Food Microbiology

Introduction

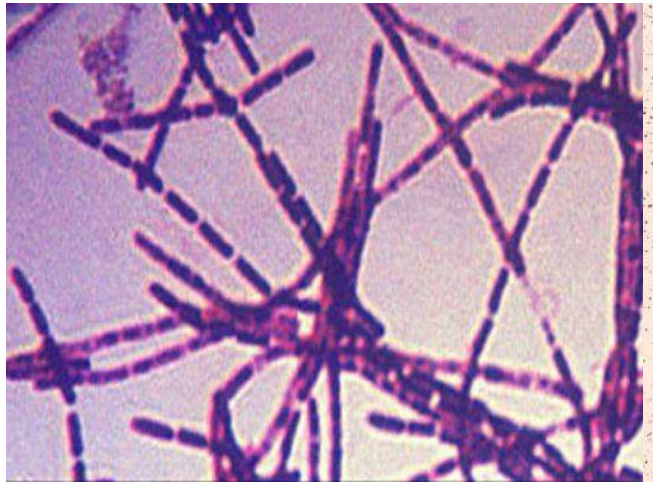
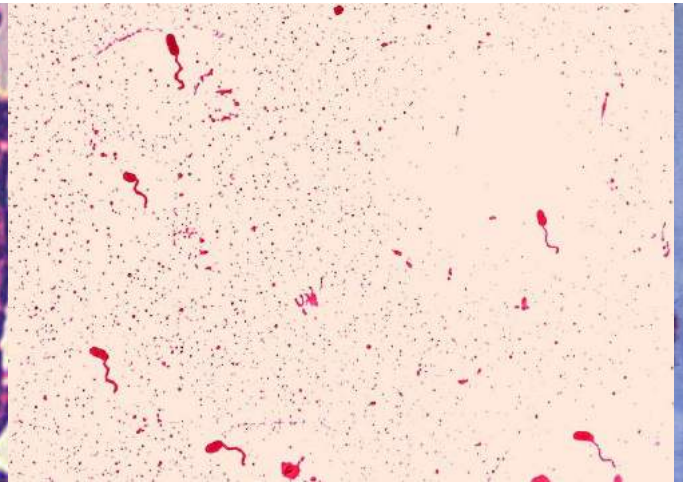
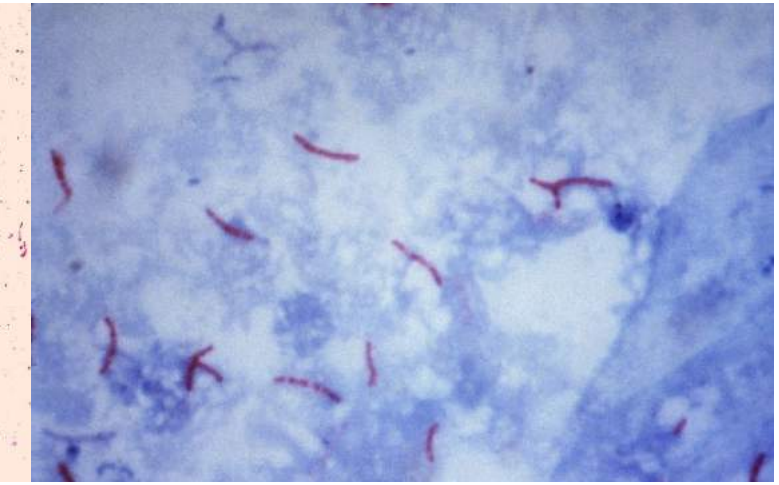
Food materials considered as a good environment for growth of many Microorganisms which cause spoilage of large quantities that lead to large economical loosing especially if we do not follow the correct method in marketing & Storing.

Or cause **food poisoning** such as:

Bacteria: - Staphylococcus aureus, Clostridium perfringens.

Fungi: - aflatoxin poison produced by Aspergillus flavus.

Bacillus anthracis	Anthrax
Brucella melitensis	Malta fever
Vibrio cholereae	Cholera
Salmonella typhi	Typhoid disease
Mycobacterium tuberculosis	T.B

The importance of food microorganisms come from:

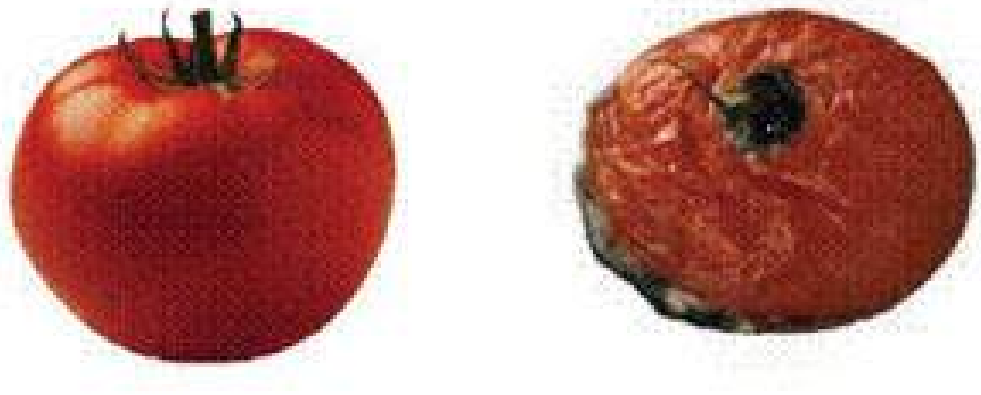
- Prevent food contamination by those dangerous microorganisms
- Control and prevent reproduction of this microorganism

The sources of food contamination:

Air, Water, soil, fertilizer (compost), insect carrier diseases & food handlers.

Causes of food spoilage:

- Microbial growth.
- Insects, rodents *&* birds.
- Physical changes that occurs in foods as a result of cooling & drying.
- Activity of some enzymes which normally found in foods.



Microbial growth

Physical changes

Sample collection methods

The most important roles in collecting samples are:

- The food sample must represent the whole food material.
- The sample must taking randomly.
- The sample must be taking as possible under sterile condition to prevent contamination or adding another microorganisms.
- Transfer the sample to laboratory for analysis.

Food Samples Types:

1. Liquid samples juices, milk

   Shake the bottle well before taking the sample for homogenization.

2. Solid samples Fruits , vegetables

   The sample should be taken by sterile knife or cork porer.

3. Anaerobic bacteria samples

   The sample should be taken deeply and taken possibly in the absence of air.

4. Surface samples

   A very thin layers taking from the surface of food materials.

Carrier

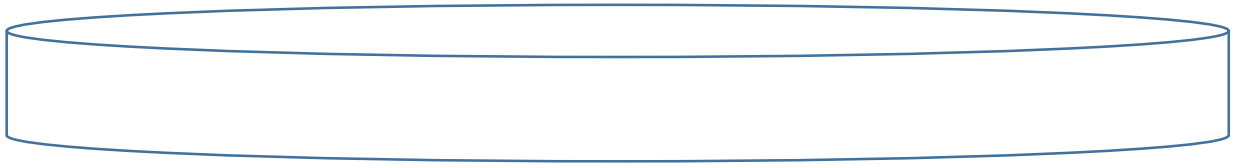
The carriers use to transport samples from food materials to culture media for:

- Protect the resident microorganisms in food materials without losing it.
- Prevent contamination with another microorganism.

Carriers Types:

1. Replica:

It is a direct method by press the food on the culture media



2. Rinse & Washes:

Rinse or wash the food in sterile diluents and shake several times then consider the washings to be stock solution. This procedure is applicable to a solid food but it is not perfect 100% **because** the microorganisms will represent only the microorganisms from the food surface.

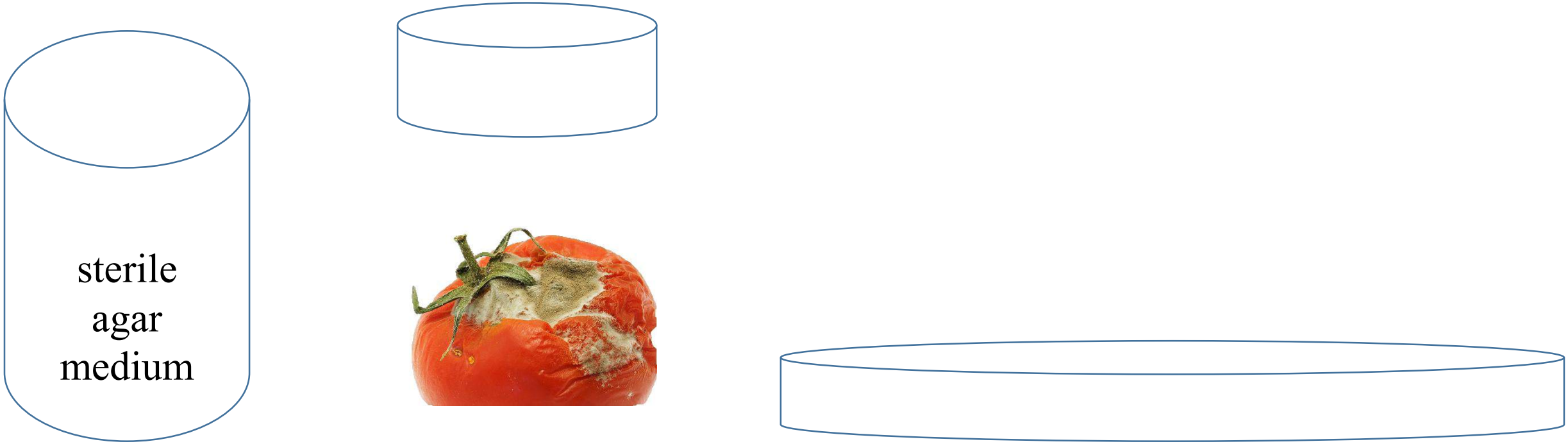
3. Adhesive tape:

Usually using self-adhesive labels because this type have the advantages that the sampling details can be written on the back of labels.

Sterile the tape by alcohol after drying pressed against food surface and then pressed against the surface of an appropriate culture medium and then removed and discarded.

4. Agar sausage

It consists of a sterile agar medium solidified inside a sterile cylindrical plastic casing, cut the end of sterile agar, casing and pressed against the food surface then placed into a sterile petri-dish.



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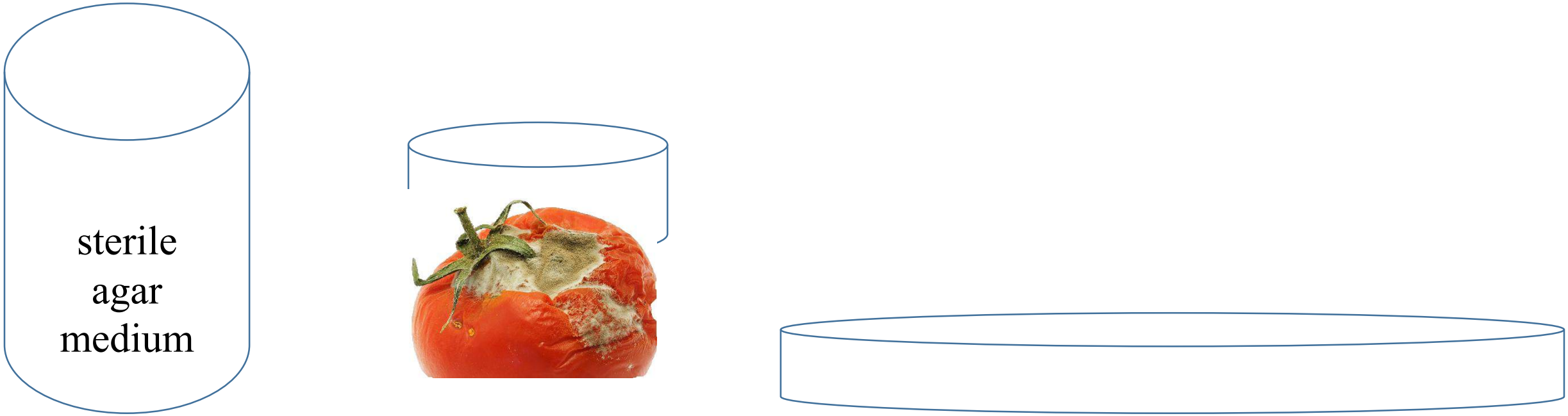

