**Mid-Term Assignment**



**(BS. MLT 6th)**

**Course title: -** Advances in Medical Laboratory  **Instructor: -** Ms. Pashmina

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# **A 50-year-old male presents with fatigue, malaise, and abdominal fullness. Clinical evaluation shows splenomegaly, anemia, and elevated white blood cell count. You suspect he might have chronic myelogenous leukemia (CML), also known as chronic myeloid leukemia:**

* **Which laboratory test would be most important in confirming a diagnosis of CML? (Briefly explain the procedure of this test)**

**1)Blood test: -**Complete Blood count CBC through hematology

**Result: -** Total leucocyte counts increase Hemoglobin and platelets also increase.

**2) Biopsy: -**Bone marrow aspiration and Bone marrow biopsy**.**

**3)Cytogenetic:-**Deduct Chromosomal abnormalities such as Philadelphia chromosome no 22 into abnormal.

**4)Imaging diagnosis: -**X-ray Lymph node, CT skin, MRI.

**5)Spinal top: -**For CSF Diagnosis to suck the blast cell.

**The initial diagnostic test is CBC**

**Procedure of CBC**

The skin is disinfected with alcohol paid. Then the needle is inserted of the area which are disinfected of the patient vein. The blood is filled from the needle by a syringe. The sample is taken to the laboratory for analysis.

**Automated Machine** sysmex XE-2100

Hematology automated analyzer used to quickly perform full blood count and reticulocyte count.it can be run on its own or connected to blood film making staning unit.

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| --- | --- | --- | --- | --- |
| **S.No** | **Blood Flaim** | **Bone Marrow** | **Biochemical** | **Immemorial marker** |
| **1** | Anemia | Hyper cellular | Uric acid increase | CD13+ |
| **2** | Neutrophils show lift shift | Increase myeloid and erythroid Ratio 10:1 | Sacrum iron increase | CD14+ |
| **3** | Eosinophilic increase |  | LDH increase | CD 15+ |
| **4** | Thrombocytosis |  | Neutrophils alkaline phosphate increase | CD 33+ |

* **What might be one potential cause for chronic myelogenous leukemia?**

The formation of new gens which are occurring in the chromosomal lead by the swapping or inserting in DNA (an oncogenes) called BCR-ABL.The abnormal genes produced the BCR-ABL protein. The specific types of protein called a tyrosine kinase. this type of protein causes Chronic myeloid leukemia cell to grow abnormal and divided out of control.

* **Cytogenetic studies show that the patient does carry the Philadelphia chromosome in his blood. What is the chance that his children have inherited this translocation?**

The chronic myeloid leukemia is very small number of patient which have BCR-ABL oncogenes but not the Philadelphia chromosome. but the mutation doesn’t pass from parent to their child. The mutation occurs during life time. They don’t have inherited by birth. Normally ABL genes present on lower end of the chromosome but when then mutation occur ABL genes convert to BCR genes and transfer to break point or center point of chromosome where two chromosomes are attached. The disorder involved hematopoiesis disorder because disease originated from single clone and slowly effect on other clone.

**Q2: - Imagine that you are working in hospital as MEDICAL LAB TECHNOLOGIST during coronavirus outbreak**

* **By which PCR Method you diagnose this novel coronavirus? What are the other applications of this technique**?

Through reverse transcription PCR (RT-PCR) We diagnose the novel corona virus because novel corona virus contains mRNA.We convert the mRNA to cDNA and then we apply the reverse transecftase PCR.

**Application of Reverse Transcription PCR**

**Research method: -**through qRT-PCR to measure the expression of gel genes in yeast cell.

**Genes Insertions:-**In the RT-PCR are also useful for the insertion of genes of eukaryotic cell to prokaryotic cells.

RT**-**PCR also useful in studying the genome of the virus whose genome are composed of RNA such as influenza and HIV.

**Genetic Disease Diagnosis: -**RT-PCR can be used to diagnosis the genetic disease like Lesch-Nyhan syndrome also use for the test of bird flu-H7N9.

**Microbiological uses: -**They are used by the microbiologist for the food safety and fermentation.

* **Briefly describe the protocol of this process?**

Reverse transcription(RT) is a technique of converting RNA to cDNA by using reverse transpectase enzyme and dNTPs.the RT step may be done from the total RNA such as that the cDNA is produced by the transcripts in the sample.

Usually have two step protocol or in a specific gene which RNA is inserted and convert to cDNA.

The following experiments can be use as the basic of RT Protocol which can be modified for the particular requirement. The cDNA is prepare using two step processes. The dilution of the cDNA adding to PCR to prepare a one-step reaction where both processes are carried out properly.

**Material**

Stranded PCR instrument.

Laminar flow which hold for RT Setup.

**Reagents**

RNA approximately 1 micro letter.

Ready script two step cDNA synthesis kit.

PCR grade water 20 ml.

* **Being a MEDICAL LAB TECHNOLOGIST what are the Laboratory Biosafety Practices Associated with coronavirus?**

**1: - Personal Protective Equipment** PPE is the last line of defense between you and harmful material in the laboratory. When you should be wearing it, and how it should be stored, cleaned, maintained and disposed.

**2: -Pipettes** Work inlaboratory contain a lot of handling and transferring liquid in small and precise volume this is mainly done by using pipettes. It is important to know that how to use properly.

**3: -Sharps** Refer to items cutting edges or cutting human skin.it contain scissors, needles.

**4: -Surface decontamination** There are many equipment that can become contaminated by biological agents or other hazardous materials. Decontamination is required to reduce the risk of infection.

**5: -Autoclave** combine heat steam, and high pressure to kill biological agents are more robust then other **for example** bacteria spores and prone and prone.

**Q3: Why we used**

1. **TAQ Polymerase in PCR** The Function of in PCR reaction is to amplify the DNA which production of multiple copies.
2. **Agarose and Loading dyes in gel electrophoresis** It Generally contain a dye to assess how to “Fast” your gel is running and a reagent to render your samples denser you remain buffer.
3. **Enzyme-labeled primary and secondary antibody in ELISA** The Primary is direct deduction that react directly react with antigens.

The secondary labeled of antibody is indirect dedication method which deduct most popular format for ELISA. The secondary antibody is specified for the primary antibodies.

1. **Blotting paper in Southern blotting** The nitro cellulose membrane have been use for the blotting in case of southern blot but in resent time nylon membrane have been implemented for the blotting processes due to their ability to attach more amount of DNA efficiently which allow the southern blot to be carried out less amount.