

① Type & number of microorganisms present

- Disinfectant activity depends on the nature & number of contaminated microorganisms & especially on the presence or absence of bacterial spores.
- Most^{of} vegetative bacteria are killed by chemical disinfectants.
- Bacterial spores are difficult to destroy but some disinfectants like aldehydes can destroy.
- *Mycobacterium tuberculosis* are destroyed by iodine, formaldehyde, alcohol & phenolic compounds.

② Time of contact.

- Sufficient time must be allowed to disinfectant for its action on microorganism.
- long time best result.

evaluation of preservatives used in pharmaceutical preparations
is very important &c.

(18)

Evacuation of disinfectants. or evaluation of bacteriostatic & bacteriocidal substances.
(Meag) (Jan-19) (May-07)

① Bacteriostatic test

- ① serial dilution in fluid media.
- ② cup plate method.
- ③ The gradient plate method.
- ④ The ditch-plate method.

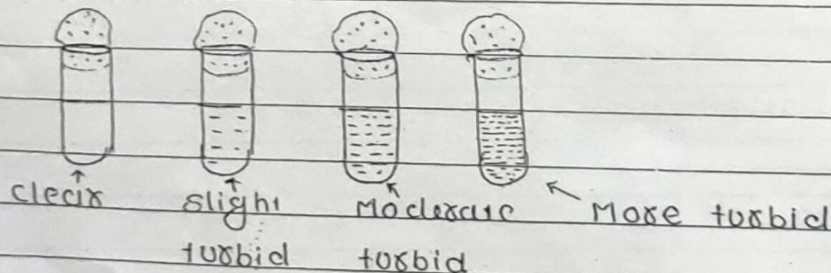
② Bacteriocidal test

- ① phenol co-efficient method.
- ② chick-martin test.

③ Bacteriostatic test:

① serial dilution in fluid media.

- The chemical agent into nutrient or agar medium & inoculated with test microorganisms.
- These tubes are incubated at 30 to 35°C for 2 to 3 days & then the results turbidity or colonies are observed. & the activity of given disinfectant is compared. as.



② cup-plate method.

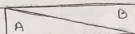
- In this method agar is melted & cooled & inoculated with test microorganism & poured into petri plate.
- In cup-plate method when inoculated agar is solidified it formed hole.
- The disinfectant agent is directly placed in holes.
- The zone of inhibition is observed after incubation at 30 to 38°C for 2 to 3 days.



Inhibition shows the activity of disinfectant.

③ The gradient plate method.

- In this method two layers of agar are poured.
- In A 1 ml of agar with antimicrobial is poured while in B only agar is taken.
- They are allow to incubate overnight.
- After overnight separation occurs which give maximum concentration on left & minimum on right.
- A & B are reversed.



④ The ditch-plate method.

- Agar is poured & allow to solidified.
- It cut out then disinfectant is poured carefully upto it & then loop of organism is streaked on agar surface.
- organism resistant to antimicrobial grow.

⑥ Bacteriocidal test.① phenol co-efficient method.

In this method, activity of disinfectant is determined by comparing with phenol so, it is known as phenol coefficient method.

It is determined by following method.

② Rideal-Gullick coefficient test (RGL)③ Chick martin test.① Rideal - Gullick coefficient test.

This method is used for evaluation of disinfectant activity by comparing with phenol.

procedure :-

- Broth is prepared by dissolving 10 g meat, 10 g peptone & 5 g sodium chloride in 500 ml water.
- Take 5 ml this solution & add test organism of *Salmonella typhi* & incubated at 38°C for 22-23 hrs.
- Again from these culture tubes, add loop of to another tube & incubated for better growth of culture.
- solution of phenol & unknown disinfectant are prepared in sterile water.
- 1 ml phenol is added to 100 ml of sterile water.
- Similarly 1 ml unknown disinfectant solution is added to 500, 600, 700 & 800 ml of water.
- 5 ml of each dilution of unknown disinfectant is added into 4 different test tubes & also 5 ml phenol dilution is added to separate test tube.
- All these tubes are kept in water bath at 19°C .
- 0.2 ml of broth culture of test organism is added to each dilution tube.
- Reaction will be occurs between test organism & dilution.