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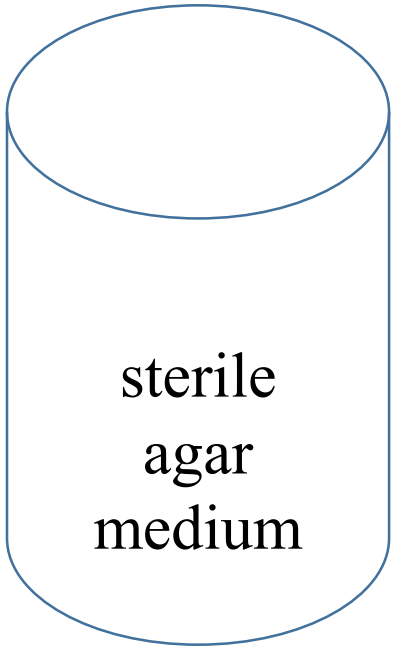
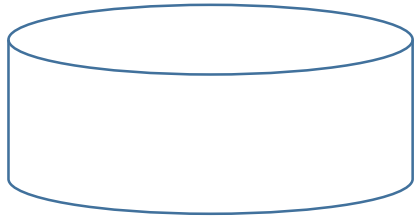
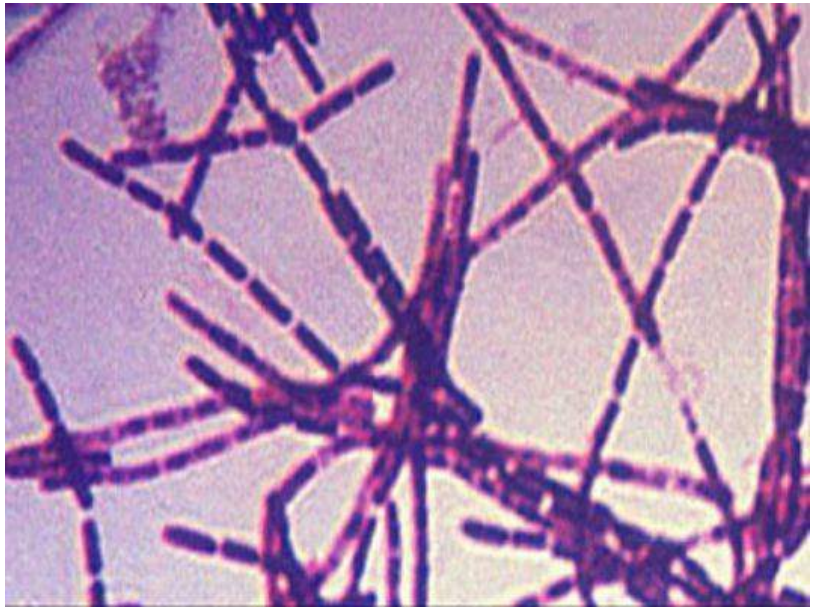




5. Contact Slide (Surface Slide):

Press a sterile glass slide against the food surface, it may then be examined microscopically after fixing & staining.

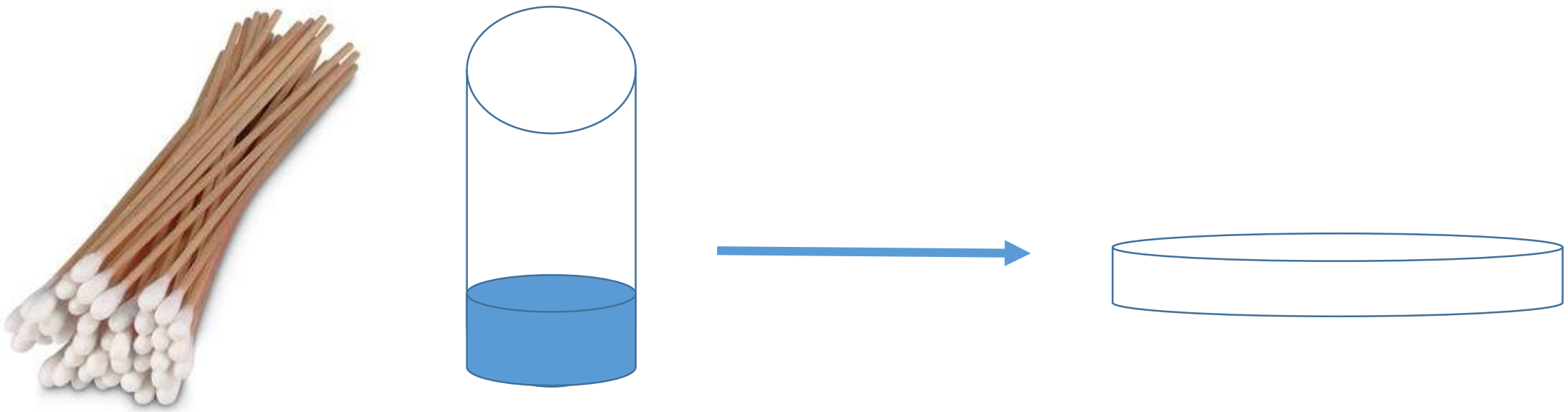


6. Swabs:

1. Cotton Wool Swab:

Are prepared from nonabsorbent cotton wool wound (role) to wooden sticks & sterilize. When taken the sample moisten the swab with sterile solution & rub firmly over the food surface & replace the swab into tube. Add 10 ml of sterile solution; agitate the swab up & down to rinsing of bacteria from the surface of swab.

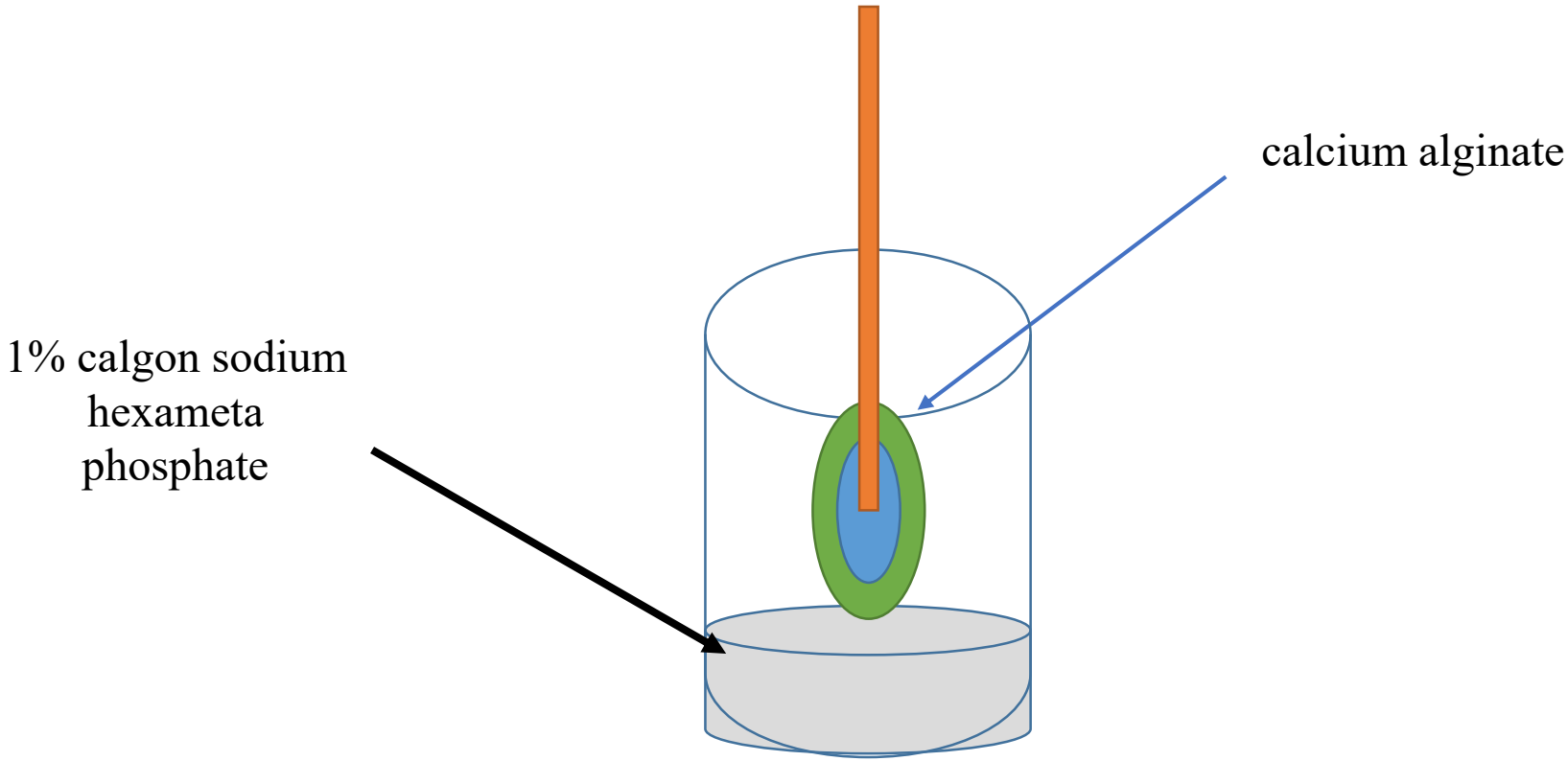
Transfer 1 ml to appropriate culture medium.



2. Algenate Swab:

Are prepared from calcium alginate. After taking the swab suspend in 1% calgon sodium hexameta phosphate shake the tube vigorously, this cause the alginate wool to dissolve (giving a suspension of all the bacteria present on the swab.

After that dilute & culture in appropriate culture medium.



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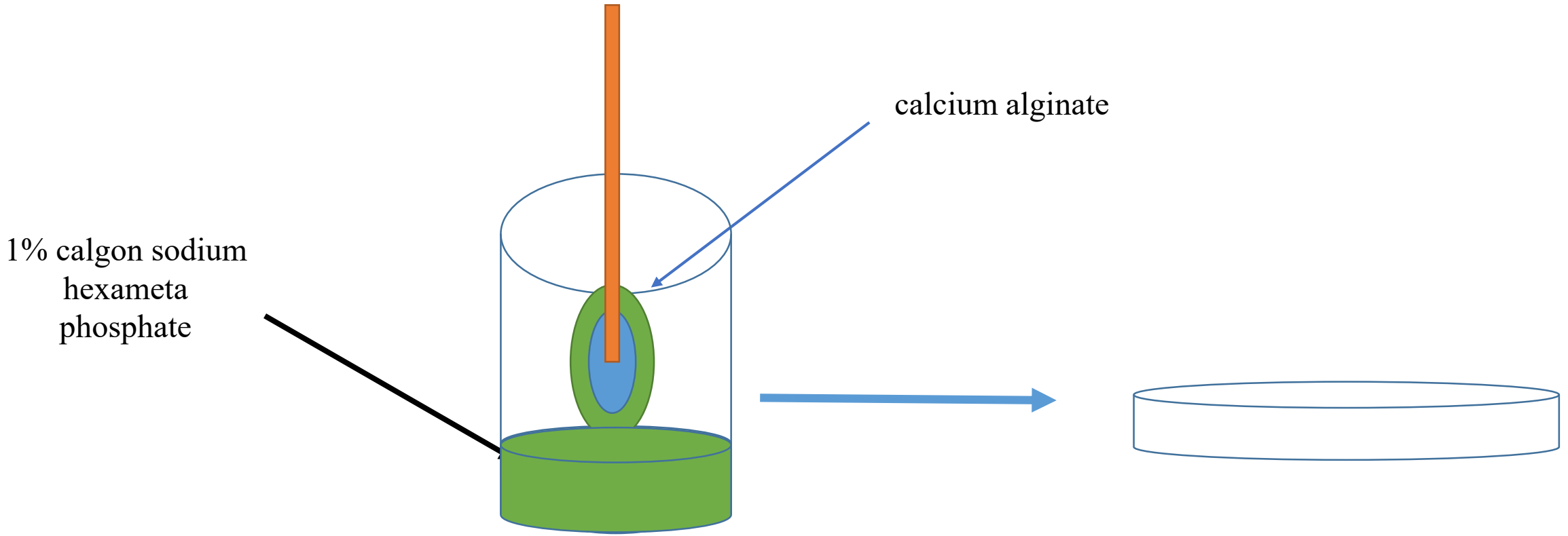

