

- These antibody are present in person who suffering from allergy, drug sensitivity, anaphylactic shock.

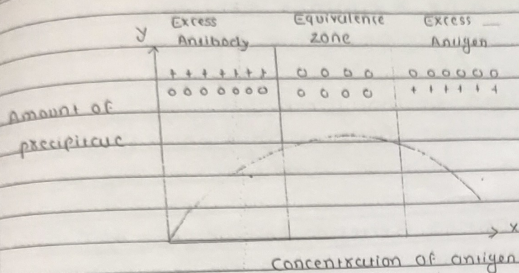
Antigen-antibody Reactions.

There are five types of antigen-antibody reaction.

- ① precipitation
- ② Agglutination
- ③ complement fixation
- ④ Neutralization
- ⑤ Immunofluorescence

① [precipitation Reaction]

- The reaction between antigen & antibody molecule in soluble form & they formed precipitate.
- This precipitate usually settles down if it is fail to settle down & remain as floccules the reaction is known as flocculation.
- precipitation occurs not only in liquid but also in gel.
- formation of precipitate layers are used to identify different between antigens & antibodies in solution.
- Factors that are affected on precipitation tests are electrolytes, pH, temperature & time.
- A precipitation reaction can be done by placing a amount of antibody in series of test tubes by addition of antigen in increase amount.
- The precipitation is allowed to form
- The tubes are then allow to precipitate to settle down & amount of precipitate in each tube is measured.
- A graph is plotted on x-axis by using increase antigen & on y-axis amount of precipitate formed.
- Excess of antibody or antigen does not formed precipitate.

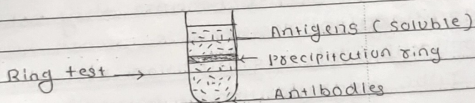


Application of precipitation Reaction :

- The precipitation test is used for qualitative & quantitative determination of both antigen & antibody.
- Qualitative tests are much more used for identify antigens.
- The following precipitation & flocculation tests are used commonly.

① Ring test :-

- This test is very simple for identify antigens.
- The antigens are over serum in narrow tube.
- The reaction is visible.
- This test is also used in detection of adulteration food.



② Slide test :-

- This is example of flocculation test.
- When drop of antigen & antiserum are placed on slide & mixed by shaking, floccules appear.
- The VDRL test for syphilis is example of slide flocculation.

③ Immunodiffusion.

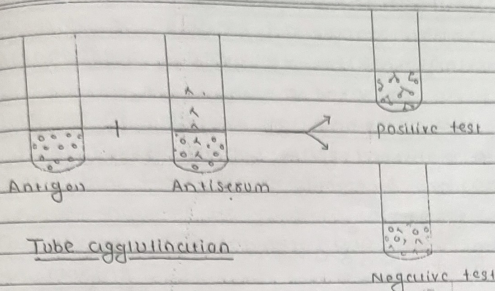
- When antibody & antigen are placed in agar gel they diffuse towards each other & formed precipitation
- Each antigen-antibody reaction gives one line precipitation therefore different antigen in mixture can be identify.

④ Agglutination Reaction.

- The agglutination reaction is similar to precipitation reaction
- The reaction between antibody & antigen formed clumping so it is called as agglutination
- The agglutination reaction is easy to perform & simple.
- When antigen is mixed with its antibody in presence of electrolytes at suitable temperature & pH, it agglutinated.
- Agglutination occurs optimally when antigens & antibodies react in equivalent.
- Incomplete antibodies do not cause agglutination with antigen. Such antibodies are called as blocking antibodies, because they inhibit agglutination
- Agglutination reaction is occurs in salt solution.
- Application of agglutination reaction.

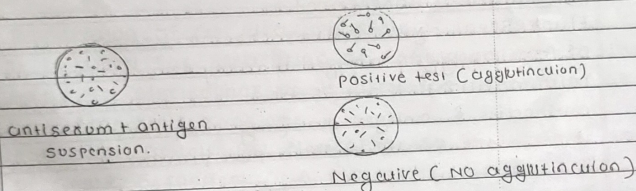
① Tube agglutination.

