BEST PRACTICE AREA: TEACHING LEARNING EVALUATION
DEPARTMENT OF ZOOLOGY

BEST PRACTICE: GOBBET AS AN EVALUATION METHOD.

1. Title of the Practice: GOBBET AS AN EVALUATION METHOD.

2. Objectives of the Practice: The main objective of this evaluation practice is to evaluate the undestanding of learning and assess the analytical skills of students.

3. The Context:
'GOBBET' refers to a passage of literature, an image, a cartoon, a photograph, a map or an Artefact which provides a context for analysis, translation or discussion in an assessment**.** The students aregiven set of instructions.

4. The Practice:
Gobbet as a mode of assessment, if effective tool to encourage the students to work as a team and analyse content of Gobbet rationally. The practice promotes leadership qualities and group collaboration / team work along with helping students understand the core concepts and applications of the same. The activities are initiated by assigning of students into groups followed by activities by giving set of guidelines and explaining the rubric of assessment. All the matter related to assessment is also upladed on CLAAP (College Moodle – Chowgule's Learn Anytime Anyplace).

Provide students the time period, guidelines and assessment criteria. Along with the photo/map/scene/artifact, series of questions can be asked (lower and higher order of Blooms taxonomy).

Ensure the students know what the objectives of the assessment are.

Inform students that the gobblet should involve evaluation of the information and not paraphrasing what is already in the piece.

students need to be told ti Include cross-references to any other primary sources, written, feel free to answer in bullet-point form.Be precise, concise and strict about only sticking to relevant information and give Rubric of Assessment:

MARKING RUBRICS	*Excellent (70% and above)*	*Average (69 – 50%)*	*Below average (49 – 30%)*	*Poor (Below 30%)*
1) Context: (5%)	Outstanding grasp and a mature understanding of the gobbet and its contexts	Comments on the nature, authorship, and other material pertinent to the context and interpretation of	Make some pertinent comments on the nature, authorship, and other relevant aspects of the	Fails to expand on the nature, authorship, and other issues relevant to the gobbet.
2)Analysis: (30%)	Clear, coherent and compelling analysis	Demonstrates familiarity with the area under discussion	Demonstrates some familiarity with the area under	May paraphrase rather than analyse the gobbet under
3) Meaning: (30%)	Comprehensive coverage. This may be achieved by citation	Identify the point of the document or the theme that it illustrates	Identify the point of the gobbet – the subject or the theme which it	Fails to identify the point or the theme of the piece
4) Citation: (5%)	Economic and effective use of all material cited	Substantiates the points that are made from evidence	Contains some citation but not appropriately used to substantiate the	Contains no citation
5) Significance: (30%)	Identifies the gobbet's significance in an independent, distinctive, and authoritative way	Explores some of the significance of the gobbet with reference to such issues as typicality, representative ness, uniqueness,	Touches on the wider significance	Fails to identify the gobbet's wider significance

5. Evidence of Success

GOBBE
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ZOO-E-5: ANIMAL CELL CULTURE AND APPLICATIONS
CA 2 (15 MKS) – TO BE SUBMITTED ON 10th February 2020

1) See the image given below. Identify the process that it describes. Explain every step/event numbered from '1 to 11', Comment on the significance of the process.

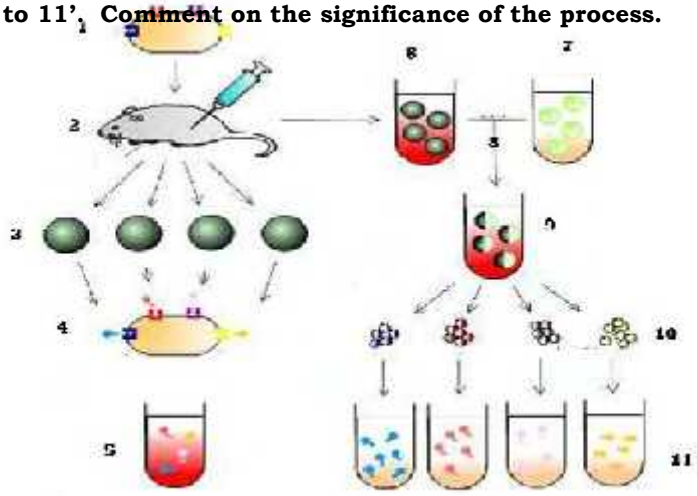


Figure 1: Gobbet

2) Analyse the image 2. What is it indicative of? Compare and contrast the 4 portions of the image viz. A,B.C and D and give justification as to when such a phenomena can occur.