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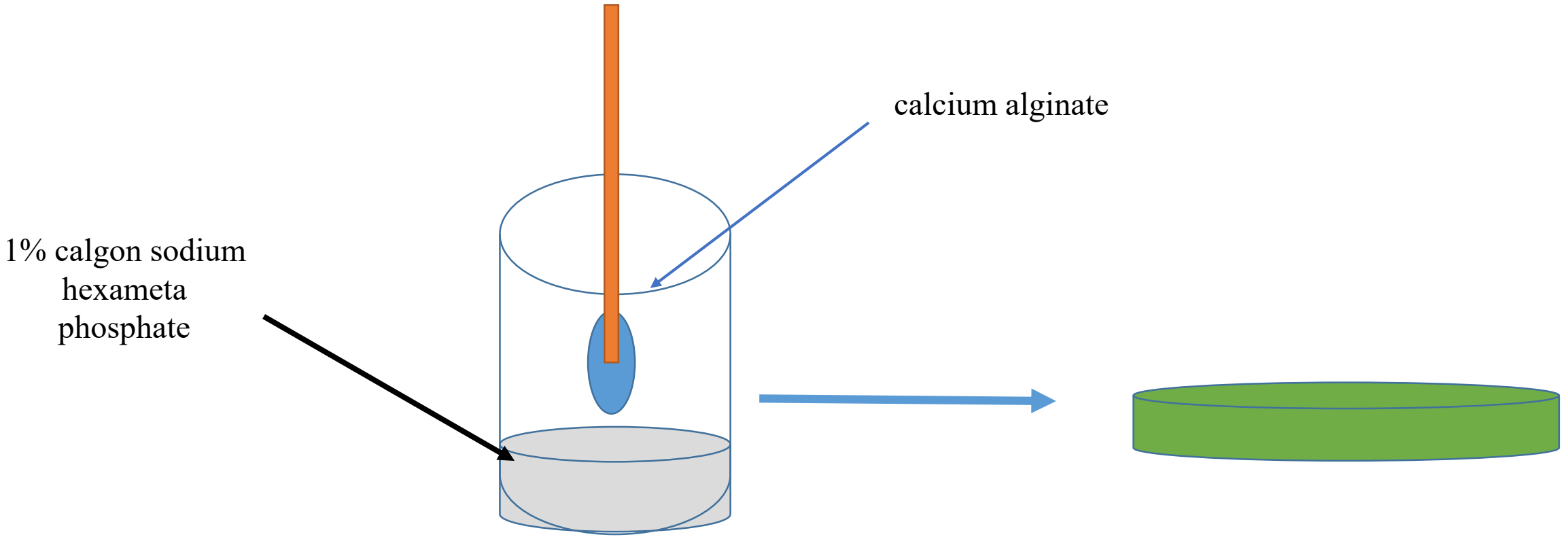


Preparation of diluents solutions:-

1) 0.1% pepton water (Ph=7) Usually use for protein samples.

2) Phosphate buffer

3) Sterile distilled water In case there is no another diluted solution

4) Anaerobic bacteria

It is the same culture media use for anaerobic bacteria growth but in liquid condition, ex: sulphid media use sulphid broth as diluted solution

5) Osmophilic microorganisms (high sugar concentration)

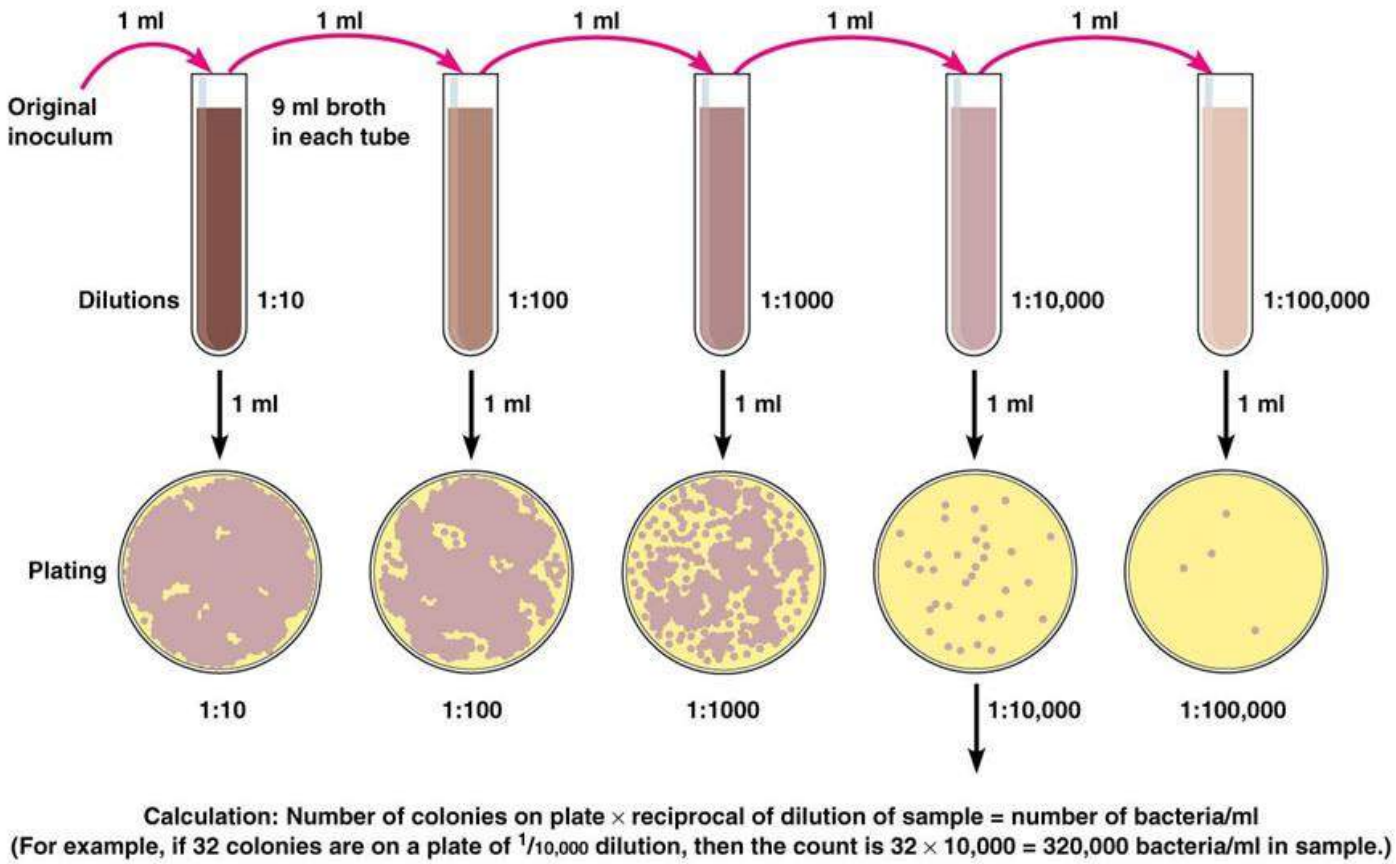
Add 15-20% sugar to solution

Add 15-20% NaCl to solution

Determination of microorganisms numbers:

1) Total count a) bread method b)haemocytometer

2) Viable count a) pouring plate method b) MPN c) spreading d) swabbing



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

Volume of Inoculum for Each Set of Five Tubes	Tubes of Nutrient Medium (Sets of Five Tubes)	Number of Positive Tubes in Set
10 ml		5
1 ml		3
0.1 ml		1

(a) Most probable number (MPN) dilution series. In this example, there are three sets of tubes and five tubes in each set. Each tube in the first set of five tubes receives 10 ml of the inoculum, such as a sample of water. Each tube in the second set of five tubes receives 1 ml of the sample, and the third set, 0.1 ml each. There were enough bacteria in the sample so that all five tubes in the first set showed bacterial growth and were recorded as positive. In the second set, which received only one-tenth as much inoculum, only three tubes were positive. In the third set, which received one-hundredth as much inoculum, only one tube was positive.

Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	9	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360

(b) MPN table. MPN tables enable us to calculate for a sample the microbial numbers that are statistically likely to lead to such a result. The number of positive tubes is recorded for each set: in the shaded example, 5, 3, and 1. If we look up this combination in an MPN table, we find that the MPN index per 100 ml is 110. Statistically, this means that 95% of the water samples that give this result contain 40–300 bacteria, with 110 being the most probable number.

Food Microbiology

Microorganisms in Meat & Meat products

(Red meat, Chickens, fish & egg)

Meat is considered as an excellent growth media for a variety of microorganisms due to many factors make it suitable for microbial growth & reproduction, such, as:

- elevated moisture,
- presence of carbohydrates & nitrogen compound, salts & minerals
- appropriate pH for microorganisms to grow and cause unwanted changes

Basically meat & its products contain microbial flora on its surface & in the; inner part which come from many different sources, Muscle tissue of healthy animals contain few of bacteria **but** cut & exposed surfaces become contaminated during and after slaughter or butchering, but bacterial count of the interior of the meat usually remains much lower

Fresh red meat:-

There are many different sources which cause raw red meat contamination:

1. Soil, Washing & drinking water, slaughter (bleeding, cutting up & handling).
2. The workers (hands & clothes).
3. Transporting & Marketing.

Kinds of microbial spoilage in fresh red meat

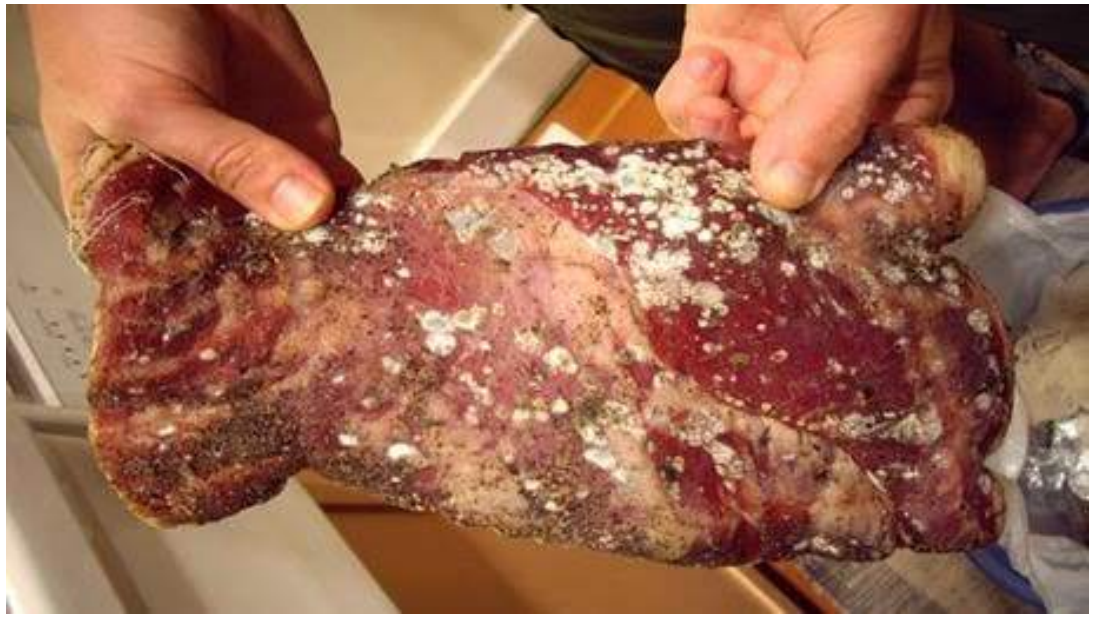
1) Off-odor & sliminess:

The first sign of meat spoilage is the appearance of odder then forming of slime materials on the surface of meat caused mainly by **Pseudomonas**.

