Sterilization :-

Some important definitions :-

- Antiseptic : A substance that inhibits or destroys microorganisms .The term is applied specifically for substances applied topically to living tissues.
- Biocide : A substance that kills all living microorganisms including spores ,both pathogenic & non pathogenic (e.g. bactericides , virucide) .
- Biostat : An agent that prevents the growth of microorganisms but does not necessarily kill them (e.g. bacteriostatic , fungi static) .
- Decontamination : The removal of microorganisms with no quantitative implication . The term is relative & th end can be achieved by sterilization or disinfection .
- Disinfection : A process that reduces or completely eliminates all pathogenic microorganisms except spores .
- Germicide : A substance that destroys microorganisms , especially pathogenic microorganisms . A germicide does not destroy spores.
- Sanitation : The process by which microbial contamination is brought to a " safe" level . This process refers primarily to the process of " cleaning" in animal objects.
- Sterilization : The use of physical and / or chemical procedures to completely eliminates or destroy all forms of microbial life .This term is absolute of indicates complete freedom from microorganisms of their spores.

A) Physical Agents : Physical agents are usually preferred over chemical agents for performing Sterilization. Heat , in one form or another occupies the most important place as physical agent so sterilize.

1. Moist Heat :

This is heating in the presence of water & can be employed in the following ways :

- Temperature below 100° C
- Temperature around 100° C
- Temperature above 100° C

Temperature below 100° C :- This includes holder method of Pasteurization where 60° C for 30 mins is employed for sterilization & in its flash modification wherein objects are subjected to a temperature of 71.1° C for 15 Sec. This method does not destroy spores .

Temperature around 100° C :- Tyndalization is an example of this methodology in which steaming of the object is done for 30 mins on each of the 3 consecutive days .

The principle is that spores which survived the heating process would germinate before the next thermal exposure & would then be killed.

Temperature above 100° C :- Dry saturated steam acts as an excellent agent for sterilization .

Autoclaves have been designed on the principles of moist heat .

Time - Temperature relationship in heat sterilization

Process	Temperature	Holding Period
Moist Heat (Autoclaving)	121° C 126° C 134° C	15 mins 10 mins 3 mins
Dry Heat	160° C 170° C 180° C	120 mins 60 mins 30 mins

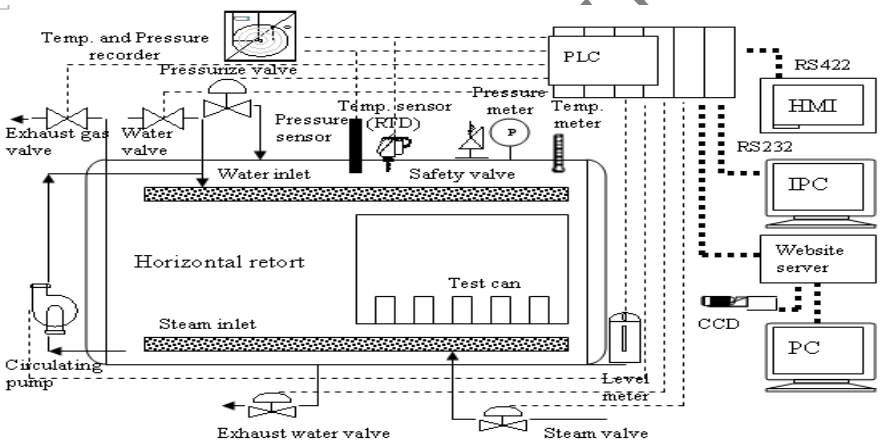
AUTOCLAVE :

Structure :- A laboratory consists of vertical or horizontal cylinder of gun metal or stainless steel in a supporting iron case .The lid is fastened by screw clamps & made airtight by washer. There is discharge tap for air & steam ,a pressure gauge of a safety value on the lid . Heating is done by electricity or can also be done by gas .

Functioning of an Autoclave :-

1. Put sufficient quantity of water in the cylinder .
2. Put the materials to be sterilized on the tray .
3. Start the heating .
4. Screw tight the lid ,keeping the discharging tap open .
5. Adjust the safety value to the required pressure .
6. Allow the steam air mixture to escape freely till all the air has been displaced . this can be tested by leading the escaping steam into a pail of water through a rubber tubing close the discharge tap when no more air bubbles come through .
7. Count the holding time from the time when the safety value opens & the excess steam escapes .
8. Turn off the heater when the holding time is complete .
9. Allow it to cool ,till the pressure gauge indicates that the pressure inside is equal to the atmospheric pressure .
10. Open the discharge tap slowly & let the air into autoclave .

