PHARMACEUTICAL SPOILAGE

Definition

- **Spoilage of pharmaceutical products and drugs are referred as the changes in the physical and chemical properties in such a way that the formulation or therapeutic agents gets deteriorate and become not suitable for use.**
- **Microbial spoilage includes the contamination of pharmaceutical products with the microbes which leads to spoilage of the products affecting Drug safety and quality, and it not intended for use.**
- **Shortly defined as deterioteration of pharmaceutical products by the contaminant microbes.**
- There are several **factors that cause spoilage of pharmaceuticals, like**

PHYSICAL **CHEMICAL** **MICROBIAL**

Deterioration of pharmaceutical factors like heat, temperature, evaporation, etc.	**Deterioration of pharmaceuticals because of chemical reactions like oxidation, reduction, hydrolysis, protolysis, ionization,etc.**	**Deterioteration of pharmaceuticals due to of contamination microbial any cell. i,e bacteria, fungi, mould, etc.**

why to study??

- To determine the microbial contaminants present in any formulation
- To avoid health hazard related to the microbial infections
- To acquire knowledge about sources and different types of microbial contaminants related to different dosages and their deterioration action
- To avoid the financial problems for the manufacturer due to the loss of products

- To find out the limits of microbial contents in the pharmaceutical products.
- Parenteral, oral and topical preparation causes infections after microbial contamination, serious condition can be seen in case of typhoid fever by the ingestion of contaminated thyroid tablets.

Types of microbial spoilage of pharmaceuticals

Physico-chemical spoilage

- Viable Growth
- Gas Production
- Physical spoilage
  - Olfactory
  - Colouration

Chemical spoilage

- Hydrolysis
- Acetylation
- Depolymerisatio n
- Degradation

Biological spoilage

- Release of Toxins
- Microbial metabolites

Physico-chemical spoilage

- In this kind of spoilage, there are some changes are **caused by microbial species and due to these changes the physical properties are also gets altered or deteriorate,** thus it is called physico-chemical spoilage.
- **Viable growth:** Microbial cells form a viable layer over the surface of pharmaceutical formulation. This layer or the presence of microbial cell can be clearly seen by naked eyes.

Examples: layer of moulds over syrups or sugar containing products, tablets, creams, ointments.

- **Colouration:** occurs due to the **alteration in the components chemical nature and these changes are caused by change in pH of the formulation, redox of the product, production of some other metabolites by microorganism.**

Examples: Pseudomonas species microorganism metabolize wide range of metabolites that cause colouration of blue- green, brown. Surface decolouration of tablets containing biological products by some mould.

- **Gas production:** some **microorganism contaminants in pharmaceutical formulations produces gases by their metabolic activities and form gas bubbles and broth/foam over the formulation. Products containing carbohydrates or starchy material are more susceptible for gas production**

Examples: Production of CO_2 in syrups caused by osmo-tolerant moulds and yeasts **(Aspergillus spp. and Zygosaccharomyces rouxii).** Klebsiella produces gas in creams and ointments containing vitamins and proteins. Desulfovibrio oxidize simple organic compounds and produces hydrogen sulphide in suspensions.

- **Physical spoilage:** These are some changes in physical appearance caused by microbial call activities.

Examples: In emulsion, some microbial cells cause hydrolysis of oil phase, cause change in oil-water equilibrium and make emulsion unstable. Hydrolysis of oils also affect pH of formulation and alter the stability of emulsions. In syrups, microbial cells metabolise sugar molecules and due to which the concentration of syrup change and product become unstable.

- **Olfactory spoilage:** spoilage by some microorganism and moulds generates unpleasant smell from the products. This kind of spoilage mainly caused by microbial cells that produces sulphur containing gases (sulphur dioxide, hydrogen sulphide) and fishy smell due to formation of fatty acids along with odour generates by amines and alcohol production.

Examples: **Contamination in syrup of Tolu by penicillium species produces toluene like smell. Actinomycetes produces smell of geosmin in water phase use for formulations.**

Chemical spoilage

- These are various types of chemical spoilage in pharmaceutical compounds, these occurs due to various types of chemical reactions, mediated by contaminating microorganism.