2) Discoloration:

The appearance of colored spots on the surface of meat as a result of microbial growth

Pseudomonas	Green spots	Bacteria
Serratia	Red spots	
Cladosporium	Black spots	Molds
Sporotrichum	White spots	
Penicillium	Green spots	
Rhodotorula	Red-pinkish	Yeast



3) Putrification & Rancidity:

Putrification: growth of anaerobic microorganisms producing protease enzyme causing analysis of proteins to NH_3, H_2S & other putrid compounds.

Rancidity: analysis of lipid components of meat to fatty acids & glycerol giving rancid odder.

Both these cases are caused by ***Pseudomonas***.

4) Meat Souring:

Occurs when meat is stored at room temperature, **Mesophilic bacteria** (Such as: Coliform & Lactobacillus) grow causing oxidation of carbohydrates in meat to organic acids.

- The bacterial condition of meat is determined by taking both superficial and deep tissue sample.
- The microbial condition of surface can be assessed most rapidly by microscope examination of contact slides stained by gram method but detailed cultural examination are best achieved by taking superficial sample as very thin slices using sterile scalpels and forceps.
- Cooking will destroy a very large proportion of the microflora of the raw meat, even inumber, although important storage after cooking can allow proliferation so such survivors.

Meat preserved by use of sodium chloride and sodium nitrate may contain salt tolerant microorganisms.

Hash meat

Marked by high microbial contents?

using of hash meat machines,

- increase the exposed surface area,
- mixing the contaminated parts with uncontaminated ones and
- addition of contaminated vegetable, grains and spices; increase the contamination of hash meat with large number of microorganisms.

physiological methods in preservations (cooling, radiation, addition of preserving material such as latic acid and acetic acid to decrease pH).

Fish meat

It is spoiled faster than red meat, because of

1. Increase of moisture.
2. Increase of pH.
3. Lipid components in fish oxidize faster than the lipid components in red meat.
4. The tissues fish are softer than red met.

The microbial flora of fish = microbial flora of the water

- greater number of psychrotrophs, fewer bacteria with an optimum growth temperature of 37 °C
- many of bacteria are halophilic or at least salt tolerant compared with the usual flora of meat

Ex: ***Pseudomonas, Achromobacter, Vibrio, Flavobacterium.***

❖ Rivers are more **contaminated** than seas and oceans because of

their high content of organic compound in form of industrial wastes, so the microbial flora of rivers is more various and contain addition to the previous genera

Clostridium, E.coli, Lactobacillus, Bacillus.

- preserve fish meat

1. it should not exposed high temperature degree. It's butter to be cooled
2. addition of salts or acids to decrease pH and prevent microbial activity
3. the place of sales should be clean and supplied with equipments keep the temperature degree low.

Chickens

As a result, chickens' environment that is full of different kinds of microorganisms from different sources, the microbial flora of chickens will be so various, like:

Staphylococcus, Streptococcus, E.coli, Pseudomonas, Clostridium, Lactobacillus and Salmonella.

The most important genus is **Salmonella** that causing food poisoning, the first source of contamination is the field and its contents of drinking water, wastes and fodder.

Eggs

❖ They represent a perfect growth media ?

Enriched with proteins, lipids & vitamins encourage the growth & reproduction of microorganisms

✓ **physical properties prevent their spoilage**

1. The solid calcic shell of eggs prevent the entrance of microorganisms inside the eggs but sometimes microorganisms could reach to the contents of eggs when the calcic shell is broken.

2. The bacteria that are able to penetrate & cross the albumen (egg white) found another hindrance. A thin membrane surrounding the egg yolk.

✓ Chemical properties prevent their spoilage

the albumen (egg white) which is not appropriate

for their growth, because of:

1. Alkaline of albumen (egg white)(pH=9.6).
2. It contains enzymes like lysozyme that cause lyses of the cell wall of Gram negative bacteria.
3. Stickiness & gelatinous (jellylike) that prevent the movement and spread of bacteria..

The spoilage of egg yolk occurs with Gram negative bacteria, such as:

Pseudomonas	→	**Green putridity of egg contents.**
Ackromobacter	→	**Colorless spoilage of egg contents**
Proteus	→	**Black putridity with dislike odor**

- A surface spoilage of egg shell occurred by large number of molds especially when eggs exposed to moisture causing colored spots on the shell, such as: ***Penicillium, Cladosporium, Sporotrichum, Mucor***
- Eggs also could be contaminated with Salmonella; **food poisoning** bacteria; as a result of contamination of the egg-shell with feces, dust.

Food Microbiology

Bacterial Indicators of Food Contamination

These bacterial groups are found in human & animal feces, so their presence in food indicates the probability of contamination with feces & the bearing of pathogenic bacteria.

1. Coliform.
2. Fecal Streptococci.
3. Gas producing Closteridia.

I. Coliform bacteria (E.coli):

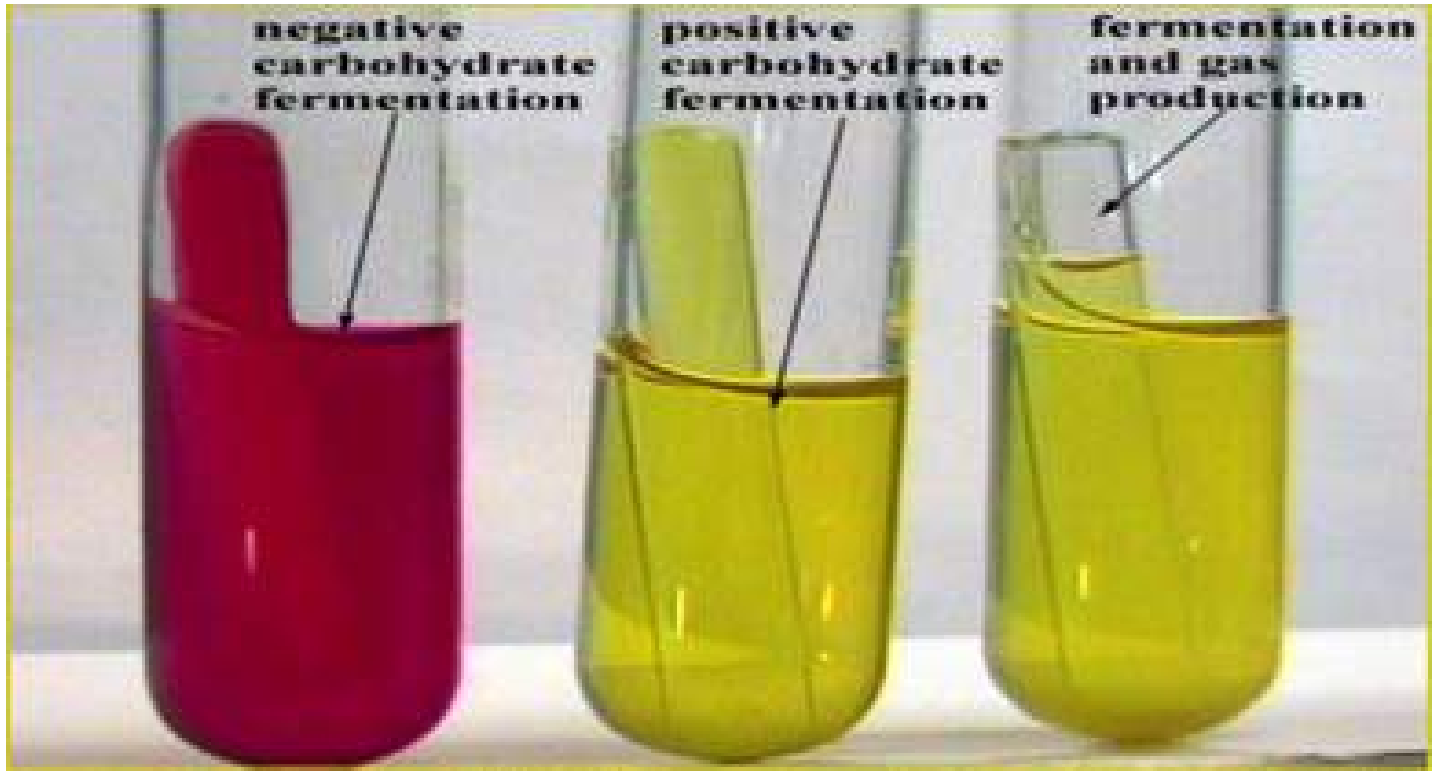
Coccobacilli, Gm-ve, non- spore forming, lactose fermentor, gas producer when grow at 37°C for 48 hrs. & presence in high number in human & warm blooded animals feces.

Working Method:

Take food sample (minced meat), and make a serial dilutions with peptone water

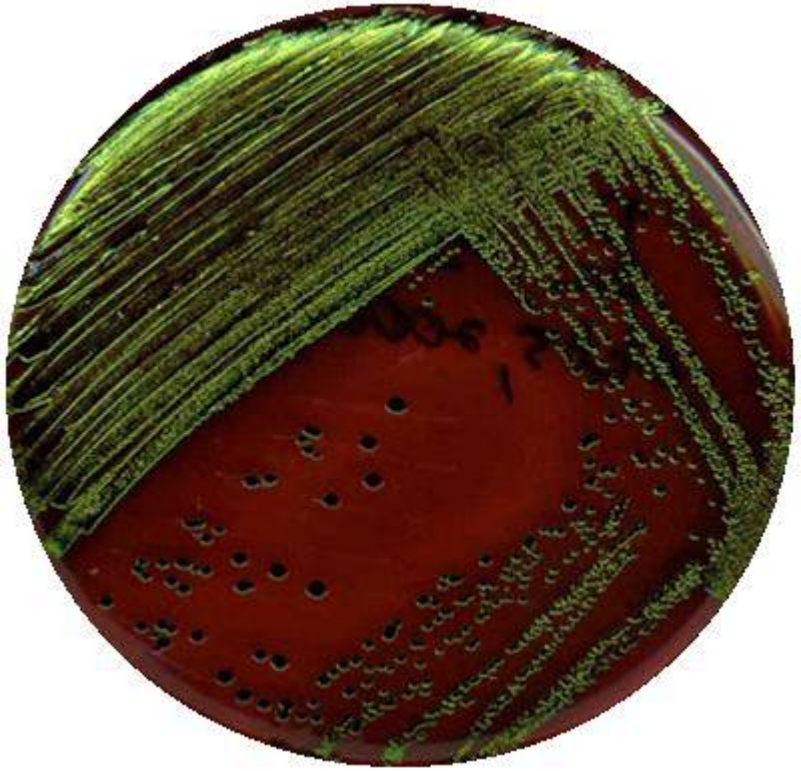
1. Presumptive Test:

Inoculate lactose broth from the serial dilution, incubate at 37°C for 48hrs. Positive results appear as gas production (as a bubble in durham tube).