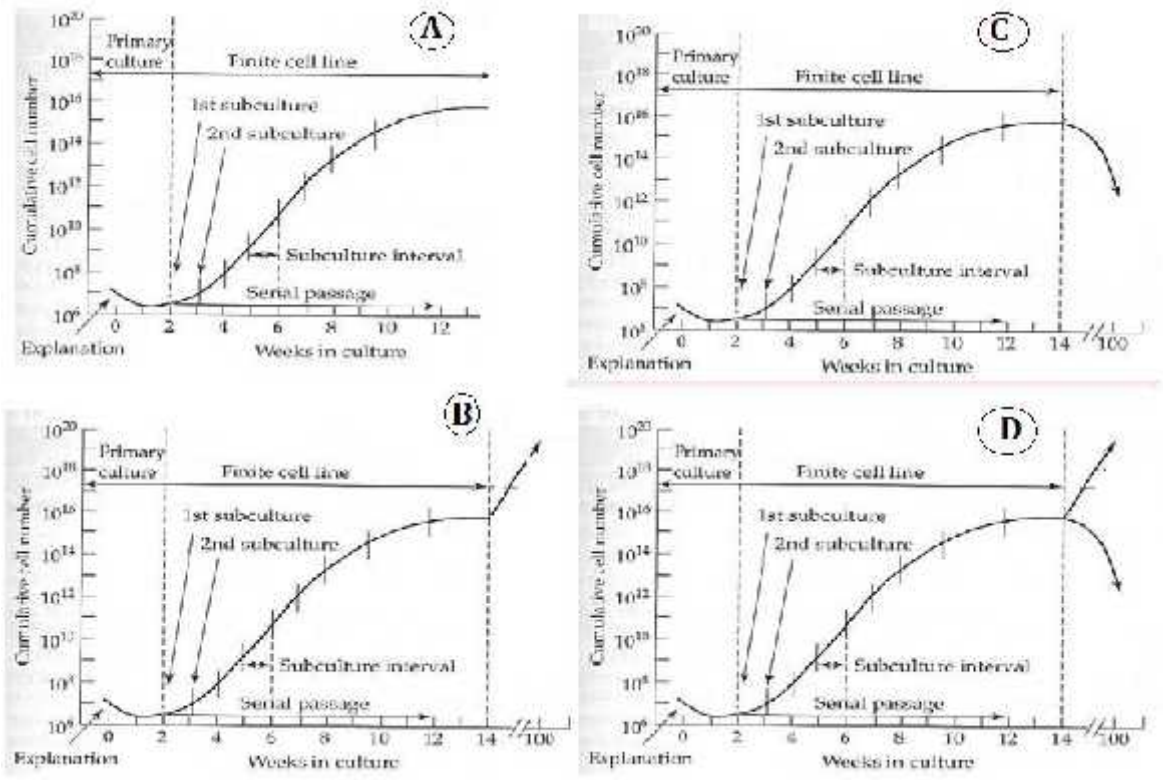


Figure 2:
Gobbet

3) Given below is an image with clippings of lab and the procedure conducted. Looking at the sequence of events from A to F, describe the procedure conducted and steps incolved.