- After 2 minutes take 1 ml mixture from each five dilution test tube & labeled as set-1 similarly repeat 4 times.
- Total 4 sets are prepared.
- duration of time for each set of test tubes from zero as follow.
- These sets of tubes are incubated at 37°C for 2-3 days & growth of organism is observed by turbidity.
- The compounds with phenol coefficient value below 1 are weak disinfectant.
- The compounds with phenol coefficient value above 1 are strong disinfectants.

phenol coefficient is calculated by this formula.

$$p. \text{ coefficient} = \frac{\text{volume of disinfectant that does not kill in 5 min but kill in 7.5 min}}{\text{volume of phenol that does not kill in 5 min but kill in 7.5 min}}$$

$$p. \text{ coefficient} = \frac{700}{100} = 7$$

② Chick - martin test :-

- Similar to phenol coefficient difference is just test contain suspended organic matter like yeast & the time period for bactericidal action is 30 minutes.



penicillin was 1st antibiotic which used in treatment of pathogenic disease cause by Gram positive microorganism. Penicillin highly affected against Gram positive organism.

49

Chemical changes during fermentation process.

- There is no change in pH at initial stage of fermentation process.
- The pH is rises upto 7 to 7.5. the components of media are utilize & ammonia gas is released from carbon source liquid. the temperature of medium is maintain at 22°C to 25°C.
- The fermentation process come to stop when pH of media rises upto 8.0 the pH can be maintained at 7.0 by adding calcium carbonate to medium.
- The O₂ level must be maintained.
- After 40 hrs penicillin secreted fungus.
- The process continues for 6 to 8 days.
- At the end of fermentation, penicillin is excreted.

Isolation & purification :-

- Mycelium & cells are generally removed from the fermentation medium by filtration.
- Mycelium is washed again to remove the residual penicillin & then used as animal feed or fertilizer.
- After remove of mycelium, the ^{penicillin} antibiotic is remove by extraction, adsorption or precipitation.
- To begin the process, pH is maintain 2 to 2.5 by addition of sulphuric or phosphoric acid. & temperature is maintain 0°C to 3°C so penicillin is not damage.
- The impurities from the penicillin is removed by activated charcoal.
- The next step is precipitation of penicillin in presence of sodium or potassium acetate.
- Next purification of penicillin by distillation.
- purified penicillins are collected by filtration.
- Addition of certain component to penicillin which

(19) Ideal properties of disinfectants. (M-02)

- should be effective wide variety of microorganism
- should be nontoxic to human & animals
- should be stable on storage
- should be odourless or have pleasant odour.
- should have low surface tension.
- should be easily available & inexpensive.
- should be fast acting
- should be active in presence of organic matter.
- should have detergent action which increase activity of disinfectant.

Classification of disinfectant.

Chemical agents are most commonly used as disinfectant.

These chemical agents are.

- (1) Acids & alkalis
- (2) Halogens
- (3) Heavy metals
- (4) Phenol & its derivatives
- (5) Alcohols
- (6) Aldehydes
- (7) Quaternary ammonium.
- (8) Dyes.
- (9) Detergent & soaps.

① production of penicillin by fermentation method

- penicillium chrysogenum is used for commercial production of penicillin.

Fermentation medium :-

- These all elements are required for synthesis of cell & formation of product.

① carbohydrates

② Nitrogen

③ calcium carbonate

④ potassium dihydrogen phosphate

⑤ phenylacetic acid & phenylacetamide

⑥ magnesium sulphate, ammonium sulphate.

⑦ Antifoam agents like tributyl acetate, lard oil.

Biomass production

- Before starting the process, the fermenters must be sterilize with steam.
- The production media & inoculum are added to fermenter.
- During initial vegetative growth the spores are allowed to grow for 40 hrs for development of biomass.
- penicillins form filamentous broth which increase the viscosity & so prevent the O_2 transfer.
- Therefore, the fermenter must have enough O_2 supply because penicillium is aerobic organism.
- The pH of medium should be maintained at 6.5.
- From the growth phase of culture, penicillin production phase starts.
- The penicillin production phase can be increase by adding carbon source.

1st naturally occurring penicillin was benzylpenicillin, benzathine penicillin G. obtained by fermentation of fungus *penicillium notatum*.