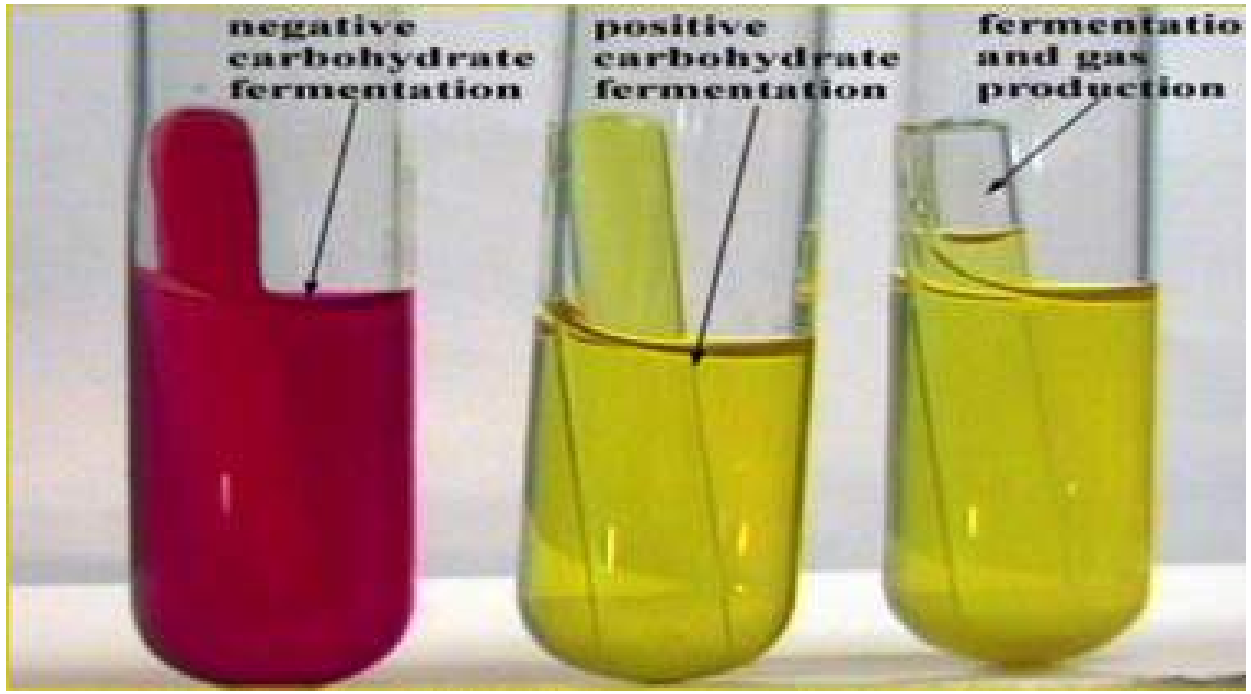
2. Confirm Test:

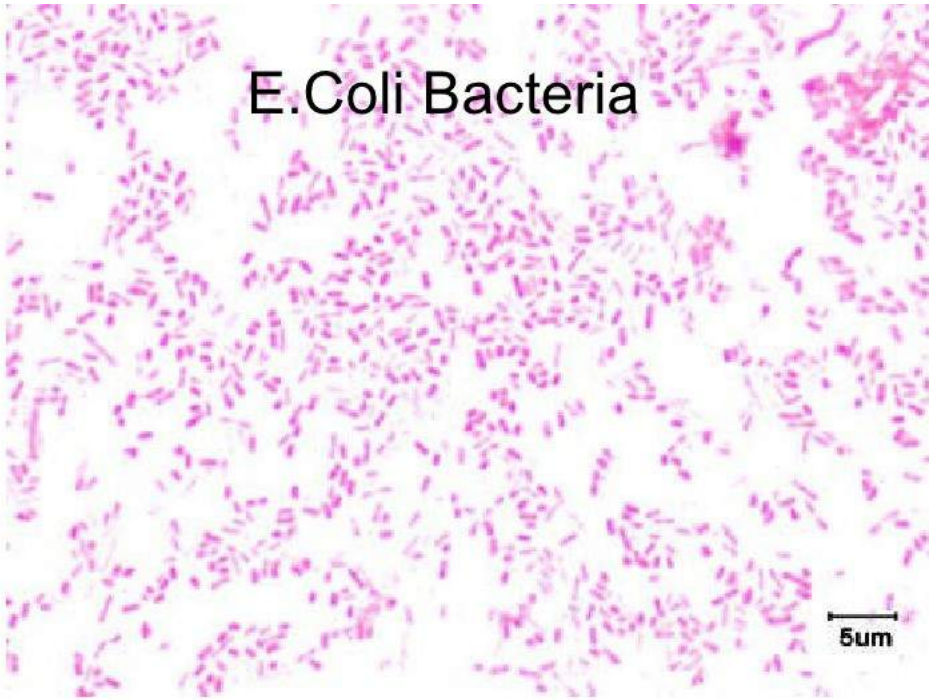
Streaking from the positive result of Presumptive test to Endo agar or EMB (Eosin Methylene Blue). Incubate at 37 C for 48hrs. Positive results appear as pink colonies on Endo agar & GMS (Green Metalic Sheen colonies) on EMB.



3. Complement Test:

Transfer from the **positive colonies of confirm test** to lactose broth. Incubate at 37°C for 48hrs. Positive results appear as gas production and then examine microscopically the positive result.





The presumptive test confirms to as the presence of coliform bacteria while the confirm & complement tests confirm the presence of *E.coli*

Because *E.coli* is found in different places in nature other than human & animals intestine, so we use more specific test to detect the source of bacteria (Eijkman Test) by inoculating the doubt samples in lactose broth and incubating it at 44.5 °C. Just the fecal *E.coli* can grow in this temperature because they can ferment lactose to acid & gas while other normal *E.coli* cannot ferment this sugar.

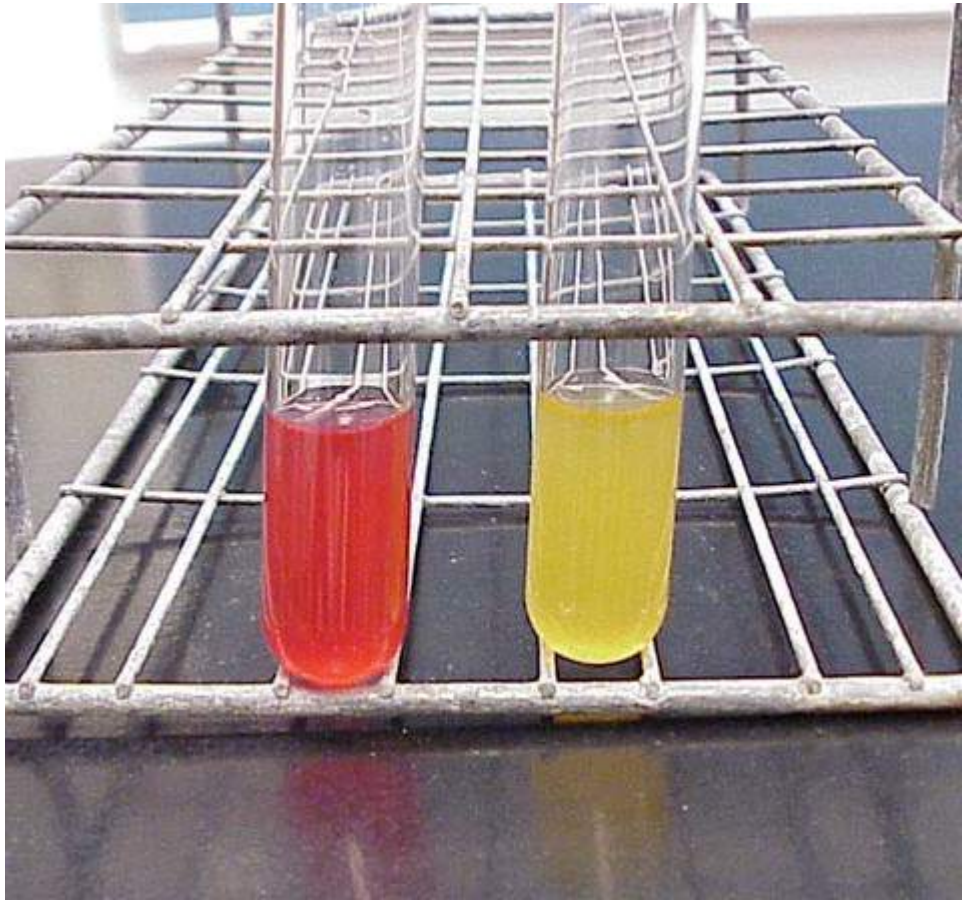
II.Fecal Streptococci:

This test is used as a complement test for the coliform indication tests and it confirms the food fecal contamination.

Working method:

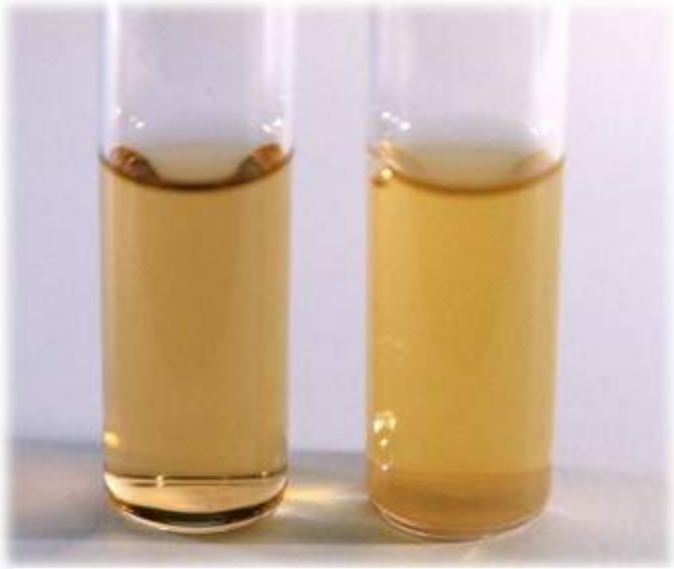
1. Take the food sample and make a serial dilution, ex.: cheese dilute with Na acetate. Milk dilute with pepton water.
2. Inoculate azid dextrose broth from the serial dilution. Incubate at 37 C for (24- 48hrs.)

Positive results appear by change the media color to yellow as a result of acid production.



3. Transfer from the positive tubes to Ethyl Violet Azid broth. Incubate at 37°C for 24 hrs.

The positive results appear as a **violet ring** in the bottom of the tube or as heavy (extensive) turbidity. For more confirm examine the cells under microscope.



Izda: Negativo. Dcha: Positivo, depósito naranja.

III.Gas producing Clostridia (*Ctosterium perifringenes*)

This bacteria colonize human & warm blooded-animals' intestine (normal flora), so the presence of this bacteria in food indicates food' contamination with stool-because their spores resist some thermal treatment.

The indicator of these bacteria is uncommon in use **because** of the difficilty of cultivation, but it is considered as a complement test for *E.coli, Streptococcus faecalis* tests.

Working Method:

1. Take food samples. Make a serial dilution & then heat the serial dilution at 80C for 15 mm, (to kill the vegetable cells & survive the spores). Inoculate **milk broth** and then incubate at **37 °C for 5 days** indicate the presence of stormy fermentation (positive result). This bacteria can produce large amount (quantity) of gas & acids.

2. Inoculate the special culture media (selective) D.R.C.M (Differential reinforced Closteridial Media). Incubate under **anaerobic condition** at for 24-hrs. To test the result put NaOH on the colonies for 20-30 Sec. the positive colonies appear pink color. We can use other media containing antibiotics such as: Polymixm B & Cycloserine (to prevent contamination with other bacterial species).

Food Microbiology

Microorganism's in Fruits & Vegetables

❖ The different kinds of microorganisms attach (infect) the crops of fruits & vegetables during their growth in plant, harvesting stages, storage, transport and marketing.

Pre-mature fruits and veggies & before collection: some bacteria & molds may attack it and cause spoilage; the spoilage degree depends on the following:

- Suitable control and active-mode-of cultivation.
- The premature fruits contain some acids and inhibitor-materials which prevent microbial activity.
- The internal components of the fruits may still healthy if the outer layer (skin) was undamaged (it prevents the entrance of microorganisms)

Post- mature & after collection:

the degree of spoilage depends on

- the way of dealing with fruits from the harvesting stage to the consumption by consumers.
- If the outer layer have been scratched or damaged the microorganisms can enter from water, air, soil, fertilizer. So it can grow quickly, reproduce and spoil the fruits (Some microorganisms can normally enter (penetrate) the fruits from the natural pores on the fruit surface).
- After harvesting the fruits many changes happen in their chemical structure as a result of respiration and enzymes activity which reduce acidity and degrade some inhibitors components that will lead to microbial activation.

The pH range determined the nature & type of microorganisms which is responsible for the spoilage of vegetable & fruits.

- In **fruits** it ranges from **2.5-5**. The molds & yeasts are responsible for the spoilage and the source mostly the soil. These microorganisms need mono and disaccharides to grow **because** they cannot utilize polysaccharides **because** they lack the necessary enzymes
- also molds & yeasts can grow in high sugar concentration (**65-70%**) which most bacteria cannot grow in this sugar concentration,
- whereas the bacteria is responsible for vegetable spoilage **because** the pH range is (4.5- 7) so it is responsible for 36% of vegetable spoilage.