Hydrolysis: Some bacteria cells contains catalyse enzymes that hydrolysis of pharmaceuticals.

Examples: **Atropine hydrolysed by Pseudomonas bacteria, Aspirin hydrolysed by bacillus and clostridium species.**

Acetylation: Some microorganism cellular enzymes cause acetylation of drugs and cause loos of activity

Examples: Chloramphenicol acetylation caused by staphylococci and streptococccci gram +ve bacteria by the enzyme chloramphenicol acetyl-transferase.

Depolymerisation: Depolymerisation is a process in which the polymers are degraded to their monomers. A lots of polymers are used in the formulation of many types of pharmaceutical preparations as diluents, binders, thickening, suspending agents, etc.

Examples:

Starch (Glucose): Depolymerize by bacteria amylase

Pectin(Galactourinic acid): Depolymerize by bacteria pectinase

Dextran(Glucose): Depolymerize by bacteria dextranase

Cellulose (Glucose): Depolymerize by bacteria cellulase

- **Degradation:** Due to the microbial contamination, the active therapeutic agents or the formulation ingredients can be degraded or metabolized.

Examples:

Penicillin degraded by betalactamase containing bacteria cells,

Prednisolon degraded by Aspergillus species

Biological spoilage

- Some bacteria cells contaminated the pharmaceuticals and utilizes the various compounds present in that formulation to perform their metabolic activities. Due to theses metabolic activities, the microbial cells produces certain chemicals which they release in the pharmaceutical preparation. This is called biological spoilage.
- Mainly two types of chemicals release by the microorganisms

Microbial toxins: Several microorganisms produce toxic molecules that may cause spoilage of pharmaceutical formulations. Such as endotoxins produces by some grain –ve bacteria like *E.coli*

Microbial metabolites: Bacterial metabolites are the biosynthetic products from microbial cells. Bacterial cells produces various metabolites which cause product spoilage because of these metabolites are toxic to humans.

Examples: **Different types of amines and organic acids from bacteria cells. Metabolites from fungi and moulds. Fungi and mould are more specifically grow on the formulations having Talc, Kaolin and Starch.**

Tabulation of some formulation/Ingredients and their agent which cause

Formulation/Chemical compounds/Drugs	Contaminating species/agents
Polysaccharides (Agar, Starch,Cellulose)	Bacillus, pseudomonas, clostridia spp.
Proteins and Gelatin	Aspergillus spp. Penicillium spp.
Creams and emulsions	moulds
Aromatic compounds	Pseudomonas spp. Gram negative bacteria
Acetyl salicylic acid	Acinetobactor spp.
Syrup of Tolu	Penicillium spp.
Atropine eye drop	Corynebacterium spp.
Prednisolon tablet	Aspergillus spp.
Hydrocortison tablet	Clostridium herbarum
Rose-Hip preparation	Anaerobic bacteria

Factors effecting microbial spoilage

- **By understanding the influence of environmental parameters on microorganisms, it may be possible to manipulate formulations to create conditions which are as unfavourable as possible for growth and spoilage, within the limitations of patient acceptability and therapeutic efficacy.**

1. ***Size of contaminant inoculum***

- Low levels of contaminants may be present in a product but it would cause low rates of deterioration.
- Ingredients contaminated by a high level of microorganisms cause appreciable microbial degradation.
- However, inoculum size alone is not always a reliable indicator of likely spoilage potential
- A very low level of pseudomonas in a weakly preserved solution may suggest a greater risk than tablets containing high numbers of fungal and bacterial spores.

2. *Nutritional factors:*

- Gross microbial spoilage of a pharmaceutical products generally required appreciable growth of the contaminating microorganisms within the dosage forms.
- Most of the organic and inorganic ingredients act as potential carbon or nitrogen substrates for microbial growth
- The complexity of many formulations offers considerable nutritional variety for a wide range of microorganisms.
- The use of crude vegetable or animal products in a formulation provides an additionally nutritious environment. Even demineralised water prepared by good ion-exchange methods will normally contain sufficient nutrients to allow significant growth of many waterborne Gram-negative bacteria such as Pseudomonas spp.