SCHEMATIC DIAGRAM OF AUTOCLAVE :



2. Dry Heat :

Dry heat is less efficient process than moist heat & bacterial spores are most resistant to it . Spores may require a temperature of 140° c for there to be get killed . Dry heat can be used by following means :

- Incineration:- This is an efficient method for sterilization & disposal of contaminated materials at a high temperature .
- Red Heat :- Inoculating wires , loops & points of forceps are sterilized by holding them in the flame of a Bunsen burner until they are red hot
- Flaming :- Direct exposure for a few seconds may be used for scalpels & the neck of flasks , but it is of uncertain efficacy.
- Hot Air sterilizers (Ovens) :- Dry heat sterilization is usually carried out in hot air ovens in which a number of time – Temperature combinations can be used:

Temperature	Holding Period
160° C	120° C
170° C	60° C
180° C	30° C

Dry heat is employed for sterilization of glassware ,glass

syringes ,oils & oily injections as well as metal instruments .

3. Ionizing Radiations :

Ionizing radiations include X – rays ,gamma rays & beta rays kill microbes by damaging the DNA. The Ionizing radiations are used for the sterilization of single use disposable medical items .

4. Ultraviolet Radiations :

The UV rays of wave length 240 – 280 nm have been found to be most efficient in sterilizing .

Exposure of UV damages DNA .This results into noncoding cessions in DNA & bacterial death .

5. Filtration :

Filters have been used since long to purify water & sewage .

Types of filters :-

- Unglazed ceramic filters (chamberland & Doulton filters) .
- Asbestos filters (Seitz ,Carlson & Sterimat filters) .
- Sintered glass filters .
- Membrane filters .

Membrane filters are widely used now a days .These are made up of cellulose ester & are most suitable for preparing sterile solutions .

The range of pore size in which these are available is 0.05 – 12um whereas the required pore size for sterilization is in range of 0.2 – 0.22 um .

B) Chemical Agents : Chemical disinfections ,unlike chemotherapeutic agents ,are highly toxic for all types of cells

1. Oxidizing Agents :
   - Chlorine & Iodine :- These are most useful disinfections .Iodine as a skin disinfectant .They are effective against sporulating organisms.

Mixture of various surface acting agents with iodine are known as Iodophores & these are used for the sterilization of dairy products .

Chlorine is one of the most potent bactericidal agent ,its activity is markedly by the presence of organic matter & hence it becomes necessary to ascertain .The chlorine damaged of the water before it is disinfected.

   - Hydrogen peroxide :- In a 3% solution is a harmless but very weak disinfectant whose primary use is the cleaning of a wound .
   - Potassium permanganate :- It is another oxidizing agent which is used in the treatment of urethritis .

2. Alkylating Agents :Damage produced by such agents is irreversible resulting in inhibition of enzymatic activity .
   - Formaldehyde :- Is one of the least selective agents acting on proteins .It is a gas that is usually employed as its 37 percent solution , Formalin .

When used in sufficiently high concentration it destroys the bacteria & spores .

   - Ethylene oxide :- It is employed in gaseous sterilization .It is active against all kinds of bacteria & spores .

Ethylene oxide is being used to sterilize fragile ,heat sensitive equipments ,powders as well as components of space crafts .

3. Organic Solvents :-

Alcohol :- Alcohol disorganizes the lipid structure of the membrane by penetrating into the hydrocarbon region .

It also denatures the protein of the cell .

Chloroform & Toluene :- are used to keep the solutions sterile & to disrupt permeability barriers .

Ethanol :- is used to sterilize the skin prior to cutaneous injections .It is active against gram – positive & gram – negative & acid – fast organisms & acts best at concentration Of 50 – 70 percent .

4. Acids & Alkalies :- These agents releases their free H^+ & OH^- ions & then through undissociated molecules or by altering the pH of the medium they denature the proteins of the organisms .