Frozen fruits:

- also the freezing leads to absence of O2 & Co2 which leads to the disappear of aerobic microorganisms. The most important molds & yeasts that cause frozen fruits spoilage are:

Yeasts: *Candida, Torulopsis, Rhodotorula*
Molds: *Cladosprium, Botrytis*

The most important spoilage on fruits & vegetables

Type of spoilage	Causative agent	Spoilage
Bacterial Soft rot	*Erwinia carotovora.*	Lyses of pectin, watery soft figure with off-odder on vegetable
Souring & Slimness	*Pseudomonas* *Coliforms* *Lactobacillus*	Vegetables souring
Rhizopus Soft rot	*Rhizopus*	Cottony growth with black spots & sliminess
Alternaria rot	*Alternaria*	Black or brown coloration
Gray mold rot	*Botrytis*	Gray spots on vegetable; & fruits
Blue mold rot	*Penicillium*	Bluish- green coloration
Black mold rot	*Aspergillus niger*	Back growth

Dried (drying fruits):

Xerophilic molds and osmophilic yeasts are responsible for spoilage of drying fruits.

- *Aspergillus glaucus* can grow in low aw reach to 0.7 and according to
- yeasts *Candida*, *Hanseniaspora*, *Zygosaccharomycs* can grow on dried dates & figs causing spoilage, they grow in moisture not exceeding 25% and temperature between (20-37 ˚C) and causing souring of dried dates & figs.

Food Microbiology

Bread & Cereal grains microorganisms

Rice & wheat arc the most grains used & utilized by humans, the sources of microbial contamination may begin from cultivation in the (agricultural) field, water, air, soil, insects, birds & rodents.

There are **two factors** which control the microbial growth & reproduction in cereal grains:

1. Moisture
2. Storage temperature

So **do not leave out** cereal grains in a wet atmosphere or store it in a wet place, and when moisture exceeded 14% must be dried grains industrially before storing **because this high ratio of moisture encouraged fungal growth** especially those toxin production such as: *Aspergillus flavus.* The indigenous flora of grain includes coli-aerogenes organisms;

therefore coliform counts on flours may be advisable when these are being incorporated into food products on which coliform counts are normally conducted, although usually the flour incorporated into products will receive a heat treatment sufficient to kill these organisms.

Since flour is usually to be subjected to a heat treatment, the most significant microorganisms to be sought are species of *Bacillus* & *Clostridium*.

Very rarely, species of *Bacillus* (especially *Bacillus subtilius* may cause a defect in bread known as ropiness due to the production of capsularmaterial.

The commercially marketing flour contain spores of molds & bacteria especially thermophilic bacteria such as: ***Bacillus subtilius, B. mesentericus***, so they transfer to dough which bread is done from it

Bread microbial Spoilage:

Spores of bacteria, molds & yeast transfer from flour to dough & when adding the water, spores begin to grow & cause **acidic fermentation** like lactic acid production & alcoholic fermentation like ethanol production & CO_2 so these gases are produced due to, fermentation that causes bubbles inside dough, oven temperature kill all microbes' presence in bread dough expect spores that resist this temperature.

Bread contaminated after baking from tables, workers & insects; if we put hot bread inside the polyethelene sacs, thus lead to cause wet sacs (moisture) so spores growth may be encouraged

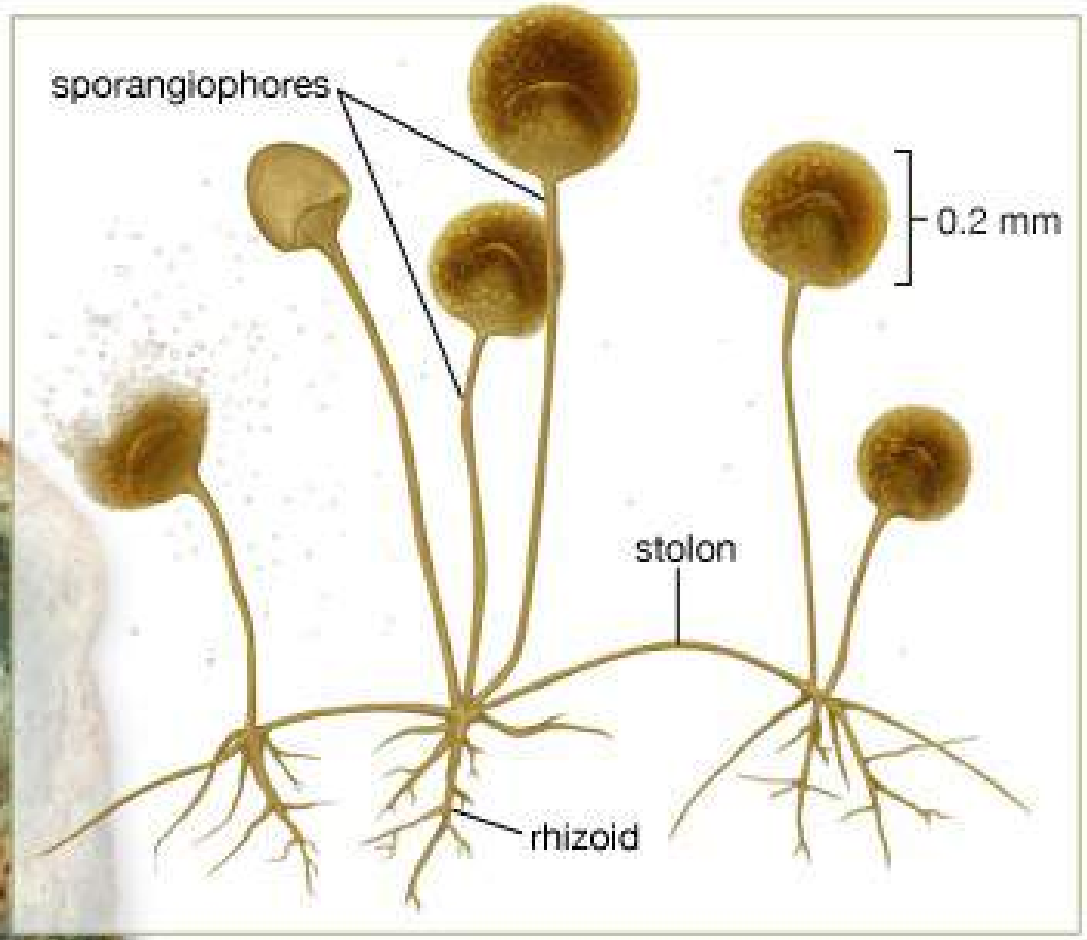
Types of bread microbial spoilage:

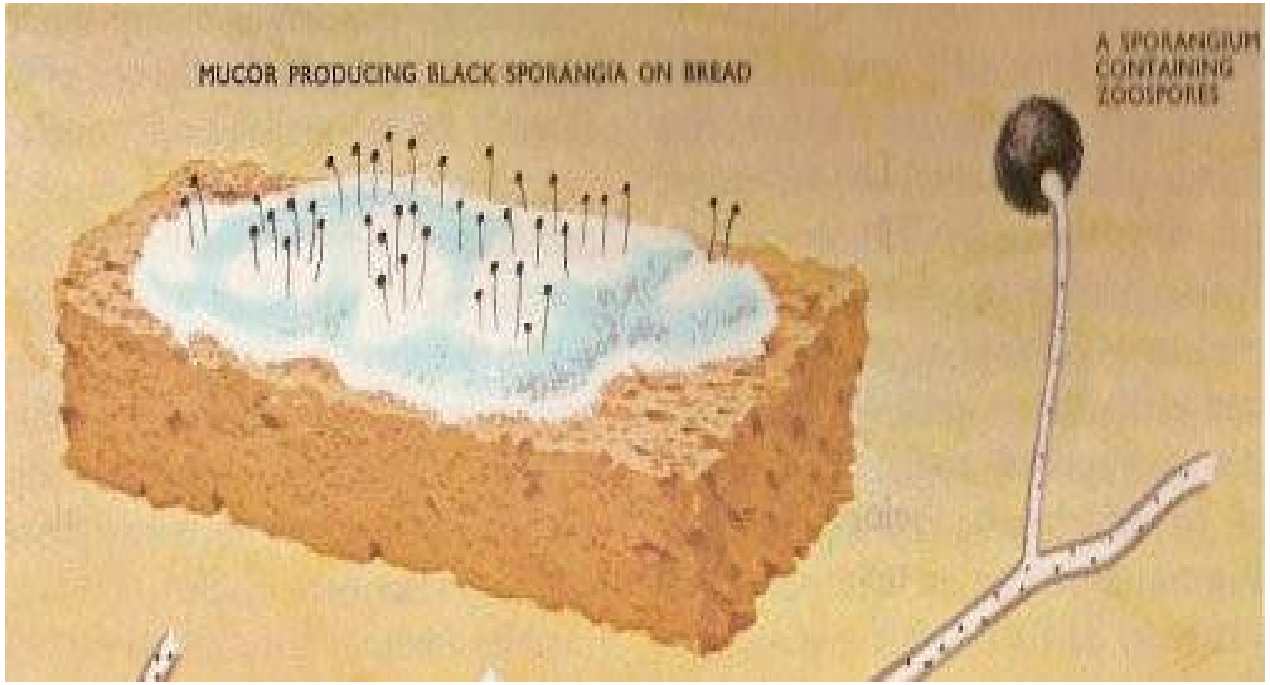
a) Bread moldiness:

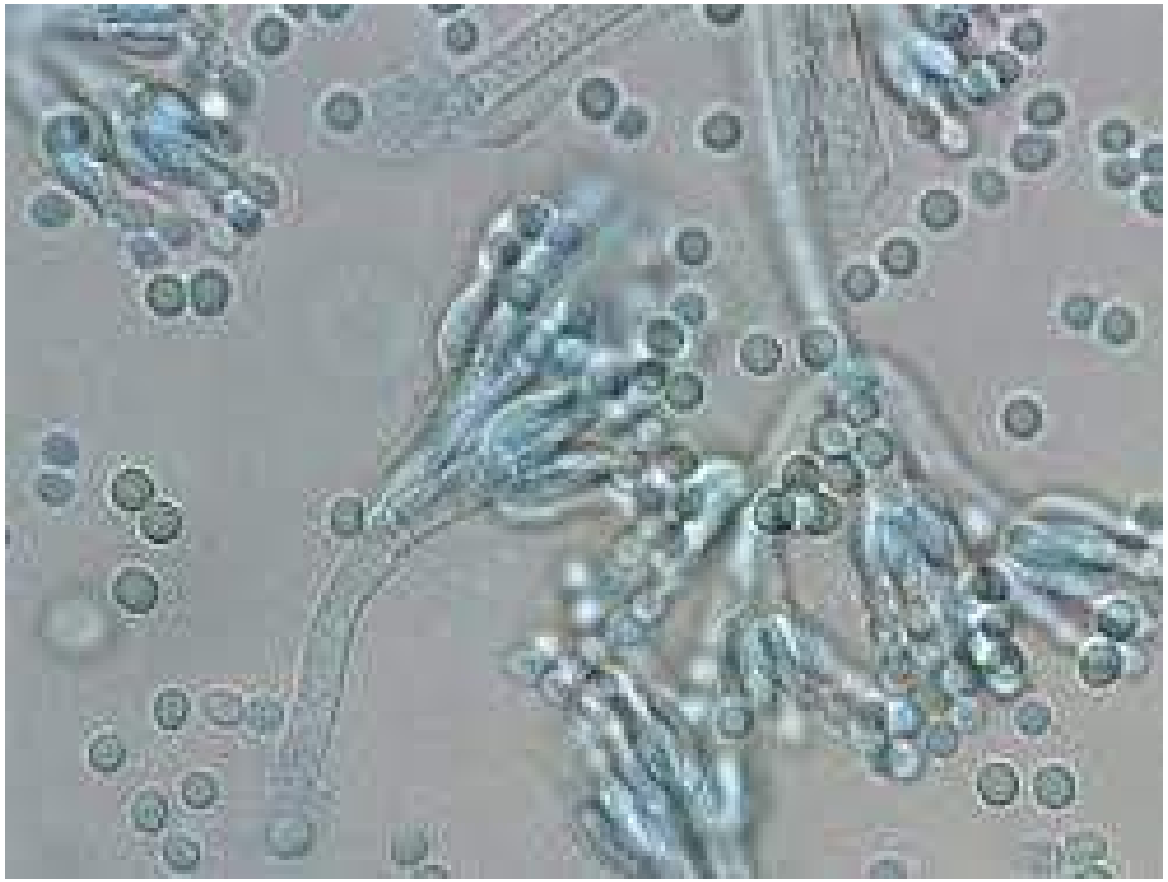
Happen due to molds growth on bread, such as:

Molds	Appearance of growth
Rhizopus	نمو ابيض منقط بالاسود
Aspergillus niger	نمو اسود بشكل دبابيس
Monilia	نمو احمر وردي Bloody bread
Mucor	نمو زغبي ابيض
Penicillium	نمو اخضر زيتوني

Rhizopus stolonifer




MUCOR PRODUCING BLACK SPORANGIA ON BREAD
A SPORANGIUM CONTAINING ZOOSPORES



b) Bread ropiness:

Bacteria cause this type of spoilage are spore forming bacteria *Bacillus subtilius* & *Bacillus mesentricus*, they are resistant to the heat of the oven, grow in bread &

1. produce rop & slimy materials this can cause glutan proteolysis & produce slimy peptids,
2. this bacteria also analyze the starch in to simplified sugars & undesirable organic acids which cause bread acidity.