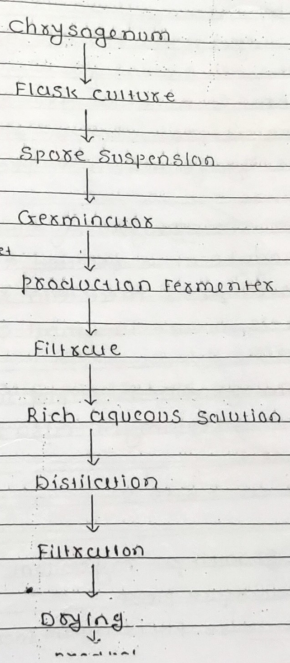
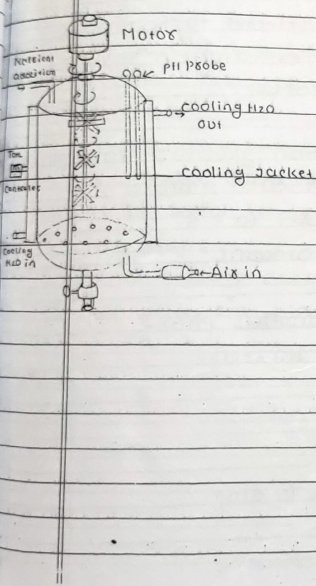
In present it obtained from *chrysogenum*.
Increase yield of penicillins.

Examples :-

- 1. phenylacetamide is responsible for growth of fungus
- 2. phenylethylamine is increase yield of penicillin G
- 4% phenylacetic acid or 1% cystine in increase penicillin yield three time high.

Semi-synthetic penicillins

- The semi-synthetic penicillins such as Ampicillin, Methicillin, Oxacillin, propicillin are prepared by chemical cyclization of 6-aminopenicillanic acid (6-APA).
- The penicillin nucleus attached with 6-APA & synthesized



③ production of streptomycin by fermentation

- Streptomycin is produced by soil organism, *Streptomyces griseus*.

④ Fermentation medium

- ① Glucose as carbon source
- ② Soyabean meal as nitrogen source.
- ③ KH_2PO_4
- ④ NaCl
- ⑤ $(NH_4)_2SO_4$
- ⑥ $CaCO_3$

Biomass production

- Before starting the process, the fermenters must be sterilized by steam.
- The *Streptomyces griseus* are grown in sporulation medium.
- This inoculum & nutrition are added to fermenter.
- The commercial production of streptomycin involves three phases by fermentation process.

① phase - 1 :-

- In this phase *Streptomyces* mycelium growth is take place.
- NH_3 from soyabean is release & also glucose.
- The pH of medium is maintain 6.7 to 7.5.
- Streptomycin is occurs in small amount.

② phase - 2 :-

- The mycelium growth is rapid in this phase.
- production of streptomycin also increase.
- glucose is used.
- pH maintain at 7.6 to 8.

③ phase - 3 :-

- In this phase growth of mycelium is stop.
- Autolysis of cell take place.
- Ammonia is release. pH is again increase.

- The duration of fermentation process is 6 to 7 days, to get high yield of Streptomycin.
- The temperature of fermentation should be maintained at 25°C to 30°C .
- should be more O_2 supply.
- concentration of inorganic phosphate should be low.
- should be given sufficient carbon & nitrogen.

Isolation & purification

- The mycelium is separated from broth by filtration.
- Mycelium is filtered in filter press.
- Next step washing with water & 1% citric acid.
- precipitated by methanol.
- purification by activated charcoal.
- Further purification by column chromatography.
- It is 1st aminoglycoside antibiotic used as therapeutic.
- It was isolated from actinomycetes bacteria Streptomyces griseus.
- It is an antibiotic & active against many gram positive bacteria especially bacilli.
- It is used in treatment of TB, pneumonia, gastrointestinal infections.
- Now a day Streptomycin is administered with penicillin antibiotic to provide best action.

- (3) production of vit. B₁₂ by Fermentation (M-03)
- vitamin B₁₂ is commercially produced by fermentation process by utilizing the microorganism.
 - production of vit B₁₂ from propioni bacterium shermanii

Fermentation medium.

- Three types of media used for the production of B₁₂ with P. shermanii

- ① Maintenance medium.
- ② seed culture medium.
- ③ main culture medium.

① Maintenance medium.

- components of maintenance medium.

- Tryptone
- Yeast
- tomato juice
- Agar

- All these components are dissolved in 1-lit. of water & incubate at 30°C for 96 hrs.
- Maintain pH at 7.2

② seed culture medium.

- two types 1) First Stage medium
2) Second Stage medium.

① First Stage medium

- components similar to maintenance medium excepting of agar.
- Incubate at 30°C for 48 hrs.

