This will ensure maximum visibility of the tonsillar bed.
- Either depress the tongue with a spatula or ask the child to say “aahh”. The procedure is likely to cause gagging and the tongue will move to the roof of the mouth. This can prevent accurate sampling, therefore it is important to quickly but gently rub the swab over the ansillar fossa (tonsillar bed) or area where there is exudate or a lesion.
- Care should be taken not to contaminate the swab by contact with the tongue or the oral mucosa on removal.
- Return swab to the container with transport medium.

Procedure for throat swab

- Place the child in a position with a good light source. This will ensure maximum visibility of the tonsillar bed.
- Either depress the tongue with a spatula or ask the child to say “aahh”. The procedure is likely to cause gagging and the tongue will move to the roof of the mouth. This can prevent accurate

sampling, therefore it is important to quickly but gently rub the swab over the ansillar fossa (tonsillar bed).

- Care should be taken not to contaminate the swab by contact with the tongue or the oral mucosa on removal.
- Return swab to the container with transport medium.

Patients that have previously been screened positive for MRSA should in addition to the nose and throat swabs also have swabs taken from the following sites:

- hairline (swab along outline of scalp hair)
- axillae
- groin
- perineum
- any wounds or skin lesions
- umbilicus (only in neonates)
- insertion sites of devices (eg tracheostomy, gastrostomy, central venous catheter)

When taking samples from intact skin the swab should be moistened with sterile 0.9% saline solution before sampling as this assists in the transfer of bacteria from the sampling site to swab and can increase the number of micro-organisms collected.

Sputum

Good quality sputum samples are essential for accurate microbiological diagnosis of pneumonia but also acute tracheitis and bronchitis. Samples contaminated with oro-pharyngeal secretions and saliva are difficult to interpret and can be misleading.

- Encourage the child to cough especially after sleep and expectorate into a container. Alternatively, a poorer sample may be obtained as from naso-pharyngeal/tracheal suction using a sputum trap can be undertaken.
- Physiotherapy may help to facilitate expectoration.
- Ensure the material obtained is sputum and not saliva

Biopsy material (TISSUE SAMPLE)

Specimens such as skin, muscle, kidney, liver, jejunal, tissue or brain biopsies are generally obtained by medical staff either under general or local anaesthetic according to the site. A sterile technique is required for all these procedures. All biopsy specimens must be discussed with the relevant laboratory personnel in order that:

- The most appropriate specimen and laboratory tests are undertaken. If the specimen is small it may be necessary to limit the range of tests.
- Check if the specimen is to be fixed in formalin. DO NOT use formalin if the specimen is for microbiological investigation. In many cases both histopathological and microbiological/virological analysis will be required and it is critical that separate specimens be sent for these purposes so they are processed and transported appropriately.

Wound swabs

Interpretation of results must be in conjunction with clinical signs. In the absence of clinical signs of infection wound swabs will provide little if any useful information and simply reflects colonisation.

- Obtain the specimen prior to any dressing or cleaning procedure of the wound. This will maximise the material obtained and prevent killing of the organism by the use of antiseptics.
- Use a sterile swab and gently rotate on the area to collect exudate from the wound and place into transport medium. Where there is pus collect as much as possible in a sterile syringe or sterile container (do not use a swab) and send to the laboratory.

For detection of *Mycobacterium tuberculosis*, pus collected neat in to a pot or tissue biopsy is preferred, however a calcium alginate swab can be used. The alginate swab gradually dissolves maximising the isolation of the organism as the numbers of organisms are usually small

Fungal samples of hair, nail and skin

Special containers (Dermapak®) may be obtained from the microbiology department:

- Samples of infected hair should be removed by plucking the hair with forceps or gloves. The root of the hair is infected not the shaft.
- Samples of the whole thickness of the nail or deep scrapings should be obtained.
- The skin should be cleaned with an alcohol swab. Epidermal scales scraped from the active edge of a lesion or the roof of any vesicle should be obtained.

Blood samples

Blood sampling should be performed by a healthcare worker trained and competent in the procedure. As there are many haematological, biochemical, immunological and microbiological blood tests the person should seek information as to the appropriate laboratory containers required for specific tests and the amount of blood required.

This information is available on PiMS or in the individual departmental user manuals. Protective clothing such as gloves and aprons (and facial protection when appropriate) must be used along with the aseptic non-touch technique.

The 'Broken Needle Technique' (breaking the hub of the needle to obtain blood from small infants) poses an additional risk of injury to the child and user and must NOT be used.

Blood culture

Detection of microorganisms by culture of blood is essential in the diagnosis of bloodstream infections, including infective endocarditis, infections presenting as pyrexia of unknown origin, prosthetic material infections and intravenous catheter infections. Blood culture may also detect bacteraemia associated with primary infections such as pneumonia and septic arthritis. Accurate positive results provide valuable information to guide optimal antibiotic therapy early on which can improve outcome from these conditions.

On the other hand, contaminated blood cultures can cause considerable diagnostic confusion and lead to unnecessary or sub-optimal antimicrobial therapy.

Contamination may be prevented by careful collection of the blood using the aseptic non-touch-technique. The specimen should also preferably be taken during pyrexial episodes as more bacteria may be present at that time.

Blood cultures should be taken when there is a clinical need to do so in response to any of the following clinical signs suggestive of sepsis and a deteriorating clinical picture including:

- abnormalities in
    1. heart rate
    2. core temperature
    3. leucocyte count
- presence of rigors or chills
- other focal signs of infection, such as pneumonia, septic arthritis, meningism, urinary tract infection including pyelonephritis and acute abdominal pathology.

  Procedure (Department of Health (DH) 2007):
- Use both blood culture bottles (aerobic and anaerobic) and scrub the bung with a 2% chlorhexidine/70% alcohol wipe (eg Clinell®) for 15 seconds and allow to dry prior to inoculation.

- Prior to venepuncture soap and water should be used to clean any visibly soiled skin. The skin must then be decontaminated with a 2% chlorhexidine/70% alcohol applicator (eg Chloraprep®Sepp 0.67 ml) and allowed to dry. Do not re-palpate vein (even with gloved hand) after decontamination.
- After withdrawing the blood, insert the blood into the container with a new sterile needle. There is a risk of contamination of skin organisms on the needle used to withdraw the blood.
- When inoculating the blood culture bottles, the **anaerobic culture bottle should be inoculated first** and then the aerobic culture bottle, so that oxygen trapped in the syringe will not be transferred to the anaerobic bottle.
- Volume of blood is the most critical factor in the detection of blood stream infections. Place up to 4ml in the aerobic bottle (priority) and up to 10 ml in the anaerobic bottle, but ensure that when using both bottles, the anaerobic bottle is inoculated first. For neonates 1-2 mls of blood is recommended. However the sensitivity of neonatal blood cultures is increased if more blood is cultured.
- Inoculation of the blood into the blood culture bottles should be performed first before inserting blood into other bottles as many of these bottles are not sterile and accidental contamination may occur.
- Children with suspected central venous line sepsis, blood for culture may be taken from a peripheral vein stab and also from the appropriate intravascular lines to enable identification of colonisation of the line. In cases of suspected bacterial endocarditis three blood cultures should be taken from separate venepunctures to optimise recovery of bacteria which may be present low in numbers.
- Blood sampling for culture from a peripheral cannula: cultures from should only be taken from newly inserted peripheral cannulae if there is no alternative to obtain a blood sample for culture through a separate venepuncture. Strict asepsis must be maintained. The specimen must be clearly labelled indicating that the blood sample was taken from a peripheral cannula, as the risk of contamination is high.
- A poster with detailed illustration of the blood culture technique is available to GOSH staff internally on the GOSHweb intranet site.

Analysis of antibiotic levels

The relationship between drug dose, drug concentration in biological fluid and the individual child‟s metabolic process must be understood for interpreting results. The results may be affected by the route of administration, age of the child and disease process such as liver and renal disease. The analysis involves testing levels in blood serum or plasma in direct relationship to drug administration.

Routine in house drug level assay is available for amikacin, gentamicin, tobramycin and vancomycin. Other levels are referred and should be discussed.

- Individual antibiotic policies are on the Medicine and Pharmacy intranet web page (available to GOSH staff internally on the GOSHweb intranet site) and available upon request.
- Record on the laboratory form/sample the drug, dose and mode of administration, the time the drug is given and whether the sample is a 'peak' or 'trough' level.
- Trough levels are taken immediately prior to the time a drug is due; peak levels are taken one hour after bolus or infusion finished.
- Blood for antibiotic assay must not be taken through the same catheter which has been used to give the antibiotic at any time. Antibiotics bind to plastic and the drug may release intermittently giving false results.

- Spurious low or high level results may occur if blood is drawn from any central venous catheter and ideally levels should not be drawn from a central venous catheter.

Cerebrospinal fluid

Sampling of cerebrospinal fluid is essential for the accurate diagnosis of infective meningitis and may aid in the diagnosis of encephalitis.

Cerebro-spinal fluid (CSF) is most commonly obtained via a lumbar puncture performed by medical staff. A sterile technique is required as there is a risk of introducing infection itself causing meningitis. Specimens of CSF should be dispatched to the laboratory immediately.

Outside normal working hours it is essential that the on-call laboratory staff are contacted when the sample is being transported. It is important not to store the specimen in a refrigerator as this may cause the cells to deteriorate or lyse giving rise to misleading results.

It is common practice to send three separate collection tubes of CSF when investigating for evidence of sub-arachnoid haemorrhage, as the initial part of the sample may be contaminated with blood from outside the sub-arachnoid space.

If this is performed, it is important to label the tubes as such and specifically request counts on the first and third samples. It is also important to remember that a CSF glucose level (sent separately to chemical pathology) can only be accurately interpreted in conjunction with a simultaneous plasma glucose level.

Urine

Bedside urine testing for the presence of blood, protein and other analytes is usually undertaken with reagent strips the results of which indicate that further laboratory investigation is required.

Most urine samples sent to the microbiology laboratory are for bacteriological investigation. The same collection techniques also apply to samples sent for virological investigation.

Urine samples should be dispatched to the laboratory as soon as possible or no more than four hours if kept at room temperature or up to 24 hours if kept at four degrees centigrade to avoid overgrowth of organisms and misleading results.

All methods used to collect urine samples can result in contamination with bacteria from outside the bladder. This can lead to an inaccurate diagnosis, involve unnecessary treatment or require a sample to be repeated which has implications for patient care and cost effectiveness.

Normal social hygiene, such as washing the genitalia with soap and water and drying thoroughly is considered sufficient to minimise contamination from the skin prior to collection of the specimen. Assess the clinical and psychosocial needs of the child as to whether cleaning the genitalia is necessary. The nurse must be sensitive to the cultural issues surrounding touching intimate parts of the body.

It can be difficult to obtain a urine sample from incontinent or non-toilet trained children and the most popular non-invasive method is the midstream or „clean catch“ specimen.

Midstream or 'clean catch' specimen

The 'clean catch' method is the most popular non-invasive method.

- Ensure that the child“s/young person“s genitalia have been washed with soap and water and dried thoroughly. Ask the child/young person to wash hands with soap and water.
- In the female encourage separation of the labia whilst passing urine.
- In the male encourage retraction of the prepuce, if appropriate, whilst passing urine.
- Ask the child/young person to void a small amount of urine into the toilet first.
- Then ask the child/young person to urinate 10-20 ml directly into the specimen container.
- Instruct the child/young person that the remaining urine can passed into the toilet.

- Place the lid securely on the specimen container. Wipe the outside of the container with a sanitising wipe and place container in polythene transport bag.

Pad or bag specimen

Urine collection pads or urine collection bags are often used for incontinent or non toilet trained children, but are more susceptible to contamination due to close contact with the anogenital area. Urine collection pads as the next best option to clean catch. When using urine collection pads, manufacturer‟s instructions should be followed. Cotton-wool balls, gauze- or sanitary pads should not be used.

Urine collection pads

- Remove nappy and clean perineum or prepuce of the infant with soap and water. Do not apply any creams.
- Place the urine collection pad across vulva or penis in a lengthwise fashion.
- Remove the adhesive backing from the pad and secure to nappy.
- Change urine collection pad every 30-45 minutes and also when the child has passed stool, to reduce the risk of contamination with skin-or faecal flora.
- Once the child has passed urine remove the nappy with the urine collection pad in it.
- Lay the pad down wet side up on an appropriate clean surface.
- Take sterile 5 ml syringe and place the tip on the pad. Extract the urine by pulling up the plunger.
- Repeat until required amount of urine is obtained.
- Empty syringe into a sterile container.

Urine collection bags:

- Select the correct size sterile urine bag to avoid leakage or contamination with faeces.
- Remove nappy and clean the perineum or prepuce of the infant with soap and water.
- Dry area thoroughly and do not apply any creams.
- Remove the protective backing from the bag, then
  - For the female, place the bag over the vulva, starting from the perineum and working upwards, pressing the adhesive to perineum and symphysis.
  - For the male, insert penis and scrotum into the opening of the bag and press adhesive to perineum and symphysis.
- Cut a hole in the diaper and pull the urine bag through the opening.
- Once the child has passed urine, perform hand hygiene, put on gloves and remove the bag.
- Hold the bag over a sterile urine specimen container and cut off the tip of the bottom corner of the bag. Empty the urine into the container.
- Wash the genitalia after the procedure to prevent soreness of the skin.

Suprapubic aspirate

Collection of urine by a supra-pubic aspirate should be considered when a sterile sample is required. Ultrasound guidance should be used to indicate the presence of urine in the bladder before a suprapubic aspirate is attempted.

Catheter specimen

This is collected from the self-sealing bung of the urinary drainage tubing in a child who is already catheterised. Do not disconnect the closed drainage system as infection may be introduced nor take the sample from the urinary drainage bag as the specimen maybe contaminated.

- Using an aseptic non-touch technique, clean the catheter sampling site with 2% chlorhexidine/70% alcohol wipe (eg Clinell®) and allow to dry.

- Collect the urine using appropriate sterile equipment appropriate to access port ie either using a sterile syringe and needle and inserting the needle into the bung at an angle of 45 degrees; this will minimise penetration of the wall of the tubing and subsequent needle stick injury, or a needle-less system.
- Gently withdraw the urine into the syringe.
- Remove the needle and syringe, wipe the area with the alcohol swab and allow to dry. The rubber bung will self-seal.
- Place the urine in a sterile container.
- Discard the needle and syringe into a sharps container.

Obtaining urine from a Mitroffanof stoma

The specimen should be obtained by a nurse who is familiar with the Mitroffanof operation and the specific anatomy of the area on the child.

- The specimen should ideally be taken in conjunction with normal bladder emptying.
- A new sterile catheter of the child‟s normal catheter size should be used.
- Clean the stoma with soap and water and dry.
- Gently insert the lubricated sterile catheter into the stoma and collect the urine into a sterile container. A water-soluble lubricant should be used.
- Ensure the bladder is completely empty before withdrawing the catheter.
- Wipe the area dry with a tissue.

Stool samples

- Please specify whether the sample is a routine (admission) screening sample or an investigation for suspected intestinal infection.
- If viral gastroenteritis (eg norovirus, rotavirus) is suspected, the stool specimen should be sent to the virology laboratory. To exclude a bacterial cause a second stool specimen can be sent to the microbiology laboratory.
- A faecal specimen is more suitable than a rectal swab.
- A specimen can be obtained from a nappy or clean potty/bedpan.
- Use the scoop attached to the inside of the lid of the specimen container to place faecal material into the container.
- Where diarrhoea is present, a small piece of non-absorbent material lining the nappy can be used to prevent material soaking into the nappy.
- Examine the sample for consistency, odour or blood and record observations to monitor changes.
- If segments of tapeworm are seen, send to the laboratory. Tapeworm segments can vary from the size of rice grains to a ribbon shape, one inch long.
- For the identification of *Enterobius vermicularis* (threadworm/pin worm) material should be obtained first thing in the morning on awakening by using a clear adhesive tape (eg Sellotape®) slide. Place the sticky side of a strip of tape over the anal region to obtain the material and stick the tape smoothly onto a glass slide. The worm can then be identified under the microscope. Thread worm lay their ova on the perianal skin at night and therefore will not be seen in a faecal specimen.
- Where acute amoebic dysentery is suspected, the specimen of stool must be freshly dispatched to the laboratory. The parasite causing amoebic dysentery exists in a free-living motile form and in the form of non-motile cysts. Both forms are characteristic in their fresh state but the motile form cannot be identified when dead. „Hot faeces‟ should be discussed with the laboratory prior to collection.

Microbiologic Examination

Direct Examination and Techniques: Direct examination of specimens reveals gross pathology. Microscopy may identify microorganisms. Immunofluorescence, immuno-peroxidase staining, and other immunoassays may detect specific microbial antigens. Genetic probes identify genus- or species-specific DNA or RNA sequences.

Culture: Isolation of infectious agents frequently requires specialized media. Nonselective (noninhibitory) media permit the growth of many microorganisms. Selective media contain inhibitory substances that permit the isolation of specific types of microorganisms.

Microbial Identification: Colony and cellular morphology may permit preliminary identification. Growth characteristics under various conditions, utilization of carbohydrates and other substrates, enzymatic activity, immunoassays, and genetic probes are also used.

Serodiagnosis: A high or rising titer of specific IgG antibodies or the presence of specific IgM antibodies may suggest or confirm a diagnosis.

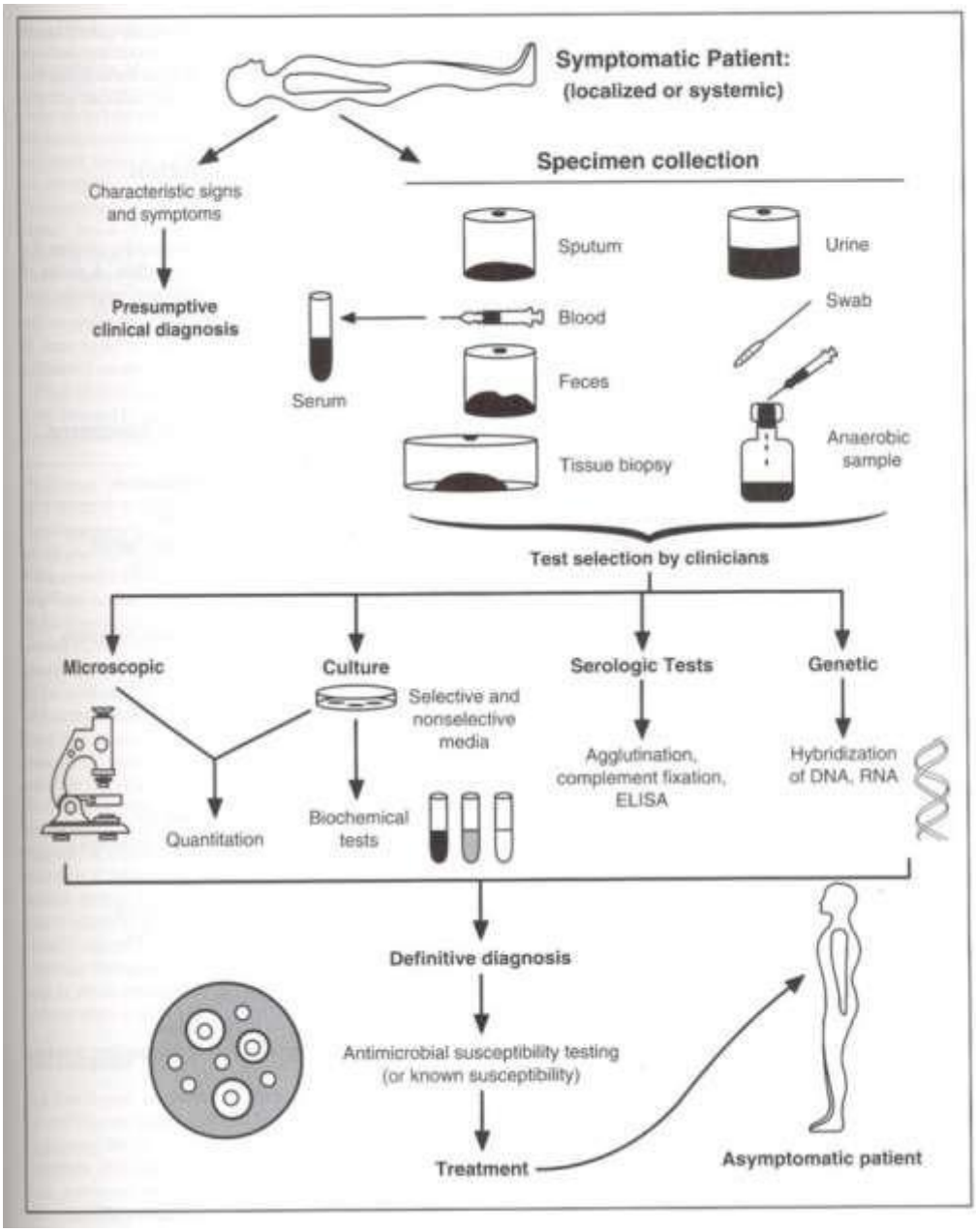
Antimicrobial Susceptibility: Microorganisms, particularly bacteria, are tested in vitro to determine whether they are susceptible to antimicrobial agents.

Introduction

Some infectious diseases are distinctive enough to be identified clinically. Most pathogens, however, can cause a wide spectrum of clinical syndromes in humans. Conversely, a single clinical syndrome may result from infection with any one of many pathogens. Influenza virus infection, for example, causes a wide variety of respiratory syndromes that cannot be distinguished clinically from those caused by streptococci, mycoplasmas, or more than 100 other viruses.

Most often, therefore, it is necessary to use microbiologic laboratory methods to identify a specific etiologic agent. Diagnostic medical microbiology is the discipline that identifies etiologic agents of disease. The job of the clinical microbiology laboratory is to test specimens from patients for microorganisms that are, or may be, a cause of the illness and to provide information (when appropriate) about the in vitro activity of antimicrobial drugs against the microorganisms identified.

Laboratory procedures used in confirming a clinical diagnosis of infectious disease with a bacterial etiology. The staff of a clinical microbiology laboratory should be qualified to advise the physician as well as process specimens. The physician should supply salient information about the patient, such as age and sex, tentative diagnosis or details of the clinical syndrome, date of onset, significant exposures, prior antibiotic therapy, immunologic status, and underlying conditions. The clinical microbiologist participates in decisions regarding the microbiologic diagnostic studies to be performed, the type and timing of specimens to be collected, and the conditions for their transportation and storage. Above all, the clinical microbiology laboratory, whenever appropriate, should provide an interpretation of laboratory results.



The manifestations of an infection depend on many factors, including the site of acquisition or entry of the microorganism; organ or system tropisms of the microorganism; microbial virulence; the age, sex, and immunologic status of the patient; underlying diseases or conditions; and the presence of implanted prosthetic devices or materials. The signs and symptoms of infection may be localized, or they may be systemic, with fever, chills, and hypotension. In some instances the manifestations of an infection are sufficiently characteristic to suggest the diagnosis; however, they are often nonspecific.

Microbial Causes of Infection

Infections may be caused by bacteria (including mycobacteria, chlamydiae, mycoplasmas, and rickettsiae), viruses, fungi, or parasites. Infection may be endogenous or exogenous. In endogenous infections, the microorganism (usually a bacterium) is a component of the patientapos;s indigenous flora. Endogenous infections can occur when the microorganism is aspirated from the upper to the

lower respiratory tract or when it penetrates the skin or mucosal barrier as a result of trauma or surgery. In contrast, in exogenous infections, the microorganism is acquired from the environment (e.g., from soil or water) or from another person or an animal. Although it is important to establish the cause of an infection, the differential diagnosis is based on a careful history, physical examination, and appropriate radiographic and laboratory studies, including the selection of appropriate specimens for microbiologic examination. Results of the history, physical examination, and radiographic and laboratory studies allow the physician to request tests for the microorganisms most likely to be the cause of the infection.

Specimen Selection, Collection and Processing

Specimens selected for microbiologic examination should reflect the disease process and be collected in sufficient quantity to allow complete microbiologic examination. The number of microorganisms per milliliter of a body fluid or per gram of tissue is highly variable, ranging from less than 1 to 10^8 or 10^{10} colony-forming units (CFU). Swabs, although popular for specimen collection, frequently yield too small a specimen for accurate microbiologic examination and should be used only to collect material from the skin and mucous membranes.

Because skin and mucous membranes have a large and diverse indigenous flora, every effort must be made to minimize specimen contamination during collection. Contamination may be avoided by various means. The skin can be disinfected before aspirating or incising a lesion. Alternatively, the contaminated area may be bypassed altogether. Examples of such approaches are transtracheal puncture with aspiration of lower respiratory secretions or suprapubic bladder puncture with aspiration of urine. It is often impossible to collect an uncontaminated specimen, and decontamination procedures, cultures on selective media, or quantitative cultures must be used (see above).

Specimens collected by invasive techniques, particularly those obtained intraoperatively, require special attention. Enough tissue must be obtained for both histopathologic and microbiologic examination. Histopathologic examination is used to distinguish neoplastic from inflammatory lesions and acute from chronic inflammations. The type of inflammation present can guide the type of microbiologic examination performed. If, for example, a caseous granuloma is observed histopathologically, microbiologic examination should include cultures for mycobacteria and fungi. The surgeon should obtain several samples for examination from a single large lesion or from each of several smaller lesions. If an abscess is found, the surgeon should collect several milliliters of pus, as well as a portion of the wall of the abscess, for microbiologic examination. Swabs should be kept out of the operating room.

If possible, specimens should be collected before the administration of antibiotics. Above all, close communication between the clinician and the microbiologist is essential to ensure that appropriate specimens are selected and collected and that they are appropriately examined.

Microbiologic Examination

Direct Examination

Direct examination of specimens frequently provides the most rapid indication of microbial infection. A variety of microscopic, immunologic, and hybridization techniques have been developed for rapid diagnosis.

TABLE 10-1 Rapid Tests Commonly Used to Detect Microorganisms in Specimens

Specimen	Test	Application
Blood	Giemsa	Plasmodia, microfilarie
	EIA	Hepatitis A and B virus, human immunodeficiency virus
Cerebrospinal fluid	Gram stain	Bacteria
	LA; COA	Haemophilus influenzae, Neisseria meningitidis
	India ink wet mount or LA	Streptococcus pneumoniae, Cryptococcus neoformans
Wound exudates, pus	Gram stain	Bacteria
Respiratory secretions	Gram stain	Bacteria
	Acid-fast stain	Mycobacteria, nocardiae
	IFA or genetic probe	*Legionella* species, *Streptococcus pyogenes*
	KOH wet mount	Fungi
	Gomori methenamine silver stain	Fungi, *Pneumocystis carinii*
	FA, EIA	Respiratory syncytial virus
Urine	Gram stain	Bacteria
Urethral or cervical scrapings or exudates	Gram stain, EIA, IFA, EIA, or genetic probe	Neisseria gonorrhoeae *Chlamydia trachomatis*, papillomaviruses
Genital ulcer	FA, EIA, or genetic probe	Herpes simplex virus
Feces	Methylene blue stain	Leukocytes
	Eosin wet mount, trichrome stain	Parasites
	EM, LA, EIA	Rotaviruses
	EIA	Adenoviruses, *Clostridium difficile*

Abbreviations: COA, coagglutination; EIA, enzyme immunoassay; IFA, immunofluorescent antibody; LA, latex agglutination.

Rapid Tests Commonly Used to Detect Microorganisms in Specimens.

Sensitivity and Specificity

The sensitivity of a technique usually depends on the number of microorganisms in the specimen. Its specificity depends on how morphologically unique a specific microorganism appears microscopically or how specific the antibody or genetic probe is for that genus or species. For example, the sensitivity of Cram stains is such that the observation of two bacteria per oil immersion field (X 1,000) of a Gram-stained smear of uncentrifuged urine is equivalent to the presence of $\geq 10^5$ CFU/ml of urine. The sensitivity of the Gram-stained smear for detecting Gram-negative coccobacilli in cerebrospinal fluid from children with *Haemophilus influenzae* meningitis is approximately 75 percent because in some patients the number of colony-forming units per milliliter of cerebrospinal fluid is less than 10^4. At least 10^4 CFU of tubercle bacilli per milliliter of sputum must be present to be detected by an acid-fast smear of decontaminated and concentrated sputum.