Microorganisms living in Adverse Condition Foods
(Pickles & Sugar Foods)

Foods with high sugar concentration are not appropriate for the growth of many microorganisms, therefore they are considered less dangerous & slower to be contaminated with harmful microorganisms than other kinds of food.

The term osmophilic refers to the microorganisms which could be isolated from sugar foods because they prefer high sugar concentration for their growth & reproduction

Honey

It can't be spoiled normally **because** of its sugar, concentration that reach to 80%.

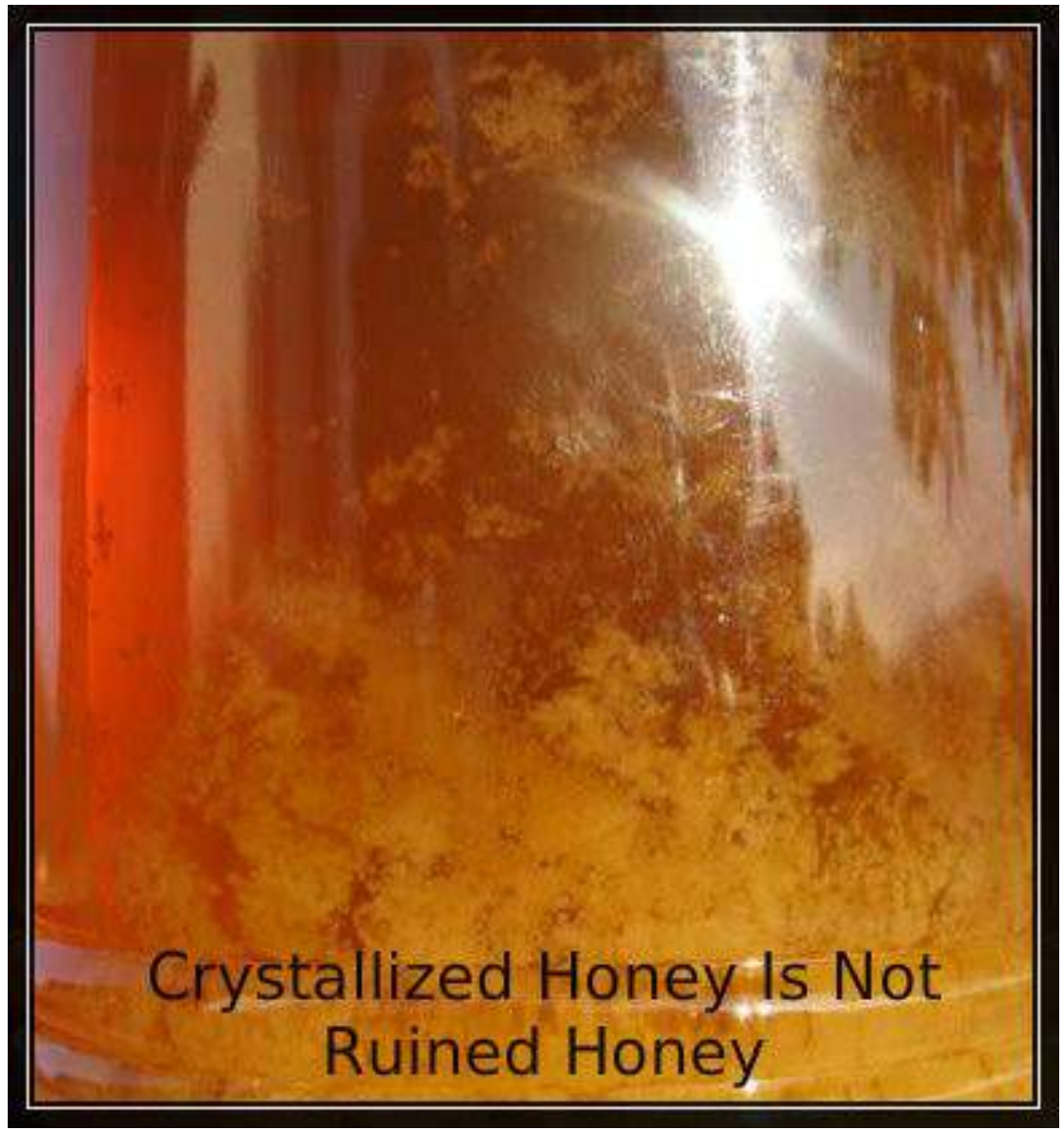
Although spoilage could occur when humidity is elevated to 10% **because** of accumulation of water between sugars molecules of honey & its other components, this condition is referred to (**crystallization**), then the honey acquires an alcoholic yeasty flavor when **ethanol** is produced because of a fermentative reaction which occurs when temperature is elevated, this condition is referred to (**yeasty honey**).

Finally a rapid spoilage may occur when crystallization increases as well as humidity to more than **20%** especially in an adulterated honey.

The microorganisms that are responsible of honey spoilage are osmophilic

- **Yeasts**: *Saccharomyces cerevisiae*, *Saccharomyces rouxii*
- While **molds** spoil the honey by growing on the surface because of the ability of surface of the Honey to absorb humidity from the atmosphere & presence of O_2 which is necessary for the growth of molds: ***Aspergillus***, *Penicillium* & *Mucor*. So in order to preserve honey pasteurization must be done for 30 min. at 60° C.


One Green Tomato


Crystallized Honey Is Not
Ruined Honey

Debbis;

It is produced from dates, contain a high percentage of sugar (70-80-%) therefore the osmophillic yeasts which are grown in 75% concentration of sugar such as ***Saccharomyces rouxii*** that is isolated from local samples can spoil the debbis by **forming gases, alcohols & acids that change the taste.**

Jams & Candies:

❖**Jams** contain high sugar concentration reach to 70% but they are more tendency to be contaminated **because**

1- they are made of different kinds of fruits that they are mixture of good & spoiled fruits,

2- also during preparation of jams heat cannot reach to the whole big fruits to kill all the spores that may be presented in the depth of spoiled fruit.

❖**Candies & Chocolate** are rare to be spoiled **unless** they will be filled with contaminated vegetarian stuff or contaminated milk with spores of bacteria. In anaerobic conditions spores of ***Clostridium*** are activated & grown in candies

Also using contaminated, nuts with bacterial spores & fungal toxins in the filling of (Baklawa- Arabian Sweets) leading to contamination of this kind of sweet with dangerous microorganisms.

Food microbiology

Microorganisms in Pickles

Microorganisms in Pickles

Pickles arc made by lactic acid fermentation by lactic acid bacteria, for the good vegetables that chapped into small pieces with 2-15% of NaCl (salt), the ratio of salt varies according to the type of the vegetables in which the acidity reduces 1.5% (lactic Acid).

- This acid gives

1. a special flavor to the pickles
2. besides it works as a preservative.

The Role of Lactic Acid Bacteria in Pickles

cabbage pickles

leuconostoc mesenteroides

- first stage

acidity rate 0.1-1%

lactobacillus plantarum

- becomes more active because it tolerates the acidity than the first bacterium

acidity rate 2%.

lactobacillus brevis

- change the remaining sugar into lactic acid

acidity rate 2.4%.

olive pickles

fermentation lasts for many months

lactobacillus plantarum

bacteria dominates on the last stage of fermentation; also it is found that this bacteria play a major role in the fermentation of cucumber pickles.

Pickles spoilage

Unpasteurized pickles, spoil in large quantities by microorganisms such as *bacillus*, *Clostridium*, Enterobacterecae, yeasts as well as molds. Generally pickles spoilage are as follows:

1. Pickles Spoilage by Oxidative film yeasts

lactic acid $\xrightarrow[\text{oxidize}]{\text{Candida}}$ **Co_2 & H_2O + a thin white film on pickles**

- make it easy for other kinds of spoilage to take place.

2. Pickles Spoilage by Fermentative Yeasts

grow inside pickles —*Torulopsis*→ **large quantities of gases**

- which make pasteurization difficult outside the fruit leading to floated pickles

3. Pickles Spoilage with *Leuconostoc* bacteria

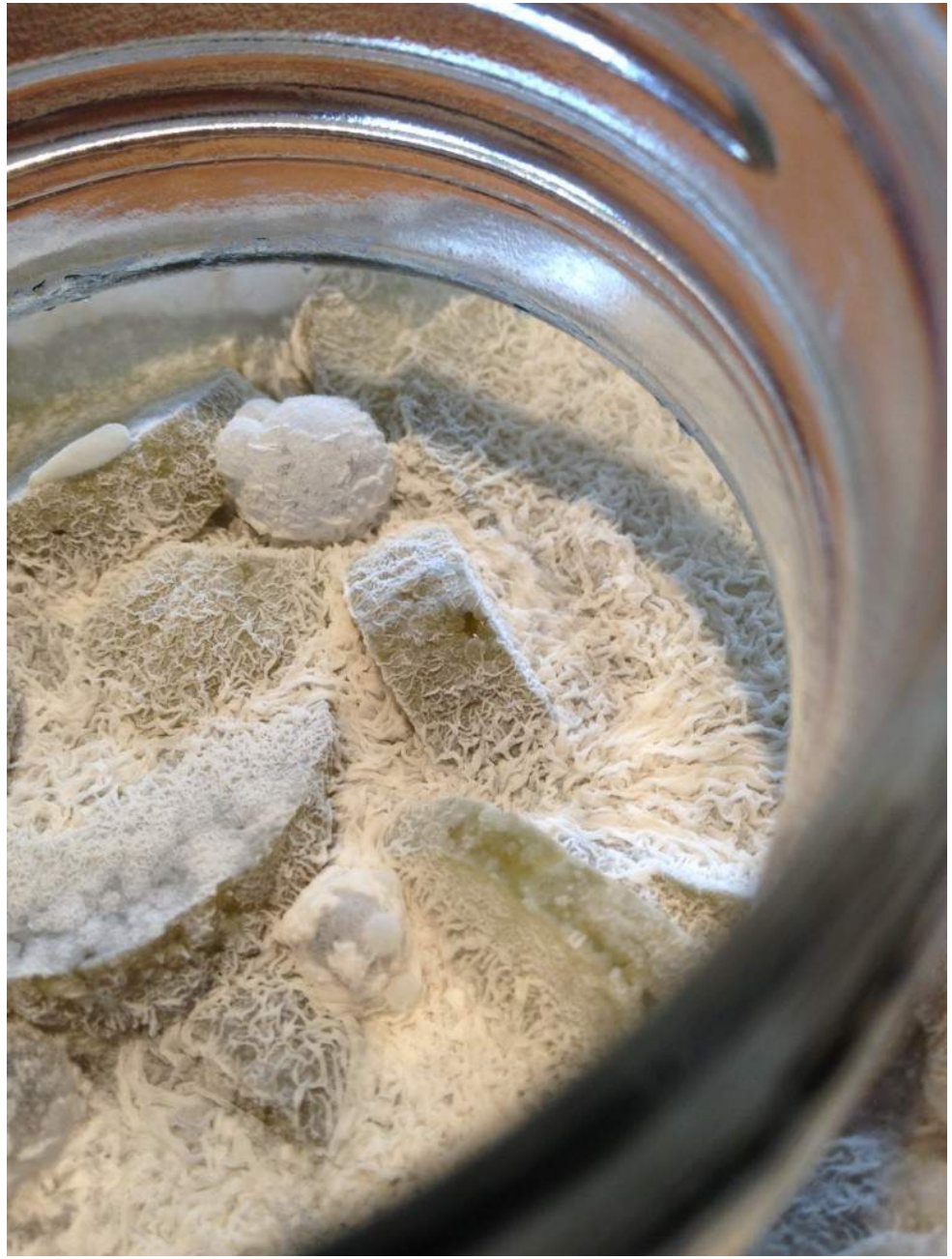
Which makes a slime layer on the pickles forming slimy pickles