② second stage medium.

- corn steep liquor solid.
- Glucose.

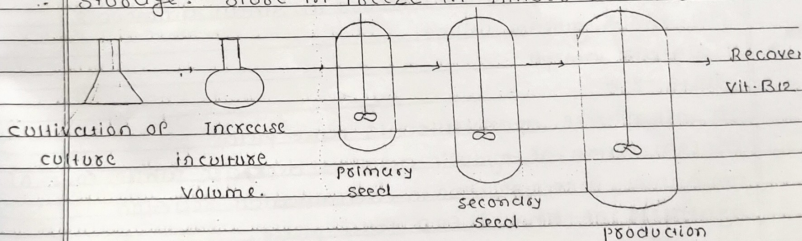
- All components dissolve in 1-lit of water & incubate at 30°C for 24 hrs.
- Maintain pH at 6.5.

③ Main culture medium.

- corn steep liquor solids
- glucose
- cobalt chloride
- propionibacterium is a microaerophilic bacteria. they required O_2 for growth but at low concentration.
- They are unable to grow at high O_2 concentration.

Isolation & purification.

- cells are separated from the culture broth by heating at 80-120°C for 10-30 minutes.
- purification is done with silica gel & other substance by dilution with water alcohol or water phenol mixture.
- Storage:- Store in freeze in Amber colour container.



Uses:- In treatment of anemia.

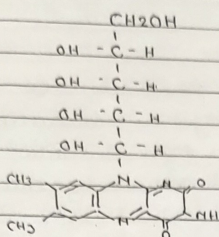
- In deficiency of B12
- In humans, vit. B12 is synthesized by microorganism in lower intestinal tract which can't be absorbed. It is required as external source.

(SEP-08) (Feb-11)

- (a) production of vit. B₂ (Riboflavin) by fermentation
- Vitamin B₂ is commercially produced by fermentation process by utilizing microorganism.

Fermentation medium

- Glucose - Semipurified sugar
- Lipid - Corn oil
- pH - 6 to 7.5
- Temperature - 26°C to 28°C
- Duration of incubation is 4 to 5 days.
- Inoculum & fermentation media are added to fermenter.
- Aeration must be avoided for more riboflavin yields.
- Riboflavin production by candida sps should be done in fermenters which coated with plastic not in steel fermenters. This is because ascomycetes is very sensitive to iron.
- The process of fermentation in three phases.
- Phase - 1 :-
 - During 1st phase growth of mycelium take place
 - Production of riboflavin occurs in small amounts.
 - Maximum glucose utilize.
 - Maintain low pH
- Phase - 2 :-
 - In this phase sporulation is take place
 - Concentration of pyruvic acid is decrease while conc. of ammonia increase due to decarboxylation reaction.
 - Synthesis of FMN & FAD take place.
- Phase - 3 :-
 - Autolysis of cells take place
 - Free riboflavin is discharged into medium.
 - Higher yield can be obtained by addition of glycine & pancreatic extract.



Riboflavin

- Vitamin B₂ is called riboflavin due to presence of sugar alcohol, ribitol group in its structure.
- Microbiologist isolated riboflavin as coenzyme from yeast.
- Almost all green plants, fungi & bacteria can synthesize Vitamin B₂.
- Chemically vit. B₂ is yellow water soluble.
- Milk, dairy products, eggs, meat contain vit. B₂.

(Feb-10) (M-08) (Feb-12)

57

24

Principle & procedure in antibiotic assay

Principle :-

- Microbiological assay of antibiotics involves determination of potency of test antibiotic.
- Test antibiotic is tested that it inhibits the growth of microorganism or not & comparing with standard antibiotic preparation.
- The microbiological assay of antibiotic is based on following tests.

(1) Serial dilution test

(2) Diffusion test.

- This two methods also used in evaluation of disinfectants.

(1) Serial dilution test.

- In this method serial dilution of antibiotic is prepared as test & incubate & tested as it inhibits the growth of microorganism with comparing standard preparation of antibiotic.

(2) Diffusion test.

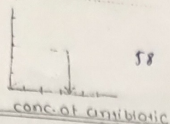
- In this method agar is pouring into petri-plates with agar nutrient & inoculated with test organism.
- The plates are dried at 37°C for 30 minutes to solidify.

Now antibiotic solution under test is applied on solid medium of agar by making cavities in the medium.