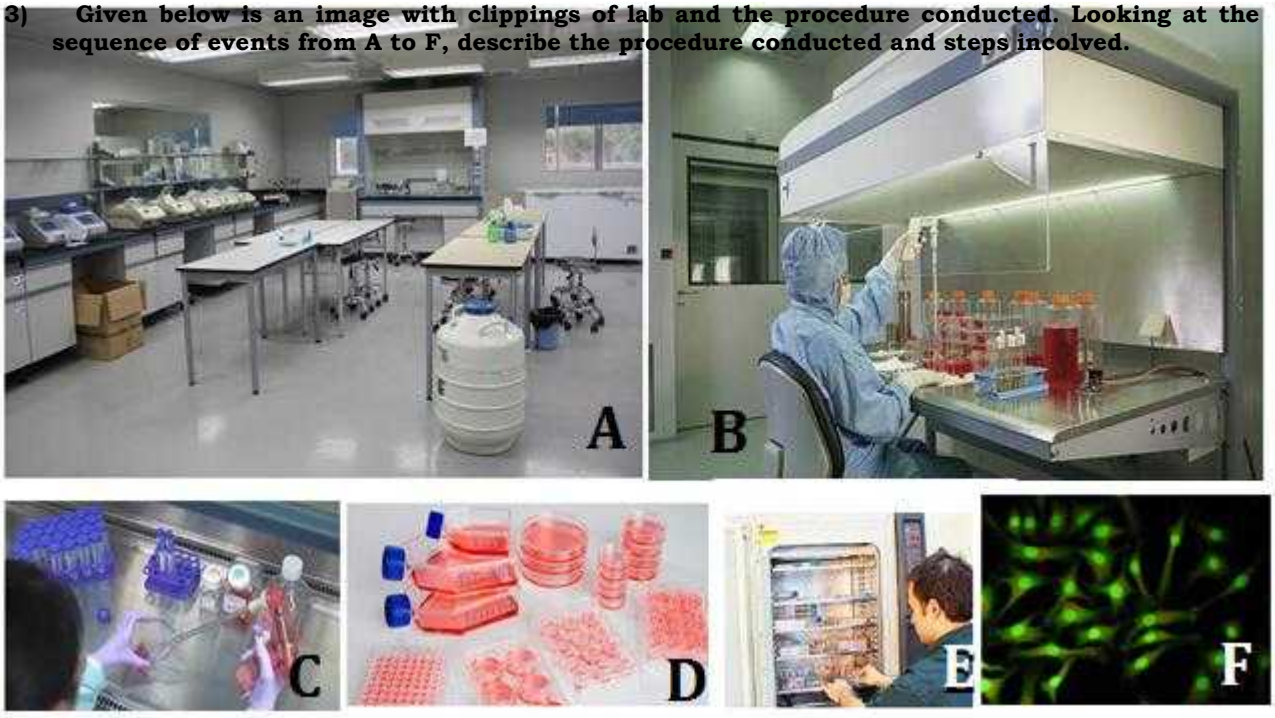


Figure 3:
Gobbet

4) What do you understand by the term hybridoma technology? What are the valuable products obtained from Hybridomas as of today. Suggest an alternative method to obtain valuable products instead of hybridoma technology.

Course faculty: Dr. Nandini Vaz Fernandes Ms. Madhu Balekai Ms. Prasanna Naik Gaonkar

The Answers Submitted by students:

GOBBET

CA-2

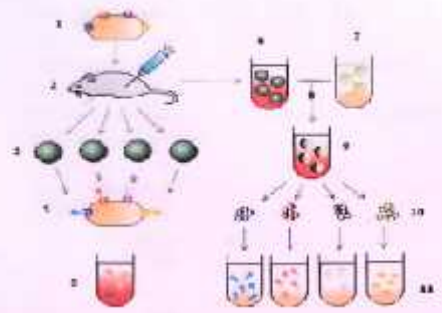
COURSE TITLE- ZOOIV-E-5- ANIMAL CELL CULTURE AND APPLICATIONS

GROUP 6

SEMESTER IV

CLASS – SYBSC

1) See the image given below. Identify the process that it describes. Explain every stepevent numbered from 1-11. Comment on the significance of the process.



➢ The process described below is production of monoclonal antibodies (mAB) using hybridoma technology. Monoclonal antibodies are antibodies made from identical immune cells that are clones of unique parent cell (Kaul T, 2007). They are derived from a single B- cell clone that recognise and bind to a single, unique epitope of an antigen. The steps involved in the production are as listed below-

1- Selecting an antigen having multiple epitopes- This antigen is responsible to producing multiple antibodies

2- Immunization of mice- the mouse is injected with the specific antigen that acts as an immunogenic protein for testing the antiserum. This is done via intraperitoneal immunization or subcutaneous immunization. This will trigger the immune system to produce antibodies against that specific antigen. These antibodies are processed are isolated from the spleen.