An increase in the sensitivity of a test is often accompanied by a decrease in specificity. For example, examination of a Gram-stained smear of sputum from a patient with pneumococcal pneumonia is highly sensitive but also highly nonspecific if the criterion for defining a positive test is the presence of any Gram-positive cocci. If, however, a positive test is defined as the presence of a preponderance of Gram-positive, lancet-shaped diplococci, the test becomes highly specific but has a sensitivity of only about 50 percent. Similar problems related to the number of microorganisms present affect the sensitivity of immunoassays and genetic probes for bacteria, chlamydiae, fungi and viruses. In some instances, the sensitivity of direct examination tests can be improved by collecting a better specimen. For example, the sensitivity of fluorescent antibody stain for *Chlamydia trachomatis* is higher when endocervical cells are obtained with a cytobrush than with a swab. The sensitivity may also be affected by the stage of the disease at which the specimen is collected. For example, the detection of herpes simplex virus by immunofluorescence, immunoassay, or culture is highest when specimens from lesions in the vesicular stage of infection are examined. Finally, sensitivity may be improved through

the use of an enrichment or enhancement step in which microbial or genetic replication occurs to the point at which a detection method can be applied.

Techniques

For microscopic examination it is sufficient to have a compound binocular microscope equipped with low-power (1OX), high-power (40X), and oil immersion (1OOX) achromatic objectives, 10X wide-field oculars, a mechanical stage, a substage condenser, and a good light source. For examination of wet-mount preparations, a darkfield condenser or condenser and objectives for phase contrast increases image contrast. An exciter barrier filter, darkfield condenser, and ultraviolet light source are required for fluorescence microscopy.

For immunologic detection of microbial antigens, latex particle agglutination, coagglutination, and enzyme-linked immunosorbent assay (ELISA) are the most frequently used techniques in the clinical laboratory. Antibody to a specific antigen is bound to latex particles or to a heat-killed and treated protein A-rich strain of *Staphylococcus aureus* to produce agglutination. There are several approaches to ELISA; the one most frequently used for the detection of microbial antigens uses an antigen-specific antibody that is fixed to a solid phase, which may be a latex or metal bead or the inside surface of a well in a plastic tray. Antigen present in the specimen binds to the antibody The test is then completed by adding a second antigen-specific antibody bound to an enzyme that can react with a substrate to produce a colored product. The initial antigen antibody complex forms in a manner similar. When the enzyme-conjugated antibody is added, it binds to previously unbound antigenic sites, and the antigen is, in effect, sandwiched between the solid phase and the enzyme-conjugated antibody. The reaction is completed by adding the enzyme substrate.

Agglutination test in which inert particles (latex beads or heat-killed *S aureus* Cowan 1 strain with protein A) are coated with antibody to any of a variety of antigens and then used to detect the antigen in specimens or in isolated bacteria.

Genetic probes are based on the detection of unique nucleotide sequences with the DNA or RNA of a microorganism. Once such a unique nucleotide sequence, which may represent a portion of a virulence gene or of chromosomal DNA, is found, it is isolated and inserted into a cloning vector (plasmid), which is then transformed into Escherichia coli to produce multiple copies of the probe. The sequence is then reisolated from plasmids and labeled with an isotope or substrate for diagnostic use. Hybridization of the sequence with a complementary sequence of DNA or RNA follows cleavage of the double-stranded DNA of the microorganism in the specimen.

The use of molecular technology in the diagnoses of infectious diseases has been further enhanced by the introduction of gene amplication techniques, such as the polymerase chain reaction (PCR) in which DNA polymerase is able to copy a strand of DNA by elongating complementary strands of DNA that have been initiated from a pair of closely spaced oligonucleotide primers. This approach has had major applications in the detection of infections due to microorganisms that are difficult to culture (e.g. the human immunodeficiency virus) or that have not as yet been successfully cultured (e.g. the Whipple's disease bacillus).

Culture

In many instances, the cause of an infection is confirmed by isolating and culturing microorganism either in artificial media or in a living host. Bacteria (including mycobacteria and mycoplasmas) and fungi are cultured in either liquid (broth) or on solid (agar) artificial media. Liquid media provide greater sensitivity for the isolation of small numbers of microorganisms; however, identification of mixed cultures growing in liquid media requires subculture onto solid media so that isolated colonies can be processed separately for identification. Growth in liquid media also cannot ordinarily be quantitated. Solid media, although somewhat less sensitive than liquid media, provide isolated

colonies that can be quantified if necessary and identified. Some genera and species can be recognized on the basis of their colony morphologies.

In some instances one can take advantage of differential carbohydrate fermentation capabilities of microorganisms by incorporating one or more carbohydrates in the medium along with a suitable pH indicator. Such media are called differential media (e.g., eosin methylene blue or MacConkey agar) and are commonly used to isolate enteric bacilli. Different genera of the Enterobacteriaceae can then be presumptively identified by the color as well as the morphology of colonies.

Culture media can also be made selective by incorporating compounds such as antimicrobial agents that inhibit the indigenous flora while permitting growth of specific microorganisms resistant to these inhibitors. One such example is Thayer-Martin medium, which is used to isolate *Neisseria gonorrhoeae*. This medium contains vancomycin to inhibit Gram-positive bacteria, colistin to inhibit most Gram-negative bacilli, trimethoprim-sulfamethoxazole to inhibit *Proteus*species and other species that are not inhibited by colistin and anisomycin to inhibit fungi. The pathogenic *Neisseria* species, *N gonorrhoeae* and *N meningitidis*, are ordinarily resistant to the concentrations of these antimicrobial agents in the medium.

The number of bacteria in specimens may be used to define the presence of infection. For example, there may be small numbers ($\leq 10^3$ CFU/ml) of bacteria in clean-catch, midstream urine specimens from normal, healthy women; with a few exceptions, these represent bacteria that are indigenous to the urethra and periurethral region. Infection of the bladder (cystitis) or kidney (pyelone-phritis) is usually accompanied by bacteriuria of about $\geq 10^4$ CFU/ml. For this reason, quantitative cultures of urine must always be performed. For most other specimens a semiquantitative streak method over the agar surface is sufficient. For quantitative cultures, a specific volume of specimen is spread over the agar surface and the number of colonies per milliliter is estimated. For semiquantitative cultures, an unquantitated amount of specimen is applied to the agar and diluted by being streaked out from the inoculation site with a sterile bacteriologic loop The amount of growth on the agar is then reported semiquantitatively as many, moderate, or few (or 3+, 2+, or 1+), depending on how far out from the inoculum site colonies appear. An organism that grows in all streaked areas would be reported as 3+.

Chlamydiae and viruses are cultured in cell culture systems, but virus isolation occasionally requires inoculation into animals, such as suckling mice, rabbits, guinea pigs, hamsters, or primates. Rickettsiae may be isolated with some difficulty and at some hazard to laboratory workers in animals or embryonated eggs. For this reason, rickettsial infection is usually diagnosed serologically. Some viruses, such as the hepatitis viruses, cannot be isolated in cell culture systems, so that diagnosis of hepatitis virus infection is based on the detection of hepatitis virus antigens or antibodies.

Cultures are generally incubated at 35 to 37°C in an atmosphere consisting of air, air supplemented with carbon dioxide (3 to 10 percent), reduced oxygen (microaerophilic conditions), or no oxygen (anaerobic conditions), depending upon requirements of the microorganism. Since clinical specimens from bacterial infections often contain aerobic, facultative anaerobic, and anaerobic bacteria, such specimens are usually inoculated into a variety of general purpose, differential, and selective media, which are then incubated under aerobic and anaerobic conditions

General procedure for collecting and processing specimens for aerobic and/or anaerobic bacterial culture.

The duration of incubation of cultures also varies with the growth characteristics of the microorganism. Most aerobic and anaerobic bacteria will grow overnight, whereas some mycobacteria require as many as 6 to 8 weeks.

Microbial Identification

Microbial growth in cultures is demonstrated by the appearance of turbidity, gas formation, or discrete colonies in broth; colonies on agar; cytopathic effects or inclusions in cell cultures; or detection of genus- or species-specific antigens or nucleotide sequences in the specimen, culture medium, or cell culture system.

Identification of bacteria (including mycobacteria) is based on growth characteristics (such as the time required for growth to appear or the atmosphere in which growth occurs), colony and microscopic morphology, and biochemical, physiologic, and, in some instances, antigenic or nucleotide sequence characteristics. The selection and number of tests for bacterial identification depend upon the category of bacteria present (aerobic versus anaerobic, Gram-positive versus Gram-negative, cocci versus bacilli) and the expertise of the microbiologist examining the culture. Gram-positive cocci that grow in air with or without added CO_2 may be identified by a relatively small number of tests (seeCh.12). The identification of most Gram-negative bacilli is far more complex and often requires panels of 20 tests for determining biochemical and physiologic characteristics. The identification of filamentous fungi is based almost entirely on growth characteristics and colony and microscopic morphology. Identification of viruses is usually based on characteristic cytopathic effects in different cell cultures or on the detection of virus- or species-specific antigens or nucleotide sequences.

Interpretation of Culture Results

Some microorganisms, such as *Shigella dysenteriae*, *Mycobacterium tuberculosis*, *Coccidioides immitis*, and influenza virus, are always considered clinically significant. Others that ordinarily are harmless components of the indigenous flora of the skin and mucous membranes or that are common in the environment may or may not be clinically significant, depending on the specimen source from which they are isolated. For example, coagulase-negative staphylococci are normal inhabitants of the skin, gastrointestinal tract, vagina, urethra, and the upper respiratory tract (i.e., of the nares, oral cavity, and pharynx). Therefore, their isolation from superficial ulcers, wounds, and sputum cannot usually be interpreted as clinically significant. They do, however, commonly cause infections associated with intravascular devices and implanted prosthetic materials. However, because intravascular devices penetrate the skin and since cultures of an implanted prosthetic device can be made only after incision, the role of coagulase-negative staphylococci in causing infection can usually be surmised only when the microorganism is isolated in large numbers from the surface of an intravascular device, from each of several sites surrounding an implanted prosthetic device, or, in the case of prosthetic valve endocarditis, from several separately collected blood samples. Another example, *Aspergillus fumigatus*, is widely distributed in nature, the hospital environment, and upper respiratory tract of healthy people but may cause fatal pulmonary infections in leukemia patients or in those who have undergone bone marrow transplantation. The isolation of *A fumigatus* from respiratory secretions is a nonspecific finding, and a definitive diagnosis of invasive aspergillosis requires histologic evidence of tissue invasion.

Physicians must also consider that the composition of microbial species on the skin and mucous membranes may be altered by disease, administration of antibiotics, endotracheal or gastric intubation, and the hospital environment. For example, potentially pathogenic bacteria can often be cultured from the pharynx of seriously ill, debilitated patients in the intensive care unit, but may not cause infection.

Serodiagnosis

Infection may be diagnosed by an antibody response to the infecting microorganism. This approach is especially useful when the suspected microbial agent either cannot be isolated in culture by any known method or can be isolated in culture only with great difficulty. The diagnosis of hepatitis virus and Epstein-Barr virus infections can be made only serologically, since neither can be isolated in any

known cell culture system. Although human immunodeficiency virus type 1 (HIV-1) can be isolated in cell cultures, the technique is demanding and requires special containment facilities. HIV-1 infection is usually diagnosed by detection of antibodies to the virus.

The disadvantage of serology as a diagnostic tool is that there is usually a lag between the onset of infection and the development of antibodies to the infecting microorganism. Although IgM antibodies may appear relatively rapidly, it is usually necessary to obtain acute- and convalescent-phase serum samples to look for a rising titer of IgG antibodies to the suspected pathogen. In some instances the presence of a high antibody titer when the patient is initially seen is diagnostic; often, however, the high titer may reflect a past infection, and the current infection may have an entirely different cause. Another limitation on the use of serology as a diagnostic tool is that immunosuppressed patients may be unable to mount an antibody response.

Antimicrobial Susceptibility

The responsibility of the microbiology laboratory includes not only microbial detection and isolation but also the determination of microbial susceptibility to antimicrobial agents. Many bacteria, in particular, have unpredictable susceptibilities to antimicrobial agents, and their susceptibilities can be measured in vitro to help guide the selection of the most appropriate antimicrobial agent.

Antimicrobial susceptibility tests are performed by either disk diffusion or a dilution method. In the former, a standardized suspension of a particular microorganism is inoculated onto an agar surface to which paper disks containing various antimicrobial agents are applied. Following overnight incubation, any zone diameters of inhibition about the disks are measured and the results are reported as indicating susceptibility or resistance of the microorganism to each antimicrobial agent tested. An alternative method is to dilute on a $\log_2$ scale each antimicrobial agent in broth to provide a range of concentrations and to inoculate each tube or, if a microplate is used, each well containing the antimicrobial agent in broth with a standardized suspension of the microorganism to be tested. The lowest concentration of antimicrobial agent that inhibits the growth of the microorganism is the minimal inhibitory concentration (MIC). The MIC and the zone diameter of inhibition are inversely correlated. In other words, the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. Conversely, the more resistant the microorganism, the higher the MIC and the smaller the zone of inhibition.

Two methods for performing antibiotic susceptibility tests. (A) Disk diffusion method. (B) Minimum inhibitory concentration (MIC) method. In the example shown, two different microorganisms are tested by both methods against the same antibiotic. The MIC (A) Disk diffusion method. (B) Minimum inhibitory concentration (MIC) method. In the example shown, two different microorganisms are tested by both methods against the same antibiotic. The MIC of the antibiotic for the susceptible microorganism is 8 μg/ml. The corresponding disk diffusion test shows a zone of inhibition surrounding the disk. In the second sample, a resistant microorganism is not inhibited by the highest antibiotic concentration tested (MIC $\geq$ 16 μg/ml) and there is no zone of inhibition surrounding the disk. The diameter of the zone of inhibition is inversely related to the MIC.

The term susceptible means that the microorganism is inhibited by a concentration of antimicrobial agent that can be attained in blood with the normally recommended dose of the antimicrobial agent and implies that an infection caused by this microorganism may be appropriately treated with the antimicrobial agent. The term resistant indicates that the microorganism is resistant to concentrations of the antimicrobial agent that can be attained with normal doses and implies that an infection caused by this microorganism could not be successfully treated with this antimicrobial agent.

Method of transport of clinical samples to laboratory and storage

Abscess material may be collected using the Culturette System, Port-A-Cul system or a sterile syringe and needle. The Culturette System is used only for superficial lesions. This type of specimen will not be cultured for anaerobes. Abscess specimens will only be processed for anaerobes if collected using either the Port-A-Cul system or a syringe and needle. Specimens collected using a sterile syringe and needle must not be transported with the needle on the syringe. Needles must be carefully removed and properly discarded and the syringe cap aseptically replaced on the syringe tip. All specimens must be properly labeled with the patient's name, medical record number, location and the time and date the specimen was collected. Specimens are transported at room temperature and must reach NHRMC lab within 8 hours of collection.

Specimens are collected into a sterile, screw-cap container. Specimen containers must be labeled with the patient's name, medical record number, location, and the date and time the specimen was collected. Specimens transported at room temperature must reach NHRMC lab within 2 hours of collection. Alternatively, specimens are transported at refrigeration temperatures and must reach NHRMC lab within 8 hours of collection.

Catheter Tips (Intravascular)

Catheter tips are collected into a sterile, screw-cap container. Specimen containers must be labeled with the patient's name, medical record number, location, and the date and time the specimen was collected. Specimens transported at room temperature must reach NHRMC lab within 2 hours of collection. Alternatively, specimens are transported at refrigeration temperatures and must reach NHRMC lab within 8 hours of collection.

Cerebrospinal Fluid (CSF)

Cerebrospinal fluid is collected into sterile, screw-cap tubes or containers. A minimum of 2 mL is required. Specimen containers must be labeled with the patient's name, medical record number, location, and the date and time the specimen was collected. Specimens are transported at room temperature and must reach NHRMC lab within 4 hours of collection.

Stool

Specimes are collected into a clean, leak-proof container (tight fitting lid or screw - cap). All specimens must be properly labeled with the patient's name, medical record number, location and the time and date the specimen was collected. Specimens are transported at refrigeration temperature and must reach NHRMC lab within 8 hours of collection.

Specimes are collected into a clean, leak-proof container (tight fitting lid or screw - cap). All specimens must be properly labeled with the patient's name, medical record number, location and the time and date the specimen was collected. Specimens are transported at refrigeration temperature and must reach NHRMC lab within 8 hours of collection. This test is molecular and only one test per patient is needed since it is highly specific and highly sensitive. Testing will only be performed on samples that meet the criteria for toxigenic *C. difficile* infection (diarrheal or unformed stools only).

Intravascular Catheter Tips - please refer to Catheter Tips (Intravascular) Sputum

Specimens are collected in a sterile, screw-cap container. A minimum of 2 mL must be collected. Recommended volume is between 5 and 10 ml. All specimens must be properly labeled with the patient's name, medical record number, location and the time and date the specimen was collected. Specimens transported at room temperature must reach NHRMC lab within 2 hours of collection. Alternatively, specimens transported at refrigeration temperature must reach NHRMC lab within 8 hours of collection.

Throat (Group A Strep antigen detection)

Throat swabs are collected using the Culturette system. Following collection, the moisture ampule must be crushed. All specimens must be properly labeled with the patient's name, medical record number,

location and the time and date the specimen was collected. Specimens are transported at room temperature and must reach NHRMC lab within 8 hours of collection.

Specimens are collected using the Culturette system. Following collection, the moisture ampule must be crushed. All specimens must be properly labeled with the patient's name, medical record number, location and the time and date the specimen was collected. Specimens transported at room temperature must reach NHRMC lab within 2 hours of collection. Alternatively, specimens transported at refrigeration temperature must reach NHRMC lab within 8 hours of collection.

URINE

Specimens are collected using the Culturette system. Following collection, the moisture ampule must be crushed. All specimens must be properly labeled with the patient's name, medical record number, location and the time and date the specimen was collected. Specimens are transported at room temperature and must reach NHRMC lab within 8 hours of collection.

Specimens are collected using the LCx STD Swab Specimen Collection and transportation Kit. Do not use the large-tipped cleaning swab for specimen collection. Swab specimens that are moderately bloody or grossly mucoid must not be tested as these levels of blood and mucus may interfere with the assay. Each specimen tube must be properly labeled with the patient's name, medical record number, location and the time and date the specimen was collected. Specimens are transported at either room temperature or refrigeration temperature and must reach NHRMC lab within 8 hours of collection.

Specimens are collected in a sterile, screw-cap container. A minimum of 10 mL is required. Each specimen tube must be properly labeled with the patient's name, medical record number, location and the time and date the specimen was collected. For additional formation, please see: Collection, Transportation and Handling of Urine Specimens.

Unpreserved specimens are transported at room temperature and must reach the laboratory within 2 hours of collection.

Chemically preserved specimens are transported at room temperature and must reach the laboratory within 24 hours of collection.

Specimens may be preserved refrigerated and must reach the laboratory within 24 hours of collection.

Possible Questions

Part B

1. Define medical diagnosis
2. Say about human body systems
3. What are clinical samples?
4. What are pathogens and pathogenicity?
5. What is PCR?

Part C

1. Explain in detail about the collection of samples
2. Describe the lab diagnosis of UTI.
3. Illustrate the morphology and pathogenesis of URT pathogens Write about the transport media and its importance.
4. Discuss about the investigation by culturing.
5. Describe various sources and methods of infection.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB **COURSE NAME: MICROBIAL DIAGNOSIS IN HEALTH CLINIC**

COURSE CODE: 18MBU304B **UNIT: II** **BATCH-2018-2021**

Unit II Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
The sputum specimen must be stored at _____	10^0c	5^0c	4^0c	8^0c	4^0c
The blood samples should be collected at the_____stage of the disease.	Late	Early	Before Diagnosis	After Symptom	Early
When a parasite is growing and multiplying within a host, it is said have_____	Pathogen	Infection	Organism	Diagnose	Infection
For microbiological examination urine must be collected as a specimen_____	Good	Sterile	Normal	Clean Catch Mid-Stream	Clean Catch Mid-Stream
Cary-Blair medium is used for the transportation of_____ specimen.	Blood	Csf	Stool	Urine	Stool
A parasite organism or agent that produce such a disease is a	Pathagon	Parasite	Bacteria	Virus	Pathagon
The ability of an organism to cause disease is called	Diagnosis	Symptoms	Pathogenicity	Disease	Pathogenicity
The most important step in the diagnosis of an infection is the proper collection of_____.	Specimen	Organism	Symptoms	Prophylaxis	Specimen
In case of meningeal irritation or affected cerebrum _____ specimen is collected.	Blood	Cerebrospinal Fluid	Urine	Pus	Cerebrospinal Fluid
In blood specimens____s separated and used for serological techniques.	Haemoglobin	Serum	Iron	Protein	Serum
The collected CSF should be stored at	Room Temperature	4^0c	2^0c	5^0c	Room Temperature
_____ is a good transport medium for stool sample.	Agar Medium	Peptone Water	Cary-Blair Medium	Alkaline Medium	Cary-Blair Medium
An alternate transport medium for stool sample is_____	Alkaline-Peptone Water	Peptone Water	Alkaline Medium	Water	Alkaline-Peptone Water
A sterile____is used for transportation of specimens to labs.	Container	Plastic Container	Screw-Cap Container	Metal Container	Screw-Cap Container
Specimens must be collected before administration of _____	Vaccines	Antimicrobial Drugs	Symptoms	Culturing	Antimicrobial Drugs
To eliminate normal flora skin surface is cleansed with_____.	Germicide	Soap	Vaseline	Cotton	Germicide
Pus swabs collected in transport medium should reach lab within _____hours	2	4	6	8	6

During blood collection the skin should be vein punctured by cleansing with_____.	Isopropyl Alcohol	Antiseptic	Soap	Antibiotic	Isopropyl Alcohol
About_____ml of blood should be collected.	10	20	30	40	20
The blood samples should be incubated at_____.	25^0c	37^0c	4^0c	10^0c	37^0c
_____ml of urine sample should be collected.	10	40	30	20	20
In case of_____only few millilitres of urine specimen can be collected.	Arthritis	Gas Gangrene	Renal Failure	Paralysis	Renal Failure
If the urine sample is delayed for more than an hour _____ can be added.	Amino Acid	Boric Acid	Sodium Chlloride	Calcium Chloride	Boric Acid
In case of suspected renal tuberculosis the urine should be collected for_____successive mornings	1	2	3	4	3
The CSF should be collected from _____	Cerebrum	Cerebellum	Ventricle	Arachnoid Space	Arachnoid Space
In case of infants the CSF should be collected by puncturing _____.	Ventricle	Arachnoid Space	Cerebrum	Cerebellum	Ventricle
About_____ml of CSF should be collected in sterile tubes.	10	30	40	20	20
For collecting nasopharyngeal aspirates_____is passed gently through one nostril.	Sterile Catheter	Syringe	Swab	Cotton	Sterile Catheter
_____is collected in case of eye specimens.	Pus	Mucous	Conjuctival Scrapings	Conjunctival Tissue	Conjuctival Scrapings
Respiratory secretions should be transported to laboratory within _____.hours	1	2	3	4	2
The natural secretions of eye contains_____enzymes	Antibiotic	Toxins	Antibacterial	Amylase	Antibacterial
If not possible to obtain stool a specimen is collected by inserting _____	Cotton Wool Swab	Syringe	Catheter	Cotton	Cotton Wool Swab
Salmonella, Shigella and Vibrio survive in Cary Blair medium for_____hours.	24	48	32	76	48
Campylobacter can survive in C-B medium for__hours.	2	4	6	8	6
If cholera is suspected the stool sample should be sent to the lab within _____	1	3	5	8	8
_____is the transportation medium used in case of cholera.	CB Medium	Boric Acid	Alkaline Peptone	Phosphate Buffered	Alkaline Peptone Water

			Water	Saline	
For suspected viral enteritis the stool sample is transferred to 9ml of _____	Phosphate Buffered Saline	Alkaline Peptone Water	CB Medium	Boric Acid	Phosphate Buffered Saline
About_____ml of sterile phosphate buffered saline is needed for suspected viral enteritis	3	6	9	12	9
_____is the transportation medium used for the collection of synovial, pleural or asceptic fluid samples	Trisodium Citrate	Sodium Chlloride	Boric Acid	Calcium Chloride	Trisodium Citrate
About_____ml of synovial, pleural and ascitic samples were collected.	2 To 3	5 To 6	3 To 5	4 To 6	2 To 3
Synovial, pleural and ascitic fluids should be mixed with _____.	Antibiotic	Antiseptic	Anticoagulant	Antibacterial	Anticoagulant
In case of sputum sample the sample should be collected best in _____.	Morning	Evening	Mid Night	Noon	Morning
_____bacilli requires entirely different method of identification	Rod Shaped	Spindle Shaped	Club Shaped	Acid-Fast	Acid-Fast
In suspected anthrax the pus sample should be collected from _____.	Lesions	Scrapings	Edema	Necrosis	Lesions
For darkfield examination pus from syphilitic ulcers are suspected in 0.2ml of_____.	Salt Solution	Saline Solution	Anticoagulant	Antiseptic	Saline Solution
In case of tuberculosis_____specimen is collected	Stool	Urine	Blood	Sputum	Sputum
The sputum sample collected in paper towel should be soaked in	Saline Solution	Anticoagulant	Formalin Solution	Salt Solution	Formalin Solution
_____technique is used to stain acid fast bacilli	Ziehl Neelsen	Gram Staining	Endospore Staining	Quellung	Ziehl Neelsen
In bacilliary dysentry_____yields more positive cultures than stools.	Smears	Swabs	Rectal Swabs	Renal Smears	Rectal Swabs
In case of salmonellosis_____sample is collected.	Urine	Stool	Sputum	Blood	Blood
In case of thyphoid_____sample should be collected.	Sputum	Blood	Faeces	CSF	Blood
In order to avoid drying of stool sample the swabs should be placed in_____.	Thioglycollate Semisolid Medium	Sodium Citrate	Buffered Glycerol Water	Para Amino Benzoic Acid	Thioglycollate Semisolid Medium

A n alternative for Thioglycollate semisolid medium is_____.	Sodium Citrate	Buffered Glycerol Water	Para Amino Benzoic Acid	Thioglycollate Semisolid Medium	Buffered Glycerol Water
_____should be incorporated in all blood culture media	Trisodium Citrate	Formalin Solution	Sodium Citrate	Para Amino Benzoic Acid	Para Amino Benzoic Acid
_____is added to the blood sample to prevent coagulation	Sodium Citrate	Thioglycollate Semisolid Medium	Antiseptic	Antibacterial	Sodium Citrate
Once the specimen is collected the container should be_____.	Labelled	Opened	Diluted	Defined	Labelled
Samples of blood and CSF for culture should not be _____	Labelled	Refrigerated	Processed	Incubated	Refrigerated
The sputum sample should be free from _____	Blood	Pus	Saliva	Tissue	Saliva
When the patient has symptoms of cystitis_____should be collected.	Blood	Pus	Sputum	Urine	Urine
In case of suspected septicemia_____sample should be collected	Blood	Pus	Sputum	Urine	Blood

UNIT-III
SYLLABUS

Examination of sample by staining – Gram stain, Ziehl-Neelson staining for tuberculosis, Giemsa-stained thin blood film for malaria. Preparation and use of culture media - Blood agar, Chocolate agar, Lowenstein-Jensen medium, MacConkey agar, distinct colony properties of various bacterial pathogens.

Staining Techniques

Because microbial cytoplasm is usually transparent, it is necessary to stain microorganisms before they can be viewed with the light microscope. In some cases, staining is unnecessary, for example when microorganisms are very large or when motility is to be studied, and a drop of the microorganisms can be placed directly on the slide and observed. A preparation such as this is called a **wet mount.** A wet mount can also be prepared by placing a drop of culture on a cover-slip (a glass cover for a slide) and then inverting it over a hollowed-out slide. This procedure is called the **hanging drop.**In preparation for staining, a small sample of microorganisms is placed on a slide and permitted to air dry. The smear is heat fixed by quickly passing it over a flame. **Heat fixing** kills the organisms, makes them adhere to the slide, and permits them to accept the stain.

Simple staining technique.

Staining can be performed with basic dyes such as crystal violet or methylene blue, positively charged dyes that are attracted to the negatively charged materials of the microbial cytoplasm. Such a procedure is the **simple stain procedure.** An alternative is to use a dye such as nigrosin or Congo red, acidic, negatively charged dyes. They are repelled by the negatively charged cytoplasm and gather around the cells, leaving the cells clear and unstained. This technique is called the **negative stain technique.**

Differential staining technique.