- It is most widely used agglutination test
- It is done in round-bottom test tubes
- When antigen suspension is added to antiserum in test tubes the agglutination of serum can be estimated.
- The Tube agglutination is used for diagnosis of typhus fever



② Slide agglutination

- A drop of antiserum is mixed with antigen suspension by platinum loop & a mixed antigen suspension is examined by naked eye or under low power microscope for agglutination.
- positive result shows clumping

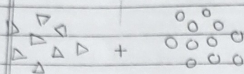


③ Complement fixation test

- Complement is a normal component of serum & it is activated when it joins with antigen-antibody complex.
- Complement fixation reaction is used to identify certain viral infections such as small pox, influenza & poliomyelitis.
- It is also used for identification of many microorganisms.

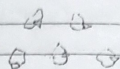
④ Neutralization Reaction.

- Neutralization reactions are antigen-antibody reaction. which are used to determine activity of toxin or virus which neutralized by antibody.
- This also known as toxin neutralization.
- Toxin neutralization can be tested in vivo or in vitro.

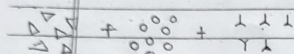


Toxic molecules

cells



cell damaged by toxic.



Toxic

cells

Antitoxic molecule

Y

Toxin

neutralize

cell undamaged

⑤ Immunofluorescence

- Fluorescent dye or fluorochrome used in estimation of antibody or antigen binding to cell
- Immunofluorescence is used for identify tissue antigen antibodies & antigen-antibody complexes.
- It is more sensitive than precipitation & complement fixation test.
- There are two types Direct fluorescent-antibody technique & indirect fluorescent antibody technique
- Direct immunofluorescence is used for identification of bacteria, viruses
- In this method antigen is taken on the slide & antibody is mixed with fluorescent dye, which is then added to slide & incubated for short duration. next wash the slide & observe under fluorescence microscope
- antigen appear yellow-green fluorescent

Application of Southern blotting technique & gel electrophoresis. (Aug-10) (Feb-10) (Aug-03)

Application of Southern blotting technique.

It is first nucleic acid blotting procedure.

Southern blot technique is simple & most sensitive technique. Some of its application are as follows.

- ① This technique is important for confirmation of DNA cloning.
- ② It is useful for mapping restriction sites around a single copy gene.
- ③ Southern blot technique is invaluable method in gene analysis.
- ④ It is forensically applied for identify minute quantities of DNA example identify parenthood etc.
- ⑤ It is mainly used for determination of RFLP.

Application of Gel electrophoresis.

- It is used for separation of fragments DNA mixture.
- Agarose gel electrophoresis is used for separation of DNA fragments ranging
- poly acryl amide gel electrophoresis is used for separation of single stranded DNA molecules.
- Gel electrophoresis is used for understanding the molecular configuration of DNA molecules.
- Gel electrophoresis is also used for separation of RNA molecules.
- A widely used technique for identification of DNA or RNA fragments is blotting techniques.

(31)

Evaluation of preservatives. (Aug-12) (Aug-11) (Aug-10) (Feb-10)

- preservatives are substances which are added to dosage forms to protect from microbiological growth or from microorganism during manufacturing process.
- In sterile articles packaged or containe -- antimicrobial preservatives are added to inhibit the growth of microorganism
- The function of preservatives is protection from microorganism
- pharmaceutical products are used for long time or short time it should be protect from pathogenic microbes so preservatives are used to protect them
- Antimicrobial substances are added to keep quality & activity as pure.
- The evaluation of preservatives is done to checked the activity of preservatives which inhibit the growth of microorganism which compared with standard preservatives.
- Make the test solution add test organisms, say, bean casein digest, in agar medium, incubate at 30°C - 35°C for 12-24 hrs. & add antimicrobial agent after inoculation observe the medium & activity of added ^{ant} microbial agent.
- pharmaceutically useful preservatives.
 - Sorbic acid
 - Benzoic acid
 - propionic acid.
 - Boric acid
 - Phenol
 - cresol

- preservatives should be able to kill all microbes & not be toxic to the patient.
- preservatives are widely used in pharmaceutical dosage forms such as emulsions, suspensions, parenteral preparation etc.
- The main function of preservatives is to prevent the growth of unwanted microorganism in pharmaceutical preparation.
- Chloroform is most widely used preservatives in oral formulation.
- preservatives are widely used in cosmetic preparation, lotions, creams & shampoos.
- Preservative effective test :-
- Test is applied to the formulated medicine to determine preservative effectiveness against microorganism.
- Test is applied to injectable dose such as nasal & ophthalmic products.
- Medium :-
- For initial cultivation of test microorganism, use soyabean casein digest agar medium or any other medium.
- Choice of test microorganisms.
- Choice, microorganisms which are easily grow in the raw materials or which occur in manufacturing environment & causing health problems if they grow in the product.
- A preservative should be active against microorganism.
- Test microorganism is subcultured on the surface of soyabean casein digest agar medium.
- Incubate the bacterial cultures at 30 to 35°C for 24 hrs.
- The bacteria & antimicrobial agent are diluted with saline solution to count.