4. Pickles Spoilage with bacillus bacteria

Pickles appear black (Black Pickles) because this bacteria has the ability to **produce H_2S** that reacts with the metal of cans forming a black residue of Fe_2SO_3

5. Pickles Spoilage by Molds

It occurs as a result for the growth of molds that secretes pectinase enzyme causing the tearing of the pickles tissue of the pickles giving them soft appearance (Soft Pickles) such as *Penicillium*, *Cladosporium*.



Food Microbiology

Microorganisms in Milk

Q\ Milk consider as a typical media for the growth of many Microorganisms?

Because

- it is rich in: important proteins, carbohydrates, lipids, minerals and vitamins.
- In addition to its optimal pH (6.7) and optimal moisture for microbial activity.

There are two important points for the healthy and microbial side of the milk

1- If the milk hadn't been good pasteurized it would transfer some diseases to human.

- ex: fever, Malta fever , Food poisoning by Enterotoxins of *Streptococcus pyogens .*

2- The milk speedily spoils because it is considered as a perfect culture media for the growth of bacteria, molds and yeasts.

Raw milk

The fresh raw milk which is taken from cow breast contains a low number of bacteria: *lactobacillus, streptococcus lactis, coliform, Micrococcus.*

1- The number of bacteria begins to decrease for a short period called (Bactericidal phase) **because** the raw milk contains antibacterial materials which are: **Lysozyme, Lactenin, Leucocytes** and **Lactoferrins**.

The lactenin is considered as the most effective (extensive effect) material against the bacteria ,

it consists of three compounds act together against the bacteria **(Hydrogen peroxidase, Thiocyanates** and **Lactoperoxidase).**

- **Note:** these three compounds are found in large quantities in human mother

2- *Streptococcus lactis*

Sugar milk (lactose) → Lactic acid

Acidity ratio reaches 1 %
pH = 4.6

3- *Lactobacillus*

will be active in this Stage because it is known to resist more acidity and this leads to increase the acidity ratio to 2% which will stop the growth of normal flora in milk.

4- *Geotrichium &* membranous yeasts (on the surface of the milk)

Lactic acid —Oxidation→ water + Co2

leads to decrease acidity and will disappear the acid.

5- *Bacillus, proteus, Achromobacter & Pseudomones.*

Putrefied & rancid bacteria will be active on the remaining's for the lasting lipids & proteins in the milk & convert them to putrefied & rancid liquid.

Sources of milk contamination

breast surface, soil, water, air, cattle , mechanical milking, worker , insects & flies

a) Raw Milk Spoilage :

The fresh raw milk contain between 10^2 - 10^3 bacteria/ml. The required bacterial number that is necessary to cause undesirable changes in color & taste is 10^7 cell/ml. The important microbiological problems which happen in raw milk are:-

Causative agent	spoilage
Bacillus cereus	Coagulate like cheese production of Renin and precipitate casein
Clostridium, Coliform	Gassiness or frothiness (gas production)
Alcaligenes	Viscous in milk (production capsule')
Pseudomonas fluorescence	Undesirable taste (F.A. (Fatty acid lysis)
Serretia marcescens	Red color in milk

B- Pasteurized milk spoilage:

Pasteurization means: the milk must be exposed to 72°C for 15 second or 63 °C for 30 min. to **control the pathogenic bacteria** like *Mycobacterium tuberculosis, Salmonella, Brucell, Listeria* & to **prolong the storage period**.

- The pasteurized milk spoilage happen **because** of the resistance of some vegetative thermophiles like *Lactobacilus*, *Microbacterium*, *B. subtilis*

Dried milk (powder):

Made by the removal of part of water in milk and heat treatment pre or post-canning takes place to prevent the spoilage. During the microbial examination of the dried milk we can see,

- if the viable growth was a pure culture that means the contamination was by thermophilic bacterial spores,
- while the mixed culture indicates that the contamination was caused by insufficient heat treatment or the contamination happened during examination or using it.

Sterilized milk

Milk sterilizes by using high temperature 121°C for 15-20 min, packed in a glass bottle or metal bottle, in this manner all microbes will be killed, which cause the spoilage during the storage under natural conditions. They may be found in small number of sterilization heat resistant & spore forming bacteria like *Bacillus, Clostridium.*

Laboratory Work:

1- Direct total count by using Breads methods

2- Coliform bacterial count by using violet red agar or MaCconkev

3- Dye reduction test

This test is applied to see the biological activities of bacteria in the milk (**metabolism**), the activity proportional to the number of bacteria & respiration rate to prepare the anaerobic conditions, so the dyes are reduced.

In this test we can use two types of stains methylene blue & Resazorine.

The methylen blue convert during reduction to colorless, while Resazorine reduced by two stages : When it is **oxidized**, the color appears blue and converts to pink & then the last stage convert is colorless.

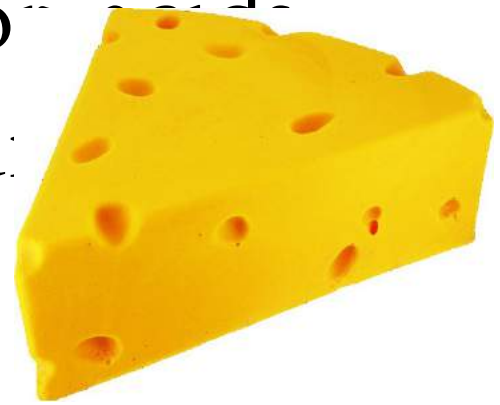
1. Add 1ml of methylen blue to 9 ml of milk.
2. Mix very well with confirmed absence of gas bubbles (to prevent the dye Oxidation).
3. Incubate the tubes at 37°C & calculate the necessary time to convert the dye to colorless, examine the tubes every 30 min.

When we use resazorine dye, the milk color convert to pink during 10 min, the milk should be refused (you shouldn't expose (milk + dye) to high which prevents dye oxidation

Micro-organisms in Dairy product

A- Cheeses

Cheese is the hard product of milk , It is produced by the addition of lactic-acid bacteria as **astarter** or the addition of enzymes o[illegible] followed by processes to give the textu[illegible] flavour of cheese.



Steps of making cheese

1- Treatment of raw milk:

Sterilize the milk to decrease the micro-organisms that have spoiled the cheese. The temperature is different either pasteurization or boiling, it depends on the type of cheese.

2- Adding of bacterial starter or Rennete:

The target of adding a starter to produce a **sour flavor** and **precipitate the casein protein** to make cheese therefore the bacterial starter should be the good one to produce a good cheese.

(Rennete): Is a raw extract from the four stomach of a calf. It contains an enzyme (Renine) that reacts with casein and make it precipitate.

3- Treating the cheese material

By pressing and salting the cheese or ripened. There are various kinds of cheese depend on the varieties of a starter, temperature or time of incubation and ripening method to make the cheese different in taste , flavour, texture and color.

Dividing Cheeses depending on softness

Soft cheeses	moisture content 40-80%
Semi-soft cheeses	moisture content 30-40%
Hard cheeses	moisture content 30%

Cheeses can be divided into

Ripened and **Unripened** cheese

Ripening: It is a process by which the cheese take the texture and flavour by using of enzymes (like Protease and Lipase) or by adding the bacteria and mold which are responsible for producing the type of cheese.

Spoilage of cheese depend on

1. type of cheese.
2. The moisture content.
3. Temperature.
4. Time of storage.

The stages of cheese spoilage

A- Through production :

Coliform	produce acids , gases and alchols
Streptococcus lactis	cheese with sour flavour
Bacillus & clostridium	lipolyzation & proteolyzation of cheese

B– Through Ripening

Micrococcus	Embittered flavour
Lactobacillus planetarium	Dark- color (H_2S production)

C– After production :

Geotrichum	coloration on the surface of cheese & lysis of lactic acid
Cladosporium	green-chrysolite color
Pseudomonas , Proteus	Slime & Off-oder

Lactobacillus	Rogosa or M.R.S. medium
Coliform	Macconkey agar medium
Staphylococcus	Staph 110 medium

the Listeria

Listeriosis

1- Meningitis.
2- Abortion and died infants in pregnant women .
3- Inflammation of animals udder .

Bacteria can easily grow on culture media and appear in Nutrient agar , Blood agar , Trypton agar in circulars transparent colonies like (**dew drops**) with sour odder or buttermilk like odder. It grows better in the presence of glucose in medium.

Fermented Milks (Yoghurt)

- *Lactobacillus bulgaricas* and *Streptococcus thermophilus*

Sterilization is very impotent to prevent the contaminated bacteria and inhibit the enzymes, to producer a yoghurt which is more sour or have an

temperature or the time of incuba

Lipid dairy product

1- butter

2- Cream

1- butter

Butter is manufactured from sterilized milk

- by the addition of a starter containing **citrate-fermenting organisms** like

1. *Streptococcus lactis*
2. *Streptococcus cremoris*

to produce lactic acid and decrease the pH to make ripened cream butter .

- The flavor of butter is made by adding a starter in addition of two kinds of organisms

(*Streptococcus paracitrovorus and Streptococcus citrovorus*)

incubated at 22C° for 24hr then shaking in churns, the floated butter drops are carried out, washed sometimes salted to produce salted butter.

Margarines: It is animal's or vegetable oils inoculated by a starter of butter to smell like butter

Spoilage of butter

Butter **is less spoiled by micro-organisms** as result of lipid content **but** the higher concentration of protein and moisture **cause fast and more spoilage of the butter**.

Refrigeration and storage of the butter at low temperatures also retards microbial growth

Spoilage of butter is therefore most likely to arise from the activity of micro-organisms capable of growing at low temperatures,

particularly those capable of

1. lipolysis,
2. proteolysis or
3. loss of flavour
4. causing discoloration

, like *Geotrichum* fungi and. *Pseudomonas fluorescens* , *Pseudomonas fragi* and *Achromobacter* bacteria which excrete lipolytic enzymes (lipase) that produce short chain of fatty acids causing rancidity of butter.

Caseolytic micro-organisms.

- Milk agar medium (Nutrient agar + 30% Skim milk)
- positive result appears by **a clear zone around the colonies**

Lipolytic micro-organisms

- Oil agar medium (Nutrient agar + 5% olive oil)
- Reagent*:* Saturated copper sulphate solution
- a bluish-green colours zone appears

Halophilic micro-organisms

- using medium (Nutrient agar + 15%Nacl)

2- Cream

Sterilize the milk and cool it, the lipid layer will appear on the surface of the milk thickness of lipid layer depends on the lipid content that will be too much in buffalo milk this layer also contain quantity of protein , mineral's salt, sugar of milk.

The sterilized cream has a low microbial content, and the microbial spoilage may occur **because** of the microorganisms that is already present in the original milk.

The end