The **differential stain technique** distinguishes two kinds of organisms. An example is the **Gram stain technique.** This differential technique separates bacteria into two groups, Gram-positive bacteria and Gram-negative bacteria. Crystal violet is first applied, followed by the mordant iodine, which fixes the stain. Another differential stain technique is the **acid-fast technique.** This technique differentiates species of *Mycobacterium* from other bacteria. Heat or a lipid solvent is used to carry the first stain, carbolfuchsin, into the cells. Then the cells are washed with a dilute acid-alcohol solution. *Mycobacterium* species resist the effect of the acid-alcohol and retain the carbolfuchsin stain (bright red). Other bacteria lose the stain and take on the subsequent methylene blue stain (blue). Thus, the acid-fast bacteria appear bright red, while the nonacid-fast bacteria appear blue when observed under oil-immersion microscopy. Other stain techniques seek to identify various bacterial structures of importance. For instance, a special stain technique highlights the **flagella** of bacteria by coating the flagella with dyes or metals to increase their width. Flagella so stained can then be observed.

A special stain technique is used to examine bacterial **spores.** Malachite green is used with heat to force the stain into the cells and give them color. A counterstain, safranin, is then used to give color to the nonsporeforming bacteria. At the end of the procedure, spores stain green and other cells stain red. Other stain techniques seek to identify various bacterial structures of importance. For instance, a special stain technique highlights the flagella of bacteria by coating the flagella with dyes or metals to increase their width. Flagella so stained can then be observed.

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Materials Required:

1. Clean glass slides
2. Inoculating loop
3. Bunsen burner
4. Bibulous paper
5. Microscope
6. Lens paper and lens cleaner
7. Immersion oil
8. Distilled water
9. 18 to 24 hour cultures of organisms

Reagents:

1. Primary Stain - Crystal Violet
2. Mordant - Grams Iodine
3. Decolourizer - Ethyl Alcohol
4. Secondary Stain - Safranin

Procedure:

Preparation of the glass microscopic slide

Grease or oil free slides are essential for the preparation of microbial smears. Grease or oil from the fingers on the slides is removed by washing the slides with soap and water. Wipe the slides with spirit or alcohol. After cleaning, dry the slides and place them on laboratory towels until ready for use.

Labeling of the slides

Drawing a circle on the underside of the slide using a glassware-marking pen may be helpful to clearly designate the area in which you will prepare the smear. You may also label the slide with the initials of the name of the organism on the edge of the slide. Care should be taken that the label should not be in contact with the staining reagents.

Preparation of the smear

- Bacterial suspensions in broth: With a sterile cooled loop, place a loopful of the broth culture on the slide. Spread by means of circular motion of the inoculating loop to about one centimeter in diameter. Excessive spreading may result in disruption of cellular arrangement. A satisfactory smear will allow examination of the typical cellular arrangement and isolated cells.
- Bacterial plate cultures: With a sterile cooled loop, place a drop of sterile water or saline solution on the slide. Sterilize and cool the loop again and pick up a very small sample of a bacterial colony and gently stir into the drop of water/saline on the slide to create an emulsion.
- Swab Samples: Roll the swab over the cleaned surface of a glass slide**.**

Heat Fixing

Heat fixing kills the bacteria in the smear, firmly adheres the smear to the slide, and allows the sample to more readily take up stains.

- Allow the smear to air dry.

- After the smear has air-dried, hold the slide at one end and pass the entire slide through the flame of a Bunsen burner two to three times with the smear-side up.

Gram Stain Procedure

1. Place slide with heat fixed smear on staining tray.
   Gently flood smear with crystal violet and let stand for 1 minute.
2. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
3. Gently flood the smear with Gram‟s iodine and let stand for 1 minute.
4. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
5. Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize.
6. Immediately rinse with water.
7. Gently flood with safranin to counter-stain and let stand for 45 seconds.
8. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
9. Blot dry the slide with bibulous paper.
10. View the smear using a light-microscope under oil-immersion.

Ziehl Neelsen Staining for tuberculosis

Ziehl-Neelsen (ZN) method of Acid Fast staining technique is used to stain *Mycobacterium*species including *M. tuberculosis, M. ulcerans, and* **M. leprae** and nontuberculous mycobacteria (NTM).
Detection of acid-fast bacilli (AFB) in stained and acid-washed smears examined microscopically may provide the initial bacteriologic evidence of the presence of mycobacteria in a clinical specimen. Smear microscopy is the quickest and easiest procedure that can be performed.
The cellwall of Mycobacteria contain high concentration of lipid making them waxy, hydrophobic, and impermeable to routine stain such as the **Gram Stain.** They are also resistant to acid and alcohol and is described as Acid Fast Bacilli (AFB) or Acid Alcohol Fast Bacilli (AAFB).
There are two procedures commonly used for acid-fast staining:

1. Carbolfuchsin methods which include the Ziehl-Neelsen and Kinyoun methods (Light /bright field microscope)
2. **Fluorochrome procedure using auramine-O or auramine-rhodamine dyes** (Fluorescent microscope).

Principle of Ziehl-Neelsen method of acid-fast staining

Mycobacteria, which do not stain well by Gram stain, are stained with **carbol fuchsin combined with phenol**.

1. In the „**hot'** ZN technique, the phenol-carbol fuchsin stain is heated to enable the dye to penetrate the waxy mycobacterial cell wall.
2. In the „**cold**‟ technique known as **Kinyoun Method**, stain are not heated but the penetration is achieved by increasing concentration of basic fuchsin and phenol and incorporating a „wetting agent‟ chemical.

The stain binds to the mycolic acid in the mycobacterial cell wall. After staining, an acid decolorizing solution is applied. This removes the red dye from the background cells, tissue fibres, and any organisms in the smear except mycobacteria which retain (hold fast to) the dye and are therefore referred to as acid fast bacilli (AFB).
Following decolorization, sputum smear is counterstained with malachite green, or methylene blue which stains the background material, providing a contrast colour against which the red AFB can be seen.

Among the *Mycobacterium* species, *M. tuberculosis* and *M. ulcerans* are strongly acid fast. When staining specimens for these species, a **3% v/v acid alcohol** is used to decolorize the smear, where as *M. leprae* is only weakly acid fast. 0.5-1% v/v decolorizing solution is therefore used for *M. leprae* smears and also different staining and decolorizing time.

Note: *0.5% Acid alcohol or 5% Sulphuric acid is used for Atypical AFB because they (eg. Mycobacterium leprae, Nocardia asteroides) are much less acid and alcohol fast than Mycobacterium tuberculosis bacilli.*

Sample Collection & Preparation : Due to overnight accumulation of secretions, first morning specimens are more likely to yield better recovery of AFB.

- **Direct Smear:** Smear prepared directly from a patient specimen prior to processing.
- **Indirect Smear:** Smear prepared from a processed specimen after centrifugation (is used to concentrate the material)

Reagents required:

1. Carbol fuchsin stain (filtered)
2. Acid alcohol 3% v/v (or 20% sulfuric acid)
3. Malachite green 5 g/l (0.5% w/v) or Methylene blue, 5g/l

Ziehl-Neelsen Staining procedure

1. Spread the sputum evenly over the central area of the slide using a continuous rotational movement. The recommended size of the smear is about **20 mm by 10 mm.**
2. Place slides on dryer with smeared surface upwards, and air dry for about 30 minutes.
   Fig. Heat Fixation of smear (Upper: using electric heater, lower: using burner)
3. Heat fix dried smear.
4. Cover the smear will carbol fuchsin stain
5. Heat the smear until vapour just begins to rise (i.e. about 60 degree Celsius). Do not overheat (boil or dry). Add additional stain if necessary. Allow the heated stain to remain on the slide for **5 minutes.**
6. Wash off the stain with clean water.
7. Cover the smear with **3% v/v acid alcohol for 2-5 minutes** (or 20% sulfuric acid) or until the smear is sufficiently decolorized, i.e. pale pink.
   Note: *Check to see that no more red color runs off the surface when the slide is tipped. Add a bit more decolorizer for very thick slides or those that continue to "bleed" red dye.*
8. Wash well with clean water
9. Cover the stain with **malachite green stain for 1-2 minutes**
10. Wash off stain with clean water
11. Wipe the back of the slide clean, and place it in a draining rack for smear to air dry (DO NOT BOLT DRY).
12. Examine the smear microscopically, using the 100x oil immersion objective (*10X eye piece for a total of 1000X magnifi cation*) and scan the smear systematically.

Procedural note:

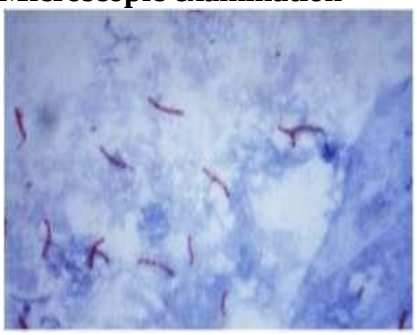
- Heat fixation of untreated specimen may NOT kill *M. tuberculosis (exercise care when handling slides)* whereas alcohol fixation is bactericidal.
- Acid alcohol is flammable, therefore use it with care.
- Take great care while heating carbol fuchsin (as staining rack may contain flammable chemicals) to reduce the fire risk.
- Slides must not touch each other when placed on staining rack to prevent transfer of material from one slide to another.

Results:

Reagent	Acid Fast	Non-Acid Fast
Carbol Fuchsin with heat	Red (Hot Pink)	Red (Hot Pink)
Acid Alcohol	Red	Colorless
Methylene Blue/Malachite Green	Red	Blue/Green

1. **AFB:** Red, straight or slightly curved rods, occurring singly or in small groups, may appear beaded.
2. **Cells:** Green
3. Background material: Green

Microscopic examination



- **GIEMSA STAINING OF MALARIA BLOOD FILMS - MALARIA**
  GIEMSA STAINING OF MALARIA BLOOD FILMS for MALARIA
- **PURPOSE AND SCOPE**
  - To describe the procedure for properly staining malaria blood films with Giemsa stain. This procedure is to be modified only with the approval of the national coordinator for quality assurance of malaria microscopy. All procedures specified herein are mandatory for all malaria microscopists working in national reference laboratories, in hospital laboratories or in basic health laboratories in health facilities performing malaria microscopy.
  - **BACKGROUND**
  - A properly stained blood film is critical for malaria diagnosis, especially for precise identification of malaria species. Use of Giemsa stain is the recommended and most reliable procedure for staining thick and thin blood films. Giemsa solution is composed of eosin and methylene blue (azure). The eosin component stains the parasite nucleus red, while the methylene blue component stains the cytoplasm blue. The thin film is fixed with methanol. De-haemoglobinization of the thick film and staining take place at the same time. The ideal pH for demonstrating stippling of the parasites to allow proper species identification is 7.2. Methods of staining The two methods for staining with Giemsa stain are the rapid (10% stain working solution) and the slow (3% stain working solution) methods. The rapid (10% stain working solution) method This is the commonest method for staining 1–15 slides at a time. It is used in outpatient clinics and busy laboratories where a quick diagnosis is essential for patient care. The method is efficient but requires more stain. The need for speed justifies the additional cost. The slow (3% stain working solution) method The slow method is used for staining larger numbers of slides (≥ 20). It is ideal for staining blood films collected during cross-sectional or epidemiological surveys and field research and for preparing batches of slides for teaching. It is

less appropriate when a quick result is needed. The slow method is less expensive than the rapid method because it requires much less stain (3% rather than 10% stain solution).

- **SUPPLIES AND MATERIALS**
- For the rapid (10% stain working solution) method • Giemsa stain (10% solution) (See MM-SOP-04 for method of preparation); • a small container or beaker for Giemsa working stain; • absolute methanol, acetone-free; • a Pasteur pipette with a rubber teat; • a small container or beaker for methanol; • a curved plastic staining tray, plate or staining rack; • a timer; • a slide-drying rack; • a small electric hair-dryer; 2 MM–SOP–07A: GIEMSA STAINING OF MALARIA BLOOD FILMS • protective latex gloves, powder-free, disposable and • Distilled or deionized water buffered to pH 7.2. For the slow (3% stain working solution) method • Giemsa stain (3% solution) (See MM-SOP-04 for the method of preparation); • a small container for Giemsa working stain; • absolute methanol, acetone-free; • a Pasteur pipette with a rubber teat; • a small container or beaker for methanol; • staining troughs that can hold 20 slides placed back to back; • a timer; • a slide-drying rack; • protective latex gloves, powder-free, disposable, and • distilled or deionized water buffered to pH 7.2. 4.
- **SAFETY PRECAUTIONS**
- 1. Methanol (methyl alcohol) is inflammable and highly toxic if inhaled or swallowed; it can cause blindness and even death if swallowed in any quantity. Avoid contact and inhalation. When it is not in use, it should be stored in a locked cupboard.
- 2. Universal precautions – including use of relevant personal protective equipment such as gloves, safety glasses and a laboratory coat or gown must be practiced.

Preparation and use of culture media

Culture Technique

Bacteria will grow on practically any source of organic food which provides **carbon** compounds to be respired for **energy**, and **nitrogen** compounds to be incorporated into **proteins** for **growth**. These substances are normally provided dissolved in water. However, in nature, bacteria can break down solid and insoluble substances by releasing **enzymes** into the substrate in which they are growing. These substances are thus broken down or digested to simpler substances and the process is called **extracellular digestion** because it takes place outside the bacterial cells.

The two normal **media** used in bacteriology are a clear soup-like liquid **nutrient broth**, usually in tubes, and **nutrient agar**, which is set into a jelly by the addition of a seaweed extract called agar, and when melted poured into glass or plastic **Petri dishes - also known as "plates"**.

Sometimes, substances are mixed into media, in order to suppress growth of other types of bacteria. There are many such selective media.

A standard **carbon source** is **glucose**, and **nitrogen** is often provided by **peptones** (partially digested **proteins**), or **inorganic salts**. Minerals and vitamins may also be provided, according to the growth requirements of the bacteria. Combinations of chemicals (buffers) may be used to keep the pH stable. Measured amounts of the concentrates are added to water, and dissolved to reconstitute the media.

These media must then be **sterilised** by heating in an autoclave (like a pressure cooker) at 121°C (pressure 1 bar or 15 lb/sq. in.) for 15 minutes, which kills all living organisms, including spores.

All apparatus used from this point onwards must be sterilised by heat (glassware - 160 °C for 2 hrs) or exposure to radiation.

Aseptic techniques must be used to reduce the likelihood of bacterial contamination. This usually involves **disinfection** of working areas, minimising possible access by bacteria from the air to exposed media, and use of **flames** to kill bacteria which might enter vessels as they are opened.

PREPARATION OF BROTH, SLANT & STAB CULTURES

Nutrient Broth Culture:

- Light your Bunsen burner.
- In one hand hold both the stock culture and the broth culture to be inoculated. Loosen the tube caps
- In your other hand hold the inoculating loop.
- Flame the inoculating loop to redness by holding it pointed down into the flame, starting near the handle and then moving the loop into the flame. This technique sterilizes the loop and, if wet with a culture, heats up the loop without spattering bacteria into the air and onto the surrounding area.
- Let the loop cool a minute. A hot loop will damage the bacteria cells.
- Using the fingers of the "loop hand" remove the cap from the stock culture tube and flame the tube mouth. DO NOT set the tube top down on the table.
- Insert the cooled sterilized loop into the culture tube being careful to not touch the sides of the tube. Touch the loop to the culture. You need not scrape a visible amount from the culture. Hold the tube as horizontal as possible to preclude particles from the air settling into the tube BUT do watch out for any condensate in the bottom of slant cultures. Don't let this fluid wash across the face of the culture.
- Remove the loop being careful again to not touch the tube sides.
- Flame the tube mouth and replace the cap.
- Remove the cap of the broth tube. Flame the top. Remember to hold the top in your fingers.
- Insert the loop into the broth and shake to remove the bacteria.
- Withdraw the loop, flame the tube mouth and replace the cap.
- Resterilize the inoculating loop and place it on the table. NEVER place a contaminated loop on the table.
- Return the stock culture to the rack or holder.
- Gently shake your broth culture. Label it with: today's date, microbe's name, your last name & section letter. The label should be placed so that it will not be in the way of later observations.

Inoculate TWO broths, one each from the two different stock cultures provided.

Incubate your broth cultures as directed. Most bacteria grow fully in broth cultures in 24-48hrs. The tops should be loosened when incubated to allow gas exchange. Observe & record observations at least once each day for the next three (3) days.

Nutrient Agar Slant Culture:

- Hold the tubes, flame the tops, and transfer the culture as outlined for the broth culture. Use the inoculating needle instead of the loop. Remember to sterilize it before and after each use.
- Inoculate your slant by moving the needle gently up the surface of the agar in a snake-like fashion. Be careful to not gouge the agar surface. If there is any liquid in the bottom of the slant tube avoid sticking the needle into this condensate.
- Label it with: today's date, microbe's name, your last name & section letter. The label should be placed so that it will not be in the way of later observations.

Inoculate TWO slants, one each from the two different stock cultures provided.

Incubate your slant cultures as directed. The tops should be loosened when incubated to allow gas exchange. Observe & record observations at least once each day for the next three (3) days.

Nutrient Agar Stab Culture:

- Handle the tubes and inoculating needle as outlined above.
- Inoculate your stab by thrusting the needle straight down into the agar center.

•Label your stabs with: today's date, microbe's name, your last name & section letter. The label should be placed so that it will not be in the way of later observations.
Inoculate TWO stabs, one each from the two different stock cultures provided.
Incubate your stab cultures as directed. The tops should be loosened when incubated to allow gas exchange. Observe & record observations at least once each day for the next three (3) days.

RECIPES

Nutrient Broth (100mL):

•Into a 250mL beaker place 0.3g beef extract, 0.5g peptone, 50mL distilled water
•Gently heat (stir) until dissolved (will be colored).
•Bring the volume to 100mL with distilled water.
•Adjust the pH to 7.0 using 0.1N NaOH or).1M HCl as needed.

Nutrient Agar:

Prepare exactly like nutrient broth BUT in step 1 add 1.5g of Agar.
Marine Agar (100mL):
1. Into a 250mL beaker place 0.5g peptone, 0.1g yeast extract, 0.01g FePO4, 1.5g Agar.
2. Bring the volume to 100mL with aged seawater.
3. Gently heat, (stir) until dissolved.
4. Adjust the pH to 7.5 – 7.8.

TYPES OF CULTURE MEDIA

Media are of different types on consistency and chemical composition.

A. On Consistency:

1. Solid Media. Advantages of solid media: (a) Bacteria may be identified by studying the colony character, (b) Mixed bacteria can be separated. Solid media is used for the isolation of bacteria as pure culture. 'Agar' is most commonly used to prepare solid media. Agar is polysaccharide extract obtained from seaweed. Agar is an ideal solidifying agent as it is : (a) Bacteriologically inert, i.e. no influence on bacterial growth, (b) It remains solid at 37°C, and (c) It is transparent.
2. Liquid Media. It is used for profuse growth, e.g. blood culture in liquid media. Mixed organisms cannot be separated.

B. On Chemical Composition :

1. Routine Laboratory Media
2.Synthetic Media. These are chemically defined media prepared from pure chemical substances. It is used in research work.

ROUTINE LABORATORY MEDIA

These are classified into six types: (1) Basal media, (2) Enriched media, (3) Selective media, (4) Indicator media, (5) Transport media, and (6) Storage media.

1.BASAL MEDIA. Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. Staphylococcus and Enterobacteriaceae grow in these media.

2.ENRICHED MEDIA. The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar and Lowenstein-Jensen media. Streptococci grow in blood agar media.

3.SELECTIVE MEDIA. These media favour the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: MacConkey agar, Lowenstein-Jensen media, tellurite media (Tellurite inhibits the growth of most of the throat organisms except diphtheria bacilli). Antibiotic may be added to a medium for inhibition.

4.INDICATOR (DIFFERENTIAL) MEDIA. An indicator is included in the medium. A particular organism causes change in the indicator, e.g. blood, neutral red, tellurite. Examples: Blood agar and MacConkey agar are indicator media.

5.TRANSPORT MEDIA. These media are used when specie-men cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.

6. STORAGE MEDIA. Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth.

COMMON MEDIA IN ROUTINE USE

Nutrient Broth. 500 g meat, e.g. ox heart is minced and mixed with 1 litre water. 10 g peptone and 5 g sodium chloride are added, pH is adjusted to 7.3. Uses: (1) As a basal media for the preparation of other media, (2) To study soluble products of bacteria.

Nutrient Agar. It is solid at 37°C. 2.5% agar is added in nutrient broth. It is heated at 100°C to melt the agar and then cooled.

Peptone Water. Peptone 1% and sodium chloride 0.5%. It is used as base for sugar media and to test indole formation.

Blood Agar. Most commonly used medium. 5-10% defibrinated sheep or horse blood is added to melted agar at 45-50°C. Blood acts as an enrichment material and also as an indicator. Certain bacteria when grown in blood agar produce haemolysis around their colonies. Certain bacteria produce no haemolysis. Types of changes : (a) beta (p) haemolysis. The colony is surrounded by a clear zone of complete haemolysis, e.g. Streptococcus pyogenes is a beta haemolytic streptococci, (b) Alpha (a) haemolysis. The colony is surrounded by a zone of greenish discolouration due to formation of biliverdin, e.g. Viridans streptococci, (c) Gamma (y) haemolysis, or, No haemolysis. There is no change in the medium surrounding the colony,

Chocolate Agar or Heated Blood agar. Prepared by heating blood agar. It is used for culture of pneumococcus, gonococcus, meningococcus and Haemophilus. Heating the blood inactivates inhibitor of growths.

MacConkey Agar. Most commonly used for enterobacteriaceae. It contains agar, peptone, sodium chloride, bile salt, lactose and neutral red. It is a selective and indicator medium :

(1) Selective as bile salt does not inhibit the growth of enterobactericeae but inhibits growth of many other bacteria.

(2) Indicator medium as the colonies of bacteria that ferment lactose take a pink colour due to production of acid. Acid turns the indicator neutral red to pink. These bacteria are called 'lactose fermenter', e.g. Escherichia coll. Colourless colony indicates that lactose is not fermented, i.e. the bacterium is non-lactose fermenter, e.g. Salmonella. Shigella, Vibrio.

Mueller Hinton Agar. Disc diffusion sensitivity tests for antimicrobial drugs should be carried out on this media as per WHO recommendation to promote reproducibility and comparability of results.

Hiss's Serum Water Medium. This medium is used to study the fermentation reactions of bacteria which can not grow in peptone water sugar media, e.g. pneumococcus, Neisseria, Corynebacterium.

Lowenstein-Jensen Medium. It is used to culture tubercle bacilli. It contains egg, malachite green and glycerol. (1) Egg is an enrichment material which stimulates the growth of tubercle bacilli, (2) Malachite green inhibits growth of organisms other than mycobacteria, (3) Glycerol promotes the growth of Mycobacterium tuberculosis but not Mycobacterium bovis.

Dubos Medium. This liquid medium is used for tubercle bacilli. In this medium drug sensitivity of tubercle bacilli can be carried out. It contains 'tween 80', bovine serum albumin, casein hydrolysate, asparagin and salts. Tween 80 causes dispersed growth and bovine albumin causes rapid growth.

Loeffler Serum. Serum is used for enrichment. Diphtheria bacilli grow in this medium in 6 hours when the secondary bacteria do not grow. It is used for rapid diagnosis of diphtheria and to demonstrate volutin granules. It contains sheep, ox or horse serum.
Tellurite Blood Agar. It is used as a selective medium for isolation of Cotynebacterium diphtheriae. Tellurite inhibits the growth of most secondary bacteria without an inhibitory effect on diphtheria bacilli. It is also an indicator medium as the diphtheria bacilli produce black colonies. Tellurite metabolized to tellbrism, which has black colour.
EMB (Eosin-methylene blue) Agar. A selective and differential medium for enteric Gram-negative rods. Lactose-fermenting colonies are coloured and nonlactose-fermenting colonies are nonpigmented. Selects against gram positive bacteria.
XLD (Xylose Lysine Deoxychoiate). It is used to isolate Salmonella and Shigella species from stool specimens. This is a selective media.
SS (Salmonella-Shigella) Agar. It is a selective medium used to isolate Salmonella and Shigella species. SS Agar with additional bile salt is used if Yersinia enterocolitica is suspected.
DCA (Desoxycholate Citrate Agar). It is used for isolation of Salmonella and Shigella. The other enteric bacteria are mostly inhibited (a selective medium). It is also a differential (indicator) medium due to presence of lactose and neutral red.
Tetrathionate Broth. This medium is used for isolating Salmonella from stool. It acts as a selective medium. It inhibits normal intestinal bacteria and permits multiplication of Salmonella.
Selenite F Broth. Uses and functions are same as that of tetrathionate broth.
Thiosulphate-Citrate-Bile-Sucrose (TCBS) Agar. TCBS agar is a selective medium used to isolate Vibrio cholerae and other Vibrio species from stool.
Charcoal-yeast agar. Used for Legionella pneumophila. Increased concentration of iron and cysteine allows growth.
Tellurite-Gelatin Agar Medium (TGAM). It may be used as transport, selective and indicator medium.
Alkaline peptone water. See under Vibrio. (Chapter 51).
Campylobacter Medium. This selective medium is used to isolate Campylobacter jejuni and Campylobacter coli from stool.
Cary-Blair Medium. It is used as a transport medium for faeces that may contain Salmonella, Shigella, Vibrio or Campylobacter species.
Amies medium is used for gonococci and other pathogens.
Peptone Water Sugar Media. These indicator media are used to study 'Sugar fermentation'. 1 % solution of a sugar (lactose, glucose, mannitol etc) is added to peptone water containing Andrade's indicator in a test tube. A small inverted Durham tube is placed in the medium. The media are colourless. After culture, change of a medium to red colour indicates acid production. Gas, if produced collects in Durham tube.
Motility Indole Urea (MIU) Medium. This is used to differentiate enterobacteria species by their motility, urease, and indole reactions.
TSI (Triple sugar iron) Agar-
KIA (Kligler Iron Agar). This is a differential slope medium used in the identification of enteric bacteria. The reactions are based on the fermentation of lactose and glucose and the production of hydrogen sulphide
Christensen's Urea Medium. This is used to identify urea splitting organisms, e.g. Proteus. A purple pink colour indicates urea splitting
Bordet-Gengou Medium. This medium is used for culture of Bordetella pertussis. Increased concentration of blood allows growth. It contains agar, potato, sodium chloride, glycerol, peptone and 50% horse blood. Penicillin may be added to it.

Distinct colony Properties of various Bacterial Pathogens

1. Main difference between Gram + and Gram – bacteria
2. Examples of intracellular and extracellular bacteria
3. Flagella and type III secretion – 2 related bacterial multi-protein machines
4. 2 examples of manipulation of host actin cytoskeleton: bacterial entry and movement
5. 3 mechanisms of horizontal gene transfer
6. Genome diversity and evolution

Bacteria

Small and unicellular

- No internal membrane-bound organelles
- Generally haploid
- Some are mobile- use of flagella
- Typical size- 1µm
- Cocci- spherical
- Bacilli- rod shaped
- Spirilli- spiral
- Vast majority are harmless or beneficial (COMMENSAL), but some are pathogenic

Gram Negative (GN)

Examples: -

Escherichia coli- there are many different types; some harmless ones in the gut and some pathogenic ones(EPEC - diarrhea, EHEC - dysentry and kidney failure) - Salmonella (typhimurium - food poisoning, typhi - typhoid) Shigella (dysentry) - Vibrio cholerae (cholera) - Neisseria (meningitidis- meningitis, gonorrhoeae- gonorrhea)

Gram Positive (GP)

Examples:

Staphylococcus aureus (skin diseases, endocarditis, bacteraemia, joint diseases, - pneumonia) - Streptococcus pneumoniae (pneumonia, meningitis, otitis media) - Streptococcus pyogenes (tonsilitis, necrotizing fasciitis, bacteremia, scarlet fever) Mycobacteria (MB)¬

Examples: -

Mycobacterium tuberculosis (TB)

Mycobacterium leprae (leprosy)

Identifying Bacteria

Gram stain: distinguishes between two different kinds of bacterial cell walls

- Bacteria are stained with a violet dye and iodine, rinsed in alcohol and then stained with a red dye
- Stain indicates whether bacteria is GN or GP
- MCD Microbiology Alexandra Burke-Smith 2 GP: PEPTIDOGLYCAN in cell wall retains¬ dye- appears DEEP VIOLET ◊Structure of GP cell wall GN: outer membrane resists the dye. The¬ cells absorb counter stain- appear PINK

Pathogens

1. COLONIZE in host, e.g. on mucosal surface
2. PERSIST: find unique niche and avoid host defences e.g. resist complement, phagocytosis
3. REPLICATE- using nutrients from the host
4. DISEEMINATE throughout tissues (this is the manifestation of disease)
5. CAUSE DISEASE

Extracellular (EC)

Replicates outside cells, e.g. Staphylococcus, Streptococcus, Yersinia, Neisseria

Intracellular (IC)

Replicate inside cells - Initially taken up by phagocytosis, forming a PHAGOSOME- membrane bound compartment.