Collection of Specimens :

1. Collection of Urine :-
   - I. Urine for general examination may be collected in a bottle ,properly cleaned with soap & water .It must be properly labeled .
   - II. For culture mid steam urine should be collected.
   - III. For routine examination about 100 ml of urine (morning sample) should be collected in a cleaned & dry container .
2. Collection of cerebro spinal fluid (CSF) :
   - I. CSF is collected by puncturing the space between 3rd & 4th lumbar vertebrae .It is called Lumber puncture .
   - II. Specimen collection may be done by expert doctor under sterilized conditions .
   - III. About 3 – 5 ml of CSF can be collected in 3 tubes (5 ml each) .
   - IV. It is sent immediately to the laboratory for tests .
3. Stool examination :
   - I. Stool should be collected in sufficient quantity .
   - II. Stool must be collected in a proper container ,e.g ,waxed card board box ,a wide mouthed plastic bottle with a lid etc .

(Diagram of Wide mouthed plastic Bottle with lid)

3. Stool should be sent & processed in the laboratory within 1 hour of its passing by the patient .

4. Collection of sputum specimen :
   - I. Patient should rinse the mouth with water before coughing out specimen .
   - II. Collect the sputum in the morning .
   - III. Sputum must be collected in glass screw top jars or waxed paper cup with lid .

INFECTION : Infection is the invasion of a host organism's bodily tissues by disease causing organism , their multiplication ,& the reaction of host tissues to these organisms & the toxins they produced .

Infections are caused by micro – organisms such as viruses ,prions ,bacteria & viroid ,& larger organisms like parasites & fungi .

Infectious Disease : A disease condition caused by the presence or growth of infectious micro – organisms or parasites .

Disease : Any deviation from a condition of good health & well being .

Pathogens : Microbes capable of causing infectious that results in disease are called pathogens .

This characteristic is called as pathogenicity of the organisms .

Virulence : Degree of pathogenicity of microbe is referred to its virulence .Virulence is a quantitative term .

Dynamics of infectious disease : For an infectious disease to perpetuate there has to be a reservoir of microorganism from where the causative agent should be transmitted to a susceptible host either directly or through the agency of a vehicle or a vector .

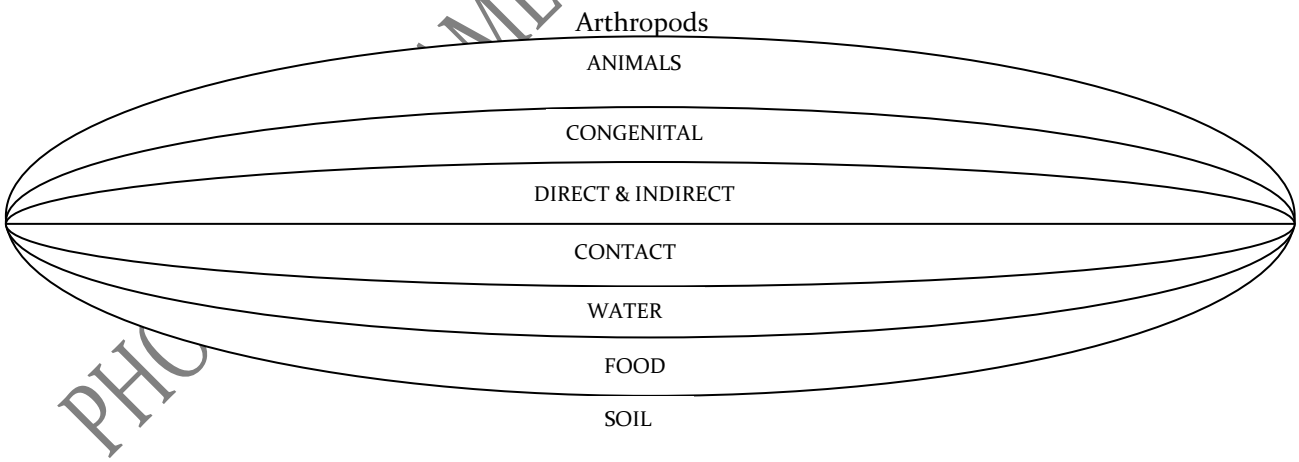
Source & reservoir : The source of infection is the person ,animal ,object or substance from which an infectious agent passes or disseminates to the host .

Where as a reservoir is defined as any person ,animal ,arthropod ,plant ,soil or substance (or combination of these) in which an infectious agent lives & multiplies .

These can be of three types :

- Humans
- Animals
- Non – living substance

I	II	III
Source of infection	Mode of transmission	Susceptible person



SOURCES AND VEHICLES OF INFECTION.

Modes of transmission: The micro – organisms can be transmitted to human beings directly or indirectly .

