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**PAPER ADVANCEMENT IN MLT**

**BS MLT**

**Q1:**

Fish is a cytogenetic technique which can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes

It is mainly used to visualized the specific cytogenetic abnormalities

Its result must be correlate with Pathologic , clinical and molecular information

We used it mostly for finding specific features in DNA and those features can used in genetic counseling , medicine and species identification

**PRINCIPLE:**

1. FISH technique is used to identify specific sequence on chromosomes
2. The DNA sequences are identify through fluorescent labeled probes
3. That probe is initially fluorescent labeled but after it become denature
4. DNA of chromosomes are also denature
5. Counter staining is perform or done to observe the cells or chromosomes
6. The stained slide are observe using the required filter

**PROCEDURE:**

1. Slide preparation for cell morphology and fixation using standard cytogenetic procedure
2. Slide review and targeted area marking at department of histopathology
3. The probe labeling ( direct and indirect labeling)
4. Hybridization
5. Fluorescence staining
6. Examine (microscopic examination )

**Q2(a)**

**DIFFERENT TYPES OF PROBES:**

**CENTROMERS PROBES:**

 This probe is mainly design to hybridize centromere

 Its fluorescence is bright because of large number of repeats in centromere

 Also used to find out the exact copies of specific chromosomes

**TELOMERIC PROBES:**

 It is only specific for the human chromosomal arm

 It contain a locus estimated the end of chromosome

**WHOLE CHROMOSOME PAINT PROBE:**

 Used to determine or find the composition of marker chromosomes

**GENE SPECIFIC PROBE:**

 It is known as locus specific probe

 Used to detect the presence and absence of location in a particular gene

**Q2(b)**

1. **INTERMITTENT FLOW CENTRIFUGE:**

 Performed in cycles

 Blood is collected from individual to prevents clotting , anticoagulant added to tubing blood pumped into centrifuge bowl through inlet port

Bowl rotates and components separated according to specific gravity

RBCs packed against outer rim of bowl

Followed by WBCs, platelets and plasma separated components flow from bowl through outlet port into separate collection bags

Undesired components are diverted into reinfusion bag and return to the individual

Reinfusion completes one cycle

1. **CONTINIOUS FLOW CENTRIFUGATION :**

It withdraw, process and return blood to individual simultaneously

This is contrast to IFC procedure , which completes a cycle before beginning a new one

Always done with 2 venepuncture sites

1. **MEMBRANE FILTRATION TECHNIQUE:**

 Blood that pass over membrane with specific pore sizes allow passage of plasma through membrane while cellular portion passes over it

Limited to study plasma collection

**Q4**

**APHARESIS:**

 It is a Greek word which means that to take away

It is a medical technology in the blood of a donor or patient is passed through on apparatus that separates out one particular constituent and returns the reminder to the circulation

It was invented by American technologist HEREB CULLIS 1972

**STATIONARY PHASE:**

The solid or liquid phase of chromatography system on which the materials to be separated are selectively adsorbed

Adsorption or retention or partition or both or any other principle of a substance on the stationary phase

**RADIOACTIVITY:**

A bonding assay in which the binder is an antibody which uses radioactivity to measure the amount of bound and or free antigen radioactively labeled antigen is called tracer. Radioactive isotopes are usually H beta or gamma is called radioactively and radioisotopes are one of the main factors for immunodiagnostic technology

**RAST:**

Radio immuno sorbent test

A test used primary for quantifying total serum immune globin E level in blood serum

A radio allegro sorbent test is a blood test using radioimmunoassay test to detect specific igE antibodies to determine the substance a subject is a allergic to . This is different from a skin allergy test, a person suffers from severe skin condition such as widespread eczema

**LECAPHRESIS:**

The leukocytes are specifically the granulocytes can be heaviest from a donor to supply granulocytes to help fight against infection in patients such as neonates

Leucocytes in some case of leukemia with very high white blood cells removal of increase WBCs , may prevent complications of thrombosis , severe neutropenia

**Q3**

**DNA SEQUENCING:**

DNA sequences is used to determine the sequence of individual genes

Used to determine the sequence of entire all genome

Identification of mutation

Detect different genes that cause disease

**FISH:**

It is used for find specific factor in DNA for use in genetic counseling, medicine and species identification

It is to visualized a specific cytogenetic chromosomes

It is used to chromosomes detection

Genes rearrangements, markers chromosomes identification

Fish is a genetic technique used to diagnose Congenital diseases such as Edward syndrome , down syndrome and also diagnose infectious disease

**CHROMATOGRAPHY :**

Chromo mean color , grapy mean to write or draw

A laboratory technique for the separation of compound of mixture

Chromatography technique is also be used in the separation of vitamins , proteins , lipids

**PAPER CHRPMATOGRAPHY:**

It is used to determine from type of sugar , amino acid , body fluids which are associated hereditary metabolic disorder

**RADIO IMMUNO ASSAY:**

It is a primarily used to analyze antigens

It is used to analyze of vitamin , hormones , metabolites and diagnostic markers

For example FISH T3 T4 ACTH TESTESTERON AND VITMINS B12 ETC

It is a technique used for detecting infection HIV , Hepatitis B , A etc

**NORTHERN BLOTTING:**

Observe a particular gene

Used to show over expression of oncogenes and down regulation of tumor suppressor genes in cancerous cells

Also used for studying mRNA splicing

RNA molecules detected

**Q5**

**BLOTTING TECHNIQUE:**

It is a technique which is used to transfer DNA , RNA, and Proteins

The visualization of specific DNA , RNA and proteins among thousands of contaminating molecules requires the convergence of number of techniques which are collectively called blotting

**TYPES:**

1. **SOUTHERN BLOTTING:**

Sir Edwin southern professor of biochemistry was develop this method

This method involves separation , transfer , and hybridization

This method is routinely use in the molecular biology for the detection of specific DNA sequence or DNA samples and can detect single gene and can be a large part as well as piece of DNA such as viral genome

Southern blotting is combines with agarose gel electrophoresis for size separation of DNA with the method of hybridization

**APPLICATIONS:**

1. Identification of the transferred genes in transgenic individuals
2. Southern blots are used in gene discovery , mapping , evolution and developmental studies , diagnostics and forensic
3. Analyze the genetic patterns which appears in a persons’ DNA

**NOUTHERN BLOTTING:**

It is a technique used for the detection of specific RNA sequence and it was develop by James and George at stand ford university 1979

**APPLICATION:**

1. Detection of mRNA transcript size
2. Study RNA degradation
3. Study of gene expression is specific for mRNA
4. It is used to confirm and check transgenic or knockout mice animals

**WESTHERN BLOTTING:**

It is an immune blotting technique which rely on the specificity of binding between a protein of interest and a probe antibody raised against and that particular protein allow detection if protein of interest in a mixture of many similar molecules

It was discovered in 1981

**Applications:**

1. The confirmatory HIV test
2. Western blotting is also used as the definitive test for bovine spongiform encephalopathy
3. Some form of Lyme disease testing can be performed in western blotting