- The plates are incubated.
- After observe the inhibition growth of organism by developed of circular zone of inhibition.
- By this way ^{test} antibiotic is determine.

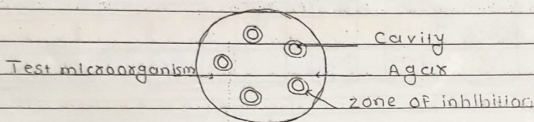
The microbiological assay of antibiotic is carried out by following two methods. (1) cup-plate method &

(2) Turbidity method.



① Cup-plate method.

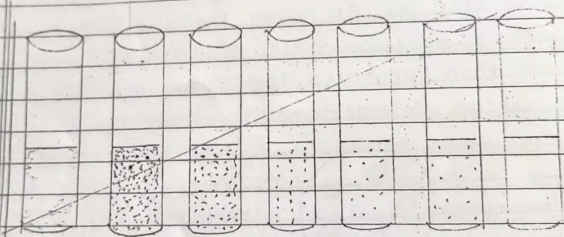
- This method depends on diffusion of antibiotic from cavity through solidified agar to extent growth of added microorganism is prevented in circular area around the cavity containing solution of antibiotic.
- Standard preparation & test antibiotic are prepared.
- These solution are applied on surface of solid medium.
- Solution added to cavity must be sufficient to fill holes.
- Then plates are left standing for 1 to 4 hrs. at room temperature.
- This plates then incubated for 18 hrs at 37°C & measure areas of circular inhibition zones.



② Turbidimetric method.

- This method depends on the growth of microorganism in solution of antibiotic.
- This method has advantage.
- This method take short incubation time for growth of test microorganism in 3 to 4 hrs.
- However, the presence of residues or other inhibitory substances are affected to this assay more than cup-plate method.
- 5 different concentration of standard solution are prepared.
- Test antibiotic solution is adjusted by dilution.
- 1 ml of standard solution & sample solution are added in each test tube.

- To each tube 9 ml of test organism is added.
- 3 control tubes are also prepared one containing inoculated culture, second containing identical & third containing uninoculated culture.
- All the tubes are placed in incubator at the specific temperature for 4 to 5 hrs.
- After incubation add 0.5 ml formaldehyde to each tube.
- The growth of test microorganism is measured by determination against the blank solution at 530 nm.



Amount of growth.

Microbiological assay of vit. B12 (Feb-11) (M-03)

- culture media used for assay of vit. B12
- usually culture media is prepared in the form of solution because cyanocobalamin is dissolved uniformly
- cyanocobalamin is added to test tube which contains culture media & regarded as standard tubes.
- Test sample is added to another test tube
- Lactobacillus is used as test organism which added into each test tube, (both standard & test)
- All test tubes are incubated at specific temperature
- After incubation all the standard tubes are examined for microbial growth
- Spectrophotometer is used to measure transmittance of incubated tubes.
- Microbiological assay of B12 may be done by following method.

① Titrimetric method.

② Turbidimetric method.

① Turbidimetric method. :-

- Take 10 test tubes add 0.0 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 4.0 ml, 4.5 ml & 5.0 ml of standard cyanocobalamin solution.
- To each test tube add 5 ml basal medium stock soln.
- Adjust final volume by using water.
- In 4 test tube add 1.0 ml, 2.0 ml, 3.0 ml & 4.0 ml test solution
- Adjust final volume by water.
- Sterilize all test tube in autoclave
- After sterilization cool all test tube at room temp. & add drop of inoculum (lactobacillus) & incubate
- Titrate each tube with 0.05N NaOH & determine

transmittance for standard & test sample.

② Turbidimetric method.

- Apparatus, reagents & procedures are same as titrimetric method but this method contain two more test tubes which neither standard nor test nor indicator.
- Incubated cu test tubes
- By using blank tube adjust the transmittance at 640 nm in photoelectric colorimeter & record the transmittance reading.

26

Microbiological assay of streptomycin. (Aug-11)

procedure :-

Medium :-

peptone

Beef extract

Yeast extract

Agar

Distilled water

PH after sterilization

- The bacillus subtilis is sensitive to streptomycin so it is used as test organism.
- Streptomycin is an antibiotic
- Antibiotic are the substance which kill or inhibits the growth of microorganism
- Method of Assay :- cup-plate method.
 - "Zone of inhibition" is observed
 - Calculate the diameter

CH-08] (Aug-04) (Aug-11) (Oct-07)

(21)

Standardization of vaccines & sera.