3. *Moisture content: water activity (A_w)*

- Microorganisms require readily accessible water in appreciable quantities for growth to occur.
- By measuring a product's water activity (A_w), it is possible to obtain an estimate of the proportion of uncomplexed water that is available in the formulation to support microbial growth, using the formula: A_w = vapour pressure of formulation/vapour pressure of water under similar conditions.
- Most microorganisms grows best at high water activity.
- Hence, formulation can be protected from microbial attack by lowering their water activity with addition of suitable levels of sugars, polyethylene glycols or sodium chloride or by drying.

4. *pH*

- Extremes of pH prevent microbial attack.
- Around neutrality, bacterial spoilage is more likely, with reports of Pseudomonads and related Gram-negative bacteria growing in antacid mixtures, flavoured mouthwashes and in distilled or demineralised water.
- Above pH 8 (e.g. with soap-based emulsions) spoilage is rare. In products with low pH levels (e.g. fruit juice-flavoured syrups with a pH 3–4), mould or yeast attack is more likely.
- Yeasts can metabolize organic acids and raise the pH to levels where secondary bacterial growth can occur.

Minimum water activity that supports growth of some microorganisms

Microorganism	Water activity
Clostridium botulinum,	0.95
Bacillus cereus,	0.95
Pseudomonas aeruginosa,	0.95
Salmonella spp.	0.95
Staphylococcus aureus (anaerobic),	0.90
Candida spp., *Saccharomyces*	
Staphylococcus aureus (aerobic)	0.86
Penicillium spp.	0.82
Most spoilage yeast	0.88
Most spoilage molds	0.80
Osmotic yeast	0.70

5. Storage temperature

- Spoilage of pharmaceuticals could occur potentially over the range of about -20°C to 60°C
- The particular storage temperature may selectively determine the types of microorganisms involved in spoilage.
- A deep freeze at -20°C or lower is used for long-term storage of some pharmaceutical raw materials and short-term storage of dispensed total parenteral nutrition (TPN) feeds prepared in hospitals.
- Reconstituted syrups and multi-dose eye-drop packs are sometimes dispensed with the instruction to 'store in a cool place' such as a domestic fridge (8°–12°C), partly to reduce the risk of growth of contaminants inadvertently introduced during use.
- Conversely, Water for Injections (EP) should be held at 80°C or above after distillation and before packing and sterilization to prevent possible regrowth of Gram negative bacteria and the release of endotoxins.

6. *Redox potential*

- The ability of microbes to grow in an environment is influenced by its oxidation-reduction balance (redox potential), as they will require compatible terminal electron acceptors to permit their respiratory pathways to function.
- The redox potential even in fairly viscous emulsions may be quite high due to the appreciable solubility of oxygen in most fats and oils.

7. *Packaging design*

- Packaging can have a major influence on microbial stability of some formulations in controlling the entry of contaminants during both storage and use.
- Considerable thought has gone into the design of containers to prevent the ingress of contaminants into medicines for parenteral administration, owing to the high risks of infection by this route.
- Self-sealing rubber wads must be used to prevent microbial entry into multi-dose injection containers following withdrawals with a hypodermic needle.
- Where medicines rely on their low A_w to prevent spoilage, packaging such as strip foils must be of water vapour-proof materials with fully efficient seals.
- Cardboard outer packaging and labels themselves can become substrates for microbial attack under humid conditions, and preservatives are often included to reduce the risk of damage.

SOURCES OF CONTAMINATION

- Contaminants can gain entry into a production process stream from several sources such as, Personnel, Poor facility design, Incoming ventilation air, Machinery and other equipment for production, Raw material and semi-finished material, Packaging material, Utilities, Different media used in the production process as well as for cleaning and Cleanroom clothing.

Personnel

- Personnel who are supervising or performing drug manufacturing or control can be a potential source of microbiological contamination and a vector for other contaminants.

The main reasons for contamination from the personnel include:

- Lack of training
- Direct contact between the operator's hands and starting materials, primary packaging materials and intermediate or bulk product
- Inadequate personnel cleanliness
- Access of unauthorized personnel into production, storage, and product control areas
- Inadequate gowning and personnel protective equipment, and
- Malpractices like eating food, drinking beverages, or using tobacco in the storage and processing areas.

