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**ANSWER NO QUESTION 1**

Case Study #2

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populations can overgrow and produce their enterotoxins. These toxins released are ultimately

responsible for the diarrhea symptoms of the host.

6. What is the major virulence factor for this microorganism?

**Fluorescent in situ hybrization**

It is a cytogenetic technique used for the detection of some duplication and deletion 0f nucleotides in a chromosome with in a morphological preserved tissue or cells by using fluroscent complementary DNA probes; it is used for the detection chromosomal abnormalities.

**Principle of FISH**

The complementary DNA probes will hybridize to the complementary regions of chromosome for visualizing for specific location on chromosomes.

**Procedure of FISH**

1. Denaturation the double standard DNA strands into single standards DNA molecules by heating.
2. The probes are denatured and then added to the sample.
3. If a small duplication is present that is complementary to the probes, it will hybridize and via the fluorescent dye it will be visualize them to be seen.
4. Examine (microscope examination)
5. Fluorescent staining
6. The probe labeling (direct and indirect labeling)
7. Slide preparation for call morphology and fixation using standard cytogenetic procedure.
8. Slide review and targeted area making at department of histopathology.

**AMSWER NO QUESTION 2**

1. **Diffrentiate between diffrent types of probes**

* **Centromere**
* **Telomerase**
* **Whole chromosome point**
* **Locus**

1. **Centromere Probes**

Centromere probes target centromeric region of a particular chromosome.

This type of probe allows us to determine the quantities and qualitative number of centromere. Centromere probes bind to repetitive alpha satellite DNA sequence eg centromere 17 is used as a reference probe for HER 2/ new.

Also use for to determining the copies of particular chromosomes.

1. **Telomerase probes**

It is specific for a single human chromosome arm they contain a locus automated the end of the chrosome.

Telomerase are DNA structure at the end of eukaryotic chromosome that protects them from degeneration of DNA repair activities.

1. **Whole chromosome pointing probes**

It is used for determination of composition of marker chromosome and for confirmation of the presence of chromosome rearrangements.

1. **Locus probes**

it is used for the determination of the presence or absence or location of the particular gene .Locus specific probes target a specific gene sequence of interest . These probes can determine whether a gene is amplified deleted or present in a normal copy number.

1. **Diffrentiate between diffrent type of centrifugation methods**
2. **Intermediate flow centrifugation**
3. Performed in a cycle and blood is collected from individual to prevent clotting .
4. Blood pumped into centrifuge through Intel part of component are separated specific gravity
5. With 1 venipuncture blood is withdrawn and vein fused through the some needle with two venipuncture one for phlebotomy and 1 for reinfusion.
6. CONTINUOUS FLOW CENTRIFUGE
7. In this technique the blood is processed and return the blood to individual simultaneously .
8. It is contrast to IFC procedure which complete the cycle before beginning the new one.

Always need 2 venipuncture

**ANSWER NO QUESTION 3**

What are the applications of the following technique?

* DNA Sequencing
* FISH
* Northern blotting
* Radio immune assay
* Chromatography

**DNA Sequence**

* DNA sequence is used to determine the sequence of the individual Gene.
* DNA sequence is used to determine the sequence of entire all genome.
* Is used for the Identification of Mutation.
* Is used to detect the different gene that is cause disease.

**FISH**

* It is used to find the specific factor and DNA for used in genetic counseling ,Medicine and species identification
* Fish it is a technique used to visualize a specific cytogenetic chromosome.
* It is used for chromosome micro deletion and detection.
* Gene rearrangement.
* Marker chromosome identification.
* Fish is a genetic technique used to diagnose can genital disease such as Edward syndrome, down syndrome and also diagnose infection disease.
* Detection of gene deletion and gene amplification.

**CHROMATOGRAPHY**

* Chromo means cooler and grapy means to write OR draw a graph.
* A laboratory technique is used for the separation of compound of mixture.
* Chromatography technique is also be used for the separation of vitamins, protein and lipids etc.
* Paper chromatography is used to determine some types of sugar, amino acid , body fluid which are associated with hereditary metabolic disorder .