Then there are three pathways: - ESCAPE from the phagosome and replicate in the cytoplasm, e.g. listeria, shigella - MODIFY the appearance of the phagosome so host cells cannot recognise the foreign pathogen and prevent fusion with lysosomes, e.g. salmonella mycobacteria - SURVIVE in the phagolysosome formed when the phagosome fuses with a lysosome, e.g. coxiella

Motility and Invasion Requires two related multi-protein machines:

FLAGELLA-

Protein structures protrude through membrane, and the rotation of these cause movement - Rings of protein subunits form in the inner membrane on the cytoplasmic face - This forms a channel for the assemble of other rod-like proteins - Rod-like proteins and CAPPING PROTEINS (which integrate the new subunits) form a hook structure- this is the precursor to the flagella - The hook is of fixed length. Capping proteins are then lost and new protein subunits are added to form the base of the flagella - Protein subunits form the flagella, again involving capping proteins - Rotational plate at base of hook structure forces the flagellum to rotate- TORQUE provides the movement Type III Secretion System¬ - Similar to flagella, but DELIVERS VIRULENCE PROTEINS into host cells MCD Microbiology Alexandra Burke-Smith 3 E.g. Salmonella invasion of epithelial cells (GN) Effectors interfere with signalling pathways and induce actin polymerisation, membrane ruffling and• bacterial internalisation – i.e. the bacteria engineer their own uptake into the cell Needle tip structure secretes effector proteins• through TRANSLOCASE COMPLEX which forms a channel into the host cell This provides the transport mechanism for the• virulence proteins, e.g. toxins The type of effector proteins determine the• result of the injection E.g. Listeria manipulation of actin (GP) Listeria - causes food poisoning and more• serious diseases in the immunocompromised, elderly and pregnant women Invasion: phagocytosis by zipper mechanism,• escapes from phagosome using toxins Intracellular movement: polymerises actin on one end of bacterium- forming COMET TAILS which drives the• movement of the bacterium around the cell Cell-to-cell spread: bacterium can protrude from host cell and be engulfed into neighbouring one

• Bacterial Genomes

Encode 500-4500 proteins: approx 90% are “uninteresting” from pathogenic point of view as they have other• functions. Pathogenic genes (VIRULENCE GENES) are mainly selected by immune system There is unexpected variation in the genomes of similar pathogens (Core genes + accessory genes = gene• repertoire) The more strains of pathogens you sequence, the number of new genes remains the same, although you• would expect it to decline. This is due to STRAIN SPECIFIC DNA Accessory genes: genes for pathogenesis and virulence – huge variation•

Replication

◊Bacteria replicate by binary fission Horizontal Transmission

Transformation

The uptake of exogenous DNA and its integration by homologous recombination - E.g. neisseria, streptococcus

Transduction

Phage replicates its DNA in bacterium and cuts bacterial DNA into small pieces - Some bacterial DNA may be packaged ine phage heads. Bacterium lyses and new phage particles are released (LYTIC INFECTION) - Phage particle injects bacterial DNA into new bacterial cell - Injected DNA may be incorporated into bacterial chromosome

Bacteriophage conjugation

Transfer of plasmid through CONJUGATIVE MATING BRIDGE – enables DNA transfer The vast majority of accessory genes acquired by horizontal transfer from (mainly) unknown sources• If DNA confers selective advantage, they remain in the bacterial chromosomes as PATHOGENICITY ISLANDSgiving• the chromosome a mosaic like structure of core "housekeeping" genome with interspersed islands that confer virulence properties Pathogenicity islands are the driving force of evolution, but there origin is frequently unknown• incredible source of genetic variation, rapid generation time + selective pressure, approx 1000,000,000 years.

Possible Questions

Part A

1. Explain wet mount.
2. What is meant by hanging drop method?
3. Define heat fixation.
4. Write about simple staining method.
5. Define Capsule.
6. Define flagella.
7. Define spore.
8. Write about negative staining method.
9. Explain types of staining.
10. Write about extracellular digestion.
11. Define culture media.
12. Define selective media.
13. Define differential media.
14. Define transport media.
15. Define basal media.
16. Define indicator media.

Part B

1. Discuss the roles of stain and its properties.
2. Write the protocol for simple staining technique.
3. Comment on differential staining.
4. Explain in detail gram staining method.
5. Explain in detail about acid fast staining.
6. Discuss the staining principle of spore.
7. Explain the preparation of nutrient agar slant.
8. Explain the preparation of nutrient broth.
9. Explain the preparation of nutrient agar deep.
10. Write about media and its types.
11. Comment on selective media.
12. Comment on properties of differential media.
13. Write about various transport media.
14. Give the importance of basal media.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB **COURSE NAME: MICROBIAL DIAGNOSIS IN HEALTH CLINIC**

COURSE CODE: 18MBU304B **UNIT: III** **BATCH-2018-2021**

Unit III Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
One angstrom is equal to_____.	One tenth of a micrometer	One thousandth of a micrometer	One thousand of a nanometer	One tenth of a nanometer	One tenth of a micrometer
With the increase in the value of numerical aperture of an objective lens the resolving power_____.	Remains the same	Increases indefinitely	Decreases	Increases up to a limit	Increases indefinitely
Endospores and inclusion bodies can be best detected with the help of_____.	Fluorescence microscope	Dark field microscope	Phase field microscope	Bright field microscope	Bright field microscope
Treponema pallidum can be best identified using_____.	Fluorescence microscope	Dark field microscope	Bright field microscope	Phase contrast microscope	Dark field microscope
The generally preferred method of observing wet mounts of bacteria is_____.	Phase contrast microscopy	Interference microscopy	Dark field microscopy	Fluorescence microscopy	Phase contrast microscopy
The colonies produced by Pseudomonas on Mac Conkey's medium are	Purple colored	Pink colored	Pale colored	Green colored	Pale colored
Staining material of gram positive bacterium is	Fast green	Haematoxylon	Crystal violet	Safranin	Crystal violet
In which type of microscopy the microorganism is likely to show varying degrees of darkness within the cell?	Dark-field	Bright field	Phase contrast	Electron	Electron
Which of the following is a supravital fluorescent stain?	Acridine orange	Congored	Neutral red	Safarinin	Acridine orange
Which of the following is not used as a fixative?	Ethanol	Glacial acetic acid	Formaldehyde	Conc.HCI	Conc.HCI
Magnification of the microscope depends on which of the following?	light source	Magnifying power of the eyepiece	Wire	body tube	Magnifying power of the eyepiece
Fixation means _____	Preserves internal and external structures of microorganisms	Colouration	Modent	smearing	Preserves internal and external structures of microorganisms
The most commonly used fixation in light microscopy for observing microorganisms is	Osmic acid	Aldehydes	Heat	Chloroform	Heat

Stains are used to_____.	Store cultures	To reveal their shape and size	pathogen	Antiseptic	To reveal their shape and size
Which is the coloring agent used in biological?	Stain	Dye	Chromophore	Auxochrome	Chromophore
Dye is an organic compound containing _____.	Ionophore and chromophore	Chromophore and chromogen	Chromophore and auxochrome	Auxochrome and ionophore	Chromophore and auxochrome
Basic stains are absorbed at_____.	pH values higher than the isocletric point	Isolelectric point	pH values lower than the isoleletric point	Very low pH	pH values higher than the isocletric point
Which of the following is not a basic stain?	Crystal violet	Methyl violet	Safranin	Nigrosin	Nigrosin
Which of the following is a neutral stain?	Picric acid	Giemsa	Neutral red	Malachite green	Neutral red
Rhodamin is a _____	Dye	Stain	Decolariser	None	Dye
Which of the following is not a mordant?	Tannic acid	Salts of aluminium	Salts of chromium	Salts of sodium	Salts of aluminium
Which of the following is not a differential staining?	Gram staining	Acid fast staining	Spore staining	Capsule staining	Gram staining
Spore staining involves the use of _____.	Malachite green and safranin	Lactophenol cotton blue	Crystal violet and safranin	Methylene blue and carbol fuchin	Malachite green and safranin
Acridine dyes are more effective against	Gram positive	Gram negative	Ricke tsia	Mycoplasma	Gram positive
Gram staining is an example for	Simple staining	Differential staining	Capsule staining	Negative staining	Differential staining
Cell wall of gram negative bacteria is	Thick	Lipids are more	Teichoic acids are absent	Polysaccharide	Lipids are more
Ultra violet light has shorter wavelenth of	100-200 nm	100-300nm	100-400nm	100-500nm	100-300nm
Light may pass through a substance, a phenomenon known as _____	Diffraction	Transmission	Fluorescence	Absorption	Diffraction
Substance that absorbs light of one wavelength will emit light of different wavelength. This phenomenon is called_____	Diffraction	Transmission	Fluorescence	Absorption	Fluorescence

The_____is the ratio of the speed of light in a given medium to the speed of light in a vacuum.	Refractive index	Diffraction	Transmission rate	Scattering	Diffraction
Distortion based on the shape of the lens is called _____	Chromatic aberration	Spherical aberration	reflection	refraction	Chromatic aberration
Distortion based on the color of light is called	Chromatic aberration	Spherical aberration	reflection	refraction	Chromatic aberration
Modern microscopic lenses called_____	Convex lenses	Concave lenses	Flat field lenses	Lenses	Convex lenses
Cytoplasmic streaming is present in	Prokaryotes	Animals	Eukaryotes	Plants	Eukaryotes
The motile bacteria is	S. typhi	K. pneumoniae	B. anthracis	Shigella	S. typhi
The differences between Gram positive and Gram negative bacteria is shown to reside in the	Cell wall	Nucleus	Cell membrane	Mesosomes	Cell wall
Commonly used stains include_____	Crystal violet	Nigrosin	Methylene blue	Saffranin	Saffranin
Which of the following used in negative staining _____	Crystal violet	Nigrosin	Methylene blue	Saffranin	Crystal violet
A substance that fixes the primary stain in the bacterial cells is known as a _____	Primary agent	Mordant	Colourant	Coolant	Mordant
The percentage of alcohol used in Gram staining is	75%	95%	60%	25%	95%
Fluorescent dye binds to the_____of the cell walls of mycobacteria.	Mycolic acid	Dipimelic acid	Dipicolinic acid	Naladic acid	Mycolic acid
Capsule formation occurs in the presence of	Albumin	Charcoal	Serum	Starch	Serum
Fluorescent dye_____is illuliminated with blue light, it emits green light.	Nigrosin	Methylene blue	Flurescein isothiocyanate	Safranin	Flurescein isothiocyanate
The darkfield_____lenses focus light on the specimen at an oblique angle.	Concave lens	Convex lens	Convex-convex lens	Condensor lens	Condensor lens
Triponema pallidum that causes syphilis is viewed by_____microscopy.	Light	Bright field	Dark field	Electron	Dark field
Gram positive bacteria appear as	Pink	Violet	Orange	Green	Violet
Gram negative bacteria appear as	Pink	Violet	Orange	Green	Pink
Acid fast bacteria are	Neisseria		Mycobacteria	E.coli	Mycobacteria

		Staphylococci			
When observing a specimen using the power or oil immersion objective one should focus with the_____knob only.	Fine adjustment	Coarse adjustment	Mechanical stage	Rotator	Coarse adjustment
The foundation of the microscope is _____.	Arm	Lens	Base	Condenser	Lens
If the specimen is in focus using one object and requires very little adjustment when switching to another objective, the microscope is described as_____.	Par focal	Focus	Wet mount	Scanning	Par focal
_____microscope that utilizes electrons to illuminate the specimen being viewed.	Dark field	Bright field	Electron	Flurescence.	Electron
_____microscope that illuminate the specimen in a blackened field.	Dark field	Electron	Bright field	Simple	Dark field
The_____knob is used first when focusing with scanning or low power objectives.	Fine adjustment	Coarse adjustment	Eye piece	objective	Fine adjustment
The part of the microscope that is also called the eye piece.	Ocular	Objective	Arm	Diapharm	Ocular
Immersion oil prevents the_____of light.	Refraction	Absorption	Transmission	reflection	Refraction
The low power objective has a magnification of_____x.	10x	40X	1000x	100 x	40X
Mycobacteria are stained with	Gram's staining	Simple staining	Capsule staining	Ziehl – Neelsen's staining	Ziehl – Neelsen's staining
The source of electron is a hot _____ filament in an electron gun.	Copper	Tungsten	Platinum	Gold	Tungsten
In scanning electron microscopy the emitted light is converted to a_____.	Heat	Electrical current	Sound	magnet	Electrical current
_____Used as an indicator in Mcintosh anaerobic jar for preparation of anaerobic culture.	Methylene blue	Phenol red	Bromothymol blue	Neutral red	Methylene blue
_____is a simple media	Blood agar	Robertson's medium	Mac conkey's agar	Nutrient agar	Nutrient agar

_____media contain additives that enhance the growth of the desired organism by inhibiting other organisms.	Simple	Transport	Selective	Indicator	Selective
When S.typhi is grown in Wilson and Blair medium, containing sulphite, the bacterial colonies become due to reduction of sulphite.	Black	Green	Pink	Yellow	Black
Mac Conkey's agar is a _____ medium	Selective & Differential	Differential	Transport	Enrichment	Selective & Differential
_____is a transport medium	Mac Conkey	Blood agar	Stuart's Media	Nutrient agar	Stuart's Media
In Mac conkey's Medium, lactose fermenters produce_____colonies.	Pink	Yellow	Black	Green	Pink
Assay medium is also known as	Selective media	Complex media	Production media	Indicator media	Production media

UNIT-IV
SYLLABUS

Serological Methods - Agglutination, ELISA, immune fluorescence, Nucleic acid based methods – PCR, Nucleic acid probes, Typhoid, HBV, HCV, HIV and Dengue.

Serological Methods

Agglutination Reactions

The interaction (immune reaction) between antibody and a particulated antigen resulting a visible clumping called **agglutination.** Antibodies that produce such reactions are called **agglutinins.** Agglutination reactions are similar in principle to precipitation reactions; they depend on the cross linking of polyvalent antigens. The excess of antibody concentration inhibits precipitation reactions, also inhibit agglutination reactions; this inhibition is called the **prozone effect (prozone phenomenon)**. Hence serial dilutions are usually prepared in many similar immunological techniques to avoid this prozone effect by reaching the dilution of the optimum antibody concentration. All type of agglutination reactions are simple to performe (easy), can detect small amounts of antibodies (concentrations as low as nanograms per milliliter) and of low cost.

General feature of antigen antibody reactions

The reaction is **specific**; an antigen combines only with its homologous antibody and vice versa

The specificity however is not absolute and cross reactions may occur due to antigenic similarity or relatedness.

1. Entire molecules react and not fragment
2. There is no denaturation of the antigen or the antibody during the reaction.
3. The combination occurs at the surface, therefore it is the surface antigens that are immunologically relevant.
4. The combination is firm and irreversible. The firmness of the union is influenced by the affinity and avidity of the reaction.

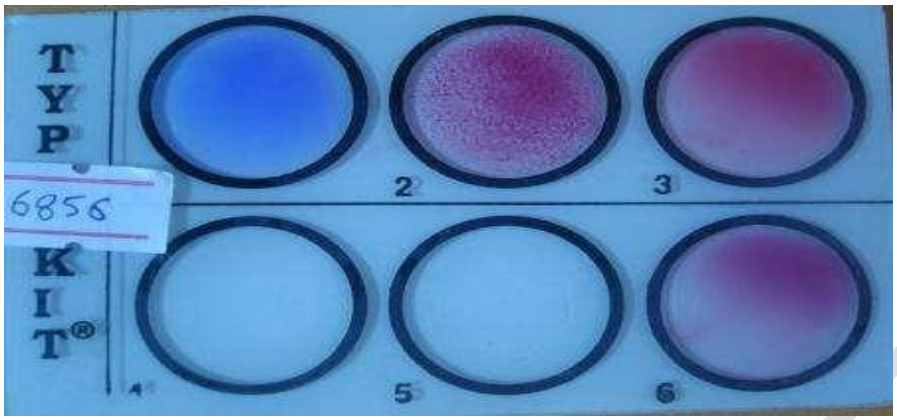
1. Affinity refers to the intensity of attraction between the antigen and antibody molecules. It is a function of the closeness of fit between an epitope and the antigen combining region of its antibody.
2. Avidity is the strength of the bond after the formation of the antigen antibody complexes. It reflects the overall combining property of the various antibody molecules in an antiserum, possessing different affinity constants with the multiple epitopes of the antigen.

5. Antigens and antibodies can combine in varying proportions, unlike chemicals with fixed valencies. Both antigens and antibody are multivalent, antibodies are generally bivalent, though IgM molecules may have five or ten combining sites. Antigens may have valencies up to hundreds.

1. . Types of agglutination

1. Slide agglutination: Serotyping.
2. Tube agglutination: e.g. Widal test.
3. Indirect (passive agglutination): where soluble antigens are coated on vehicle particle e.g. latex particle, RBCs.

Slide agglutination.



Widal Test: Sample showing H positive in screening test

- When a drop of the appropriate antiserum is added to a smooth, uniform suspension of a particulate antigen in a drop of saline on a slide or a tile, agglutination takes place.
- A positive result is indicated by the clumping together of the particles and the clearing of the drop. Depending up on the titre of the serum, agglutination may occur instantly or with in seconds.
- Clumping occurring after a minute may be due to drying of the fluid and should be disregarded.
- It is essential to have on the same slide a control consisting of the antigen suspension in saline, without the antiserum, to ensure that the antigen is not autoagglutinable.
- Slide agglutination is a routine procedure for the identification of many bacterial isolates from clinical specimens. It is also the method used for blood grouping and cross matching.

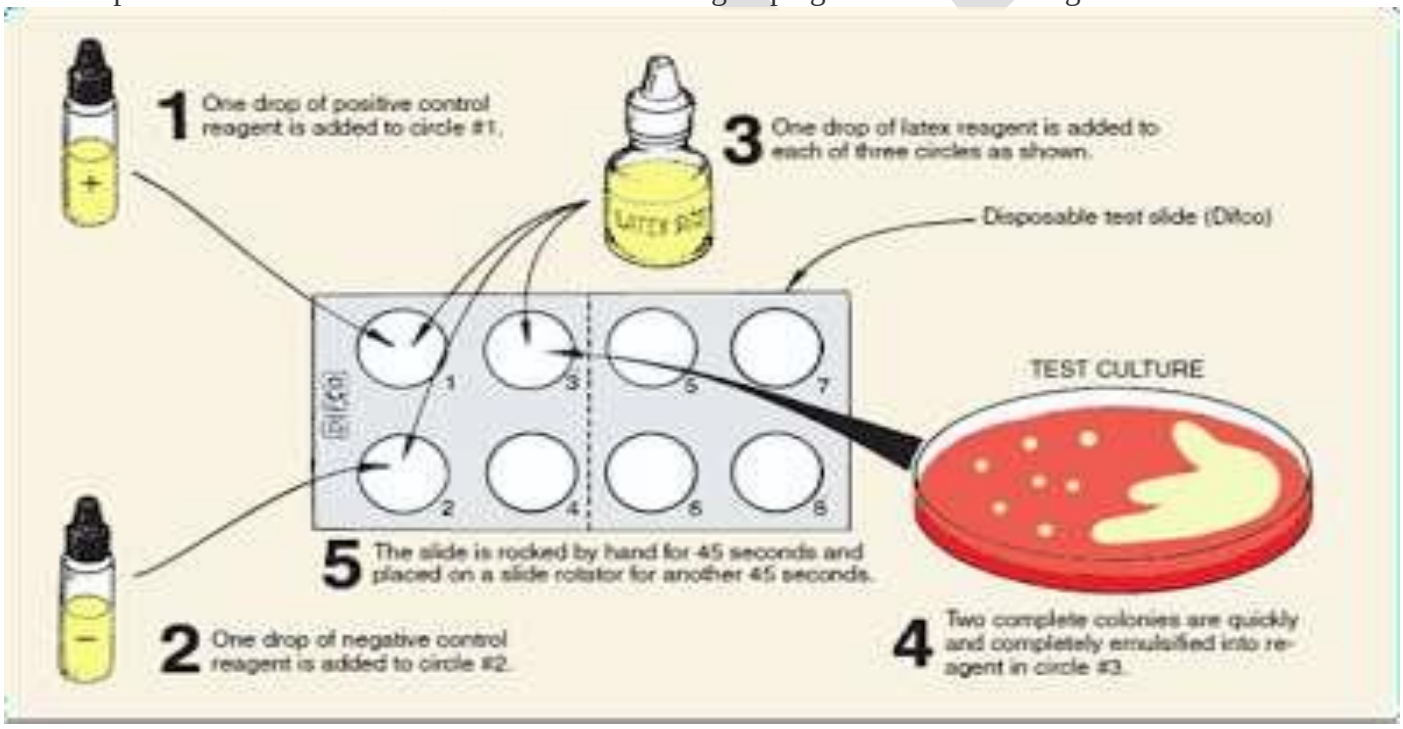


Fig: How to perform slide agglutination

Tube agglutination

This is the standard quantitative method for the measurement of antibodie

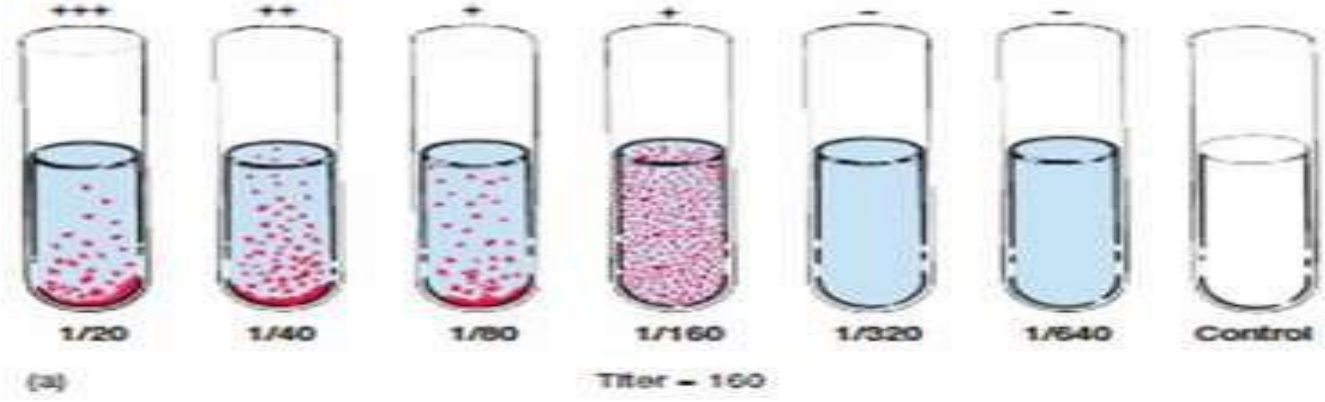


Fig. (a).Tube agglutination test for determining antibody titer.

- When a fixed volume of a particulate antigen suspension is added to an equal volume of serial dilutions of an antiserum in test tubes, the agglutination titre of the serum can be estimated.
- Tube agglutination is routinely employed for the serological diagnosis of typhoid, brucellosis and typhus fever (weil- felix reaction).

Widal test

The procedure involves adding a suspension of dead typhoid bacterial cells to a series of tubes containing the patient's serum, which has been diluted out to various concentrations. After the tubes have been incubated for 30 minutes at 37° C, they are centrifuged and examined to note the amount of agglutination that has occurred. The reciprocal of the highest dilution at which agglutination is seen is designated as the **antibody titer** of the patient's serum. For example, if the highest dilution at which agglutination occurs is 1:320, the titer is 320 antibody units per milliliter of serum. Naturally, the higher the titer, the greater is the antibody response of the individual to the disease.

Two types of antigens are used, the **H or the flagellar antigen** and the **O or the somatic antigen** of the typhoid bacillus.

Find details information about WIDAL TEST here

Round bottomed felix tubes are used for agglutination. Agglutinated bacilli spread out in a disc like pattern at the bottom of the tubes. The tube agglutination test for brucellosis may be complicated by the prozone phenomenon and the presence of blocking antibodies. Several dilution of the serum should be tested to prevent false negative results due to prozone.

The **weil- felix reaction** for serodiagnosis of typhus fever is a heterophile agglutination test and is based on the sharing of a common antigen between typhus rickettsiae and some strains of proteus bacilli. Another example of the heterophile agglutination test is the streptococcus MG agglutination test for the diagnosis of primary atypical pneumonia.

Examples of agglutination tests using red blood cells as antigens are the Paul Bunnel test and the cold agglutination test. The cold agglutination test is positive in primary atypical pneumonia. The patient's sera agglutinate human O group erythrocytes at 4°C the agglutination being irreversible at 37°C.

Hemagglutination is used in blood typing

Agglutination reactions are routinely performed to type red blood cells. In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient blood is the basis for matching blood types for transfusions.

Particle agglutination

Numerous procedures have been developed to detect antigen via the agglutination (clumping) of an artificial carrier particle such as a latex bead with antibody bound to its surface.

Latex agglutination

Antibody molecules can be bound in random alignment to the surface of latex (polystyrene) beads. Antigen present in a specimen being tested binds to the combining sites of the antibody exposed on the surfaces of the latex beads, forming cross- linked aggregates of latex beads and antigen.

- The size of the latex bead (0.8µm or larger) enhances the ease with which the agglutination reaction is recognized.
- Levels of bacterial polysaccharides detected by latex agglutination have been shown to be as low as 1.0 ng /ml because the pH, osmolarity and ionic concentration of the solution influence the amount of binding that occurs, conditions under which latex agglutination procedures are carried out must be carefully standardized.
- Additionally, some constituents of body fluids such as rheumatoid factor, have been found to cause false- positive reactions in the latex agglutination systems available. To counteract this problem. It is recommended that all specimens be treated by boiling or with ethylenediaminetetraacetic acid (EDTA) before testing.
- Commercial test systems are usually performed on cardboard cards or glass slides; manufacturers recommendations should be followed precisely to ensure accurate results.
- Reactions are graded on a 1+ to 4+ scale, with 2+ usually the minimum amount of agglutination seen in a positive sample.
- Control latex (coated with antibody form the same animal species from which the specific antibody was made) is tested alongside the latex. If the patient specimen or the culture isolate reacts with both the test and control latex, the test is considered non specific and therefore uninterpretable.
- Latex tests are very popular in clinical laboratories to detect antigen to *Cryptococcus neoformans* in CSF or serum and to confirm the presence of beta- hemolytic streptococcus form the culture plates. Latex tests are also available to detect *Streptococcus agalactiae*, *Clostridium difficile* toxins A and B and rotavirus.

Coagglutination

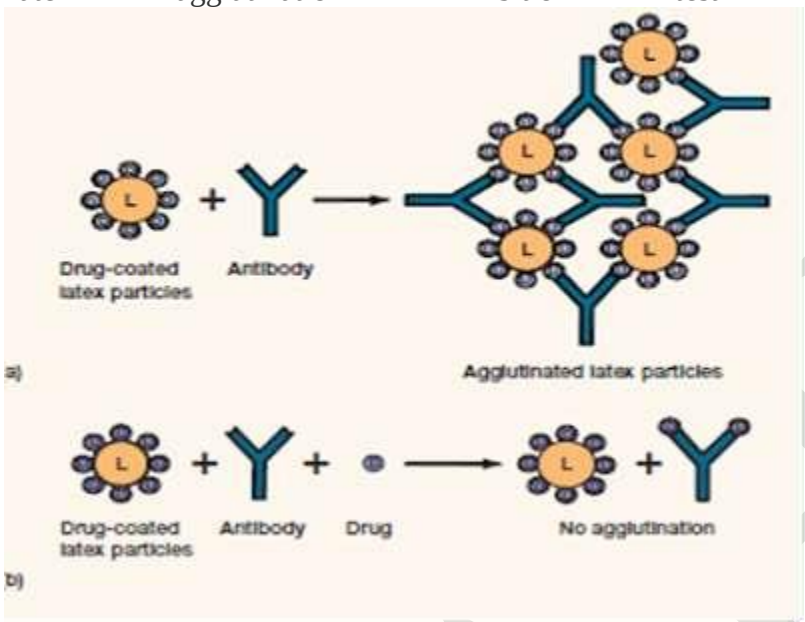
In this case the particles are killed and treated ***Staphylococcus aureus*** (cowan I strain), which contain a large amount of an antibody- binding protein, protein A, in their cell walls. In contrast to latex particles, these staphylococci bind only the base of the heavy chain portion (Fc) of the antibody, leaving both (Fab)antigen- binding ends free to form complexes with specific antigen.