Direct transmission occurs through :

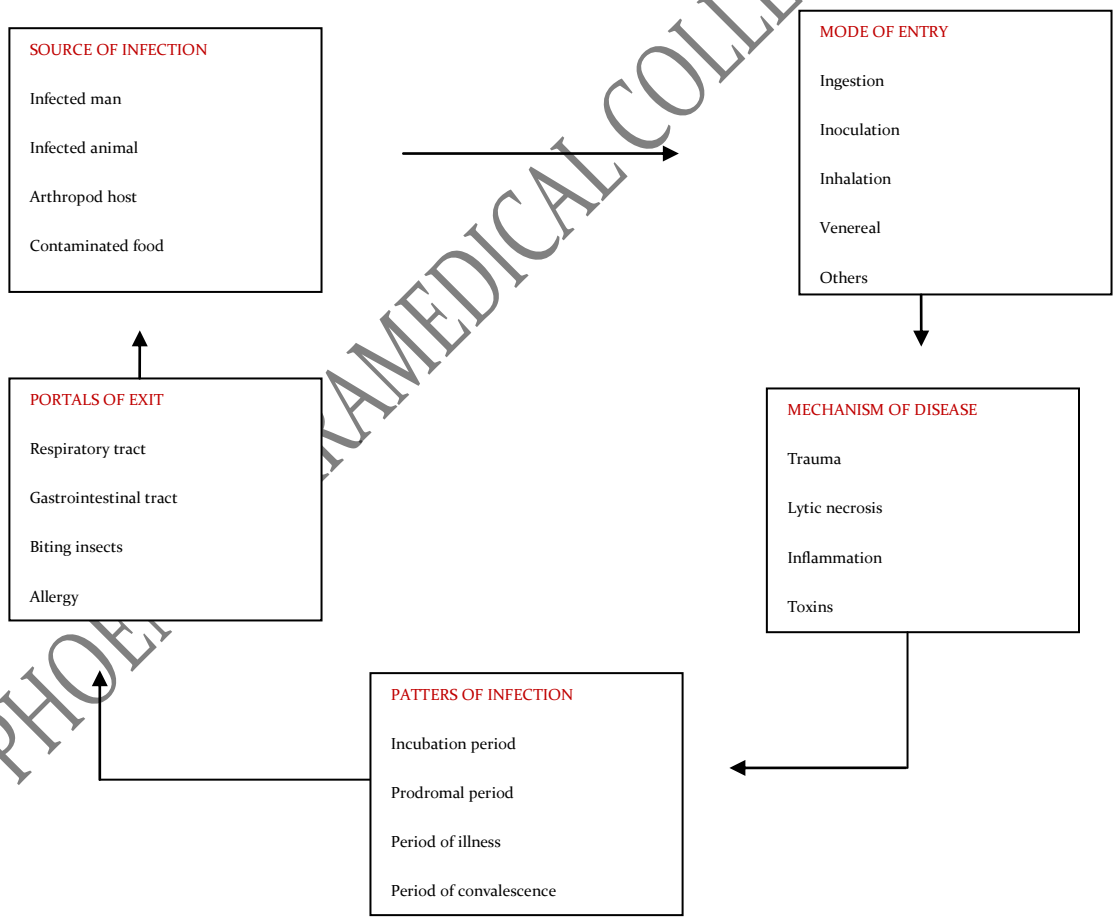
- Contact with man ,animal or inanimate objects .
- Droplet infection .
- Breach of skin or mucous membrane .
- Transplacental & congenital .

The indirect transmission is possible by any of the following mechanisms .

- Vehicles (water , food etc) .
- Vectors (mechanical or biological) .
- Air (droplet or dust)
- Fomites .
- Unclean hands & fingers .

Mechanism of infection : To produce infection in man , a microbe has to gain entry into the host . The most frequent portals of entry are the respire tract ,the gastrointestinal tract & breaks the superficial mucous membranes & skin .

From the portal of entry the parasite may spread directly through the tissues or may proceed via lymphatic channels to the blood stream ,which distributes it widely & permits it to reach tissues particularly suitable for its multiplication .



DYNAMICS OF INFECTION.

Development of disease : Once the micro – organisms overcomes the defenses of the host ,development of infectious disease follows a sequence of events .

1. Period of incubation : This is the time interval between the actual infection & the appearance of first clinical feature
2. Prodromal period : This relatively short period follows incubation period in some diseases .It is characterized by mild early symptoms such as general aches & malaise .
3. Period of illness : This is the acute phase of illness characterized by typical clinical features of the infectious disease .
4. Period of Decline : During this period the signs & symptoms subside .The fever decreases & feeling of malaise diminishes .During this period ,the person may be attacked by secondary infection .
5. Period of convalescence : The person regains strength during this period & body returns to pre diseased state .

