



Tikrit University  
College of Veterinary Medicine

## Lect.6: Practical Virology

Subject name : Molecular technique of virology

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Lecturers link

## Microscope as a method of diagnostic virology

### Light Microscope

The viruses can not be seen under the light microscope except by the labeling of the antibody with an indicator such as fluorescence or peroxide so this will consider as an indicator for the presence of the virus .

### Electron Microscope

This method is considered better than the light microscope as it provides 50,000 magnified which provide the ability to see the virus particles .

Limitation of the electron microscope :

This method characterized by lack of sensitivity which mean that we need at least of 1000,000 virus particles /1 milliliter in order to detect the virus.

## Molecular Techniques of Virus Isolation

These methods including :

1- Classical molecular methods including

A-Dot blot Method

B- Southern Blot Method

C- In situ-Hybridization

These methods dependent on using DNA or RNA probes .

Limitation or disadvantages:

- They are almost similar in their sensitivity and efficiency to classical method
- They are expensive

## Molecular Techniques of Virus Isolation

These methods including :

1- Polymerase Chain Reaction (PCR)

based on Identification of DNA

2- Reverse Transcription –Polymerase Chain Reaction (RT-PCR)

Based on identification of RNA

-These methods involves in the using of 2 primers designated to identify the target and with the presence of DNA polymerase and other important components required for the amplification , the target of interest will be amplified .

-Advantages of both PCR and RT-PCR

-Advance methods with super accuracy of efficiency.

-Commercial synthesis of oligonucleotides (primers)

-The availability of genetic sequence in the central database

## The difference between PCR and RT-PCR

### PCR

- 1- Detect DNA viruses
- 2- Does NOT need reverse transcriptase enzyme

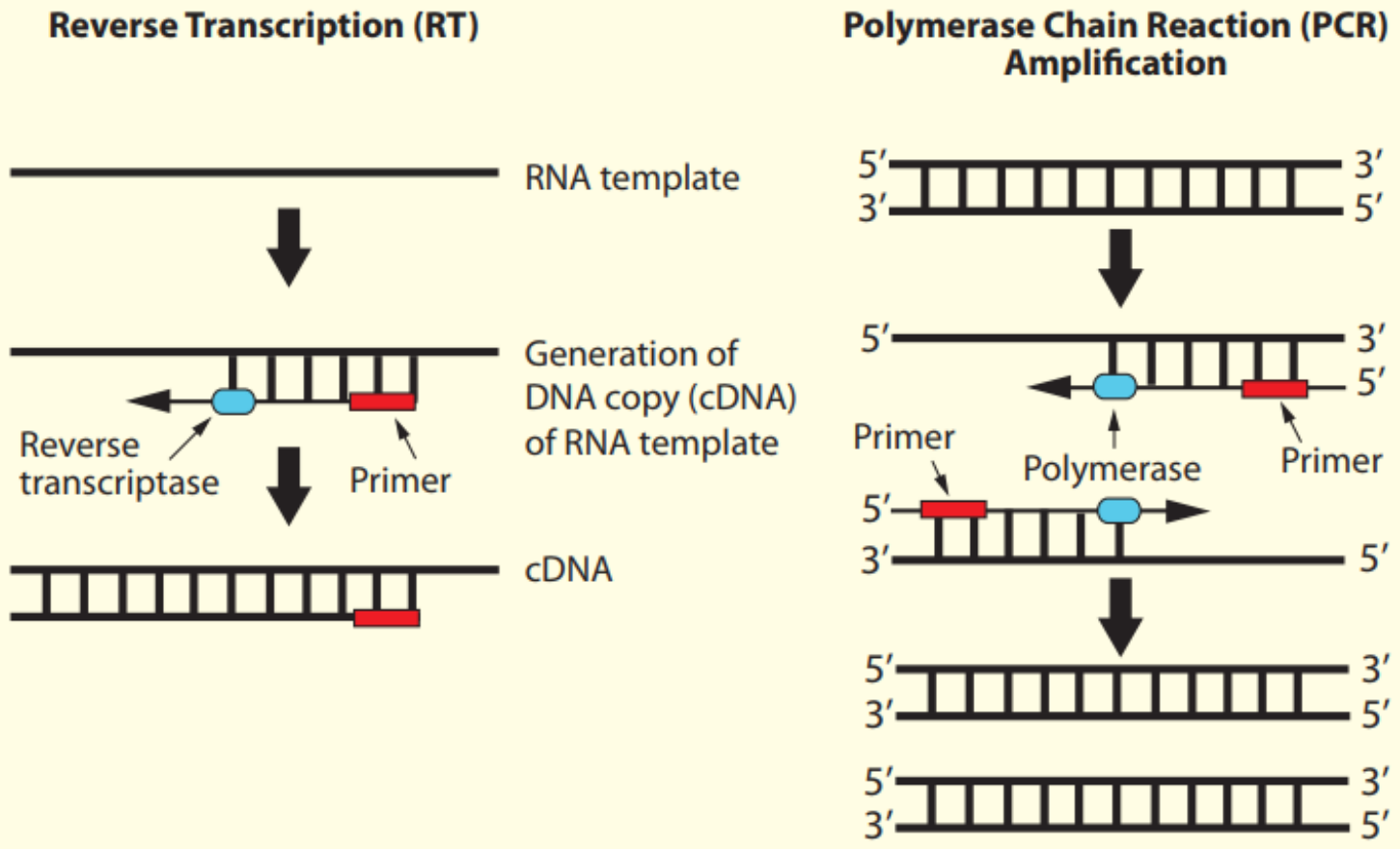
### RT-PCR

- 1- Detect RNA viruses
- 2- Need reverse transcriptase enzyme

### Function of reverse transcriptase enzyme (RT)

an enzyme which enables making a copy of DNA from RNA which generates double stranded target which is then used to identify RNA viruses .

### Reverse Transcriptase (RT) / Polymerase Chain Reaction (PCR)



Mechanism of RT PCR and PCR

4- water bath: Which is used for thawing the freeze Animal sera and required also for mini other steps .

5-centrifuge: Which considered of routers with different sizes such as 15 ml tubes and 50 ml tubes.

6- Cell counter which is required for counting the cells and checking the viability during different stages of cell culture processing .

7- refrigerator :which is required for preserving different compounds that required a temprature of 2-8 C.

8- Freezer : Which is used for storaging the cells after adding Qiazol in order for extraction of nucleic acid for PCR purposes.

9-Autoclave: autoclaving the essential parts used during the cell culture product to prevent any contamination

10-Water purification system: Which is required during the processing of cell culture.

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