Several commercial suppliers have prepared coagglutination reagents for identification of streptococci, including Lancefield groups A, B , C, D, F , G and N; *Streptococcus pneumoniae*; *Neisseria meningitides*; *N gonorrhoeae*; and *Haemophilus influenzae* types A to F grown in culture. The

coagglutination reaction is highly specific but may not be as sensitive for detecting small quantities of antigen as latex agglutination. Thus, it is not usually used for direct antigen detection.

Latex agglutination inhibition test

Latex agglutination inhibition test a) negative b) positive



The latex agglutination inhibition test relies on competition for the antibody between a latex- drug conjugate and any drug that may be present in the sample (mostly urine). A urine sample is placed in the mixing well of a slide containing antibody reagent, buffer and latex reagent.

a) If the drug is absent, the latex- drug conjugate binds to the antibody and forms large particles that agglutinate. Therefore agglutination is evidence for the absence of drugs in the urine specimen

b) If a drug is present in the urine sample, it competes with the latex conjugate for the small amount of available antibody. A sufficient quantity of the drug will prevent the formation of particles and agglutination and a positive urine sample does not change the appearance of the test mixture.

Coombs test

Direct coombs test: Detection of incomplete antibodies on patients RBCs. Antibodies attached on the surface of the RBCs (patient RBCs) + Antihuman globulin = agglutination. .1

Indirect coombs test: Detection of antibodies in patients sera. Rhesus positive RBC + Patient serum (if contains incomplete circulating Abs coats the surface of the RBC)+ Antihuman globulin which makes the bridge = agglutination .2

Hemagglutination of Blood Typing .1

Agglutination reactions (the Figure below), represent the routine laboratories test for determining blood type (blood grouping for ABO system).

Principles:

1) Recognizing the type red blood cells (RBCs) by typing A and B antigens.
2) RBCs (in the whole blood) are mixed on a slide with antisera (ready from a kit) prepared to react with A and B antigens.
3) If the antigen is present on the cells surface, they will agglutinate RBCs, forming a visible red clump on the slide.

Clinical application:
The importance of this test is that it is routinely used in matching blood types (groups) for transfusions by determination of which antigens are present on donor and recipient RBCs.

1. Bacterial Agglutination:
It is used to diagnose bacterial infection, when the immune system starts the production of serum antibodies specific for surface antigens on the bacterial cells. These Abs can be detected by bacterial agglutination reactions.

Principles:
1. Serial dilutions of serum from a patient suspected to be infected with a given bacterium in tubes.
2. Addition of bacteria to these tubes with constant concentration (same amount and concentration for all tubes).
3. If the person was infected with these bacteria, a visible agglutination will form in the tube of equivalent concentration of serum Abs. This tube is the last tube of positive (with agglutination) among all tubes. It represents the titer of serum antibodies in the patient. For example: If serial twofold dilutions of serum were prepared, and if the dilution of 1/32 shows agglutination but the dilution of 1/64 does not, then the agglutination titer of the patient's serum is 32.

4. Note: In some cases serum can be diluted up to 1/50,000 if it still shows agglutination with bacteria in all tubes (until the appearance of tubes without agglutination).

Clinical application:
The agglutinin (specific Abs) titer of an antiserum can be used to diagnose a bacterial infection. Example: Patients with typhoid fever, for example, show a rise in the agglutination titer with Salmonella typhi bacteria. Agglutination reactions also provide a way to type bacteria like different species of Salmonella can be distinguished by agglutination reactions by typing test using patient antisera

2. Passive Agglutination:
1. Usually used for soluble Antigens.
2. There are two type of this agglutination:
a. Agglutination of antigen-coated erythrocytes. It is called Hemagglutination test.
b. Agglutination of antigen-coated Latex beads. It is called Indirect agglutination test.

A. Hemagglutination test (Agglutination of antigen-coated erythrocytes)
Principles:
1. Red blood cells (sheep RBCs are mostly used) in this test are used as carriers of antigens and coloring agents. Antigen-coated red blood cells are prepared by mixing a soluble antigen with tanned red blood cells (that have been treated with tannic acid or chromium chloride to make them sticky), the Ag will be adsorbed of to the surface of the red cells.
2. Diluting serum containing antibody serial dilutions into microtiter plate wells, and the antigen-coated red blood cells are then added to each well.
3. Agglutination can be noticed by the size of the agglutinated red blood cells on the bottom of the well.

1. The formation of a red spot in the well is a marker for no agglutination.
2. There are two type of Hemagglutination tests; direct Hemagglutination and Indirect agglutination.

Clinical application:

Determine if a person is using illegal drugs, such as cocaine or heroin using agglutination inhibition assay and the suspected person urine sample.

A. Latex agglutination (Indirect agglutination test)

Principles:

1. Using synthetic particles called latex beads (polystyrene material); they act as carrier for soluble antigen in agglutination tests.
2. Absorbing Ags on beads surface, many Ags (e.g. proteins) are able to adsorb easly on latex beads surface and change soluble Ag to be a particulated Ag, they are of low cost equipment, do not interfere in Ag-Ab reaction and make the reaction more visible and can be read rapidly (3-5 min) after of mixing the beads with the test sample.

Clinical application:

1. Pregnancy test is of a highly sensitive assay for small quantities of antigen in urine samples. The latex particles coated with prepared chorionic gonadotropin antibodies (anti-HCG) in pregnancy test kits. When urine from a pregnant female, which contained HCG (work here as Ag), mixed with latex beads reagent, if agglutination occurs that will indicate for pregnancy, if absence means no pregnancy.
2. ASOT Anti-streptolysin O (ASO or ASLO) is a test that detects the antibodies formed against streptolysin O bacterial toxin during infection with Streptococcus pyogens using Latex agglutination: It is important test for the treatment of rheumatic fever and post infectious glomerulonephritis by serial dilution method or slide agglutination method.

It is a rapid latex particle agglutination test that uses latex reagent, the latex particles coated with streptolysin-O the soluble Ag, the presence of infection means the presence of antistreptolysin-O in the serum leading for agglutination of the latex particles.

Procedure:

Using the micropipette apply the followings on the test card:

1- One drop application of positive control on the first area.
2- One drop application of negative control on the second area.
3- One drop application of serum sample on the third area.
4- One drop application of latex reagent on all the three areas.
5- Sample and reagents should be mixed well using wooden sticks or electronic rotator for 2 minutes (100 rpm).
6- Results:

- Agglutination in the serum sample ----------positive.
- No agglutination in the serum sample-------- negative.
- Agglutination in negative control or no Agglutination in positive control---------- error.

1. Note: the negative ASOT result does not mean there is no infection; second test should be done after 4 weeks.

ELISA- Principle, Types and Applications

ELISA is an antigen antibody reaction. In 1971, ELISA was introduced by Peter Perlmann and Eva Engvall at Stockholm University in Sweden. It is a common laboratory technique which is usually used to measure the concentration of antibodies or antigens in blood.

ELISA is a plate based assay technique which is used for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. An enzyme conjugated with an antibody reacts with colorless substrate to generate a colored product. Such substrate is called chromogenic substrate. A number of enzymes have been used for ELISA such as alkaline phosphatase, horse radish peroxidase and beta galactosidase. Specific substrate such as ortho-phenyldiamine dihydrochloride (for peroxidase), paranitrophenyl phosphate (for alkaline phosphatase) are used which are hydrolysed by above enzymes to give colored end product.Principle

ELISAs are typically performed in 96-well polystyrene plates. The serum is incubated in a well, and each well contains a different serum. A positive control serum and a negative control serum would be included among the 96 samples being tested. Antibodies or antigens present in serum are captured by corresponding antigen or antibody coated on to the solid surface. After some time, the plate is washed to remove serum and unbound antibodies or antigens with a series of wash buffer. To detect the bound antibodies or antigens, a secondary antibodies that are attached to an enzyme such as peroxidase or alkaline phosphatase are added to each well. After an incubation period, the unbound secondary antibodies are washed off. When a suitable substrate is added, the enzyme reacts with it to produce a color. This color produced is measurable as a function or quantity of antigens or antibodies present in the given sample. The intensity of color/ optical density is measured at 450nm. The intensity of the color gives an indication of the amount of antigen or antibody.

Types of ELISA

Frequently there are 3 types of ELISA on the basis of binding structure between the Antibody and Antigen.

1. Indirect ELISA
2. Sandwich ELISA
3. Competitive

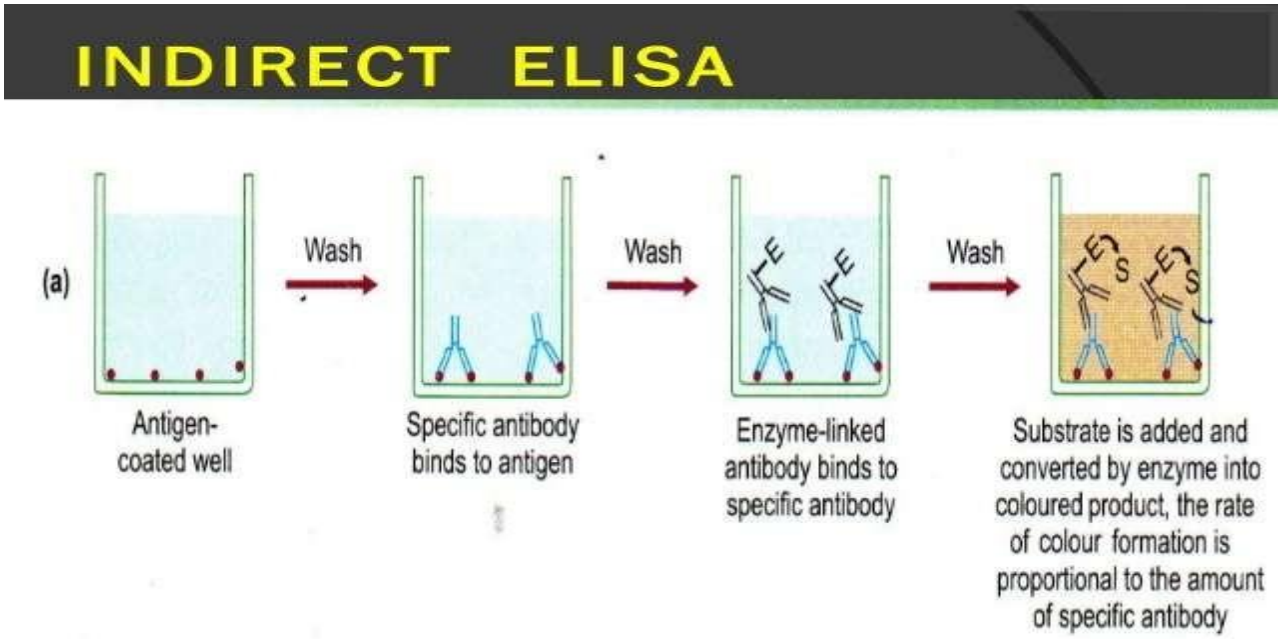
1. Indirect ELISA

Antibody can be detected or quantitatively determined by indirect ELISA. In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react with the coated antigen. Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody. Unbound secondary antibody is then washed away and a specific substrate for the enzyme is added. Enzyme hydrolyzes the substrate to form colored products. The amount of colored end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.Procedure of Indirect ELISA

1. Coat the micro titer plate wells with antigen.
2. Block all unbound sites to prevent false positive results.
3. Add sample containing antibody (e.g. rabbit monoclonal antibody) to the wells and incubate the plate at 37°c.
1. Wash the plate, so that unbound antibody is removed.
2. Add secondary antibody conjugated to an enzyme (e.g. anti- mouse IgG).
3. Wash the plate, so that unbound enzyme-linked antibodies are removed.

4. Add substrate which is converted by the enzyme to produce a colored product.

Reaction of a substrate with the enzyme to produce a colored product



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Advantages

Increased sensitivity, since more than one labeled antibody is bound per primary antibody. •

A wide variety of labeled secondary antibodies are available commercially. •

Maximum immunoreactivity of the primary antibody is retained because it is not labeled. •

Versatile because many primary antibodies can be made in one species and the same labeled • secondary antibody can be used for detection.

Flexibility, since different primary detection antibodies can be used with a single labeled secondary • antibody.

Cost savings, since fewer labeled antibodies are required. •

Different visualization markers can be used with the same primary antibody. •

Disadvantages

Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal. •

An extra incubation step is required in the procedure. •

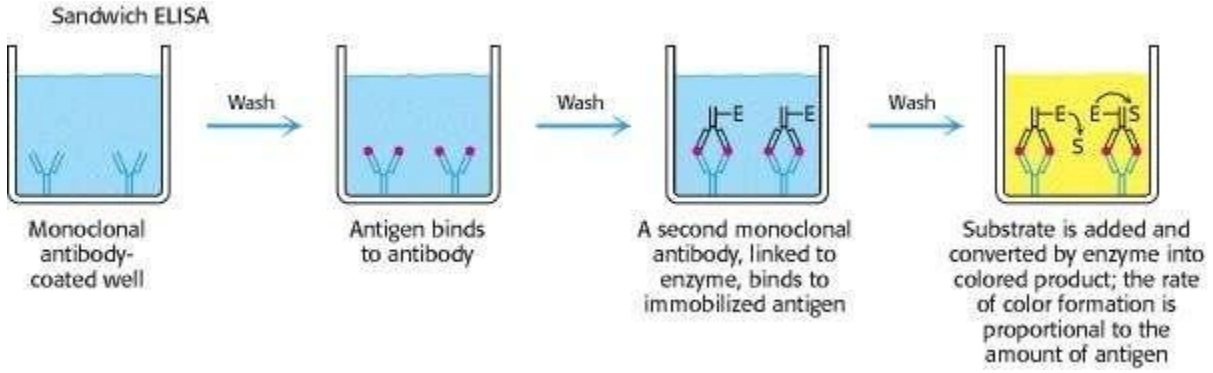
2. Sandwich ELISA

Antigen can be detected by sandwich ELISA. In this technique, antibody is coated on the microtiter well. A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. Then after unbound secondary antibody is removed by washing. Finally substrate is added to the plate which is hydrolyzed by enzyme to form colored products.

Procedure of sandwich ELISA

1. Prepare a surface to which a known quantity of antibody is bound.
2. Add the antigen-containing sample to the plate and incubate the plate at 37°c.

3. Wash the plate, so that unbound antigen is removed.
4. Add the enzyme-linked antibodies which are also specific to the antigen and then incubate at 37°c.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme to produce a colored product.
7. Reaction of a substrate with the enzyme to produce a colored product.



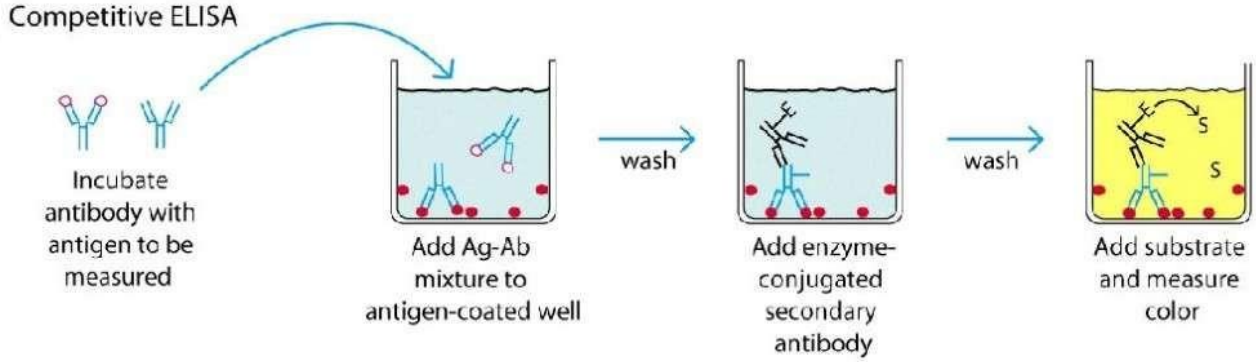
Advantages

- High specificity, since two antibodies are used the antigen is specifically captured and detected.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.
- Flexibility and sensitivity, since both direct and indirect detection methods can be used.

Competitive ELISA

- This test is used to measure the concentration of an antigen in a sample.

In this test, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to the microtitre well which is coated with antigen. The more the antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. After the well is washed, enzyme conjugated secondary antibody specific for isotype of the primary antibody is added to determine the amount of primary antibody bound to the well. The higher the concentration of antigen in the sample, the lower the absorbance.



Procedure

1. Antibody is incubated with sample containing antigen.
2. Antigen-antibody complex are added to the microtitre well which are pre-coated with the antigen.
3. Wash the plate to remove unbound antibody.
4. Enzyme linked secondary antibody which is specific to the primary antibody is added.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme into a fluorescent signal.

Advantages

- High specificity, since two antibodies are used.
- High sensitivity, since both direct and indirect detection methods can be used.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.

Application of ELISA

1. Presence of antigen or the presence of antibody in a sample can be evaluated.
2. Determination of serum antibody concentrations in a virus test.
3. Used in food industry when detecting potential food allergens.
4. Applied in disease outbreaks- tracking the spread of disease e.g. HIV, bird flu, common, colds, cholera, STD etc.

Immunofluorescence

Immunofluorescence is an assay which is used primarily on biological samples and is classically defined as a procedure to detect antigens in cellular contexts using antibodies. The specificity of antibodies to their antigen is the base for immunofluorescence.The property of certain dyes absorbing light rays at one particular wavelength (ultraviolet light) and emitting them at a different wavelength (visible light) is known as fluorescence. In immunofluorescence test, fluorescent dye which illuminates in UV light are used to detect/show the specific combination of an antigen and antibody. The dye usually used is fluorescein isothiocynate, which gives yellow-green fluorescence. Immunofluorescence tests are also termed as fluorescent antibody test (FAT). Fluorescent dyes, such as fluorescein isothiocyanate and lissamine rhodamine, can be tagged with antibody molecules. They emit blue-green and orange-red fluorescence, respectively under ultraviolet (UV) rays in the fluorescence microscope. This forms the basis of the immunological test. Immunofluorescence tests have wide applications in research and diagnostics. These tests are broadly of two types:

1. Direct immunofluorescence test
2. Indirect immunofluorescence test

Direct immunofluorescence test

Direct immunofluorescence test is used to detect unknown antigen in a cell or tissue by employing a known labeled antibody that interacts directly with unknown antigen. If antigen is present, it reacts with labeled antibody and the antibody coated antigen is observed under UV light of the fluorescence. It involves use of labeled antiviral antibody.

Method:

The specimen is placed on slide; fluorescent labeled antibody is then added to it and allowed for some time for Antigen-Antibody reaction. The preparation is then washed which will allow the removal of other components except the complex of antigen and fluorescent labeled antibody. On microscopy (Fluorescence Microscopy), Antigen- Antibody complex are observed fluorescing due to the dye attached to antibody.The need for preparation of separate labeled antibody for each pathogen is the major disadvantage of the direct immunofluorescence test.

Indirect immunofluorescence test

Indirect fluorescence is a double antibody technique. The unlabeled antibodies which have bound to the antigens are visualized by a fluorescent antiglobulin reagent directed at the unlabeled antibodies. The

indirect immunofluorescence test is used for detection of specific antibodies in the serum and other body fluids for sero-diagnosis of many infectious diseases.

Method:

Indirect immunofluorescence is a two-stage process.

First stage: A known antigen is fixed on a slide. Then the patient's serum to be tested is applied to the slide, followed by careful washing. If the patient's serum contains antibody against the antigen, it will combine with antigen on the slide.

Second stage: The combination of antibody with antigen can be detected by addition of a fluorescent dye-labeled antibody to human IgG, which is examined by a fluorescence microscope.The first step in the indirect immunofluorescence test is the incubation of a fixed antigen (e.g., in a cell or tissue) with unlabeled antibody, which becomes associated with the antigen. After careful washing, a fluorescent antibody (e.g. fluorescent labeled anti-IgG) is added to the smear. This second antibody will become associated to the first, and the antigen–antibody complex can be visualized on the fluorescence microscope.The indirect method has the advantage of using a single labeled antiglobulin (antibody to IgG) as a ‑universal reagent‖ to detect many different specific antigen–antibody reactions. The test is often more sensitive than the direct immunofluorescence test.

Indirect immunofluorescence test is used widely to:

1. Detect specific antibodies for serodiagnosis of syphilis, leptospirosis, amoebiasis, toxoplasmosis, and many other infectious diseases;
2. Identify the class of a given antibody by using fluorescent antibodies specific for different immunoglobulin isotypes;
3. Identify and enumerate lymphocyte subpopulations by employing monoclonal antibodies and cytofluorographs; and
4. Detect autoantibodies, such as antinuclear antibodies in autoimmune diseases.

Immunofluorescence may also be used to analyze the distribution of proteins, glycans, and small biological and non-biological molecules. Immunofluorescence has been widely used in biological research and medical research.

The major limitation of immunofluorescence is that the technique requires

1. expensive fluorescence microscope and reagents,
2. trained personnel
3. have a factor of subjectivity that may result in erroneous results

The biological samples include tissue and cells. Immunofluorescence aid to evaluate whether or not cells in a particular sample express the antigen in study. In cases where an immune-positive signal is found, immunofluorescence also helps to determine which subcellular compartments are expressing the antigen. Immunofluorescence can be used on cultured cell lines, tissue sections, or individual cells.

NUCLEIC ACID BASED METHODS
Polymerase chain reaction (PCR): Principle, procedure or steps, types and application
Principle:

- Polymerase chain reaction is method for amplifying particular segments of DNA. It is an enzymatic method and carried out invitro. PCR technique was developed by Kary mullis in 1983. PCR is very simple, inexpensive technique for characterization, analysis and synthesis of specific fragments of DNA or RNA from virtually any living organisms.

Steps or procedures:

PCR consists of three basic steps.

1. Denaturation:

- Two strand of DNA separates (melt down) to form single stranded DNA
- This step is generaly carried out at 92C-96C for 2 minutes.

2. Annealing:

- Annealing of primer to each strand is carried out at 45C-55C

3. Extension:

- DNA polymerase adds dNTPs complementary to templates strands at 3'end of primer.
- It is carried out at temperature of 72C.
- These three steps are repeated 20-30 times in an automated thermocycler that can heat and cool the reaction mixture in tube within very short time. This results in exponential accumulation of specific DNA fragments.
- The doubling of number of DNA strands corresponding to target sequences can be estimated by amplification number associated with each cycle using the formula.
- Amplification=2n, where n=no. of PCR cycle.
- PCR can amplify a desired DNA sequences of any origin hundred or millions time in a matter of hour, which is very short in comparison to recombinant DNA technology.
- PCR is especially valuable because the reaction is highly specific, easily automated and very sensitive.
- It is widely used in the fields like- clinical medicine for medical diagnosis, diagnosis of genetic diseases, forensic science; DNA finger printing, evolutional biology

Factors affecting PCR

i. Primer

- PCR reaction needs two primer, a forward and a reverse primer
- Primer are synthesized oligonucleotide usually ranging from 15-30 bases long
- Primers are designed such that at 3'end they donot have more than two bases complementary to each other as this results in PRIMER-DIMER formation
- The G+C contents is in the range of 40-60%
- The melting temperature (Tm) of both forward and reverse primer is usually the same.
- Low concentration of primer results in poor yield while high concentration may results in non specific amplification. Hence optimal concentration of primer is needed ie 0.1-1µ

ii. Amount of Template DNA

- Optimal amount of template DNA usually in nano gram. Higher concentration inhibit or results in non specific amplification.
- Taq DNA polymerase:
- Taq DNA polymerase is 94 KD thermostable DNA polymerase isolated from Thermus aquaticus.
- Optimal temperature for activity of Taq polymerase is 72° but it can tolerate high temperature and donot affects by denaturating temperature of 94°C.
- Taq DNA polymerase have both 5'-3' polymerase activity and 5'-3' exonuclease activity. But it lacks 3'-5' exonuclease activity (proof reading activity).

Types of PCR

1. Standard PCR:
   - Nested PCR
   - Random amplified polymorphic DNA
   - Long PCR
   - Restriction fragment length polymorphism (RFLP)
   - Amplified fragment length polymorphism (AFLP)
   - Multiplex PCR
   - Single cell PCR
   - Fast cycling PCR
   - In situ PCR
   - High fidelity PCR
   - Asymmetric PCR
   - Repetitive sequence based PCR
   - Overlap extension PCR
   - Assemble PCR
   - Mini primer PCR
   - Solid phase PCR
   - Touch Down PCR
2. Reverse transcriptase Polymerase chain reaction (RT-PCT): for RNA
   - One step RT-PCR
   - Two step RT-PCR
3. Real time PCR: for DNA or RNA
   - Dye binding to ds DNA
   - Fluorescent probes

Application:

1. Forensic science: DNA finger printing, paternity testing and criminal identification
2. Diagnosis: Molecular identification of microorganisms
3. Evolution study: evolutionary biology
4. Fossil study: paleontology
5. Gene cloning and expression

1.6. Gene sequencing

Nucleic acid probe

In molecular biology, **hybridization** (or **hybridisation**) is a phenomenon in which single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules anneal to complementary DNA or RNA. Though a double-stranded DNA sequence is generally stable under physiological conditions, changing these conditions in the laboratory (generally by raising the surrounding temperature) will cause the molecules to separate into single strands. These strands are complementary to each other but may also be complementary to other sequences present in their surroundings. Lowering the surrounding temperature allows the single-stranded molecules to anneal or ―hybridize‖ to each other.

Applications

Hybridization is a basic property of nucleotide sequences and is taken advantage of in numerous molecular biology techniques. Overall genetic relatedness of two species can be determined by hybridizing segments of their DNA (DNA-DNA hybridization). Due to sequence similarity between closely related organisms, higher temperatures are required to melt such DNA hybrids when compared to more distantly related

organisms. A variety of different methods use hybridization to pinpoint the origin of a DNA sample, including the polymerase chain reaction (PCR). In another technique, short DNA sequences are hybridized to cellular mRNAs to identify expressed genes. Pharmaceutical drug companies are exploring the use of antisense RNA to bind to undesired mRNA, preventing the ribosome from translating the mRNA into protein.

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) is a laboratory method used to detect and locate a DNA sequence, often on a particular chromosome

Researchers Joseph Gall and Mary Lou Pardue realized in the 1960s that molecular hybridization could be used to identify the position of DNA sequences *in situ* (i.e., in their natural positions within a chromosome). In 1969, the two scientists published a paper demonstrating that radioactive copies of a ribosomal DNA sequence could be used to detect complementary DNA sequences in the nucleus of a frog egg.[5] Since those original observations, many refinements have increased the versatility and sensitivity of the procedure to the extent that in situ hybridization is now considered an essential tool in cytogenetics.

TYPHOID

Typhoid is a bacterial infection that can lead to a high fever, diarrhea, and vomiting. It can be fatal. It is caused by the bacteria *Salmonella typhi.*

The infection is often passed on through contaminated food and drinking water, and it is more prevalent in places where handwashing is less frequent. It can also be passed on by carriers who do not know they carry the bacteria.Annually, there are around 5,700 cases in the United States, and 75 percent of these start while traveling internationally. Globally, around 21.5 million people a year contract typhoid.If typhoid is caught early, it can be successfully treated with antibiotics; if it is not treated, typhoid can be fatal.

Fast facts on typhoid

- Typhoid is a common bacterial infection in countries with low incomes.
- Untreated, it is fatal in around 25 percent of cases.
- Symptoms include a high fever and gastrointestinal problems.
- Some people carry the bacteria without developing symptoms
- Most cases reported in the United States are contracted overseas
- The only treatment for typhoid is antibiotics

Typhoid is an infection caused by Salmonella typhimurium bacteria that is spread from human to human.Typhoid is an infection caused by the bacterium *Salmonella typhimurium (S. typhi)*.

The bacterium lives in the intestines and bloodstream of humans. It spreads between individuals by direct contact with the feces of an infected person.No animals carry this disease, so transmission is always human to human.If untreated, around 1 in 5 cases of typhoid can be fatal. With treatment, fewer than 4 in 100 cases are fatal.*S. typhi* enters through the mouth and spends 1 to 3 weeks in the intestine. After this, it makes its way through the intestinal wall and into the bloodstream.From the bloodstream, it spreads into other tissues and organs. The immune system of the host can do little to fight back because *S. typhi*can live within the host's cells, safe from the immune system.Typhoid is diagnosed by detecting the presence of *S. typhi* via blood, stool, urine, or bone marrow sample.