Nosocomial Infections : A Nosocomial infection is contracted because of an infection or toxin that exists in a certain location ,such as a hospital .People now use nosocomial infections inter changeably with the terms healthcare Associated Infections (HAIS) & hospital Acquired Infections .

One of the most common words where HAI'S occur is the Intensive care Unit (ICU) ,where doctors treat serious diseases .

For a HAI ,the infection must occur :

- Upto 48 hours after hospital admission
- Upto 3 days after discharge
- Upto 30 days after an operation

Symptoms of Nosocomial Infections :

- Discharge from a wound
- Fever
- Cough ,shortness of breathing
- Burning with urination or difficulty urinating
- Headache
- Nausea ,vomiting ,diarrhea

These may lead to serious diseases ,

- Urinary tract infections (UTIs)
- Surgical site infections
- Gastroenteritis
- Meningitis
- Pneumonia

Causes of Nosocomial infections : Bacteria ,fungus & viruses can cause HAIS .Bacteria alone cause about 90% of these cause .

Some of the common bacteria that are responsible for HAIS are :

Bacteria	Infection type
• Staphylococcus aurens • Escherichia coli (E – coli) • Enterococci • Pseudomonas aeruginosa (P . aeruginosa)	Blood UTI Blood ,Wound ,UTI Kidney ,UTI ,Respiratory

Diagonis :- Many doctors can diagnose a HAI by sight & symptoms alone ,

- Inflammation and / or rash at the site of infection .
- Blood test & urine test as to identify the infection .

Treatment for Nosocomial Infection :

- Doctor will likely recommend antibiotics & bed rest .
- They will remove any foreign devices such as catheters .
- Doctor will encourage a healthy diet ,fluid intake & rest .

Preventions :

- Screening the ICU to see if people with HIAS need to be isolated .
- Observing hand hygiene ,which involves washing hands before & after touching people in the hospital .
- Wearing appropriate gear ,including gloves, gowns & face protection .
- Cleaning surfaces properly .
- Change catheters or bags only when medically indicated .
- Keep a closed Drainage system .

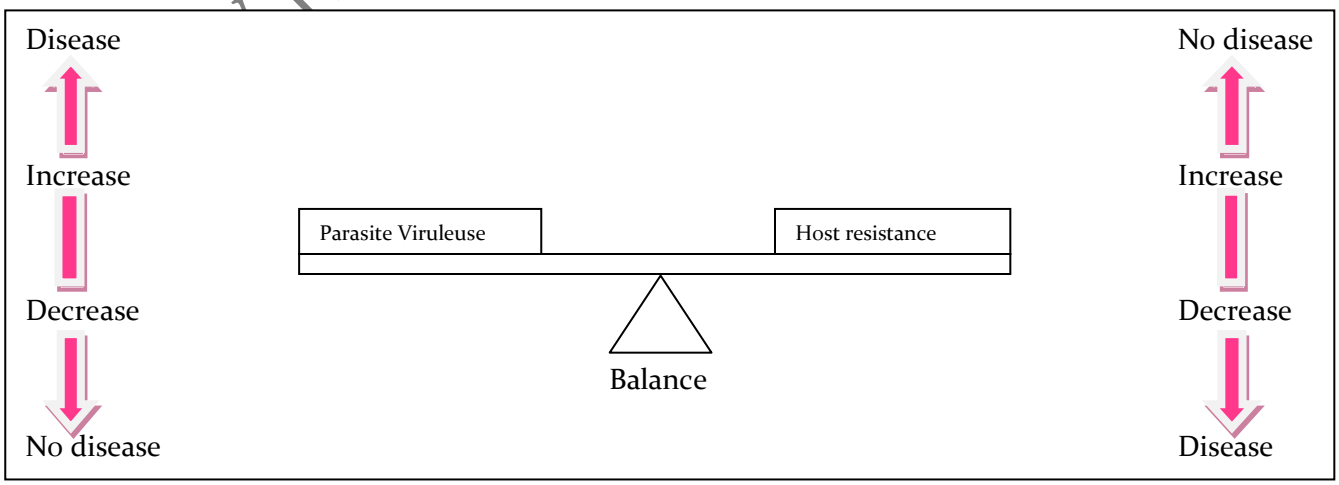
Reaction of body to infection :

Infection occurs when viruses ,bacteria or other microbes enter our body & begin to multiply .