procedure:-

- Add inoculate to each original product with standardised microbial suspension & mixed.
- Determine the number of viable microorganism by plate count method after incubate the inoculated containers.
- The preservatives is effective in product examined.

② Dick's test. (Aug-13) (May-07)

- This test used for diagnosis of scarlet fever.
- The subcutaneous injection of scarlet fever endotoxin causes redness at the site in infected person not in immune person.
- In this test subcutaneous injection which containing diphtheria toxin is given.
- A immune person does not shows any inflammation at the site of injection while infected person shows redness at the site of injections.

③ Simple Staining. (Sep-12) (Aug-11)

- Bacterial morphology can be studied by observing living preparation or by observing dead cells by staining them with dyes.
- Staining is a process of colouring to organism.
- This staining helps to observe its morphological structure under the microscope.
- Simple staining provides easy way to determine cell shape, size & arrangement.
- Common dyes are used for staining such as crystal violet, methyl blue & safranin.

④ Difference between virus & bacteria. (Sep-12) (Feb-10) (Jan-11)

- Bacteria are found in different shape & size like cocci, bacilli, spirillum, filamentous. While viruses have very simple structure & they are very small.
- Bacteria causing disease such as T.B., pneumonia, Typhoid, cholera, syphilis. While viruses causing disease such as HIV infection, polio, smallpox.
- Example of Bacteria :- *Mycobacterium tuberculosis*, *Vibrio cholerae*, *Salmonella typhi*. While viruses :- HIV.

(Feb-10) (Aug-09) (Sep-09)

(Feb-10) (Aug-10) (Feb-10) (Sep-12) (Feb-12)

(4)

plasmid & cosmid

(1) plasmid :- plasmids are self replicating, free floating circular DNA molecules.

- These are used in recombinant DNA to clone foreign DNA fragments.

- Size of plasmids 10 kb to 200 kb.

- plasmids are two types

① Relaxed plasmid

② Stringent plasmid.

- Relaxed plasmid present in multiple copies per cell.

- Stringent plasmid present in limited copies per cell.

- Artificial plasmid - plasmid pBR322.

(2)

Cosmid :- cosmids are plasmid vector which containing specific DNA called cos sites.

- The plasmid part of cosmid is enables to replicate

- The commonly used cosmids are

① cosmid pHC79

② cosmid pλ38

- cos sites can be separated by DNA

(5)

Selective media (Feb-12) (Aug-10) (Sep-08) (May-07)

- Selective media is designed as that particular microorganism grow best in it.

- It is provided nutrients for growth of particular bacteria.

- It is provided growth environment for gram-negative bacteria, some dyes such as basic fuchsin & crystal violet are added which prevent the population of gram positive bacteria.

② Disinfectant & Antiseptic (Feb-12) (Aug-10) (Feb-10) (Aug-09)

- Disinfectant it is the process of removing microorganism by applying chemical agents.
- The substance used for disinfection is called disinfectant.
- The chemical agents used for killing, inhibiting or removing microorganisms are known as disinfectants.
- Several chemical compounds are used as disinfectants.
- Chemical substances applied to ground tissue to reduce the growth of pathogenic microbe ~~but~~ without affecting host tissue are called as antiseptics.
- Antiseptic defined as the substance which prevents or inhibits or destroy the microorganism used especially on living tissue.

③ Mantoux test (Sep-12) (Sep-08)

- This test used for identify TB.
- In this test initially a very low dose 1 unit is injected intracutaneously in volume of 1 ml & if there is negative result then dose is increased to 10 units & again negative result then dose is increased to 100 units.
- The test should be done with initial low dose.
- In order to avoid repeat testing, the WHO suggest used a single dose of 5 units.

④ Widal test (Aug-11) (Feb-11)

- The Widal test is used in enteric fever.
- In case of salmonella infection it is used for agglutinating antibodies against antigen.
- It is used in diagnosis of salmonella typhi.
- Test involved agglutination of typhoid bacilli when they react with serum containing typhoid antibodies.

