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**Question no4: define the following term?**

**Answer no 4:**

**1: Aphaeresis:**

Aphaeresis is a machine invented by American medical technologist Hereb cullis in 1972.

Apheresis is a technique involve removal of blood from a donor or patient through an instrument. Removal of blood designed through a centrifuge. All component of whole blood are separated. The needed portion is withdraw and the remaining component are transfused to the donor.

**2: Stationary phase:**

In this phase the substance on which adsorption. The solid or liquid phase of a chromatography on which the material to be adsorb or separated. OR attach the simple help in dry.

**3: Radioactivity:**

Radioisotopes are the main factor of immunodiagnostic technology. Quantity of antigen made radioactive. Frequently by labeling it with gamma radioactive isotopes of iodine. Such as 125-I attached to tyrosine. The pure antigen is used as the standard or calibrator along with the specific antibody against the antigen.

**4: RAST:**

A radioallergeosorbent test (RAST) is a blood test using radioimmunoassay test for the detection specific IgE antibody, to determine the allergic substance. This is different from a skin allergy test which determine allergy by the reaction of a person skin to different substance.

**5: Leukapheresis:**

Leukapheresis is a laboratory technique in which white blood cell are separated from a whole blood. Leukopheresis may be performed to decrease a very high white blood cell count to obtain blood cell from a patient or donor. Obtain cell for research purpose.

**Question no1:**

**Answer:**

Fish (Fluorescent in situ hybridization) is a technique used for visualize specific cytogenetic abnormality. Fish is a cytogenetic technique which can be used to detect and localize the presence or absence of specific DNA sequences on chromosome.

Fluorescent probes attach on those part of the chromosome which have high sequence similarity present. Fish is often used for finding specific features in DNA. This features used in genetic counseling, medicine, and species identification.

**Principle of Fish:**

1. Sequence of DNA are identified by fluorescent labeled probe.
2. Used to detect and localize the presence or absence of specific DNA sequence on chromosome.
3. The probe is initially fluorescent labeled and then denature.
4. Denature of the DNA chromosome.
5. Counter staining is performed to observe the cell or chromosome.

**Procedure of Fish:**

* Slide preparation for cell morphology
* Denature the DNA or chromosome 5degree centigrade.
* DNA probe is labeled
* Hybridization at 37 degree centigrade
* Fluorescence staining
* Examine the slide or analyze the slide check under the microscope.

**Question no2:**

**Answers:**

Any piece of DNA which has been labeled in some way and used in hybridization it is known as probe. Designed against the sequence of interest. Size range from 20 to 40 bp.

**Differentiate between different type of probes.**

**1: centromere probe:**

This type of probe is designed for hybridize centromere. Due to large number of repeat centromere. It use for determining the number of copies of particular chromosome.

**2: Telomere probe:**

They contain a locus estimated the end of the chromosome. It have specificity for single human chromosome.

**3: whole chromosome paint probes:**

Used to determine composition of marker chromosome confirm the chromosome of rearrangement.

**4: gene/locus specific probes:**

This probe detect the presence absence and location of particular gene.

**Part 2:**

**Differentiate different type of centrifugation:**

**1: intermittent flow centrifugation:**

In this process very small volume of blood flow in cycles. Advantage of using intermittent centrifugation include use of single site venous access. The procedure time is longer and larger fluctuation in extracorporeal blood volume occur as compared to to continuous flow centrifugation.

**2: continuous flow centrifugation:**

Continuous flow centrifugation is a laboratory time saver where large volume of material can be centrifuged at high centrifugal forces without the tedium of filling and decating a lot of centrifuge tube.

**Question 3:**

**Answer:**

**Application of DNA sequencing:**

1. Determine the sequencing of individual gene
2. Determine the sequencing of entire genome.
3. Identified endangered and protected species
4. Detect the abnormal gene that are hereditary cause desease.
5. Detection gene deletion and gene amplification

**Application of Fish:**

1. Identification of marker chromosome.
2. Detection of numerical and structural chromosomal abnormally
3. Detection gene deletion and gene amplification
4. Fish can also be used compare the genome two biological species
5. Monitoring the effect of theraphy

**Application of northern blotting:**

1. Probe with radioactive DNA or RNA
2. RNA denature with formaldehyde separate by molecular weight
3. Also used for mRNA splicing
4. Can determine whether the is transcribed or not
5. Similar to southern blotting

**Application of radio immune assay:**

1. Measurement of growth hormone levels.
2. Early cancer detection
3. Analysis of hormone, vitamin, metabolites diagnostic marker.
4. It is used to measure ant-DNA antibody in systemic lupus erythematous
5. Tracking of the leukemia virus

**Application of chromatography:**

1. Separation of mixture of compound
2. Purification process
3. Helpful for the qualitative and quantitative analysis of complex mixture
4. To separate active component from plant material
5. Isolation of metabolites

**Question no 5:**

**Answer:**

**1: Northern blotting:**

Northern blot also known as RNA blot. It is a technique use in molecular biology research to study gene expression by detection of RNA in a simple.

**Application:**

1. Detection of mRNA transcription size
2. Study RNA degradation
3. Study of gene expression at the level of mRNA
4. It is used to confirm and check transgenic knockout mice and animal

**2: southern blotting:**

It is a laboratory technique used to detect a specific DNA sequence in blood or tissue. In this process use restriction enzyme cut the DNA in to fragment.

**Application:**

1. Identification of the transferred gene in transgenic individuals
2. Also used in developing, mapping, evolution and diagnostic and forensic
3. Analyze the genetic pattern which appear in a person DNA

**3: Western blotting:**

Western blot also known as protein immunoblot. it a widely used analytical technique in molecular biology and immunogenetic to detect specific protein in a sample**.** Discover in 1981.

**Application:**

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

**4: Eastern blotting:**

It is a biochemical technique used to analyze protein post translational modification including the addition of lipid phosphate and glycoconjugate. It is most uses in carbohydrates epitopes.

**Application:**

1. Detection of protein modification in bacterial species.
2. Expression of post-translated protein is important in several desease.