Symptoms

Symptoms normally begin between 6 and 30 days after exposure to the bacteria.

The two major symptoms of typhoid are fever and rash. Typhoid fever is particularly high, gradually increasing over several days up to 104 degrees Fahrenheit, or 39 to 40 degrees Celsius.The rash, which does not affect every patient, consists of rose-colored spots, particularly on the neck and abdomen.

Other symptoms can include:

- weakness
- abdominal pain
- constipation
- headaches

Rarely, symptoms might include confusion, diarrhea, and vomiting, but this is not normally severe. In serious, untreated cases, the bowel can become perforated. This can lead to peritonitis, an infection of the tissue that lines the inside of the abdomen, which has been reported as fatal in between 5 and 62 percent of cases. Another infection, paratyphoid, is caused by *Salmonella enterica*. It has similar symptoms to typhoid, but it is less likely to be fatal.

Treatment

The only effective treatment for typhoid is antibiotics. The most commonly used are ciprofloxacin (for non-pregnant adults) and ceftriaxone.Other than antibiotics, it is important to rehydrate by drinking adequate water.In more severe cases, where the bowel has become perforated, surgery may be required.

Typhoid antibiotic resistance

As with a number of other bacterial diseases, there is currently concernabout the growing resistance of antibiotics to *S. typhi*.

This is impacting the choice of drugs available to treat typhoid. In recent years, for example, typhoid has become resistant to trimethoprim-sulfamethoxazole and ampicillin.Ciprofloxacin, one of the key medications for typhoid, is also experiencing similar difficulties. Some studies have found *Salmonella typhimurium* resistance rates to be around 35 percent.

Causes

Typhoid is caused by the bacteria *S. typhi* and spread through food, drinks, and drinking water that are contaminated with infected fecal matter. Washing fruit and vegetables can spread it, if contaminated water is used.Some people are asymptomatic carriers of typhoid, meaning that they harbor the bacteria but suffer no ill effects. Others continue to harbor the bacteria after their symptoms have gone. Sometimes, the disease can appear again.People who test positive as carriers may not be allowed to work with children or older people until medical tests show that they are clear.

Prevention

Countries with less access to clean water and washing facilities typically have a higher number of typhoid cases.

Vaccination.

Before traveling to a high-risk area, getting vaccinated against typhoid fever is recommended.
This can be achieved by oral medication or a one-off injection:

- Oral: a live, attenuated vaccine. Consists of 4 tablets, one to be taken every second day, the last of which is taken 1 week before travel.
- Shot, an inactivated vaccine, administered 2 weeks before travel.

Vaccines are not 100 percent effective and caution should still be exercised when eating and drinking.Vaccination should not be started if the individual is currently ill or if they are under 6 years of age. Anyone with HIV should not take the live, oral dose.The vaccine may have adverse effects. One in 100 people will experience a fever. After the oral vaccine, there may be gastrointestinal problems, nausea, and headache. However, severe side effects are rare with either vaccine.There are two types of typhoid vaccine available, but a more powerful vaccine is still needed. The live, oral version of the vaccine is the strongest of the two. After 3 years, it still protects individuals from infection 73 percent of the time. However, this vaccine has more side effects.The current vaccines are not always effective, and because typhoid is so prevalent in poorer countries, more research needs to be done to find better ways of preventing its spread.

Eliminating typhoid

Even when the symptoms of typhoid have passed, it is still possible to be carrying the bacteria.
This makes it hard to stamp out the disease, because carriers whose symptoms have finished may be less careful when washing food or interacting with others.
People traveling in Africa, South America, and Asia, and India in particular, should be vigilant.

Avoiding infection

Typhoid is spread by contact and ingestion of infected human feces. This can happen through an infected water source or when handling food.
The following are some general rules to follow when traveling to help minimize the chance of typhoid infection:

- Drink bottled water, preferably carbonated.
- If bottled water cannot be sourced, ensure water is heated on a rolling boil for at least one minute before consuming.
- Be wary of eating anything that has been handled by someone else.
- Avoid eating at street food stands, and only eat food that is still hot.
- Do not have ice in drinks.
- Avoid raw fruit and vegetables, peel fruit yourself, and do not eat the peel.

HBV

Hepatitis B is an infection of the liver by the hepatitis B virus. It can be acute and self-resolving, or it can be chronic, leading to cirrhosis and liver cancer.
HBV is a major global health problem. Worldwide, some 887,000 people died from HBV-related liver disease in 2015. Between 850,000 and 2.2 million people in the United States (U.S.) are thought to be living with chronic HBV infection. For most adults, HBV is a short-term illness that causes no permanent damage, but 2 to 6 percent of adults infected will develop a chronic infection that can potentially lead to liver cancer. Around 90 percent of infants with the virus will develop chronic infection.There is no cure for HBV, but immunization can prevent initial infection. Antiviral medication can treat chronic infections.

Fast facts on hepatitis B

Here are some key points about hepatitis B. More detail is in the main article.

- Hepatitis B virus (HBV) is a virus that is spread through blood and other bodily fluids.

- Symptoms affect some people for a short time, but others will develop chronic symptoms and complications that can be fatal.
- Up to 2.2 million people in the United States (U.S.) have chronic HBV infection.
- Many cases go unreported or remain undiagnosed until a person shows signs of end-state liver disease.
- HBV can survive for up to 7 days outside the body at room temperature, on environmental surfaces.
- Since 1991, all infants in the U.S. have been vaccinated against HBV.

HBV can cause infection and inflammation of the liver. A person can be infected and pass on the virus without knowing it.Some individuals have no symptoms, some experience only the initial infection, but others remain chronically infected, as the virus continues to attack the liver over time without being detected. Irreversible liver damage can result. In 2014, 2,953 cases were reported to the Centers for Disease Control and Prevention (CDC), but the actual number of acute cases may have been as high as 19,200.Globally, chronic infection due to HBV is thought to affect 240 million people, and around 786,000 people die from HBV-related liver disease each year.

Causes

Hepatitis B is caused by infection of the body with the hepatitis B virus.The hepatitis B virus (HBV) is found in blood and bodily fluids. It can be transmitted through semen, vaginal fluids, and blood, and it can pass from a mother to a newborn during delivery. Sharing needles and having unprotected sex increase the risk.People tend to catch HBV when they visit a part of the world in which infection is more common.A person can spread the condition without being aware, as it may be symptomless.

Transmission

Hepatitis B is transmitted when blood, semen, or another bodily fluid from a person infected with the virus enters the body of someone who is not infected. This may be through a puncture in the skin, a shared needle, or the exchange of body fluids.

Infection can happen:

- as an infected mother gives birth
- during sexual activity
- through sharing needles, syringes, or other drug-injection equipment
- through unsafe tattoo techniques
- by sharing personal hygiene items such as razors or toothbrushes

Health workers can be at risk if they are exposed to unsafe medical practices, such as reusing medical equipment, not using personal protection, or incorrect disposal of sharps.HBV is not spread through food or water, sharing eating utensils, breastfeeding, hugging, kissing, holding hands, coughing, sneezing, or insects that bite.However, the virus can survive outside the body for at least 7 days. During this time, the virus can still cause infection if it enters the body of a person who is not protected by the vaccine.

Symptoms

Most infections occur during infancy or childhood. They are rarely diagnosed, as there may be few obvious symptoms.Symptoms of a new infection may not be apparent in children under 5 years of age and adults with a suppressed immune system. Among those aged 5 years and over, between 30 and 50 percent will show initial signs and symptoms.

These include:

- fever
- joint pain
- fatigue
- nausea
- vomiting

- loss of appetite
- abdominal pain
- dark urine
- clay-colored stools
- jaundice, or a yellowing of the skin and whites of the eyes

Acute symptoms appear from 60 to 120 days after exposure to the virus, and they can last from several weeks to 6 months.

A person with chronic HBV infection may have ongoing episodes of abdominal pain, persistent fatigue, and aching joints.

Tests and diagnosis

A blood test can diagnose acute and chronic HBV infection.Screening is available for people who have a higher risk of HBV infection or complications due to undiagnosed HBV infection.

These include:

- infants born to mothers with HBV
- sex partners of infected persons
- sexually active individuals who engage in unprotected intercourse or have multiple partners
- men who have sex with men
- injection drug users
- people who share a household with someone who has chronic HBV infection
- health care and public safety workers at risk from occupational exposure, for example, to blood or blood-contaminated body fluids
- hemodialysis patients
- anyone receiving chemotherapy for cancer
- anyone coming from a region with a high incidence of HBV, including some Asian countries
- all women during pregnancy

If a woman has HBV during pregnancy, the newborn must be vaccinated and receive hepatitis B immune globulin (HBIG) within 12 to 24 hours after birth.

Treatment

There is no specific treatment, cure, or medication for an acute HBV infection. Supportive care will depend on the symptoms.

Treatment for suspected exposure

Anyone who has unprotected exposure to another individual's potentially infected blood or body fluid can undergo a post-exposure "prophylaxis" protocol.This consists of HBV vaccination and HBIG given after the exposure and before acute infection develops.This protocol will not cure an infection that has occurred, but it decreases the rate of acute infection.

Treatment for chronic HBV infection

For chronic HBV infection, the World Health Organization (WHO) recommend treating the individual with an antiviral medication.This is not a cure, but it can stop the virus from replicating and prevent its progression into advanced liver disease.A person with chronic HBV infection can develop cirrhosis or liver cancer quickly and without warning. In low-income settings, liver cancer can be fatal within months of diagnosis.Persons with chronic HBV infection require ongoing medical evaluation and ultrasound of the liver every 6 months to monitor for liver damage or worsening disease.

Prevention

A vaccine against HBV has been available since 1982. This is a series of three injections. The first injection is given soon after birth, the second at least 1 month later, and the third dose is given at least 8 weeks after

the second dose.The CDC recommends that all children receive a birth dose of HBV vaccine and complete the series by 6 to 18 months of age.

Others who should receive the vaccine include:

- children and adolescents not previously vaccinated
- all health care workers
- anyone who may be exposed to blood and blood products through work or treatment
- dialysis patients and recipients of solid organ transplants
- residents and staff of correctional facilities, halfway houses, and community residences
- people who inject drugs
- household and sexual contacts of people with chronic HBV infection
- those with multiple sexual partners
- travelers to countries where HBV is common if they have not been vaccinated

The complete vaccine series induces protective antibody levels in over 95 percent of people vaccinated. Protection lasts for at least 20 years and is usually lifelong.

Other preventive measures

Infection can be prevented by:

- wearing appropriate protective equipment when working in healthcare settings or dealing with medical emergencies
- not sharing needles
- following safe sexual practices
- cleaning any blood spills or dried blood with gloved hands using 1:10 dilution of one part household bleach to 10 parts of water for disinfecting the area.

HCV

Hepatitis C is a contagious, viral liver disease. It is the most common blood-borne disease in the United States, and most people with hepatitis C do not realize that they have it.

The disease spreads by blood-to-blood contact, and primarily by the use of injectable drugs. There are immunizations against hepatitis A and B, but not C. To prevent infection, it is necessary to avoid exposure to the hepatitis C virus (HCV).If the virus does not clear within six months, the infection becomes chronic and only curable with medication. HCV can result in scarring, cirrhosis, cancer of the liver, and in some cases, death. There are, however, new medications that can cure chronic hepatitis C infection.This article will focus specifically on the symptoms, causes, and treatments of hepatitis C in chronic and acute forms.

Fast facts on Hepatitis C

- Hepatitis C (HCV) is a type of viral liver infection and may occur as acute or chronic.
- Acute HCV can cause abdominal discomfort, nausea, fatigue, and fever. Chronic HCV can lead to more severe scarring of the liver and liver cancers.
- HCV is the most common bloodborne disease in the United States (U.S.), and it is mainly transferred by intravenous drug use. Unsafe sex and contact with infected blood can also spread HCV.
- Direct-acting antivirals (DAAs) are now prescribed to treat HCV. They are highly effective but can cost over $100,000 for a three-month course.
- HCV is preventable through safe sex and avoiding injectable drugs.

HCV is a virus that damages the liver.

Hepatitis is an inflammation of the liver. There are several strains of viral hepatitis. The most common types are A, B, and C.HCV is the most widespread blood-borne disease in the United States (U.S.). An

estimated 2.7 to 3.9 million people have chronic HCV infection.The virus invades the cells in the liver and causes swelling and dysfunction.There is no vaccination for HCV. The presence of HCV antibodies in the immune system does not provide long-term protective immunity in the same way as a vaccine. The immune system holds no permanent record of HCV antibodies.A person can become re-infected with a different strain of the virus.

Symptoms

HCV often does not present symptoms at its acute stage. However, the condition can escalate to a chronic stage at which potentially fatal complications can develop.

Acute hepatitis C

Acute HCV infection is rarely diagnosed due to the lack of definitive symptoms. It is often referred to as a silent epidemic. The average time frame from exposure to noticeable symptoms is between 4 and 15 weeks. During this acute period, symptoms will not seem different to those caused by any other viral syndrome. People with acute HCV will experience:

- abdominal discomfort
- nausea
- fever
- joint pain
- fatigue
- jaundice, rarely
- clay-colored stools, rarely

Chronic hepatitis C

HCV becomes chronic when the virus remains in the blood for six months after the acute infection period. If the presence of HCV viruses is detected in testing at least twice over this period, a chronic diagnosis is confirmed.

The infection will not resolve unless treated with medication.

Most people experience no symptoms with chronic HCV infection. Some may experience ongoing episodes of abdominal pain, persistent fatigue, and aching joints.

After 25 to 30 years, this chronic infection may result in significant scarring, or fibrosis, of the liver. If the entire liver becomes scarred, this can progress to cirrhosis, liver failure, and possibly liver cancer.

The overall health of the infected individual and their liver will determine how quickly damage and possible progression to cirrhosis will occur. The genotype of the virus plays less of a role than the physical health of the person with HCV.

It is not until the liver is on the verge of collapse that the extent of HCV damage is apparent.

Causes and risk factors

People who inject drugs are the most at-risk group for spreading and contracting HCV.

The HCV virus causes hepatitis C. The virus is transmitted through blood-to-blood contact.

Viruses are inactive until they enter the living cell of a host. They will then hijack the hardware of the cell to make copies of themselves. Chronic HCV infection consists of millions, or possibly billions, of copies of the virus circulating within the body.

For a blood-to-blood infection to occur, blood from an infected person must enter the body of someone who is not infected. The biggest risk factor for becoming infected with HCV is sharing needles or equipment used to inject drugs.

HCV is not transmitted through casual contact, respiratory droplets, sharing food, kissing, or mosquito bites.

A speck of blood so small that it is not viewable to the naked eye can carry hundreds of hepatitis C virus particles. Cleaning a needle with alcohol, rinsing it with soap and water, and even letting the needle and syringe air-dry for several days will not kill the virus.

Once the virus is injected into the body, even if on only one occasion, exposure has occurred and infection is possible. Injecting drugs causes approximately 67 percent of global cases.

The Centers for Disease Control (CDC) have identified the factors that lead to the greatest risk of HCV exposure. These include:

- being born between 1945 and 1965
- receiving transfusions or organ transplants before 1992
- using or having used injectable drugs
- hemophilia
- long-term hemodialysis
- exposure to a needle stick, especially for people who work in healthcare
- HIV
- getting a tattoo in an unregulated setting
- risky sexual behaviors, such as unprotected intercourse with an intravenous drug user
- being born to a mother with HCV infection
- being incarcerated
- a history of long-term daily alcohol use
- unexplained liver disease
- snorting drugs

People who are at risk due to these factors can receive a screening to rule out HCV.

Tests and diagnosis

HCV is often overlooked or mistaken for a less severe viral illness. It is rare for the infection to be diagnosed during the acute phase.

The immune system of a person infected with HCV responds to the infection and, in only 20 percent of cases, will clear the virus without assistance. The remaining 80 percent of cases will turn chronic, and it can become possible to spread HCV to others without knowing.

Screening tests are available for people the CDC have determined are at risk. People can test for HCV using a simple blood test called an HCV antibody screen.

There is a rapid test approved by the Food and Drug Administration (FDA) that provides results in 20 minutes. Otherwise, the blood is drawn from a vein and processed at a lab. A negative test means that the person has not been exposed. A positive test means that the person has been exposed to HCV, but does not necessarily prove ongoing infection.

All positive HCV antibody results will lead to a second blood test called HCV RNA (PCR). This test will demonstrate whether the virus is still present.

A person with a positive PCR should see a liver specialist or a provider trained to treat HCV.

It is important to note that the positive antibody test will always remain positive, whether or not the virus is still present.

Once the chronic infection is confirmed, the genotype of the virus is established by testing. This will determine the percentile cure rate, the length of treatment, and the preferred medications.

In some instances, a liver biopsy may be recommended for grading the severity of the disease, staging the degree of fibrosis, and evaluating the extent of liver damage.

Treatment

Treatment of HCV removes the viruses from the body.

The current treatment for chronic hepatitis C is a combination of medications.
The choice of medication and duration of treatment depends on the genotype of the virus. Genotype 1a is the most prevalent in the US, and presently there are several recommended treatment options using a combination of powerful antiviral medications.
Direct-acting antivirals (DAAs) are the newest available agents to treat HCV. These medications work by targeting specific steps in the HCV life cycle to disrupt the reproduction of viral cells.
Before the availability of DAAs, the treatment for chronic HCV was lengthy and uncomfortable, with less than ideal cure rates. Now the cure rates are over 90 percent. The average duration of treatment is 8 to 12 weeks. The medications are well-tolerated, with the most common side effects being headache and fatigue.
However, new medications for HCV can cost upwards of $100,000. Check with your insurer whether they cover DAA combination therapy, and what information they need to approve coverage**.**
It is the best course of action to treat chronic HCV early before the patient develops complications or progresses to life-threatening circumstances
As education, risk-based screening, exposure prevention, and the arrival of well-tolerated treatments continue, the outlook for preventing serious liver complications and curing people who have chronic hepatitis C infection has never been better.

Prevention

Preventing HCV involves limiting exposure to the virus in the first instance.
Because HCV can only be transmitted through blood to infected blood exposure, the number one way to prevent spreading HCV is by not sharing needles and avoiding all contact with the blood of other people.
Once identified, people infected with HCV should receive both the hepatitis A and B vaccines, and make lifestyle changes to promote optimum liver health.
Obesity, smoking, diabetes and alcohol consumption can accelerate the rate of liver scarring. It is important that all individuals with HCV infection maintain good health. This involves:

- avoiding or quitting smoking
- maintaining ideal weight
- managing co-existing health problems
- abstaining from all alcohol

HIV

AIDS (acquired immunodeficiency syndrome) is a syndrome caused by a virus called HIV (human immunodeficiency virus). The disease alters the immune system, making people much more vulnerable to infections and diseases. This susceptibility worsens if the syndrome progresses.
HIV is found throughout all the tissues of the body but is transmitted through the body fluids of an infected person (semen, vaginal fluids, blood, and breast milk).
In this article, we explain HIV and AIDS, their symptoms, causes, and treatments.
HIV is a virus that attacks immune cells called CD-4 cells, which are a subset of T cells. AIDS is the syndrome, which may or may not appear in the advanced stage of HIV infection.

HIV is a virus.

AIDS is a medical condition.

HIV infection can cause AIDS to develop. However, it is possible to contract HIV without developing AIDS. Without treatment, HIV can progress and, eventually, it will develop into AIDS in the vast majority of cases

Causes

HIV is a retrovirus that infects the vital organs and cells of the human immune system.
The virus progresses in the absence of antiretroviral therapy (ART) - a drug therapy that slows or prevents the virus from developing.
The rate of virus progression varies widely between individuals and depends on many factors.
These factors include the age of the individual, the body's ability to defend against HIV, access to healthcare, the presence of other infections, the individual's genetic inheritance, resistance to certain strains of HIV, and more.

How is HIV transmitted?

Sexual transmission — it can happen when there is contact with infected sexual fluids (rectal, genital, or oral mucous membranes). This can happen while having sex without a condom, including vaginal, oral, and anal sex, or sharing sex toys with someone who is HIV-positive.

Perinatal transmission — a mother can transmit HIV to her child during childbirth, pregnancy, and also through breastfeeding.

Blood transmission — the risk of transmitting HIV through blood transfusion is extremely low in developed countries, thanks to meticulous screening and precautions. However, among people who inject drugs, sharing and reusing syringes contaminated with HIV-infected blood is extremely hazard.

Symptoms

For the most part, the later symptoms of HIV infection are the result of infections caused by bacteria, viruses, fungi, and/or parasites.
These conditions do not normally develop in individuals with healthy immune systems, which protect the body against infection.

Early symptoms of HIV infection

Some people with HIV infection have no symptoms until several months or even years after contracting the virus. However, around 80 percentmay develop symptoms similar to flu 2–6 weeks after catching the virus. This is called acute retroviral syndrome.
The symptoms of early HIV infection may include:

- fever
- chills
- joint pain
- muscle aches
- sore throat
- sweats (particularly at night)
- enlarged glands
- a red rash
- tiredness
- weakness
- unintentional weight loss
- thrush

It is important to remember that these symptoms appear when the body is fighting off many types of viruses, not just HIV. However, if you have several of these symptoms and believe you could have been at risk of contracting HIV in the last few weeks, you should take a test.

Asymptomatic HIV

In many cases, after the initial symptoms disappear, there will not be any further symptoms for many years. During this time, the virus carries on developing and damaging the immune system and organs. Without medication that stops HIV replicating, this process of slow immune depletion can continue, typically for an

average of 10 years. The person living with HIV often experiences no symptoms, feels well, and appears healthy.

For people who are taking antiretrovirals and are rigidly compliant, this phase can be interrupted, with complete viral suppression. Effective antiretrovirals arrest on-going damage to the immune system.

Late-stage HIV infection

If left untreated, HIV weakens the ability to fight infection. The person becomes vulnerable to serious illnesses. This stage is known as AIDS or stage 3 HIV.

Symptoms of late-stage HIV infection may include:

- blurred vision
- diarrhea, which is usually persistent or chronic
- dry cough
- fever of above 100 °F (37 °C) lasting for weeks
- night sweats
- permanent tiredness
- shortness of breath (dyspnea)
- swollen glands lasting for weeks
- unintentional weight loss
- white spots on the tongue or mouth

During late-stage HIV infection, the risk of developing a life-threatening illness is much greater. Serious conditions may be controlled, avoided, and/or treated with other medications, alongside HIV treatment.

HIV and AIDS myths and facts

There are many misconceptions about HIV and AIDS. The virus CANNOT be transmitted from:

- shaking hands
- hugging
- casual kissing
- sneezing
- touching unbroken skin
- using the same toilet
- sharing towels
- sharing cutlery
- mouth-to-mouth resuscitation
- or other forms of "casual contact"

Diagnosis

The CDC (Centers for Disease Control and Prevention) estimates that about 1 in every 8 HIV-positive Americans is unaware of their HIV status.

HIV blood tests and results

Diagnosis is made through a blood test that screens specifically for the virus. If HIV has been found, the test result is "positive." The blood is re-tested several times before a positive result is given.

If a person has been exposed to the virus, it is crucial that they get tested as soon as possible. The earlier HIV is detected, the more likely the treatment will be successful. A home testing kit can be used as well.

After infection with HIV, it can take from 3 weeks to 6 months for the virus to show up in testing. Re-testing may be necessary. If the moment an individual was most at risk of infection was within the last 6 months, they can have the test immediately. However, the provider will urge that another test is carried out within a few weeks.

Treatment

There is currently no cure for HIV or AIDS. Treatments can stop the progression of the condition and allow most people living with HIV the opportunity to live a long and relatively healthy life.
Earlier HIV antiretroviral treatment is crucial — it improves quality of life, extends life expectancy, and reduces the risk of transmission, according to the World Health Organization's guidelines issued in June 2013.
Currently, there is no vaccine or cure for HIV, but treatments have evolved which are much more effective and better tolerated; they can improve patients' general health and quality of life considerably, in as little as one pill per day.
It is now established that, given the right treatment, someone living with HIV can reduce his or her viral load to such a degree that it is no longer detectable. After assessing a number of large studies, the that individuals who have no detectable viral load "have effectively no risk of sexually transmitting the virus to an HIV-negative partner."
This is referred to as undetectable = untransmittable (U=U).
Emergency HIV pills (post-exposure prophylaxis)
If an individual believes they have been exposed to the virus within the last 72 hours (3 days), anti-HIV medications, called PEP (post-exposure prophylaxis) may stop infection. The treatment should be taken as soon as possible after contact with the virus.
PEP is a treatment lasting 4 weeks, a total of 28 days. Monitoring for HIV will be continued after completion of the treatment.
Antiretroviral drugs
HIV is treated with antiretrovirals (ARVs). The treatment fights the HIV infection and slows down the spread of the virus in the body. Generally, people living with HIV take a combination of medications called HAART (highly active antiretroviral therapy) or cART (combination antiretroviral therapy).
There are a number of subgroups of antiretrovirals; these include:
Protease inhibitors
Protease is an enzyme that HIV needs to replicate. As the name suggests, protease inhibitors bind to the enzyme and inhibit its action, preventing HIV from making copies of itself. These include atazanavir/cobicistat (Evotaz), lopinavir/ritonavir (Kaletra), and darunavir/cobicistat (Prezcobix).
Integrase inhibitors
HIV needs the integrase enzyme to infect T cells. This drug prevents that step. Integrase inhibitors are often used in the first line of treatment because they are effective for many people, and cause minimal side effects. Integrase inhibitors include elvitegravir (Vitekta), dolutegravir (Tivicay), and raltegravir (Isentress)
Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs)
These drugs, also referred to as "nukes," interfere with HIV as it tries to replicate and make more copies of itself. NRTIs include abacavir (Ziagen), lamivudine/zidovudine (Combivir), and emtricitabine (Emtriva)

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
NNRTIs work in a similar way to NRTIs, making it more difficult for HIV to replicate.
Chemokine co-receptor antagonists
Also known as CCRF, these drugs block HIV from entering cells. They are rarely used in America because other drugs are more effective.
Entry inhibitors

Entry inhibitors prevent HIV from entering T cells. Without access to these cells, HIV cannot replicate. As with chemokine co-receptor antagonists, they are rarely used in the United States.

A combination of these drugs will be used; the exact mix of drugs is adapted to each individual. HIV treatment is usually permanent and lifelong. HIV treatment is based on routine dosage. Pills must be taken on a regular schedule, every time. Each class of ARVs has different side effects, but some possible common side effects include:

- nausea
- fatigue
- diarrhea
- headache
- skin rashes

Complementary or alternative medicine

Although widely used, alternative or complementary medications, such as herbal ones, have not been proven to be effective. According to some limited studies, mineral or vitamin supplements may provide some benefits in overall health. It is important to discuss these options with a healthcare provider because some of these options, even vitamin supplements, may interact with ARVs.

Prevention

To prevent contracting HIV, healthcare professionals advise precautions related to:

Condomless sex - having sex without a condom can put a person at risk of contracting HIV and other sexually transmitted infections (STIs). HIV can be transmitted by having sex without a condom (vaginal, oral, and/or anal sex). It can also be transmitted by sharing sex toys with someone infected with HIV. Condoms should be used with every sexual act.

Drug injection and needle sharing - intravenous drug use is an important factor in HIV transmission in developed countries. Sharing needles can expose users to HIV and other viruses, such as hepatitis C. Strategies such as needle-exchange programs are used to reduce the infections caused by drug abuse. If someone needs to use a needle, it must be a clean, unused, unshared needle.

Body fluid exposure - exposure to HIV can be controlled by employing precautions to reduce the risk of exposure to contaminated blood. Healthcare workers should use barriers (gloves, masks, protective eyewear, shields, and gowns) in the appropriate circumstances. Frequent and thorough washing of the skin immediately after coming into contact with blood or other bodily fluids can reduce the chance of infection.

Pregnancy - some ARVs can harm the unborn child. But an effective treatment plan can prevent HIV transmission from mother to baby. Precautions have to be taken to protect the baby's health. Delivery through cesarean section may be necessary.

HIV-infected mothers can pass the virus through their breast milk. However, if the mother is taking the correct medications, the risk of transmitting the virus is greatly reduced. It is important for a new mother to discuss the options with a healthcare provider.

Education - teaching people about known risk factors is vital.