3- Isolation of different splenocytes from the spleen of mouse- these are lymphocytes capable of producing specific antibodies. They are isolated via intraperitoneal immunization

4- Formation of antigen- antibody complex- Since every antibody binds to its specific unique binding site on antigen (depicted in the picture with different colour) the splenocytes containing various paratopes fuse with antigens containing the respective epitopes and form an antigen antibody complex.

5- Blood suspension containing Antibodies-We can obtain the antibodies from the antigen-antibody complex through Blood screening. This antiserum contains polyclonal antibodies which bind to multiple epitopes of a given antigen

6- Test tube containing splenocytes having antibodies – These are normal cells having controlled growth and possess the property of producing antibodies. Since blood exists as suspension, we allow the splenocytes to centrifuge and resuspend in lysine solution. This is done to preserve the cell integrity, allow cell growth and make the cells compatible for fusion with myeloma cells.

7- Test tube containing Myeloma cells- These are cancerous plasma cells having the property of uncontrolled growth but do not produce any antibodies. These act as fusion partners.

8- Fusion of the splenocytes and myeloma cells- After ensuring they are present in appropriate ratios, mix both the cells and allow them to centrifuge. After removing the supernatant, poly ethylene glycol (PEG) is added to the pellet to allow adherence between both the cells for proper fusion.

9- Formation of hybridomas- The fusion of a normal splenocytes with cancerous blood cells called myeloma cells, results in the formation of new hybrid cells called hybridomas. They possess the property of immortal and indefinite growth as well production of the desired antibodies.

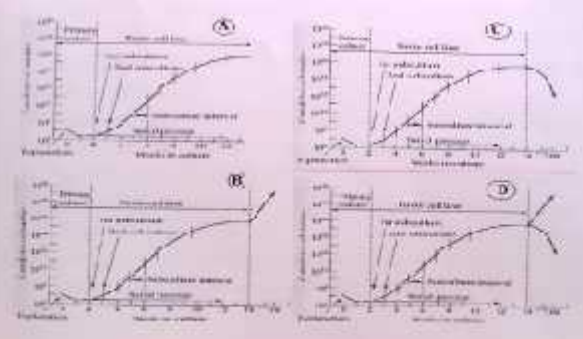
10- Selection of monoclonal antibodies- Inorder to retrieve fused cells, they are subjected to HAT (Hypoxanthine Aminopterin and Thymidine) selection. Since the fused cells are resistant to HAT medium, only these cells will grow thereby separating them from unfused cells and giving rise to pure colony of hybridomas. These hybridomas are cultured and cloned to produce identical daughter cells.

11- Production of monoclonal antibodies – After obtaining pure hybridoma cells, the identical daughter cells secrete the immune products called monoclonal antibodies. Different test tubes containing different types of antibodies, but they are derived from the same hybridoma cells hence the produced antibodies are monoclonal in nature.

➢ The significance of hybridoma technology is mentioned below-

1. In vivo diagnostics- alternative way for diagnosing and monitoring the progression of a disease through the analysis of biomarkers within the body.
2. Highly specific imaging like Positron emission tomography, magnetic resonance imaging fluorescent molecular tomography and ultrasound.
3. Used for monoclonal antibody production and exploiting the therapeutic potential in the form of cytolytic activity, inhibitors and immune-modulators.
4. mAbs are used treating autoimmune disorders like and inflammatory disorders like myocarditis, Crohn's disease, HIV and cancer as well as immunosuppression during organ transplant.
5. mAb's are conjugated with fluorophore or a drug to deliver cargo to specific targets. They can help in targeted drug delivery for immunology and oncology studies through (ADC's) and targeted biologics in the form of chimeric antigen cellec T-cell therapy(CAR T) where they target T cells to a tumor associated antigen through single chain variable fragments expressed at the surface of the T cells (Tan, 2019).
6. They are used in research purposes and analyzing human lymphocytes, MHC antigens, antigenic differences between virus and viral related proteins (Han, 2013).