Buildings and Facility

- The buildings and manufacturing facilities may also contribute to the contamination.

The main reasons of contamination due to facility issues include:

- Insufficient size and inadequate organization of the space leading to selection errors like mix-ups or cross contamination between consumables, raw materials, in-process materials, and finished products
- Inadequate filth and pest controls
- Rough floors, walls and ceilings
- Lack of air filtration systems
- Improper lighting and ventilation
- Poorly located vents, and drains
- Inadequate washing, cleaning, toilet, and locker facilities to allow for sanitary operation, cleaning of facilities, equipment, and utensils; and personal cleanliness.

Equipments

- The equipment and utensils used in processing, holding, transferring and packaging are the common source of pharmaceutical contamination.

The main reasons for contamination from the equipment include:

- Inappropriate design, size, material leading to corrosion and accumulation of static material and/or adulteration with lubricants, coolants, dirt, and sanitizing agents
- Improper cleaning and sanitization
- Design preventing proper cleaning and maintenance
- Improper calibration and irregular service, and
- Deliberate use of defective equipment

Materials

- The raw materials used for production can be a potential source of contamination.

The main reasons for contamination from the raw materials include:

- Storage and handling mistakes causing mix-ups or selection errors
- Contamination with microorganisms or other chemicals
- Degradation from exposure to excessive environmental conditions such as heat, cold, sunlight, moisture, etc.
- Improper labelling
- Improper sampling and testing, and
- Use of materials that fail to meet acceptance specifications.

Manufacturing Process

- There are various opportunities for contamination of raw material, intermediates or packaging materials throughout the manufacturing process.

The main reasons for contamination during manufacturing process include:

- Lack of dedicated facilities to manufacture a single product
- Inappropriate cleaning in-between batches to minimize the amount of product changeovers
- Use of an open manufacturing system exposing the product to the immediate room environment
- Inappropriate zoning
- Absence of an area line clearance according to approved procedures following each cleaning process and between each batch, and
- Lack of cleaning status labelling on all equipment and materials used within the manufacturing facility

To minimize the risks of manufacturing contamination

- Manufacture products in a campaign, with the appropriately qualified cleaning processes and checks performed in-between batches to minimize the amount of product changeovers
- Utilize a closed manufacturing system. This is where the product is not exposed to the immediate room environment (and vice versa)
- Perform an area line clearance according to approved procedures following each cleaning process and between each batch/campaign
- Zone the facility and
- Use Cleaning Status labelling on all equipment and materials used within the manufacturing facility

HVAC System

- HVAC stands for Heating, Ventilating, and Air Conditioning
- A poor HVAC system can be a potential source of microbes growth and a transportation mode for dispersing contaminants throughout the manufacturing facility.
- The main reasons of contamination due to HVAC issues include:
- Accumulations of organic material in or near HVAC air intakes
- Ineffective filtration of the supply air
- Insufficient magnitude of pressure differentials causing flow of reversal
- Erroneous ratio of fresh air to recirculated air
- Inabilityto access ventilation dampers and filters from

Assessment of microbial contamination and spoilage

1. Physical and chemical changes:

- It is the changes of different pharmaceutical formulations indicate microbial contamination and spoilage
- Change in viscosity, pH, emulsion stability and loss of surface activity of formulation indicates microbial spoilage.
- Measurement of oxygen consumption of the product can indicate the degree of oxidative attack and microbial growth

Sterility test

- **Testing** which confirms that products are free from the presence of viable microorganisms.
- Claim to be **sterile** or free from viable microorganisms.
- Test is conducted by competent and experienced personnel in an adequately clean room with laminar flow cabinet facilities.
- All injectables and ophthalmic preparations are sterile hence, these preparations are tested by the sterility test.

Assessment of viable microorganisms in non-sterile products

- Non-sterile products are tested for viable microorganisms for detection of pathogens and total viable counts.
- In microbiological terms, pharmaceutical products can be divided into two groups: sterile and non- sterile.
- Non-sterile drugs must satisfy the appropriate microbiological purity criteria which are included in pharmacopoeial monographs.
- Pharmacopoeial studies are prepared specifically with a view to ensuring that the medicinal product is therapeutically effective and safe for the patient.