DENGUE

Dengue fever, also known as breakbone fever, is a mosquito-borne infection that can lead to a severe flu-like illness. It is caused by four different viruses and spread by *Aedes*mosquitoes.

Symptoms range from mild to severe. Severe symptoms include dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF). These usually require hospitalization.

There are currently no vaccines. The best method of prevention is to avoid mosquito bites. Treatment is possible if diagnosis occurs before the patient develops DSS or DHF.

The Centers for Disease Control and Prevention (CDC) estimate that 400 million people are infected each year.

Dengue fever is rare in the United States (U.S.), but around 100 cases are reported each year, mostly among people traveling from outside the country. Outbreaks have occurred in Texas, Florida, and Hawaii.

Fast facts on Dengue fever

Here are some key points about dengue fever. More detail is in the main article.

- Dengue is transmitted by the mosquitoes *Aedes aegypti* and *Aedes albopictus*, which are found throughout the world.
- Around 2.5 billion people, or 40 percent of the world's population, live in areas where there is a risk of dengue transmission.
- Dengue is endemic in at least 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean.
- Symptoms usually begin 4 to 7 days after the mosquito bite and typically last 3 to 10 days.
- Effective treatment is possible if a clinical diagnosis is made early.

Signs and symptoms

Mosquitoes spread dengue fever.

Symptoms vary depending on the severity of the disease.

Mild dengue fever

Symptoms can appear up to 7 days after being bitten by the mosquito that carries the virus.

They include:

- aching muscles and joints
- body rash that can disappear and then reappear
- high fever
- intense headache
- pain behind the eyes
- vomiting and feeling nauseous

Symptoms usually disappear after a week, and mild dengue rarely involves serious or fatal complications.

Dengue hemorrhagic fever

At first, symptoms of DHF may be mild, but they gradually worsen within a few days. As well as mild dengue symptoms, there may be signs of internal bleeding.

A person with Dengue hemorrhagic fever may experience:

- bleeding from the mouth, gums, or nose
- clammy skin
- damage to lymph and blood vessels
- internal bleeding, which can lead to black vomit and feces, or stools
- a lower number of platelets in the blood
- sensitive stomach
- small blood spots under the skin
- weak pulse

Without prompt treatment, DHF can be fatal.

Dengue shock syndrome

DSS is a severe form of dengue. It can be fatal.

Apart from symptoms of mild dengue fever, the person may experience:

- intense stomach pain
- disorientation

- sudden hypotension, or a fast drop in blood pressure
- heavy bleeding
- regular vomiting
- blood vessels leaking fluid

Without treatment, this can result in death.

Treatment

Dengue is a virus, so there is no specific treatment or cure. However, intervention can help, depending on how severe the disease is.

For milder forms, treatment includes:

Preventing dehydration: A high fever and vomiting can dehydrate the body. The person should drink clean water, ideally bottled rather than tap water. Rehydration salts can also help replace fluids and minerals.

Painkillers, such as Tylenol or paracetamol: These can help lower fever and ease pain.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin or ibuprofen, are not advised, as they can increase the risk of internal bleeding.

More severe forms of dengue fever may need:

- intravenous (IV) fluid supplementation, or drip, if the person cannot take fluids by mouth
- blood transfusion, for patients with severe dehydration

Hospitalization will allow the individual to be properly monitored, in case symptoms get worse.

Rehydration salts, Tylenol, and paracetamol are available for purchase online.

Antibiotics

I. History of Antibiotics

A. –Magic Bullet‖ concept (early 1900's)

1. Paul Ehrlich proposed idea of using a drug that would selectively find and kill pathogen, but not harm human cells (early 1900's)
2. Basis of antimicrobial chemotherapy (drug therapy)

B. Discovery of Penicillin (1928)

1. Alexander Fleming noticed that the growth of *Staphylococcus aureus*was inhibited in the area surrounding a colony of mold that contaminated a Petri plate.
2. The compound was later isolated from the mold and named penicillin
3. 1st Antibiotic
4. Antibiotic – substance produced by one organism that inhibits another organism

C. Sulfanilamide compound in dyes was used (Sulfa Drugs, 1932)

1. Technically an antimicrobial and not an antibiotic, because it is not produced by an organism, i.e. synthetic drug
2. Resurrected interest in penicillin

D. Florey and Chain (1940, WWII)

1. 1st Successful clinical trials with penicillin
2. Fleming, Chain, and Florey won Nobel Prize

II. Antibiotic – Production and Range

A. Production

1. Naturally occurring – produced entirely by bacteria or fungi (true antibiotic)
2. Semi-synthetic antibiotics – part natural and part designed in lab
3. Synthetic antibiotics – designed in lab

B. Narrow Spectrum Antibiotic
1. Affect narrow range of bacteria, such as only Gram+
C. Broad Spectrum Antibiotic
1. Affect large range of bacteria, such as Gram+ and Gram-
2. Benefit vs. Disadvantage?
3. Superinfection
a) Kill good bacteria and allow opportunistic organism to flourish, such as *Candida albicans* in a yeast infection
b) Also, occurs if the strain causing the infection gains antibiotic resistanceto the antibiotic being used to treat the infection

III. Modes of Action, i.e. How do Antimicrobial Drugs Work?
A. Target differences between our cells and pathogens; selective toxicity
B. Modes of Action
1. Inhibit Cell Wall Synthesis
a. Peptidoglycan is only in bacteria cell walls
2. Inhibit Protein Synthesis – Translation at Ribosome
a. Differences in ribosomes allows for selective toxicity
b. Eukaryotes have 80S (60S and 40S) ribosomes, and prokaryotes have 70S (50S & 30S) ribosomes
*NOTE: Mitochondria contain 70S ribosomes; can affect liver and bone marrow cells
*Translation occurs at ribosomes, mRNA to protein
DNA (gene) ——transcription——-mRNA ——translation——- protein
3. Injure Plasma Membrane
4. Inhibit DNA Replication & Transcription
*NOTE: Transcription produces mRNA from gene on DNA
5. Inhibit Synthesis of Essential Metabolites
C. Drugs Used Against
1. Bacteria
2. Fungi
3. Viruses
4. Protozoans
5. Helminths

IV. Antibacterial Antibiotics
A. Inhibit Cell Wall Synthesis
1. Penicillin – prevents peptidoglycan cross-linking
a. 50 chemically related antibiotics
b. Common core of *ß-lactam ring*
c. Natural Penicillins
1) Produced from mold *Penicillium*
2) Penicillin G (injection) and Penicillin V (oral)
3) Against Gram+ (staphylococci, streptococci)
d. Semi-synthetic Penicillins
1) Part produced by mold, part produced in lab
2) Ampicillin – broad spectrum (against Gram+ and Gram-)
3) Amoxicillin and Imipenim – broad spectrum
e. Resistance to Penicillin

1) Penicillinase (a.k.a. ß-lactamase)
a) Breaks ß-lactam ring; inactivates penicillin
2) Staphyloccocus infections resistant to penicillin
a) ß-lactamase gene on plasmid
f. Methicillin (semi-synthetic penicillin) was introduced
1) Initially resistant to penicillinase, but resistance soon appeared
2) Methicillin no longer used
g. Methicillin-Resistant *Staphylococcus aureus* (MRSS)
1) Pronounced ―mersa‖
2) Now resistant to most penicillins and cephalosporins
2. Cephalosporins
a. Most common prescribed antibiotics
b. ß-lactam ring; similar mechanism to penicillins
c. 4 Generations
1. Each generation more effective against Gram- (broader spectrum)
d. Examples
1) 1st Generation – cephalexin (Keflex®), Cefazolin (Ancef®)
2) 2nd Generation – cephaclor (Ceclor®)
3) 3rd Generation – cephtriaxone (Rocephin®)
4) 4th Generation – cefepime (Maxipime®)
3. Bacitracin
a. Isolated from Bacillus from a girl named Tracy
b. Topical, against Gram+ staphylococci and streptococci
c. Neosporin®
4. Vancomycin
a. Used to treat MRSA
b. It's overuse led to Vancomycin-resistant enterococci (VRE)
c. VRE is a Gram+ pathogen
5. Antimycobacterial Antibiotics – inhibits mycolic acid synthesis
a. Mycolic acid is cell wall component of mycobacteria
a. Isoniazid (INH)
1) Against *Mycobacterium tuberculosis*
B. Inhibitors of Protein Synthesis
1. Aminoglycosides – changes 30S ribsosome, mRNA cannot be read properly
a. Streptomycin – 1st antibiotic agains Gram- bacteria (1944)
b. Neomycin – topical
c. Gentamicin – *Pseudomonas* infections
2. Tetracyclines
a. Broad spectrum antibiotic; produced by *Streptomyces*
b. Interfere with attachment of tRNA at 30S ribosome; interrupts protein synthesis
c. Against Gram+, Gram-, and intracellular rickettsias and chlamydias
d. Treat UTI's
e. Broad spectrum nature leads to suppression of intestinal flora, leading to GI upsets. Also, superinfections by *Candida albicans*
f. Can discolor teeth in children and cause liver damage in pregnant woman

g. Doxycycline is semi-synthetic tetracycline
3. Macrolides
a. Interferes with growing peptide at 50S ribosome
b. Erythromycin
1) Spectrum of activity similar to penicillin G
2) Cannot penetrate Gram- bacilli
3) Alternate to penicillin
4) Treat streptococcal and staphylococcal infections
c. Azithromycin (Zithromax®) and clarithromycin (Biaxin®)
1) Macrolides with broder spectrum and tissue penetration
2) Used to treat intracellular bacteria such as *Chlamydia*
4. Oxazolidinones (ox-a-zō-lēd-i-nōnes)
a. Interferes with interaction between mRNA and ribosomes
b. Completely synthetic
c. Used in response of VRE and MRSA
d. linezolid (Zyvox®)
C. Injury to Plasma Membrane
1. Triclosan – interrupts fatty acid synthesis
*household antimicrobial
D. Inhibit Nucleic Acid (DNA and RNA) Synthesis
1. Fluoroquinolones
a. Ciprofloxacin – Cipro®
E. Inhibit Synthesis of Essential Metabolites
1. Sulfonamides, a.k.a. sulfa drugs
2. Competitive Inhibition; binds to active site of enzymes
3. Structural analog of PABA; interferes with folic acid synthesis
4. Trimethoprim-sulfamethoxazole (TMP-SMZ)
a. Synergistic effect – much more effective when used in combination

V. Antifungal Drugs
A. Affect fungal sterols in plasma membrane; ergosterol
1. Sterol in our plasma membranes is cholesterol
B. Azoles
1. Clotrimazole and miconazole
a. Treat athlete's foot and vaginal yeast infections
C. Tolnaftate
1. Treat athlete's foot
2. Mechanism unknown
V. Antiviral, Antiprotozoan, and Antihelminthic Drugs

VI. Considerations When Choosing an Antimicrobial Drug

A. Identify Infectious Agent (pathogen causing disease)
1. Physicians direct examination; using experience will prescribe antibiotic
2. Culture and send to lab

3. Antibiotic is prescribed and if lab results show resistance to (or wrong) antibiotic prescribed, then there will be patient follow up to get them on the right antibiotic.
4. Also, even if lab (in vitro) supports that correct antibiotic was prescribed, it may not work in patient (in vivo)
a. Patient may have a few resistant cells that did not show up in lab or there could be one than one pathogen, some of which may be resistant to drug
B. Determining Pathogen Sensitivity to Drug (in lab)
1. Expose pure culture of pathogen to several types of drugs in vitro
2. Kirby-Bauer technique
a. Agar disc diffusion test
b. Look for zone of inhibition
3. Etest®
a. Used to determine Minimum Inhibitory Concentration (MIC)
b. MIC is lowest antibiotic concentration (ug/ml) that inhibits growth
4. Broth Dilution Test
a. Used to determine MIC and Minimal Bactericidal Concentration (MBC)
b. MIC and MBC minimize excessive use to decrease chance of tissue toxicity
c. Microdilution plates used in test
C. Determining Drug Safety
1. Therapeutic Index (TI)
a. Ratio of dose toxic to humans compared to minimum therapeutic dose
b. TI = Toxic Dose / MIC
D. Patient Factors
1. Take careful history of patient (check for drug contraindication)
a. Preexisting medical conditions
1) Kidney or liver diseases – metabolizing and excreting drug
b. Allergies to medications (penicillins)
c. Elderly – decreased GI absorption
d. Pregnancy – drugs can cross placenta and affect fetus (tetracycline)
e. Other medications currently being used (Drug interaction)
1) Antacids reduce absorption of isoniazid
2) Tetracycline reduces effect of oral contraceptive

Possible Questions

Part B

1. Define Serology.
2. Say about antigen
3. ELISA expand and define
4. What is diagnosis?
5. What are PCR?

Part C

1. Explain in detail about the laboratory precautions.
2. Discuss about WIDAL test
3. Comment on the clinical feature and diagnosis of UTI
4. Describe the properties and pathogenicity of LRT pathogens.
5. Give a detail note on opportunistic infection.
6. Write about the types and symptoms of HIV.
7. give a detailed note on diagnosis and its types

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB **COURSE NAME: MICROBIAL DIAGNOSIS IN HEALTH CLINIC**

COURSE CODE: 18MBU304B **UNIT: IV** **BATCH-2018-2021**

Unit IV Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
Which of the following is called serum Hepatitis?	HCV	HAV	HBV	HIV	HBV
Which of the following was a non-neural vaccine for rabies?	HEPV	Card vaccine	BPL	Simple	HEPV
Which type of antibodies will associate in blood cell coagulation?	IgE	IgA	IgM	IgG	IgM
In a antigen haptens are	Immunogenic	Non-immunogenic	Antigenic	Allergic	Non-immunogenic
The antibody that is first formed after infection is	IgG	IgM	IgD	IgE	IgM
Antibodies in our body are produced by	B-lymphocytes	T-lymphocytes	Monocytes	RBC's	B-lymphocytes
The points at which crossing over has taken place between homologous chromosomes are called	Chiasmata	Synaptonemal complex	Centromeres	Protein axes	Chiasmata
How much of globulin is present in human serum?	8%	12%	16%	4%	8%
The substance which acts as anti metabolites are called	Activators	Substrates	Inhibitor	Cofactor	Inhibitor
Enzymes are chemically	Lipids	Proteins	Carbohydrates	Vitamins	Proteins
Monoclonal antibodies are produced by	Hybridoma technology	Biotechnology	Fermentation Technology	Xeno technology	Hybridoma technology
First line of body defence is	Antibody molecules	Unbroken skin	Antigen molecules	Phagocytic cells	Unbroken skin
What is the strength of the bond between antigen and antibody?	Affinity	Avidity	Covalent	Simple	Avidity
Analysis of protein antigen is by	Southern blot	Northern blot	Western blot	Eastern blot	Western blot
Which of the following can provide naturally acquired passive immunity for the new born	IgA	IgG	IgE	IgM	IgG
Complement based agglutination reaction is known as	Haem agglutination	Coplement fixation	Conglutination	Schultz Dale Phenomenon	Haem agglutination
Reverse transcriptase is an enzyme involved in the synthesis of	DNA	Soluble RNA	m-RNA from DNA	Nucleotides	DNA
The cellular immune response is mediated by	B cells	T cell	BT cells	Endothelial cells	B cells
The major immunoglobulin present in the human serum is	IgG	IgA	IgE	IgG	IgG
Reagenic type antibody is	IgG	IgA	IgM	IgE	IgE
Blood group antigens are	Species specific	Isospecific	Autospecific	Organ specific	Isospecific

The reaction of soluble antigen with antibody is known by	Precipitation	Flocculation	Agglutination	Complement fixation	Precipitation
Interferon is composed of	Lipids	Lipoprotein	Glycoprotein	.Nucleic acid	Lipoprotein
Agglutination reaction is strongest with the immunoglobulin	IgM	IgG	IgA	IgD	IgM
The use of monoclonal antibodies is	Immunotherapy	Gene therapy	Blood transfusion	Organ transfusion	Immunotherapy
Test used for AIDS is	Widal test	ELISA	Aggluatination	CFT	ELISA
Antibody having high valency is	IgG	IgA	IgD	IgM	IgM
Intensity of attraction between antigen and antibody molecule is known as	Affinity	Avidity	Reaction	Catalyst	Affinity
Active immunity is induced by	Infection	Placental transfer of antibodies	Injection of antibodies	Injection of gamma-globulins	Infection
Pasteur developed the vaccines for	Anthrax	Polio	Cholera	Fever	Anthrax
Delayed type of hypersensitivity is seen in	Penicillin allergy	Contact dermatitis	Arthus reaction	Anaphylaxis	Contact dermatitis
The following are used for the preservation of virus, except	Freezing (–20°C–70°C)	Lyophilization	Ether	Formaldehyde	Ether
Antibody formation depends on	person	Amount of antigen	Well being	somatic ability	Amount of antigen
Immunity is lifelong following	Diphtheria	Tetanus	Measles	Yellow fever	Measles
To prepare vaccine for small pox, the material used by Edward Jenner is	Small pox material	Chicken pox material	Cow-pox material	Measles material	Cow-pox material
The character acquired by the cell due to recombination is	Inheritable	Suppressed	Dominating	Heritable	Heritable
T-cells are produced from	Bone marrow	Thymus	Spleen	Bile	Thymus
Antibodies are produced from	T-cells	â-cells	NK cells	Eosinophils	â-cells
Incomplete antigens are called	Immunogens	Epitomes	Haptens	Paratope	Haptens
The parts which filter lymph are	Lymph nodes	Spleen	Thymus	Bone marrow	Lymph nodes
The primary cells involved in immune response are	NK-cells	K-cells	Lymphocytes	BK cells	Lymphocytes
Plasma cells are the end cells of	T-cells	□-cells	Killer cells	Nk-cells	□-cells
Basophils have receptors for antibodies	IgG	IgA	IgM	IgE	IgE
Very effective, less time consuming and at a time so many samples can be detected by	ELISA	CFT	Neutralization	Agglutination	ELISA

Innate immunity is	Specific	Non-specific	Active	Passive	Specific
If more than one kind of immunizing agent is included in the vaccine, it is	Cellular vaccine	Recombinant vaccine	Mixed vaccine	Toxoid vaccine	Mixed vaccine
Vaccines are prepared from killed microbes, they are	Inactivated (killed) vaccine	Attenuated vaccines	Autogenous vaccine	Allogene vaccine	Inactivated (killed) vaccine
Vaccines used against viral infections are	Measles and Mumps vaccine	Cholera vaccine	Typhoid vaccine	Anti-rickettsial vaccine	Measles and Mumps vaccine
Vaccines prepared from toxins and chemicals are	Cellular vaccines	Sub-cellular vaccines	Attenuated vaccines	Heterologous vaccines	Sub-cellular vaccines
Example for live vaccine is	Rubella & BCG	Polio & TAB	Diphtheria & Tetanus	Hepatitis A & Rabies	Rubella & BCG
DPT is given for the prevention of	Diphtheria, Tetanus	Diphtheria, Pertusis	Diphtheria, Tetanus & pertusis	Dengue	Diphtheria, Tetanus & pertusis
Passive immunity lasts for the period of about	10 days	2 – 3 months	10 years	25 days	10 days
The markers helpful in detecting anti immunity are	Hyper gamma globulinaemia	Substrate antibodies	cortisone	hyperplasia	Hyper gamma globulinaemia
H antigen are present in	Motile organ	Non-motile organ	Blood	Semen	Motile organ
Antitoxin is used for_____immunization	Active	Passive	Complete	incomplete	Passive
Which test is used for detecting susceptibility of an individual to diphtheria toxin?	Schick tests	Dick test	V-P test	Precipitin test	Schick tests
Syndromes associated with Human T lymphotropic virus type I(HTLV-I) are	Adult T-cell lymphoma	Hairy cell leukemia	Adult T cell leukemia	B cell	Hairy cell leukemia
AIDS patients suffer from pneumonia due to	Pneumocystisis carinii	Cryptospodidium	S.pneumoniae	Toxoplasma	S.pneumoniae
Natural killer cells	Belongs to B-cell lineage	Belongs to T-cell lineage	Display cytotoxic effect on tumour cell	Require previous antigen exposure for activation	Display cytotoxic effect on tumour cell
Immunoglobulin is associated with anaphylactic delayed hypersensitivity reaction	IgE	IgA	IgD	IgM	IgE

UNIT-V

SYLLABUS

Importance, Determination of resistance/sensitivity of bacteria using disc diffusion method (Kirby Bauer Method) Determination of minimal inhibitory concentration (MIC) of an antibiotic by broth dilution method (LC_{50}, LC_{90}).

Antimicrobial Susceptibility Testing

Introduction

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem.

Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately. Inappropriate use results from physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for diagnosis of infections that potentially have a bacterial aetiology, unnecessarily prescribing expensive, broad-spectrum agents, and not following established recommendations for using chemo prophylaxis. The availability of antibiotics over the counter, despite regulations to the contrary, also fuel inappropriate usage of antimicrobial drugs in India. The easy availability of antimicrobial drugs leads to their incorporation into herbal or "folk" remedies, which also increases inappropriate use of these agents.

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in thatarea.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

Principle

The principles of determining the effectivity of a noxious agent to a bacterium were well enumerated by Rideal ,Walker and others at the turn of the century, the discovery of antibiotics made these tests(or their modification)too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field .The Oxford group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface.

With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory. Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries, it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.

3. Factors Influencing Antimicrobial Susceptibility Testing

pH

The pH of each batch of Müeller-Hinton agar should be checked when the medium is prepared. The exact method used will depend largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones, and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected. The pH can be checked by one of the following means:

* Macerate a sufficient amount of agar to submerge the tip of a pH electrode.
* Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.
* Use a properly calibrated surface electrode.

Moisture

If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

Effects of Thymidine or Thymine

Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or even no zone at all, which may result in false-resistance reports. Müeller-Hinton agar that is as low in thymidine content as possible should be used. To evaluate a new lot of Müeller-Hinton agar, *Enterococcus faecalis* ATCC 29212, or alternatively, *E. faecalis* ATCC 33186, should be tested with trimethoprim/sulfamethoxazole disks. Satisfactory media will provide essentially clear, distinct zones of inhibition 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

Effects of Variation in Divalent Cations

Variation in divalent cations, principally magnesium and calcium, will affect results of aminoglycoside and tetracycline tests with *P. aeruginosa* strains. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptably large zones of inhibition. Excess zinc ions may reduce zone sizes of carbapenems. Performance tests with each lot of Müeller-Hinton agar must conform to the control limits.

Testing strains that fail to grow satisfactorily

Only aerobic or facultative bacteria that grow well on unsupplemented Müeller-Hinton agar should be tested on that medium. Certain fastidious bacteria such as *Haemophilus* spp.,

N. gonorrhoeae, *S. pneumoniae*, and viridans and ß-haemolytic streptococci do not grow sufficiently on unsupplemented Müeller-Hinton agar. These organisms require supplements or different media to grow, and they should be tested on the media described in separate sections.

4. Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

Diffusion	Dilution	Diffusion&Dilution
Stokes method	Minimum Inhibitory Concentration	E-Test method
Kirby-Bauer method	i) Broth dilution	
	ii)AgarDilution	

Disk Diffusion

Reagents for the Disk Diffusion Test

1. Müeller-Hinton Agar Medium

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of nonfastidious bacteria for the following reasons:

* It shows acceptable batch-to-batch reproducibility for susceptibility testing.
* It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.

* It gives satisfactory growth of most nonfastidious pathogens.
* A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Although Müeller-Hinton agar is reliable generally for susceptibility testing, results obtained with some batches may, on occasion, vary significantly. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test will usually be larger than expected and may exceed the acceptable quality control limits. Only Müeller-Hinton medium formulations that have been tested according to, and that meet the acceptance limits described in, NCCLS document M62-A7- Protocols for Evaluating Dehydrated Müeller-Hinton Agar should be used.

Preparation of Müeller-Hinton Agar

Müeller-Hinton agar preparation includes the following steps.

1. Müeller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.
2. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.
3. Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
4. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).
5. Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
6. A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

2. Preparation of antibiotic stock solutions

Antibitiotics may be received as powders or tablets. It is recommended to obtain pure antibiotics from commercial sources, and not use injectable solutions. Powders must be accurately weighed and dissolved in the appropriate diluents (Annexure III) to yield the required concentration, using sterile glassware. Standard strains of stock cultures should be used to evaluate the antibiotic stock solution. If satisfactory, the stock can be aliquoted in 5 ml volumes and frozen at -20ºC or -60ºC.

Stock solutions are prepared using the formula (1000/P) X V X C=W, where P+potency of the anitbiotic base, V=volume in ml required, C=final concentration of solution and W=weight of the antimicrobial to be dissolved in V.

Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven.

The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivers ml of antibiotics to each disc.

Storage of commercial antimicrobial discs

Cartridges containing commercially prepared paper disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. Discs should be stored as follows:

* Refrigerate the containers at 8°C or below, or freeze at -14°C or below, in a nonfrost-free freezer until needed. Sealed packages of disks that contain drugs from the ß-lactam class should be stored frozen, except for a small working supply, which may be refrigerated for at most one week. Some labile agents (e.g., imipenem, cefaclor, and clavulanic acid combinations) may retain greater stability if stored frozen until the day of use.
* The unopened disc containers should be removed from the refrigerator or freezer one to two hours before use, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold disks.
* Once a cartridge of discs has been removed from its sealed package, it should be placed in a tightly sealed, desiccated container. When using a disc-dispensing apparatus, it should be fitted with a tight cover and supplied with an adequate desiccant. The dispenser should be allowed to warm to room temperature before opening. Excessive moisture should be avoided by replacing the desiccant when the indicator changes color.
* When not in use, the dispensing apparatus containing the discs should always be refrigerated.
* Only those discs that have not reached the manufacturer's expiration date stated on the label may be used. Discs should be discarded on the expiration date.

Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. A $BaSO_4$ 0.5 McFarland standard may be prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2$. $2H_2O$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.
2. The correct density of the turbidity standard should be verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standard.
3. The Barium Sulfate suspension should be transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum.
4. These tubes should be tightly sealed and stored in the dark at room temperature.
5. The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles appear, the standard should be replaced. Latex particle suspensions should be mixed by inverting gently, not on a vortex mixer
6. The barium sulfate standards should be replaced or their densities verified monthly.

Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective

Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with MIC's and the results have corroborated with clinical data. Based on study results NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

Procedure for Performing the Disc Diffusion Test

Inoculum Preparation

Growth Method

The growth method is performed as follows

1. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
2. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours)
3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for *E.coli* ATCC 25922. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Direct Colony Suspension Method

1. As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer.
2. This approach is the recommended method for testing the fastidious organisms, *Haemophilus* spp., *N. gonorrhoeae*, and streptococci, and for testing staphylococci for potential methicillin or oxacillin resistance.

Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
2. The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.
2. The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. With the exception of *Haemophilus* spp., streptococci and

   N. gonorrhoeae, the plates should not be incubated in an increased CO_2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO_2 will significantly alter the size of the inhibitory zones of some agents.

Reading Plates and Interpreting Results

1. After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed. If the test organism is a *Staphylococcus* or *Enterococcus* spp., 24 hours of incubation are required for vancomycin and oxacillin, but other agents can be read at 16 to 18 hours. Transmitted light (plate held up to light) is used to examine the oxacillin and vancomycin zones for light growth of methicillin- or vancomycin- resistant colonies, respectively,

within apparent zones of inhibition. Any discernable growth within zone of inhibition is indicative of methicillin or vancomycin resistance.

2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.

3. The sizes of the zones of inhibition are interpreted by referring to Tables 2A through 2I (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement, and the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested. Some agents may only be reported as susceptible, since only susceptible breakpoints are given.

Dilution Methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in $\log_2$ serial dilutions (two fold).

Minimum Inhibitory Concentration (MIC)

Diffusion tests widely used to determine the susceptibility of organisms isolated from clinical specimens have their limitations; when equivocal results are obtained or in prolonged serious infection e.g. bacterial endocarditis, the quantitation of antibiotic action vis-a-vis the pathogen needs to be more precise. Also the terms ‘Susceptible’ and ‘Resistant’ can have a realistic interpretation. Thus when in doubt, the way to a precise assessment is to determine the MIC of the antibiotic to the organisms concerned.

There are two methods of testing for MIC:

(a) Broth dilution method

(b) Agar dilution method.