2) Analyze the image 2. What is it indicative of? Compare and contrast the 4 portions of the image viz. A, B, C and D and give justification as to when such a phenomena can occur.



- The above images show a typical cell growth curve for cultured cells. Each image displays subculture of primary cell culture to form secondary culture. Continuous passaging of cells leads to the establishment of a cell line having finite growth for a certain period of time. But after undergoing the stationary phase, the fate of these cell lines differ except for portion A as mentioned below as mentioned below:

A- Ideal growth curve of cells showing stationary growth phase.

B- Transformed cell line-Cell line undergoes transformation to form an immortal cell line. This is, due to mutation which leads to infinite and uncontrolled cell growth and increase in cell number.

C- Finite cell line undergoes senescence- Cell death due to cessation in viable cell number as a part of natural progression of cell cycle. This phase is also called decline phase.

D- Cell culture with certain cells either undergoing senescence (finite cell line) or transformation (continuous cell line).

- The similarities between all 4 portions of the image are given below:-
- Each curve shows typical pattern of proliferation depicting a relationship between the cumulative cell number and the weeks for which cell were cultured.
- Continuous passaging of primary cells leads to the formation of finite cell lines in each curve.
- Each image shows different phases of cell growth i.e.
  a. Lag phase- The initial phase where no cell growth occurs but cells take time to get adapted to their culture environment. The length of this phase depends on the growth phase of the cell line at the time of cell culture and seeding density (in all 4 portions, nearly 2 weeks).As per the curve, this time period coincides with the time period of primary culture.
  b. Logarithmic phase- The actual phase of cell growth, where cells proliferate and cell growth exponentially increases with increase in cell density. As the cell population is most stable during this phase (in this case, around 2-12 weeks), it helps in assessing the various cell functions. As per the figure, all cell are sub cultured and passaged during initial period of this phase i.e. 1st subculture at 2 weeks(beginning of secondary cell culture), followed by 2nd subculture at 3 weeks and so on till 7 subcultures. Each subculture occurs after specific time period known as subculture intervals.
  c. Stationary Phase- As cells start attaining confluency, cell growth ceases and cells are most susceptible to injury at this phase. As per the curve, after 12 weeks, the cells undergo stationary phase.

- As mentioned above, the 4 portions of the curve differs based on the cell behaviour after establishment of finite cell line. Each curve shows different pattern of growth of cell line depending upon the type of cells cultured and environmental factors.

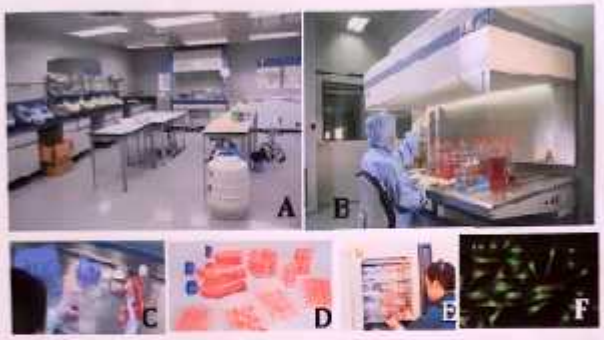
Image A- shows the cells in a stationary phase. The stationary growth phase results from a situation in which growth rate and death rate is equal. The number of new cells created is limited by the growth factor and as a result the rate of the cell growth matches the rate of cell death. The result is a smooth horizontal linear part of the curve during the stationary phase. An exponentially growing cell can enter the stationary phase due to a growth-limiting factor such as the depletion of nutrient or due to the accumulation of waste (Kolker B, 1995).

In image B, a transformed cell line is obtained when the cell line undergoes conversion to a state of unregulated growth in culture. The cells undergo transformation and acquires the ability to divide indefinitely and thus, it becomes a continuous cell line. The continuous cell lines are transformed, tumorin ans tumorigenic. It occurs spontaneously or through mutations arising due to interaction with viruses, oncogenes, radiation or drugs and chemicals. Hence the curve once again increases linearly and exponentially after finite cell line (Smith JR., 1992).

