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# **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?
* Briefly describe the protocol of this process?
* Being a MEDICAL LAB TECHNOLOGIST what are the Laboratory Biosafety Practices Associated with coronavirus?

**Answers**

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

 We can diagnose corona virus by Reverse Transcriptase PCR.

**Application:**

* R-T PCR is used in expression profiling, to determine the expression of Gene.
* R-T PCR is used to identify the sequence of RNA transcript, including transcription start and termination sites.
* Useful for diagnosis of RNA Viruses, as well as for evaluation of antimicrobial Therapy.
* Also used in diagnosed of genetic disease.

R-T PCR used to extract.

* **(B). briefly describe the protocol of this process?**

**Protocol of R-T PCR:**

 RT-PCR can be carried out by one – step RT-PCR Protocol.

 And the two – step RT-PCR Protocol.

**One – step RT-PCR Protocol:**

 This reaction carries out in one step in which all the reaction component are mixed in to one tube.

 **Two – Step RT-PCR:**

* The process occurs in two steps.
* First step involve the Reverse Transcription.

RNA into DNA or CDNA and 2nd step PCR occurred.

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

**Answer**

Eating, drinking, smoking, applying cosmetics, and handling contact lenses are not allowed laboratory working areas.

•Appropriate PPE should be worn.

• All procedures should be performed in a way that minimizes the formation of aerosols and droplets.

• All manipulations of potentially infectious materials, including those that may cause splashes, droplets, or aerosols of infectious materials (e.g. loading and unloading of sealed centrifuge cups, grinding, blending, vigorous shaking or mixing, sonic disruption, opening of containers of infectious materials whose internal pressure may be different from the ambient pressure) should be performed in appropriately maintained and

validated biological safety cabinets (BSCs). Use of Class II BSCs should be considered to protect work surface materials as well as personnel and the environment.

• Mouth pipetting must be strictly forbidden.

Personnel must wash their hands often – especially after handling infectious materials and animals, before leaving the laboratory working areas, and before eating.

• PPE must be removed before leaving the laboratory.

A controlled ventilation system maintains directional airflow into laboratory room.

# **Question 1;**

# **Case Study:**

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

 **Answers No 1**

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

 **Chronic Myeloid leukemia: (CML)**

Also Known as chronic myelogenious Leukemia.

CML is clonal Myelo proliferative disorder characterized by specific Genetic abnormality. I-e BCR-ABL fusion gene

**Diagnosis of CML:**

1. **CBC (Complete blood count):**
2. **Blood cell count:** While blood cell count will be 50 x 500 x 10⁹/L.

**Platlets Count;**

1. Thrombocytosis
2. Peripheral blood film examination.
* Normocytic, normochromic anemia.
* Neutrophils show lift shift.
* Eosinophil’s are increase.
* Bhasophilia.
* Hypo granulated myeloid cells.
1. **Bone Marrow:**
* Hyper cellular
* Increase M:E ratio i.e 10:1
* Erythroid precursors decreased
* Megakaryoblasts are increased
* Eosinophilic and basophilic granules are abnormal.
1. **Biochemical finding:**

 Serum uric acid , iron, LDH and Calcium will increase.

1. **Immunological markers:**

CD13+ , CD14+ , CD15+, CD33+,

 Chromosome analysis (cytogenetics)

Rh positive

BCR-ABL Positive

* **Answer no B: What might be one potential cause for chronic myelogenous leukemia?**

**Cause of CML:**

 The main cause of CML is the rearrangement (translocation) of Genetic material between Chromosome No 9 and Chromosome No 22. This is written as (9:22).

Part of the ABL 1 gene from Chromosme9 fuse with the part of BCR Gene from Chromosome 22, that Creating Abnormal fusion gene called BCR-ABL1.

The Abnormal Chromosome 22, Containing a piece of Chromosome 9 and the fusion Gene is often referred to as the Philadelphia chromosome.

* **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?**

**Answer**

The main cause of Chronic myloid leukemia is the translocation of genetic material between chromosome No 09 and chromosome no 22. This translocation acquired an individual lifetime and it is present in abnormal blood cell. This type of genetic changes called somatic mutation and it is not inherited.

**Question no 3**

**Why we used**

* TAQ POLYMERASE in PCR
* Agarose and Loading dyes in gel electrophoresis
* Enzyme-labeled primary and secondary antibody in ELISA
* Blotting paper in Southern blotting

**Answer:**

**(A)**

* The main function of DNA polymerase in PCR is to extent the DNA. And make multiple’s copies of DNA.
* Tag polymerase is thermo stable that can work on high temperature.

**(B).**

**Agarose:**

It is a process in molecule especially DNA , by electrophoresis

Agarose separate molecule on the basis of size, charge or shape.

 **(C).**

**Enzyme – Labeled primary;**

The enzyme is added in direct Elisa which will directly binds to the target ( antigen) that is immobilized to the plat (Solid surface).

**Secondary antibody in Elisa:**

Secondary and primary both are added in indirect Elisa.

Secondary anti body are bind to other antibody and it is then applied to the solid surface.

**Answer (D).**

**Southern blotting:**

It is laboratory technique used to detect the presence of a specific place of DNA in a sample.

* The British Biologist Edwin Southern who first published in 1975.

 **..……………. The End ……………….**