Broth Dilution Method

The Broth Dilution method is a simple procedure for testing a small number of isolates, even single isolate. It has the added advantage that the same tubes can be taken for MBC tests also:

Materials

Sterile graduated pipettes of 10ml, 5ml, 2ml and 1ml Sterile capped 7.5 x 1.3 cm tubes / small screw-capped bottles, Pasteur pipettes, overnight broth culture of test and control organisms (same as for disc diffusion tests), required antibiotic in powder form (either from the manufacturer or standard laboratory accompanied by a

statement of its activity in mg/unit or per ml. Clinical preparations should not be used for reference technique.), required solvent for the antibiotic, sterile Distilled Water - 500ml and suitable nutrient broth medium.

Trimethoprim and sulphonamide testing requires thymidine free media or addition of 4% lysed horse blood to the media

A suitable rack to hold 22 tubes in two rows i-e 11 tubes in each row.

Stock solution

Stock solution can be prepared using the formula

$$\frac{1000}{P} \times V \times C = W$$

Where P=Potency given by the manufacturer in relation to the base

V= Volume in ml required

C=Final concentration of solution (multiples of 1000)

W= Weight of the antimicrobial to be dissolved in the volume V

Example: For making 10 ml solution of the strength 10,000mg/l from powder base whose potency is 980 mg per gram,the quantities of the antimicrobials required is

$$W = \frac{1000}{980} \times 10 \times 10 = 102.04\text{mg}$$

Note:the stock solutions are made in higher concentrations to maintain their keeping qualities and stored in suitable aliquots at -20^{o}C .Once taken out,they should not be refrozen or reused.

Suggested dilution ranges of some antimicrobials are shown in Annexure II.

Method

Prepare stock dilutions of the antibiotic of concentrations 1000 and 100 µg/L as required from original stock solution (10,000mg/L). Arrange two rows of 12 sterile 7.5 x1.3 cm capped tubes in the rack. In a sterile 30ml (universal) screw capped bottle, prepare 8ml of broth containing the concentration of antibiotic required for the first tube in each row from the appropriate stock solution already made. Mix the contents of the universal bottle using a pipette and transfer 2ml to the first tube in each row. Using a fresh pipette ,add 4 ml of broth to the remaining 4 ml in the universal bottle mix and transfer 2ml to the second tube in each row. Continue preparing dilutions in this way but where as many as 10 or more are required the series should be started again half the way down. Place 2ml of antibiotic free broth to the last tube in each row. Inoculate one row with one drop of an overnight broth culture of the test organism diluted approximately to

1 in 1000 in a suitable broth and the second row with the control organism of known sensitivity similarly diluted. The result of the test is significantly affected by the size of the inoculum.The test mixture should contain 10^6 organism/ml.If the broth culture used has grown poorly,it may be necessary to use this undiluted. Incubate tubes for 18 hours at 37ºC. Inoculate a tube containing 2ml broth with the organism and keep at +4ºC in a refrigerator overnight to be used as standard for the determination of complete inhibition.

Calculations for the preparation of the original dilution.

This often presents problems to those unaccustomed to performing these tests. The following method advocated by Pamela M Waterworth is presented. Calculate the total volume required for the first dilution. Two sets of dilution are being prepared (one for the test and one for the control), each in 2ml volumes i-e a total of 4 ml for each concentration as 4ml is required to make the second dilution, the total requirement is 8ml. Now calculate the total amount of the antibiotic required for 8ml. For 64 g/l concentration, 8x64mg/l =512µg in 8 ml. Place a decimal point after the first figure (5.12) and take this volume in ml (i.e 5.12 ml) of the dilution below 512mg/l and make upto 8ml with broth. In this example given above, the series has to be started again mid way at 2 mg/l which would be obtained in the same way:

8ml of 2mg/l=16µg in 8ml.

1.6 ml of 10 mg/ l + 6.4 ml of broth

Reading of result

MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube.

Because very faint turbidity may be given by the inoculum itself, the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition.

Standard strain of known MIC value run with the test is used as the control to check the reagents and conditions.

Minimum Bactericidal Concentrations(MBC)

The main advantage of the 'Broth dilution' method for the MIC determination lies in the fact that it can readily be converted to determine the MBC as well.

Method

Dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control tube containing no antibiotic is immediately subcultured (Before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37ºC overnight. The tubes are also incubated overnight at 37ºC. Read the MIC of the control organism to check that the drug concentrations are correct. Note the lowest concentration inhibiting growth of the organisms and record this as the MIC. Subculture all tubes not showing visible growth in the same manner as the control tube described above and incubate at 37ºC overnight. Compare the amount of growth from the control tube before incubation,which represents the original inoculum. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity .These tubes are not subcultured; the purpose of the control is to confirm by its MIC that the drug level is correct,whether or not this organism is killed is immaterial.

Reading of result

These subcultures may show

- Similar number of colonies- indicating bacteriostasis only
- A reduced number of colonies-indicating a partial orslow bactericidal activity.
- No growth- if the whole inoculum has beenkilled
- The highest dilution showing at least 99% inhibition is taken as MBC

Micro-broth dilution test

This test uses double-strength Müeller-Hinton broth, 4X strength antibiotic solutions prepared as serial two-fold dilutions and the test organism at a concentration of $2x10^6$/ml. In a 96 well plate, 100 µl of double-strength MHB, 50 µl each of the antibiotic dilutions and the organism suspension are mixed and incubated at 35°C for 18-24 hours. The lowest concentration showing inhibition of growth will be considered the MIC of the organism.

Reading of result

MIC is expressed as the highest dilution which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculum itself,the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition. Standard strain of known MIC, run with the test is used as the control to check the reagents and conditions.

The Agar dilution Method

Agar dilutions are most often prepared in petri dishes and have advantage that it is possible to test several organisms on each plate .If only one organism is to be tested e.g *M.tuberculosis*,the dilutions can be prepared in agar slopes but it will then be necessary to prepare a second identical set to be inoculated with the control organism.The dilutions are made in a small volume of water and added to agar which has been melted and cooled to not more than 60ºC.Blood may be added and if 'chocolate agar' is required,the medium must be heated before the antibiotic is added.

It would be convenient to use 90 mm diameter petri dishes and add

one ml of desired drug dilutions to 19 ml of broth.The factor of agar dilution must be allowed for in the first calculation as follows.

final volume of medium in plate = 20 ml

Top antibiotic concentrations = 64mg/l

Total amount of drug = 1280µg to be added to 1 ml of water

2ml of 1280 µg /ml will be required to start the dilution = 2560µg in 2 ml

= 1.28ml of 2000µg /ml ± 0.72 ml of water.

1 ml of this will be added to 19 ml agar.

(Note stock dilution of 2000µg /ml is required for this range of MIC)

The quickest way to prepare a range of dilutions in agar is as follows:

Label a sterile petri dish on the base for each concentration required. Prepare the dilutions in water placing 1 ml of each in the appropriate dish. One ml water is added to a control plate. Pipette 19 ml melted agar, cooled to 55^{o}C to each plate and mix thoroughly. Adequate mixing is essential and if sufficient technical expertise is not available for the skilled manipulation, it is strongly recommended that the agar is first measured into stoppered tubes or universal containers and the drug dilution added to these and mixed by inversion before pouring into petri dishes. After the plates have set they should be well dried at 37^{o}C with their lids tipped for 20 to 30 minutes in an incubator. They are then inoculated either with a multiple inoculator as spots or with a wire loop or a platinum loop calibrated to deliver 0.001ml spread over a small area. In either case the culture should be diluted to contain 10^5 to 10^6 organisms per ml. With ordinary fast growing organisms, this can be obtained approximately by adding 5 µl of an overnight broth culture to 5ml broth or peptone water.

It is possible to test spreading organism such as *P.mirabilis* by this method either by cutting ditches in the agar between the inocula, or by confining each with small glass or porcelain cylinders pressed into the agar. Although swarming of *P.mirabilis* can be prevented by the use of higher concentration of agar in the medium, this is not recommended for determination of MIC because of the difficulty of ensuring adequate mixing of the drug with this very viscous medium. Selective media should not be used and electrolyte deficient media will give false results because of the effect of variation in the salt content on the action of many antibiotics.

Reading of results

The antibiotic concentration of the first plate showing ≥ 99% inhibition is taken as the MIC for the organism.

Dilution and Diffusion

E test also known as the epsilometer test is an 'exponential gradient' testing methodology where 'E' in E test refers to the Greek symbol epsilon (ε).The E test(AB Biodisk) which is a quantitative method for antimicrobial susceptibility testing applies both the dilution of antibiotic and diffusion of antibiotic into the medium.. A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this E test strip is applied onto an inoculated agar plate, there is an immediate release of the drug. Following incubation , a symmetrical inhibition ellipse is produced. The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy .

E test can be used to determine MIC for fastidious organisms like *S. pneumoniae,*

ß-hemolytic streptococci, *N.gonorrhoeae*, *Haemophilus* sp. and anaerobes. It can also be used for Nonfermenting Gram Negative bacilli (NFGNB) for eg-*Pseudomonas* sp. and *Burkholderia pseudomallei*.

Resistance of major consequence may be detected for e.g., the test is very useful in detecting glycopeptide resistant Enterococci (GRE) and glycopeptide intermediate *S.aureus* (GISA) and slow growing pathogens such as *Mycobacterium tuberculosis.* Further it can be used for detection of extended spectrum beta lactamases (ESBL). In conclusion E test is a simple, accurate and reliable method to determine the MIC for a wide spectrum of infectious agents.

5. Susceptibility of Fastidious Bacteria

DISC DIFFUSION FOR FASTIDIOUS ORGANISMS

Antibiotic susceptibility testing of *S.pneumoniae*

Media for disc diffusion

Müeller -Hinton Sheep blood agar

Standardization of inoculum.

The inocula for seeding the susceptibility media with *S.pneumoniae* is prepared from fresh pure cultures (grown overnight on Chocolate agar). Cell suspensions of the bacteria to be tested are prepared in sterile saline or Müeller-Hinton broth. The cell suspension is prepared by transferring a portion of the fresh growth with a swab or inoculating loop to the suspending medium, using caution when mixing the cells with the suspending medium so as not to form bubbles. The suspension is then compared to the McFarland standard by holding the suspension and McFarland standard in front of a light against a white background with contrasting black lines and comparing the turbidity. If the turbidity is too heavy, the suspension should be diluted with additional suspending medium. If the turbidity is too light additional cells should be added to the suspension.

For *S.pneumoniae* – Direct colony suspension is made in normal saline and turbidity adjusted to 0.5 McFarland standard. Within 15 minutes after adjusting the turbidity of the suspension the plate should be inoculated.

Inoculation of the susceptibility test media

After proper turbidity is achieved, a new sterile swab (cotton or dacron) is submerged in the suspension, lifted out of the broth, and the excess fluid is removed by pressing and rotating the swab against the wall of the tube. The swab is then used to inoculate the entire surface of the supplemented Müeller Hinton agar plate three times, rotating the plate 60 degrees between each inoculation. The inoculum is allowed to dry (usually taking only a few minutes but no longer than 15 minutes) before the discs are placed on the plates. The discs should be placed on the agar with sterile forceps and tapped gently to ensure the adherence to the agar. The plates containing the disks are incubated at 35ºC for 16 to 18 h in an inverted position in a 5% CO_2 incubator. A candle extinction jar may be used if a CO_2 incubator is not available.

Estimating the susceptibility of the strains

After overnight incubation, the diameter of each zone of inhibition is measured with a ruler or calipers. The zones of inhibition on the media containing blood are measured from the top surface of the plate with the top removed. It is convenient to use a ruler with a handle attached for these measurements, holding the ruler over the surface of the disk when measuring the inhibition zone. Care should be taken not to touch the disk or surface of the agar. Sterilize the ruler occasionally to prevent transmission of bacteria. In all measurements, the zones of inhibition are measured from the edges of the last visible colony-forming growth. The ruler should be positioned across the center of the disc to make these measurements. The results are recorded in millimeters (mm) and interpretation of susceptibility is obtained by comparing the results to the standard zone sizes. For *S.pneumoniae* the zone measurement is from top of plate with the lid removed. Faint growth of tiny colonies that may appear to fade from the more obvious zone should be ignored in the measurement.

Interpretation

Each zone size is interpreted by reference to the Table 2G (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints for *S.pneumoniae*) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement as susceptible, intermediate and resistant.

Antibiotic susceptibility of *Haemophilus* species

The medium of choice for disc diffusion testing of *Haemophilus* sp. is Haemophilus Test Medium (HTM). Müeller-Hinton chocolate agar is not recommended for routine testing of *Haemophilus* spp.

In its agar form, Haemophilus Test medium consists of the following ingredients.

* Müeller-Hinton agar,
* 15 µg/ml ß-NAD,
* 15 µg/ml bovine hematin, and
* 5-mg/ml yeast extract.

To make HTM, first a fresh hematin stock solution is prepared by dissolving 50 mg of bovine hematin powder in 100 ml of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved. Thirty ml of the hematin stock solution are added to 1 L of MHA with 5 g of yeast extract. After autoclaving and cooling to 45 to 50°C, 3 ml of an NAD stock solution (50 mg of NAD dissolved in 10 ml of distilled water and filter sterilized) are also aseptically added. The pHshould be 7.2 to 7.4.

Test Procedure

1. The direct colony suspension procedure should be used when testing *Haemophilus* sp. Using colonies taken directly from an overnight (preferably 20 to 24 hour) chocolate agar culture plate, a suspension of the test organism is prepared in Müeller-Hinton broth or 0.9% saline. The suspension should be adjusted to a turbidity equivalent to a 0.5 McFarland standard using a photometric device. This suspension will contain approximately 1 to 4 x 10^8 CFU/ml. Care must be exercised in preparing this suspension, because higher inoculum concentrations may lead to false-resistant results with some ß-lactam antibiotics, particularly when ß-lactamase producing strains of *H. influenzae* are tested. Within 15 minutes after adjusting the turbidity of the inoculum suspension, it should be used for plate inoculation.
2. The procedure for the disc test should be followed as described for nonfastidious bacteria, except that, in general, no more than 9 discs should be applied to the surface of a 150-mm plate or no more than 4 discs on a 100-mm plate.
3. Plates are incubated at 35°C in an atmosphere of 5% CO_2 for 16 to 18 hours before measuring the zones of inhibition.
4. The zone margin should be considered as the area showing no obvious growth visible with the unaided eye. Faint growth of tiny colonies that may appear to fade from the more obvious zone should be ignored in the measurement.

Zone Diameter Interpretive Criteria

The antimicrobial agents suggested for routine testing of *Haemophilus* sp. are indicated in Annexure I. Each zone size is interpreted by reference to the Table 2E (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints for *Haemophilus* sp.) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement as susceptible, intermediate and diffusion testing of *Haemophilus* spp. with other agents is not recommended.

A. Broth dilution

Broth dilution testing allows the option of providing both quantitative (MIC) and qualitative (category interpretation) results. MIC can be helpful in establishing the level of resistance of a particular bacterial strain and can substantially affect the decision to use certain antimicrobial agents.

Broth dilution can again be performed by 2 ways

1. Macro dilution: Uses broth volume of **1 ml** in standard test tubes .
2. Microdilution: Uses about **0.05 to 0.1 ml** total broth volume and can be performed in a microtiter plate or tray .

The procedure for both macro and microdilution are same except the volume of the broth.

B. Agar dilution

MIC of an antibiotic using broth dilution method is determined by using the following procedure

1. Preparation of antibiotic stock solution
2. Preparation of antibiotic dilution range
3. Preparation of agar dilution plates
4. Preparation of inoculum
5. Inoculation
6. Incubation
7. Reading and interpreting results

Preparation of antibiotic Stock solution.

Antibiotic stock solution can be prepared by commercially available antimicrobial powders (with given potency). The amount needed and the diluents in which it can be dissolved can be calculated by using either of the following formulas to determine the amount of antimicrobial powder (1) or diluent (2) needed for a standard solution:

$$(1)\text{Weight (mg)} = \frac{\text{Volume (mL)} \cdot \text{Concentration (µg/mL)}}{\text{Potency (µg/mg)}}$$

or

$$(2)\text{Volume (mL)} = \frac{\text{Weight (mg)} \cdot \text{Potency (µg/mg)}}{\text{Concentration (µg/mL)}}$$

Prepare antimicrobial agent stock solutions at concentrations of at least 1000 µg/mL (example: 1280 µg/mL) or 10 times the highest concentration to be tested, whichever is greater.

Because microbial contamination is extremely rare, solutions that have been prepared aseptically but not filter sterilized are generally acceptable. If desired, however, solutions may be sterilized by **membrane filtration**. Dispense small volumes of the sterile stock solutions into sterile glass, polypropylene, polystyrene, or polyethylene vials; carefully seal; and store (*preferably at −60 °C or below, but never at a temperature warmer than −20 °C and never in a self-defrosting freezer)*. Vials may be thawed as needed and used the same day.

Preparation of antibiotic dilution range

- Use sterile 13- x 100-mm test tubes to conduct the test. If the tubes are to be saved for later use, be sure they can be frozen.
- Close the tubes with loose screw-caps, plastic or metal closure caps, or cotton plugs.
- Prepare the final two fold (or other) dilutions of antimicrobial agent volumetrically in the broth. A minimum final volume of 1 mL of each dilution is needed for the test.

Note: For, microdilution,only 0.1 ml is dispensed into each of the 96 wells of a standard tray.

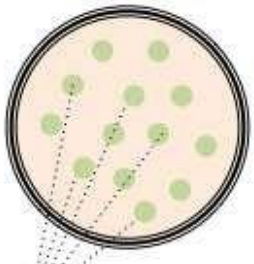
Preparation of inoculum

1. Prepare the inoculum by making a direct broth suspension of isolated colonies selected from an 18- to 24-hour agar plate *(use a non-selective medium, such as **blood agar**).*
2. Adjust the suspension to achieve a turbidity equivalent to a **0.5 McFarland turbidity standard**. This results in a suspension containing approximately 1 to 2 x 10^8 colony forming units (CFU)/mL for *Escherichia coli* ATCC®a 25922.
3. Compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.
4. Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension in broth so, after inoculation, each tube contains approximately 5 x 10^5 CFU/mL.**Note:** *This can be accomplished by diluting the 0.5 McFarland suspension 1:150, resulting in a tube containing approximately 1 x 10^6 CFU/mL. The subsequent 1:2 dilution in step 3 brings the **final inoculum to 5 x 10^5 CFU/mL.***

Broth dilution method for measuring minimum inhibitory concentration of antibiotics

Broth dilution method for measuring minimum inhibitory concentration of antibiotics

1. Obtain isolated colonies of bacterial strain to test.

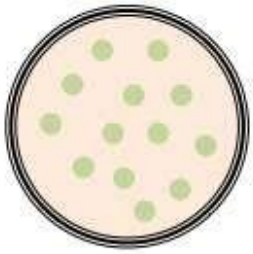


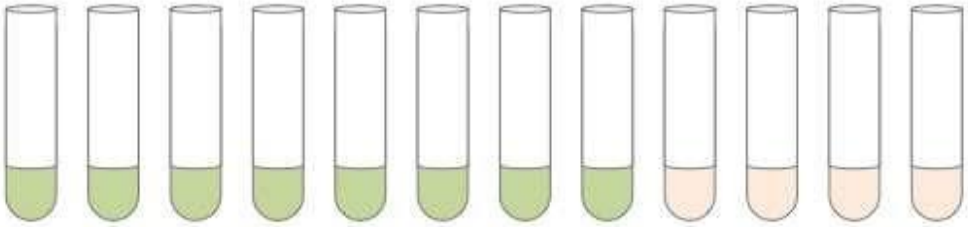
2. Combine 4-5 colonies and culture overnight in rich media broth.

3. After overnight incubation shown at left, add rich broth with appropriate dilution series of test antibiotic to test tubes. Example concentrations (mg/L) are shown below. Inoculate bacteria to a final density of 5 x 10^5 cfu/ml.

No bacteria; broth control

4. Plate aliquot of growth control (i.e., no antibiotic added) to verify cfu/ml counts of viable bacteria. Incubate overnight and count colonies.





5. After overnight incubation, check cultures for growth. The MIC is the lowest concentration of antibiotic that prevents visible growth. In this example, the MIC is 64 mg/L.

Possible Questions

Part B

1. Define pandemic and how you control it.
2. Say about circulatory system
3. What are culture media?
4. What are MIC and MBC?
5. What is Nucleic acid probes?

Part C

1. Describe the morphology, cultural properties and lab diagnosis of GI tract pathogens.
2. Explain in detail about the classification of staining methods.
3. Comment on identification and interpretation of diseases
4. State the application of ELISA
5. Write a detailed account on Kirby bauer method.
6. Elaborate the MIC and MBC procedures.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB **COURSE NAME: MICROBIAL DIAGNOSIS IN HEALTH CLINIC**

COURSE CODE: 18MBU304B **UNIT: V** **BATCH-2018-2021**

Unit V Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
The antibiotic acting on cell wall is	Bactracin	Kanamycin	Ampicillin	Tetracyclin	Tetracyclin
Aflatoxin is produced by	Aspergillus sps	Penicillium sps	Alternaria sps	Mucor Sps	Aspergillus sps
Penicillin was discovered by	Fleming	Pasteur	Koch	Hess	Fleming
Antibiotics used in combination may demonstrate	Synergism	Commensalism	Ammensalism	Parasitism	Parasitism
The drug of choice in anaphylactic shock is	Histamine	Corticosteroid	Epinephrine	Ampicillin	Epinephrine
Drugs of choice for treatment of Mycoplasma infections:	Tetracyclines	Ampicillin	Erythromycin	Penicillins	Tetracyclines
A number of viruses are known to infect mycoplasmas, called	Bacteriophages	Mycoplasma phages	Virions	Tiny strains	Mycoplasma phages
The following are true about Rickettsiae.	Unicellular organisms	Prokaryotic intracellular parasites	Presence of 70 S ribosomes	It causes hemolysis in human beings	It causes hemolysis in human beings
The causative agent of scrub typhus:	R.Quintana	R.rickettsii	R.orinetalis	R.prowazekii	R.orinetalis
Lymphogranuloma venerum (LGV) is a sexually transmitted disease is caused by	Copthalmia	C.trachomatis	C.pneumonias	C.psittasi	C.trachomatis
Intradermal test employed for diagnosis of LGV is	Frei test	Mantoux test	Schick test	Dick test	Frei test
Which algae is pathogenic to human?	Cephaloeuros	Ulothrix	Macrocystis	Prototheca	Prototheca
Erythromycin is obtained from	S.griseus	S.rimosus	S.scabies	S.erythraeus	S.erythraeus
Common cold is caused by	Adeno virus	Corono virus	Hepatitis virus	Pox virus	Corono virus
The causative agent of conjunctivitis:	Adeno virus	Corono virus	Paramyxo virus	Orthomyxo virus	Adeno virus
Antibiotics used for treatment of cholera are	Tetracyclines	Penicillins	Streptomycines	Ampicillin	Tetracyclines
Salmonella typhi is causative organism of	Undulent fever	Remittent fever	Dengue fever	Enteric fever	Enteric fever
In enteric fever, the organ lodging maximum number of the organism is	Liver	Gall bladder	Small intestine	Large intestine	Gall bladder
True about Enteric fever is	Bacteraemia in first week	Carrier in 90%	All serotypes cause the disease	Rosy spots on 18th day	Bacteraemia in first week

Gastroenteritis is caused by	Shigella	V.cholerae	V.cholera Parahaenolyticus	S.typhi	V.cholera Parahaenolyticus
E.coli produces the following toxins:	Enterotoxins	Endotoxins	Verocytotoxins	Hemolysins	Endotoxins
The following infections caused by Esch. Coli, except	Urinary tract infections	Septic infections of wounds	Diarrhoea	Meningitis	Meningitis
Diphtheria is caused by	Corynebacterium diphtheriae	C. Bovis	C. Jeikeium	C. equi	Corynebacterium diphtheriae
Causative organism of diphtheria was first demonstrated by	Robert Koch	Lois Pasteur	Klebs and Loeffler	Volhard and Fahr	Klebs and Loeffler
Coryne bacterium is	Gram positive	Resistant to Penicillin	Gram negative	Resistant to Chloramphenicol	Gram positive
C. diphtheriae consists of	Startch granules	Polymeta phosphate granules	Lipid granules	Capsule	Polymeta phosphate granules
The incubation period of diphtheriae is	Upto 2 weeks	Upto 1 week	2–4 weeks	10 week	2–4 weeks
Diphtheria virulence test is	Ascoli's thermoprecipitation test	Eleck's gel precipitation test	C.R.P test	M.R.T. test	Eleck's gel precipitation test
Diptheria toxoid is prepared by using	Aldehyde	Formalin	Phenols	Ketones	Formalin
Diphtheria is an example of	Bacteraemia	Pyaemia	Septicemia	Toxaemia	Toxaemia
Main symptom of tuberculosis is	Tubercle formation	Liquid formation	Lesion	Pus	Tubercle formation
BCG vaccine is for the prevention of	Brucellosis	Diphtheria	Botulism	Tuberculosis	Tuberculosis
Dose of BCG vaccine is	0.2–0.5 ml	0.1 ml	0.05 ml	0.2 to 0.3 ml	0.1 ml
Negative Mantoux test is important in	Pulmonary Koch's syndrome	Sarcoidosis	Carcinoma bronchus	Lymphoma	Pulmonary Koch's syndrome
Bacilli Calmette Guerin (BCG) contains the avirulent strains of	Human tubercle bacilli	Avian tubercle bacilli	Bovine tubercle bacilli	A typical mycobacteria	Bovine tubercle bacilli
Drugs used against tuberculosis (TB) are	Refampicin, Isoniazid	Ampicillin	Kanamycin	Nystatin	Refampicin, Isoniazid
The greatest number of tubercle bacilli is present in	Large sized tuberculomas	Miliary tuberculosis	Tuberculous lymphadinitis	Tuberculous cavity of the lung	Tuberculous cavity of the lung
Histoid Hansen is a veriety of	Tuberculoid Leprosy	Borderline tuberculoid	Borderline lepramatous	Lepronmetous leprosy	Lepronmetous leprosy

Streptococcus pyogens produces all of the following lesions, except	Impetigo contagiosa	Erysipeals	Boil	Paronchia	Paronchia
Causative agent of Scarlet fever:	Staphylococcus aureus	Streptococcus viridans	Stre. pyogens	Streptococcus mutans	Stre. pyogens
Rheumatic fever is most commonly caused by	S. viridans	S. pyogenes	S. aures	S. mutans	S. pyogenes
Penicillin is the drug of choice for	Scarlet fever	Whooping cough	Brucellosis	Cholera	Scarlet fever
In human being str. pneumoniae causes	Septicaemia	Paronychia	Pneumomnia	Viremia	Pneumomnia
Virulence factor for Stre. pneumoniae:	Capsular polysaccharide	Specific soluble substance	Vi-antigen	Forsmann antigen	Capsular polysaccharide
Conjunctivitis in a new born is caused by	Streptococcus	Pneumococcus	Meningococci	Staphylococcus	Streptococcus
Influenza is belonging to	Orthomyxoviridae	Retroviridae	Rio viridae	Rabhdoviridae	Orthomyxoviridae
Influenza virus contains	Eight segments of RNA	Two strands of RNA	Single RNA	Single DNA	Two strands of RNA
Influenza virus contains	Eight segments of RNA	Two strands of RNA	Single RNA	Single DNA	Two strands of RNA
'Reye's syndrome' is caused by	S.pneumoniae	S.pyogenes	H. Influenza	S. aures	H. Influenza
The commonest cause of rubella in new bornes	Congential rubella	Post natal rubella	Maternal rubella	Non rubella	Congential rubella
Mumps virus is belonging go	Retroviriae	Paramyxoviriae	Orthomyxo viridae	Retroviridae	Paramyxoviriae
Measles is characterized by	Negribodies	Babes-Ernest granules	Koplik's spots	Fever	Babes-Ernest granules
Brucella causes	Pertusis	Plague	Brucellosis	Pus	Brucellosis
Mediterranian fever is caused by	M. tuberculosis	S. typhi	C.neoformans	Brucella	Brucella
Which of the following test is specific for Brucellosis?	Frei	Weil	Castaneda strip	Rose water	Castaneda strip
Malignant pustule is caused by	Anthrax	Tetanus	Diphtheria	Plague	Anthrax
The commonest form of anthrax in man is	Alimentary	Cutaneous	Pulmonary	Hepatic	Cutaneous
The animals most frequently infected	Sheep	Cat	Dog	Pig	Sheep

with anthrax are					
Which anti rabic vaccine has been recommended by WHO as the most effective?	Duck embryo vaccine	HDCS vaccine	Sheep brain vaccine	BPL vaccine	HDCS vaccine