In image C, Cell senescence is the final, common pathway for actively dividing cells which leads to the reduction in the number of viable cells in the culture. Cell death is not due to the reduction of nutrients, but to the natural progression of the cellular cycle. By imposing a growth arrest, senescence limits the replication of the old or damaged cells. Senescent cells undergo many other phenotypic alterations such as metabolic reprogramming, chromatin rearrangement, or autophagy modulation. Senescence is a stress response that is often triggered by a persistent DNA damage response and can be induced by a wide range of intrinsic and extrinsic insults, including oncogene activation, oxidative and genotoxic stress, mitochondrial dysfunction, irradiation, or chemotherapeutic agents, hence the curve tapers down after finite cell line (Nicolas Herranz, 2018).

In image D, the curve shows 2 different growth patterns. Some cells undergo deterioration due to senescence whereas some cells continue to proliferate at an enhanced rate and show exponential growth due to cell transformation. Most of the cells will undergo fixed number of population doublings and these cells are known as finite cells while some cell lines that undergo transformation and acquire the ability to divide indefinitely, becomes a continuous cell line due to mutation (Dowd, 2019).

3) Given below is an image with clippings of lab and the procedure conducted. Looking at the sequence of events from A to F, describe the procedure conducted and steps involved.



- The events occurring in the above images take place while sub culturing adherent cells. After obtaining cells from primary cell culture, they are subcultured multiple times to obtain secondary cell culture and cell lines. The cells are obtained via cell dissociation methods(mechanical or enzymatic) followed by viable cell count , determining optimal cell density and preparation of new culture vessels for passaged cells.

  Based on the images, the steps are mentioned below:-

- ✓ This procedure takes place in a cell culture laboratory as seen in Image A. This laboratory is a single use facility and must be separated into an area specifically reserved for handling contagious material, free of contamination. The main function is to maintain sterile environment as well as appropriate temperature for producing cells in a safe and efficient manner. It must be an air conditioned room consisting of CO_2 incubators, laminar air flow, liquid nitrogen freezer, refrigerator, balance, centrifuge , inverted microscope, hemocytometer, washing sink and communicator.
- ✓ Image B shows a specialty cabinet called laminar air flow hood. It provides aseptic and sterile environment for cell culture and protects the operator from aerosol. It consists of highly specialized HEPA (high efficiency particulate air) filters that filter the airflow. As seen in the picture, there are sterilized T-flasks made out of polystyrene containing the spent culture media placed within hoods' reach. All the solutions and equipments must be sterile. Most importantly, the operator must wear sterilized gloves, masks and laboratory apron to ensure no contamination takes place while working.
- ✓ Using a sterile pipette, the laboratory worker is pouring the media in specialised flasks made out of sterile polystyrene material called T- flasks.
- ✓ As seen in image C, the spent cell culture media from the culture vessel is removed using sterilised pipettes (one time use).
- ✓ Rinse the solutions using balanced salt solution while ensuring osmolarity and pH for preserving cell integrity is maintained.
- ✓ Now remove the traces of salt solution by rinsing with wash solution.
- ✓ After discarding the wash solution from the vessel, subject it to sufficient cell dissociation reagent like trypsin or trypLE to one side of the T-flask for cell adherence and coverage of complete cell layer.
- ✓ The culture vessels mentioned in the image D are designed for storing cell culture medium. The cell culture medium is GMEM (Glasgow Modified Essential Medium, EMEM (Eagle's Minimum Essential Medium) and DMEM(Dulbecco's Modified Eagle Medium) which are supplemented by hormones and growth factors like platelet derived growth factor(PLGF) serve as nutrients and source of energy for cell growth. These include T-flask, petri plates and conical tubes of different sizes, shape, coating and loss. The coatings such as collagen, gelatine and fibronectin help in providing the cells with micro environment condition. These are made out of sterile plastic material like polystyrene, Teflon or polycarbonate that can withstand cell culture conditions and low efficient working (M.K.O, 2021). They are discarded after one time use.
- ✓ Image E shows CO_2 incubator that provides completely closed sterile environment with suitable temperature, humidity and CO_2 to the growing cells.
- ✓ Lifting the flasks in a gentle manner, such that all cells in the flask are completely dissociated. To confirm this, observe them under microscope where they appear round in shape.