- Disease occurs when the cells in our body are damaged as a result of infection & signs & symptoms of an illness appear .
- The incidence of disease among those infected varies greatly depending on the particular pathogen & individual susceptibility .
- In response to infection ,our immune system springs into action .WBC ,antibodies & other mechanisms go to work to rid our body of the foreign invader .
- Indeed ,many of the symptoms that make a person suffer during an infection – fever ,malaise ,headache ,rash result from the activities to eliminate the infection from the body .

Resistance of Host to Infection : There are a variety of mechanisms by which human beings resist attack by these can be broadly divided into :

- Natural resistance (innate immunity ,non – specific resistance or constitutive immunity)
- Acquired immunity (specific ,inducible or adaptive immunity).



Mechanism of Natural Resistance :

Natural resistance operates nonspecifically during the early phase of an immune response .

Its functions through activation of nonspecific cells whish are phagocytic cells of the mononuclear & granulocytic systems & natural killer (NK) cells .

- The most significant phagocytic cells are the macrophages & large granular lymphocytes (LGL) .
- Natural resistance is the result of the action of a NO. of systems .
- These may be differentiated into external & internal systems which are bridged with acquired immunity by the process of inflammation .
- The external system prevents the entry of microbes into the body & the internal system comes into play following injuries that breach the physical barriers .
- In addition ,inflammation also aids the lost in containing the infectious agent .

External Defense systems :

- The first line of defense against infection are the external systems .
- Major components of the external systems are the mechanical barriers ,The effectiveness of which is enhanced by various antimicrobial secretions .

External barriers to entry of microorganism :

Body site	Defense mechanism	Conditions that faster entry
1. Skin	Dryness ,acidity ,toxicity, constant shedding	wounds, excess moisture ,serous discharge
2. Respiratory tract	Ciliated cells constantly moving mucous to throat	Reduced movement of ciliated cells as in smoking chilling ,narcotics ,viral infection
3. Gastrointestinal tract	0.2% hydrochloric acid ,enzyme pepsin .	Reduced stomach acid ,ingestion of antacid .
4. Vagina	Lactobacilli during child bearing years .	Reduced numbers of Lactobacilli from douching soaps ,menopause ,antibiotic therapy .
5. Urinary tract	Flushing action of urination .	Short urethra in complete ,incomplete ,sexual intercourse .

Internal Defense systems : Microbes that by one means or another succeed in passing the external barriers encounters the second line of defense ,the internal systems .

Antimicrobial factor	Chemical	Source	Effects
Lysozyme	Protein	Most body fluids	Destroys bacterial cell wall also within phagocytes .
Beta – Lysin	protein	Serum leukocytes	Attack cytoplasmic membrane ; active against gram+ve bacteria .
Peroxidase	protein	Leukocytes ,saliva & other sources of peroxidases	Kills a variety of M/O[S] important killing mechanism in saliva & within neutrophils .
Interferon	protein	Leukocytes & tissue cells	Interferes with the multiplication of viruses by causing the formation of antiviral protein .
Complement system	Many distinct proteins	Procedure by macrophages & other host cells	Proteins acting in special sequence to produce effects such as chemotoxis , opsonization & cell lysis .

Cross Infection : A cross infection is the transfer of harmful micro – organisms .usually bacteria & viruses .

The Spread of infections can occur between people ,pieces of equipment or within the body .

Types of cross infection :

Some examples include :

1. Urinary tract infection (UTI) from an infection caused by a catheter .
2. A surgical wound infection that may cause redness ,swelling & pus .
3. An infection related to the presence of a peripheral or central venous access time .

Causes of cross infection : Cross infections can be caused by :

- Bacteria
- Fungi Parasites
- Viruses

These microorganisms can be transmitted by :

- Unsterilized medical equipment
- Coughing & sneezing
- Touching contaminated objects
- Dirty bedding
- Prolonged use of catheters ,tubes or intravenous lines.

Diagmnosis : These includes :

- Physical exam
- Blood tests
- Cultural tests
- Urine tests
- X – ray
- Health history review

Treating cross infection :

- Antibiotics are used for bacterial & parasitic infection .
- Anti – viral drugs are used to treat specific types of virus .
- Anti – fungal medications can be used to treat fungal infections .
- Dietary changes .

Preventions : A septic technique is a common process used to sterilize equipment

- Sterilized environment
- Avoiding crowd