Estimation of pyrogens

- Pyrogen a substance, typically produced by a bacterium, which produces fever when introduced or released into the systemic circulation.
- The lipopolysaccharides and lipoproteins which comprises a major part of the cell wall og Gram –ve bacteria are called endotoxins which are the most commanly called pyrogens
- To test the pyrogens presence, two tests are used

1. **RP Test (Rabbit Pyrogen Test)**
2. **LAL Test (Limulus Amoebocyte Lysate Test**

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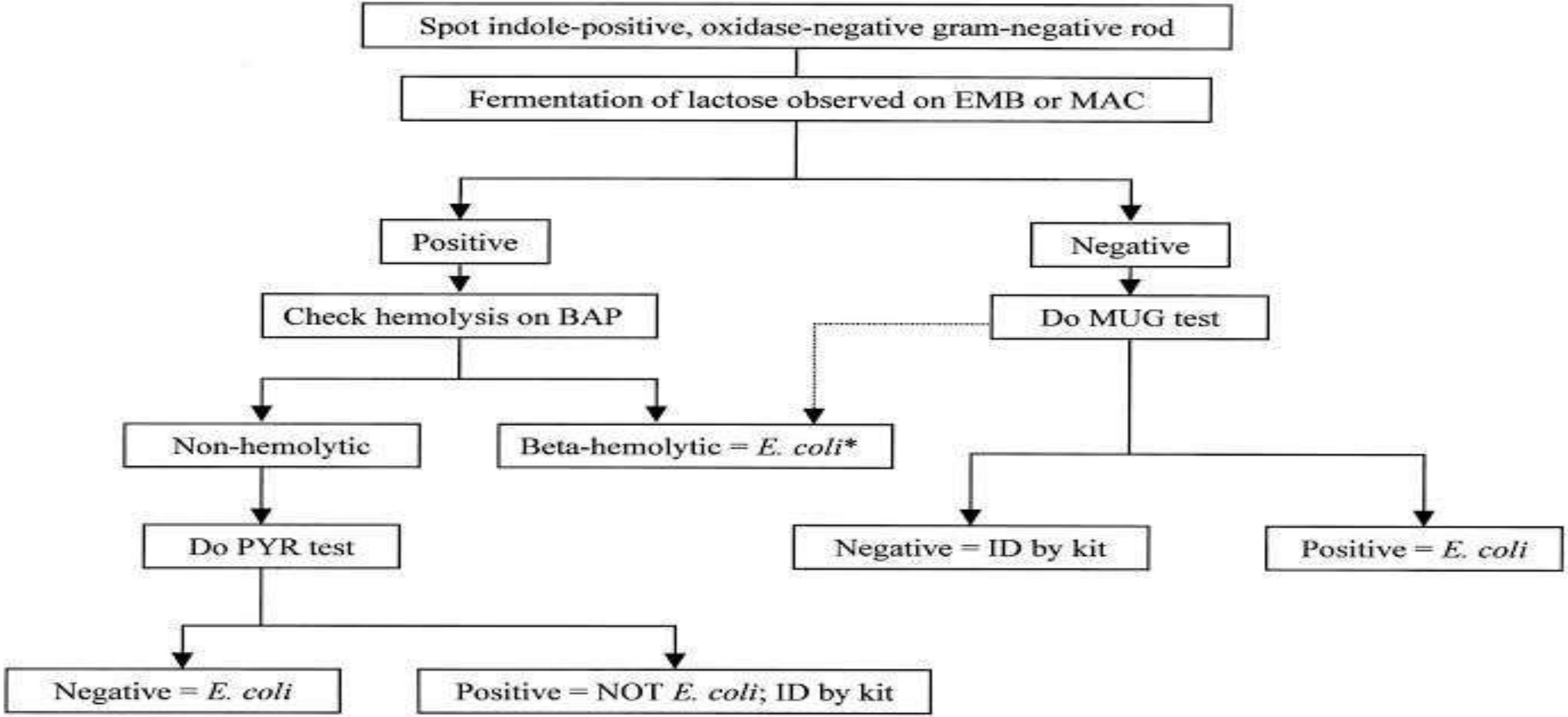
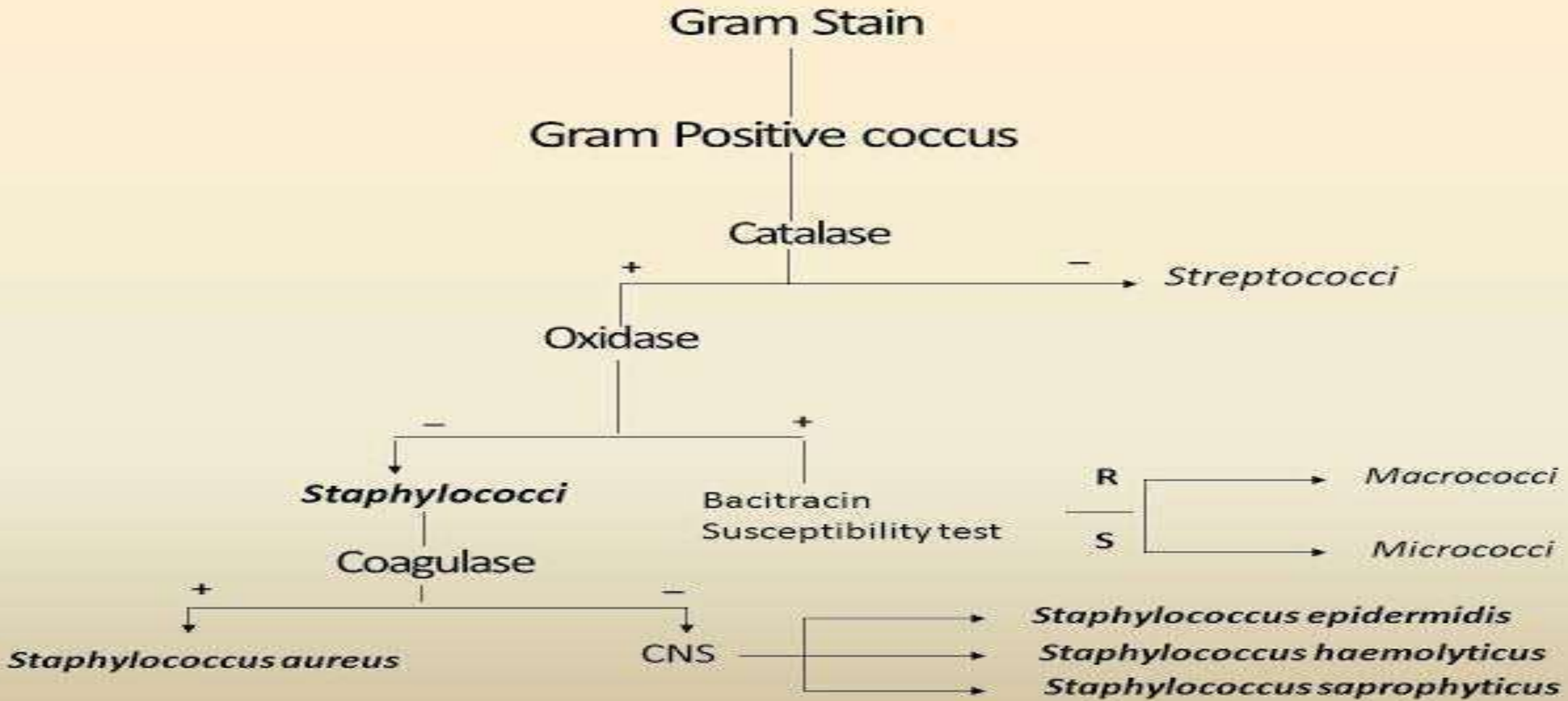
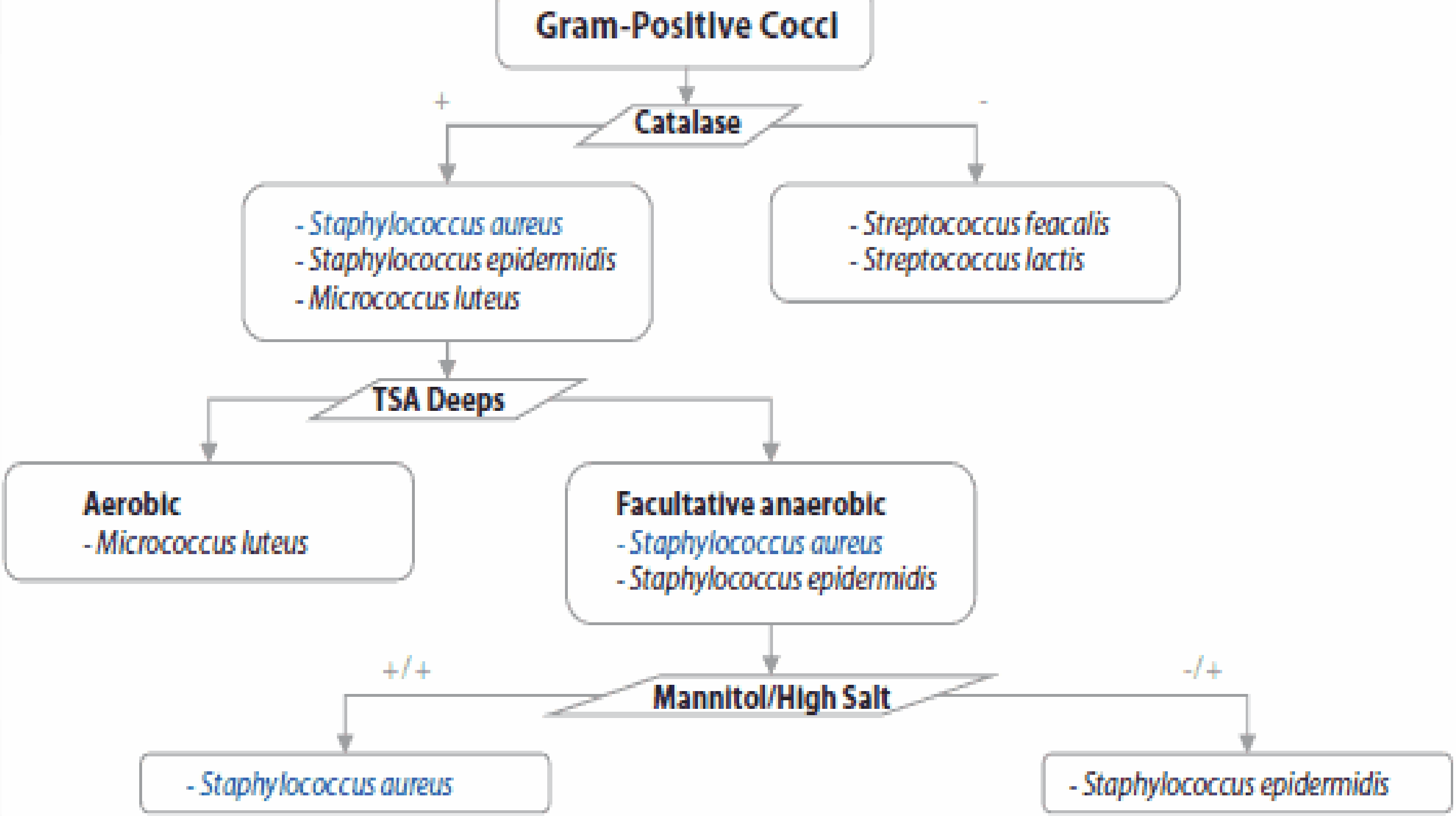
Identification of *E.coli*



Identification of *Staphylococcus aureus*




Gram-Positive Cocci
+
Catalase
-
- Staphylococcus aureus
- Staphylococcus epidermidis
- Micrococcus luteus
- Streptococcus feacalis
- Streptococcus lactis
TSA Deeps
Aerobic
- Micrococcus luteus
Facultative anaerobic
- Staphylococcus aureus
- Staphylococcus epidermidis
+/+
Mannitol/High Salt
-/+
- Staphylococcus aureus
- Staphylococcus epidermidis