- ✓ After almost 90% of cell dissociation, incubate the culture vessel at room temperature for 2 minutes, once again add complete growth medium using a new sterile pipette several times to ensure the entire cell layer covers the surface.
- ✓ Now transfer the cells to a conical tube and centrifuge them at a high speed. Discard the supernatant containing any minute traces of growth medium or dissociating agent.
- ✓ Resuspend the pellet in growth medium while gently pipetting to ensure all cells take up the medium.
- ✓ Now take a small portion of the pellet and use a hemocytometer or any cell counter to do a viable cell count. Use trypan blue stain for indicating the ratio of live to dead cells (thermofisher, 2018).
- ✓ The image E displayed screen depicts spindle shaped cells tagged with a fluorescent marker (green nuclear dye that is permeable to cell membrane thereby staining the nucleus) to detect presence of viable cells.

4) What do you understand by the term hybridoma technology? What are the valuable products obtained from hybridomas as of today. Suggest an alternative method to obtain valuable products instead of hybridoma techology.

Hybridoma technology refers to the production of antibodies in large amounts for diagnostic or therapeutic use (Ji, 2018). It features effective usage of innate functions of both immune cells and cancers, allowing production of hybridoma cells which continuously generate monoclonal antibodies specific to antigens of interest. For the generation of hybridoma cells, B lymphocytes must be successfully fused with myeloma cells using various technologies (Maschio- Tomita, 2011).

The valuable products obtained from hybridoma as of today are monoclonal antibodies. The applications of the monoclonal antibodies are as follow:

i) Diagnostic Applications-

- MAbs may be employed as diagnostic reagents for biochemical analysis or as tools for diagnostic imaging of diseases.
- Detects the protein of interest either by western blotting or immunofluorescence
- Used in cardiovascular diseases and deep vein thrombosis
- Radiolabeled MAbs can be used to locate 1° and 2° metastatic tumors
- Used in immunosuppressive therapy
- Used in pregnancy testing kits in detecting the urinary levels of human chorionic gonadotrophin.
- It also helps in the hormonal analysis of thyroxine, triiodothyronine and thyroid stimulating hormone for thyroid disorders (W. Payne, 1988).

ii) Therapeutic Applications

- MAbs are laboratory produced molecules engineered to enhance or mimic the immune system's attack on cancer cells, they are used to carry drugs and radioactive or toxic substances to cancer cells (Saljoughian, 2019).
- It is used in the immunosuppression of organ transplantation.
- In the treatment of AIDS, autoimmune diseases, malignant leukaemia, B-cell lymphoma.
- Used in the preparation of vaccines, particularly against certain viral strains or against some parasites.
- The toxins can be coupled with MAbs to form immunotoxins and is used in therapy (Aryal, 2017).

iii) Protein purification

MAbs columns can be prepared by coupling them to cyanogen bromide activated Sepharose. The immobilized MAbs in this manner are very useful for the purification of proteins by immunoaffinity method

An alternative method to obtain valuable products instead of hybridoma technology is the recombinant DNA technology. Recombinant antibodies (rAbs) can be generated in vitro through gene manipulation or production of synthetic genes (Fedarko, 2015). After collecting a gene, capable of artificially producing antibody for the given antigen, using polymerase chain reaction to increase the production, transforming a plasmid to carry the gene of interest and insert into the cancerous cell lines (myeloma or lymphoma) will directly result in uncontrolled growth of new cells giving immunology products. Recombinant DNA technology is playing vital role in improving the health conditions by developing new vaccines and pharmaceuticals. It offers new opportunities for innovations to produce a wide range of therapeutic products with immediate effect in the medical genetics and biomedicine by modifying microorganisms, animals, and plants to yield medically useful substances (Sulman Khan, 2016).

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