- Standardization of vaccines & sera done by following method.

① Identity tests② potency tests③ Safety tests.① Identity tests

- The identities of bactericidal vaccines can be checked by precipitation & agglutination reactions.
- Inactivated viral vaccines are tested by observation of antibody responses in vaccinated animals.

② potency tests.

- Vaccines containing killed microbes or their products are tested for potency.
- Vaccines containing live microorganism are generally tested for potency by counts of their visible cells eg. BCG vaccine.
- The potency of live viral vaccines is estimated by using living cells.
- potency of many vaccines are also checked by physicochemical or serological techniques.
- potency test done for:-
 - Bactericidal toxoids
 - Killed bactericidal vaccines
 - live organism vaccines
 - inactivated viral vaccines.

Ex:-

Vaccines introduced

guinea pig

After suitable time

Development of
hypersensitivity
Reaction is tested.

③ safety tests.

- Killed bactericidal vaccines must be completely free from living microbes.
- The final product must provide assurance that all microorganisms have been killed.
- Incomplete virus inactivation can be seen by infected tissues or infected animals.
- Some other tests are also used for estimation of potency & safety of bactericidal & viral vaccines.
- Such as.

① Sterility test

② Free formalin testing

③ Toxicity testing

④ Phenol concentration

⑤ presence of aluminium & calcium.

① Sterility test :- All vaccines must be bacteriologically. membrane filtration method is commonly used for sterility testing of vaccines.

② Free formalin testing :- Inactivation of bacterial toxins formed free formalin in the final product.

③ Toxicity testing :- This test is used for identify of toxic contamination in vaccines.

- In S-mice & 2-guinea pig test preparation introduces.
- All must be live without illness for 7 days.

Hybridoma techniques (11-03)production & application of monoclonal antibodies.
(Sep-12) (Feb-11) (Feb-10) (11-08) (Feb-12) (May-07) (Aug-10) (Sep-08)production

- Antibodies are produced in response to antigen which are protein or polysaccharide molecules
- Monoclonal antibody means antibody which produced by cell clone by fusion of one antibody.
- Monoclonal antibodies are mainly produced by hybridoma technology

By following steps monoclonal antibody formed.

- 1) Immunization of mice with immunogen.
- 2) Fusion of plasma cells with myeloma cells.
- 3) Selection of hybrid cells.
- 4) Growth & cloning of hybrid cells.
- 5) Purification & storage of hybridoma cells.
- 6) Human monoclonal antibody.

1) Immunization of mice with immunogen

- Immunization of mice is done by injecting immunogen which mixed with adjuvant
- The antigen is given by intradermally
- Generally, 50 μ g of antigen is administered per mouse.
- Serum of mice is assayed for antibody
- When concentration of antibody is found optimum then animal is sacrificed & spleen is dissociated into splenocytes by enzyme.

2) Fusion of plasma cell with immunogen.

- The splenocytes are fused with myeloma cells.
- The fusion agent can be defective virus, chemicals, or electrofusion.

- cell fusion is done by adding 50% polyethylene glycol solution to cell containing both spleen & myeloma cells.
- All cells are incubated at 37°C for 1 hrs. result fusion mixture contains hybrid cells & myeloma cells.

③ Selection of hybrid cells.

- Selection of hybrid cells is done in HAT medium.
- This medium allows the growth of only hybrid & myeloma cells but does not allow the growth of myeloma fusion.
- Myeloma cells are unable to synthesize HGPRT.
- So unfused myeloma cells can not live in HAT medium.
- The cells living & dividing in the HAT medium only contain myeloma cells & lymphocytes.
- These hybrid cells are called as hybridoma.

④ Growth & cloning of hybrid cells.

- Single cells secreting antibody are isolated from positive culture & increased number of cell lines.
- Limiting dilution & soft agar are mostly used for cloning techniques.
- In limiting dilution of cloning the cells in the culture are diluted result cell culture obtained only one mother cell & these cells secrete only one antibody.
- In soft agar cloning, hybrid cells are separated by growth of single cells in soft agar.
- The visible colonies are obtained from agar & then grown in 24 well plates.
- Yield of monoclonal antibody can be increase by increasing cells.

⑤ Purification & Storage of hybridoma cells.