(9) Toxoids or Toin (11-09) (Aug-11) (Oct-07) (Sep-12) (Feb-11) (Feb-10)

- Toxoids :-
- Toxoids are defined as toxins.
- Toin is treated with heat or chemical agent to destroy its toxicity without affecting its antigenic properties.
- Toxoids are produced from exotoxins.
- Tetanus toxoid is prepared from exotoxin.

(10) Mutation (11-08) (Feb-11) (May-07)

- The term mutation refers to permanent change in genetic material or nucleotide of gene. This is called as mutagenesis.
- The cell or organism shows mutation is called as mutant.
- Mutation is occurs due to change in the nucleotide of gene.
- Mutation is the ultimate source of gene which provides new raw material for evolution.
- Without mutation all genes analysis is impossible.

(11) Spirochetes (Aug-10) (Sep-12) (Feb-10)

- This types of spiral-shaped bacteria have thin flexible cell wall.
- They do not have flagella.
- Spirochetes are gram negative bacteria which causes many sexually transmitted disease.
- Spirochetes contain filaments around central axis are called axial filaments.
- Example of Spirochetes :- Treponema pallidum which cause syphilis



Spirochetes

conjugation (sep-08) (17-08)

conjugation is a process in which two live bacteria come together & transfer genetic material from donor to recipient bacteria.

In conjugation, genetic material transfer through mediator plasmid.

- conjugation is different from other gene transfer.
- conjugation requires physical contact between donor & recipient bacteria.
- Donor bacteria essentially contain plasmid while recipient bacteria does not contain plasmid.



plasmid

Donor Bacteria



Recipient bacteria

(13) Restriction endonuclease (11-09) (Feb-10)

- Restriction endonuclease also known as restriction enzyme or as molecular scissors.
- In double stranded DNA fragment, it cut the molecule sugar phosphate as a result phosphate group on 5' end & hydroxyl group on 3' end is formed.
- These enzymes named bcz they chew DNA at the restricted sites.
- Bacteria use restriction endonucleases to protect themselves against the foreign DNA.
- restriction enzymes bacteria chew or hydrolyse the foreign DNA.

11. O & H antigen - (Aug-13) (Sep-08)

It is destroyed by boiling or by treatment with classmate carbol but not formaldehyde. When mixed with H suspension formed clumps

85

12. O & H antigen - (Aug-13) (Sep-08)

O - Antigen :- This polysaccharides chain is continuous from core polysaccharides. *Salmonella typhimurium* is common bacterium which studied for Gram-negative cell wall structure.

Core polysaccharide is made up of several sugar subunits such as galactose, glucose, rhamnose, mannose.

O-antigen is unaffected by boiling, alcohol or weak acids.

13. Nutrition requirement of animal cell culture. When mixed with antiserum O antigen suspension form compact.

- ① Respiratory gas
- ② Inorganic ions
- ③ carbohydrate
- ④ Amino acids
- ⑤ protein
- ⑥ vitamins.

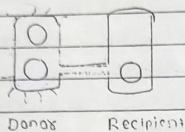
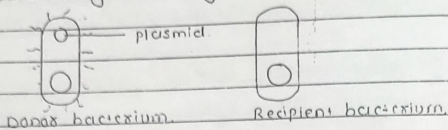
Different method of genetic recombination in bacteria or Discuss transformation & transduction or genetic exchange in bacteria - transformation (Aug-13) (M-03) (Sep-03)

- Genetic recombination is the process of formation of new genotype by exchange of genetic material between homologous chromosomes.
- Homologous chromosomes are two different chromosomes which containing similar genes.
- For organism to undergoes genetic recombination, it should contain two homologous DNA molecules.
- Most of bacteria have single chromosomes that bacteria cannot undergo recombination.
- However, bacteria undergoes recombination because of its ability to obtain extra DNA.
- Bacterial recombination following 3-gene transfer mechanisms, cis follow
 - ① Conjugation
 - ② Transduction
 - ③ Transformation.
- All three gene transfer mechanisms involves the transfer of genetic material from donor to recipient bacterium.

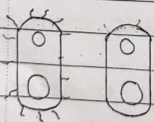
① Conjugation :-

- Conjugation is a process in which two live bacteria join together & transfer genetic material from donor to recipient bacterium.
- In conjugation, genetic material transfer by mediator plasmid.
- Conjugation requires physical contact between donor & recipient bacterium.