**RADIO IMMUNE ASSAY**

* It is primary used for the analyzed antigen notably certain hormones, protein in the serum sample.
* It is used to analyses of vitamins, hormone, metabolites and diagnostic marker for example Fish T3 ,T4, AcTH testosterone and vitamin B12 .
* It is a technique used for detecting infection HIV , Hepatitis A and B etc.

**NORTHERN BLOTTING**

* It is a technique used for gene expression detection for a particular RNA in sample .
* Northern blotting are particular useful for determine specific gene are being expressed at messenger RNA level .
* Northern blotting technique are also be used to show the expression of oncogene and tumor suppressor gene cancerous cells composed to normal tissue.
* Also used for study mRNA splicing.

**QUESTION NO 4**

Define the following terms.

* APHAERESIS
* Stationary phase
* Radioactivity
* RASTs
* Leucaphresis
* **Apheresis**
  + It is a Greek word which means to take away.
  + Apheresis “taking away” is a medical technology in which the blood of a donor or patient are passed through a apparatus that separate out one particular constituent and return reminder to the circulation.
  + These apheresis machine was invented by American biomedical technology here cullies in 1972.
  + or a procedure in which blood is collected part of the blood such as platelets or white blood cell is taken out and the rest of the blood is returned to the donor is called apheresis.

**STATIONARY PHASE**

* + The solid or liquid phase of chromatography system in which the material to the separated are selectively absorbed .
  + Absorption or retention or partition or both or any other principle of substances on the stationary phase.

**RADIOACTIVITY**

* a binding assay in which the binder is antibody which uses radioactivity to measure the amount of bound and free antigen .
* radioactivity labeled antigen is called tracer , radioactive isotopes are ussully H(beta) or (gamma)is called radioactivity .
* and radioisotopes are one of the main factor for immunodiagnostic technology .

**RAST**

* radio-immune sorbent test
* a test use primarily for quantifying total serum immunoglobulin U(IgE) level in blood serum.
* Radioallergosorbent test (RAST) is a blood test using radioimmuno assay test to detect specific IgE antibodies , to determines the substance a subject is allergic this different from a skin allergy test.
* A person suffers from severe skin conditions such as widespread eczema.

**LEUCAPHRESIS**

* The leukocyte are specifically the granulocyte can be heaviest from a donor to supply granulocyte to help fight against infection in patients such as neonate.
* Leucocyte in some cases of leukemia with very high blood cells (WBC) removal of increase WBC may help that prevent complication of thrombosis severe neutropenia.

**ANSWERR NO QUESTION 5**

**Compare and Contrast between the different types of blotting? Also give Application of each technique.**

**BLOTTING TECHNIQUE**

the visualization of the specific DNA, RNA and protein among many thousand of contain molecules required the convergence of number of technique which are collectively called blotting

**SOUTHERN BLOTTING SOLUTION**

Sir Edwin southern professor of biochemistry was developed this developed method.

This method involves separation transfer and hybridization. this method is routinely used in molecular biology for detection of specific DNA sequence in DNA sample and the dne can be dected single and can be a large part and piece of dna such as viral genome.

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

**APPLICATION OF SOUTHERN BLOTTING**

1. Identification of the transferred genes in transgenic individuals.
2. Southern blotting are used in gene discovery , mapping , evolution and development studies diagnostic and forensics.
3. Analyze the genetic patterns which appear in a persons DNA.

**NORTHERN BLOTTING**

Northern blotting is a technique used for detection of specific RNA sequences. Northern blotting was developed by James Alwine and George stark at Stanford University (1979).

**APPLICATION OF NORTHERN BLOTTING**

1. Detection of mRNA transcript size.
2. Study of mRNA degradation.
3. A standard for the study of gene expression at the level of mRNA .

It is used to confirm and check transgenic /knocked mice animals.

**WESTERN BLOTTING**

It is an immunoblotting technique which rely on the specificity of binding between a protein of interest and a probe (antibody) raised against that particular protein to allow detection of protein of interest in a mixture of many similar molecules.it was discover in (1981).

**APPLICATIONS**

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