- Antibody obtained from tissue culture or from mouse fluids can be purified by biochemical methods or salt precipitation.
- purification done for remove contaminants such as proteins, nucleic acids, endotoxins etc.
- cells are frozen at -80°C & then transferred to liquid nitrogen which having temperature -197°C
- hybridoma cells are stored for long time by this method without loss of purity.

Application :-

- Monoclonal antibodies have many application in diagnostics, therapeutics, investigations & drug targeting.

① Diagnostics application

- Monoclonal antibody used for detecting pregnancy, infection. Organisms like bacteria, protozoa, viruses.
- It is also useful to detecting diabetes & tumor cells.
- To detect early cancer.
- It is used as diagnostics agent.
- Monoclonal antibodies have important applications in cell surface diagnosis.
- It is useful in blood group detection.
- primary or metastatic tumours can be detected by radio labelled monoclonal antibody.
- It is also useful to detect breast cancer.
- Monoclonal antibody also used to study hormones & their pathophysiological roles in the body.
- hormones like GH, thyroid, TSH etc.
- pregnant women have baby with some genetic defects.

is detected by some techniques in which monoclonal antibody is also used.

(2) Therapeutic application.

- Monoclonal antibodies are used to bind antigen to neutralize its biological effects & block the activity.
- Monoclonal antibodies used against microorganism for isolation, identification, detection & classification of microbes.
- Monoclonal antibodies are used in autoimmune disease such as rheumatoid arthritis, cardiovascular disease, cancer & also used as candidates.
- HA-1A is the first human monoclonal antibodies used for clinical studies.

(3) Investigational.

- Monoclonal antibodies used in identification, characterization & separation of membrane protein.
- It is also used in purification of proteins.

(4) Drug targeting.

- In this technique, drugs are joint to antibody to form a hybrid molecule with immunological ligand

DOM

69

classmate
Date _____
Page _____

(Aug-11) (Feb-12)

(29)

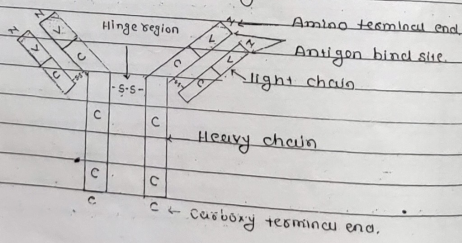
Explain Types of antibodies & describe antigen-antibody reaction. (Aug-08) (Oct-09) (Aug-11) (Oct-07)

Types of antibodies :-

- The human immunoglobulins are basically five types such as IgG, IgM, IgA, IgD, IgE.

① IgG - Immunoglobulin G

- This is the major serum immunoglobulin.
- It is contain about 80% of total immunoglobulins.
- It can cross the placenta & move freely in the blood.
- IgG contain 4 polypeptides in which 2 light weight chains & two heavy chains.
- The molecular weight of heavy chain is about 50,000 & light chain is about 25,000.
- IgG is participates in most of immunological Rxn such as fixation, precipitation & neutralization of toxins & viruses.
- Basically, there are 4 types of IgG such as IgG1, IgG2, IgG3, & IgG4.
- It is provide passive immunity to developing foetus.
- It is also provide protection against bacterial & viruses.
- In presence of antigen IgG bind to antigen & destroy so it protective our body.



② IgM - Immunoglobulin M

IgM is the largest pentameric immunoglobulin with molecular weight 900,000 daltons.

It contains 5 monomeric units

IgM is synthesized early in the immune response.

IgM has short half-life.

IgM antibodies is the first antibodies occurs in response to infection especially caused by Gram (-) bacteria.

③ IgA - Immunoglobulin A

IgA are found in saliva, tears & other body secretions.

They are also known as secretory antibodies.

They consist of two IgG-sized antibodies.

IgA is two type IgA1 & IgA2

IgA found on mucosal surface & in secretions.

IgA secretion is protect from bacteria.

IgA is second important class of antibodies which contain 10-13% of serum immunoglobulins.

④ IgD - Immunoglobulin D

IgD has molecular weight 1,80,000 daltons & contain 1% serum immunoglobulins.

IgD present on the surface of B-lymphocytes.

Its function is not clearly understood.

⑤ IgE - Immunoglobulin E

IgE is called as P-H antibody.

It is produced in respiratory & intestinal tracts.

It is present in low concentration in the serum.

IgE is responsible for anaphylactic type of hypersensitivity.

IgE generally associated with inflammatory conditions.