- Donor bacterium essential contain plasmid while recipient bacterium usually does not contain plasmid.
- plasmids in gram-negative bacteria consists genes.



- plasmids in gram positive bacteria produce sticky substance which help to direct contact between donor & recipient.
- In Gram-negative bacteria plasmids consists gene for synthesis of sex pilus.
- Bacterial conjugation occurs between donor F^+ & recipient F^- in which fertility factor is transferred from F^+ to F^- .
- F plasmid consists of 100 genes which are involved in plasmid DNA replication & production of conjugation pilus.
- After the transfer of plasmid DNA two new F^+ cells are formed & now the recipient cell can act as donor to other F^- cell. In conjugation of F^+ cells



F^+ cell F^+ cell.

with F^- cells produced $2F^+$ cells which containing F plasmid.

② Transduction :-

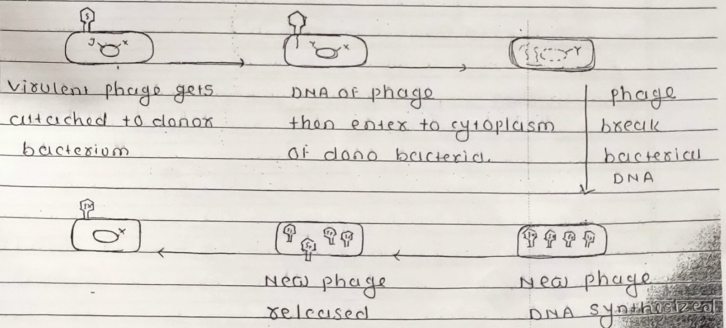
Transduction is a process in which bacteriophage DNA is transferred from donor to recipient through bacteriophage or phage. Phage is virus which composed of genetic material like DNA or RNA.

Transduction is divided into two types

- ① Generalized transduction
- ② Specialized transduction

① Generalized transduction

- In this type of transduction the phage reacts with bacterium & destroyed & formed new phages which known as virulent phage.
- The virulent phage then reacts with recipient cell & formed transduction.
- Example transduction of phage P1 on E. coli
transduction of phage P22 on Salmonella typhimurium.



• descriptive phage mechanism of generalized transduction

② Specialized transduction.

- Specialized transduction is a transfer of specific genes from donor to recipient through bacteriophage.
- Temperate phage gets attached to surface of donor bacterium.
- Specialized transduction occurs in rare case than generalized transduction.

③ Transformation :-

Transformation is the process in which recipient bacterium obtained DNA from surrounding environment such as most commonly from dead bacterial cells.

Transformation is known as important recombination technique.

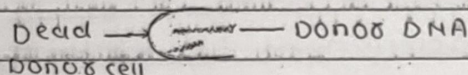
In general both gram-positive & gram-negative bacteria undergo transformation.

In natural conditions, when bacteria undergo lysis, its DNA is broken down into fragments which consist of 10-20 genes.

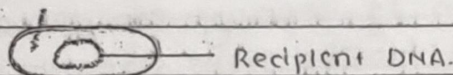
When these broken fragments come in contact with recipient bacterium, transformation takes place.

Steps of transformation.

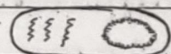
① Bacterium undergoes lysis.



② Recipient bacterium takes fragment DNA.



③ In recipient bacterium, the enzyme forms a double helix of donor DNA into a single strand.



④ The strand of donor DNA takes place into recipient strand of DNA & completes the transformation.



Recombinant cell.

(33)

Production of poliomyelitis vaccine (Sep-02)

- Inactivated poliomyelitis vaccine is aqueous suspension of poliomyelitis virus, type-1, 2 & 3 which grow in suitable cell cultures & inactivated by suitable method.

Preparation :-

- The three types of poliomyelitis virus are grown separately in suspended or fixed cell culture of monkey kidney tissue.
- Nerve cells are avoided because these cells have short life in tissue culture & sometimes causes allergic reaction in the brain.
- After virus cultivation it is tested to confirm the presence of poliomyelitis virus.
- It is passed through filters to remove remaining tissue cells & bacterial cells.
- The vaccine is inactivated by using formaldehyde under suitable conditions of pH & temperature with constant stirring.
- Inactivation is usually completed in six days but absence of active virus should be confirmed.
- The suspension is refiltered & after 9 to 12 days the suspension is retested for absence of infective virus.
- The vaccine then again tested for sterility & free from infective virus.
- Finally, formaldehyde is neutralized with sodium metabisulphite & thiomersal is added as bactericide.

poliomyelitis vaccine (live oral)

- oral poliovirus vaccine is generally given at the

birth & then at 6, 10 & 14 weeks.

- Booster doses are given between 15 to 18 months
- Oral poliovirus vaccine is used for active immunisation of children because it is simple to administer
- poliomyelitis is a disease caused by poliovirus which attacks the central nervous system & causes paralysis

C Jan-14)

(34)

production of Bacillus calmette - Guérin Vaccine (BCG vaccine)

- It is live bacterial vaccine of mycobacterium tuberculosis which developed by Calmette & Guérin in France.

preparation :-

- ① The strain is grown in suitable culture media.
 - ② After growth, they are separated by filtration in the form of cake.
 - ③ The cake is homogenised & suspended in sterile liquid medium to preserve the antigenicity of vaccine.
 - ④ The suspension is transferred into small sterile containers & freeze to prevent microbial contamination & properly sealed.
- It is available as white pellets or powder.

Storage :- BCG vaccine should be stored in sealed glass containers at temperature between 2 to 8°C.

- Dose :- single dose 0.1 ml by intracutaneous injection.
- uses :- BCG vaccine is used as immunising agent which provides protection against tuberculosis.



para → 27-28 Organ-II → 29-31

para → 27-28 Micro → 29-31

P.P → 28-29 Organ-I → 27-28

Colony → 28-29

classmate

My
D

① Louis Pasteur (Aug-10) (Dec-67)

- Louis Pasteur was French chemist & biologist.
- He was good microbiologist.
- Pasteur developed vaccine against the rabies virus which causes disease called rabies.
- He studied alcoholic fermentation & showed that O_2 concentration is affected to growth & activity of yeast cell.
- Wine, beer & milk at certain temperature most of microorganisms get killed this process is known as pasteurization.
- Louis Pasteur was first to introduce two new microorganism, aerobic & anaerobic.

② Alexander Fleming (Aug-13) (Feb-10)

- Alexander Fleming is mainly honored for development of penicillin antibiotic from mold.
- penicillin antibiotic is effective against various infection.
- The year 1945 Fleming received Nobel prize in microbiology for his discovery of penicillin.

③ Actinomycetes.

- Actinomycetes are bacteria with fungus morphology.
- They are gram positive, non motile.
- They show wide range of life cycle.
- They are rich source of secondary metabolites.
- Today antibiotics are produced by actinomycetes.
- They are also discovered production of enzymes, plant growth substances, enzyme inhibitors, etc.
- The actinomycetes are group of bacteria which occurs naturally or artificially.
- They found in soil, marine water, fresh water.

④ Rickettsia

- Rickettsia are small, gram negative bacilli which have properties between bacteria & viruses.
- They infect the vascular endothelium & reticulo endothelium cells.
- They have both DNA & RNA
- They produce endotoxin.
- Rickettsia shows two types of toxicity. they have endotoxin then kills to mice

⑤ Bacteriophages

- Bacteriophages are the viruses which infect bacteria.
- Bacteriophages are used to insert large DNA fragments into bacterial cells.
- Bacteriophages are nucleic acids
- They cannot grow without getting inside a living cell.
- With help of tail fibres bacteriophage attaches to surface of E-coli & injects its DNA into bacterium.
- Different types of bacteriophages used such as M13, P1, P₂ & λ phage.

⑥ Methyl red (MR) test

- This test is used to detect the production of acid during fermentation of glucose.
- By production of acid, pH of medium is fall
- Inoculate test microorganisms in glucose phosphate broth & incubate at 37°C for 2 to 5 days. then add 5-drops of methyl red, mixed well & observe the colour change, if red colour, positive test, while yellow colour negative test.

⑦ Voges-Proskauer (VP) test.

- Many bacteria ferment carbohydrates with production of acetoin.
- Inoculate test microorganisms in glucose phosphate broth & incubate at 37°C for 48 hrs. then add KOH & α -naphthol. positive reaction shows by developed pink colour in 2 to 3 minutes.

Barbara Calne - Queen's College
from the back