



Tikrit University
College of Veterinary Medicine

Lect.7: Practical Virology

Subject name : Diagnostic Laboratory
technique of virology

Subject year: Third-year

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Lecturers link

Diagnostic Virology:

Define as the methods that used for study of viruses and viral diseases.

Aims of using diagnostic virology :

1- detection of immune response to the virus by measuring the antibody or cell mediated mediated immune response by using immune assays.

2- Identification of the viral causative agent by using electron microscope as well as specific stain.

3-detection of viral nucleic acid .

4- Isolation and identification of the virus by using cell culture or fertile eggs.

Detection of the Immune response

-As it is not easy to detect the virus directly in general, so the indirect methods is the alternative ways to detect the causative agent such as measuring the antibody for the virus.

Indirect methods to detect the immune response

- 1- Virus Neutralization (VN)
- 2- Hemagglutination inhibition (HI)
- 3- Enzyme Linked Immune Sorbent Assay(ELISA)
- 4- Indirect Fluorescent Antibody (IFA)
- 5- Latex Agglutination (LA)
- 6- Agar Gel Immune Diffusion (AGID)
- 7- Agar Gel Precipitin (AGP)
- 8-Latex Agglutination (LA)

1- Virus Neutralization

The basic of this technique is by incubation of

- 1- Target virus
- 2- Sample of interest

in case the sample does not contain an Antibody that leading to the changes in the cell culture which will appear and called as cytopathic effect (CPE).

While if there is Antibody in the sample then the cytopathic effect will not appear as these antibodies will prevent the virus from growing in the cell culture

Hemagglutination Inhibition (HI)

This technique based on specific protein that is present on the virus leading to interact with the red blood cells (RBCs) .This technique called hemagglutination and the protein itself called hemagglutinin.

Enzyme Linked Immune Sorbent Assay ELISA

This method characterized by using plastic wells coated with the antigen (virus) or protein specific to the antigen

The sample (serum) will bind to the coated well .

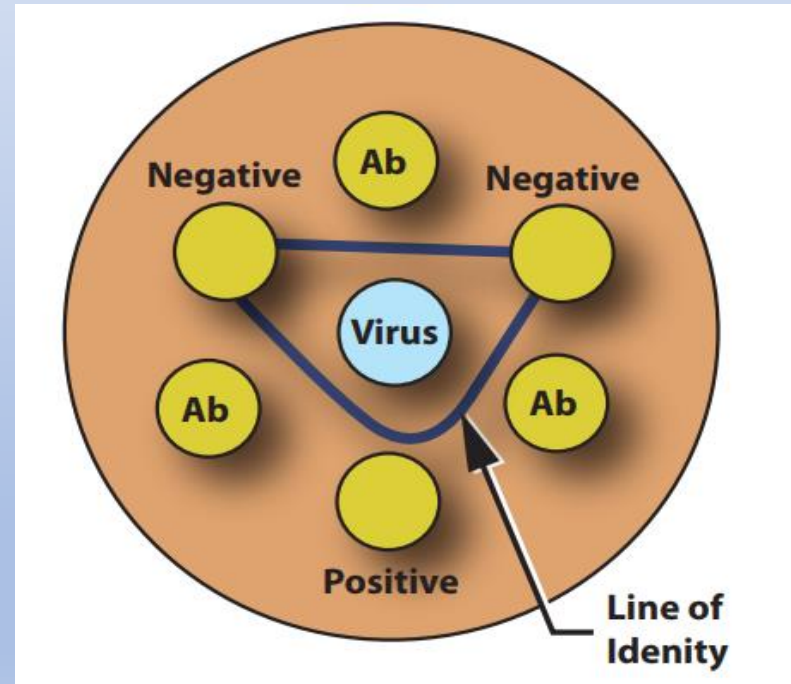
The presence of colors considered as an indicator for the presence of antibodies(positive)

The absence of color indicates for the negative samples which meaning no antibody presence

This method characterized by 1- Ease of use 2-low cost

Agar Gel Immune Diffusion (AGID)

This technique involves of diffusion of virus and antibody through an agar which will form a line of identity where the reaction between the virus and the antibody



Agar Gel Immune Diffusion Technique (AGID)

Practical Virology

Lecture 8

Molecular technique of virology

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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

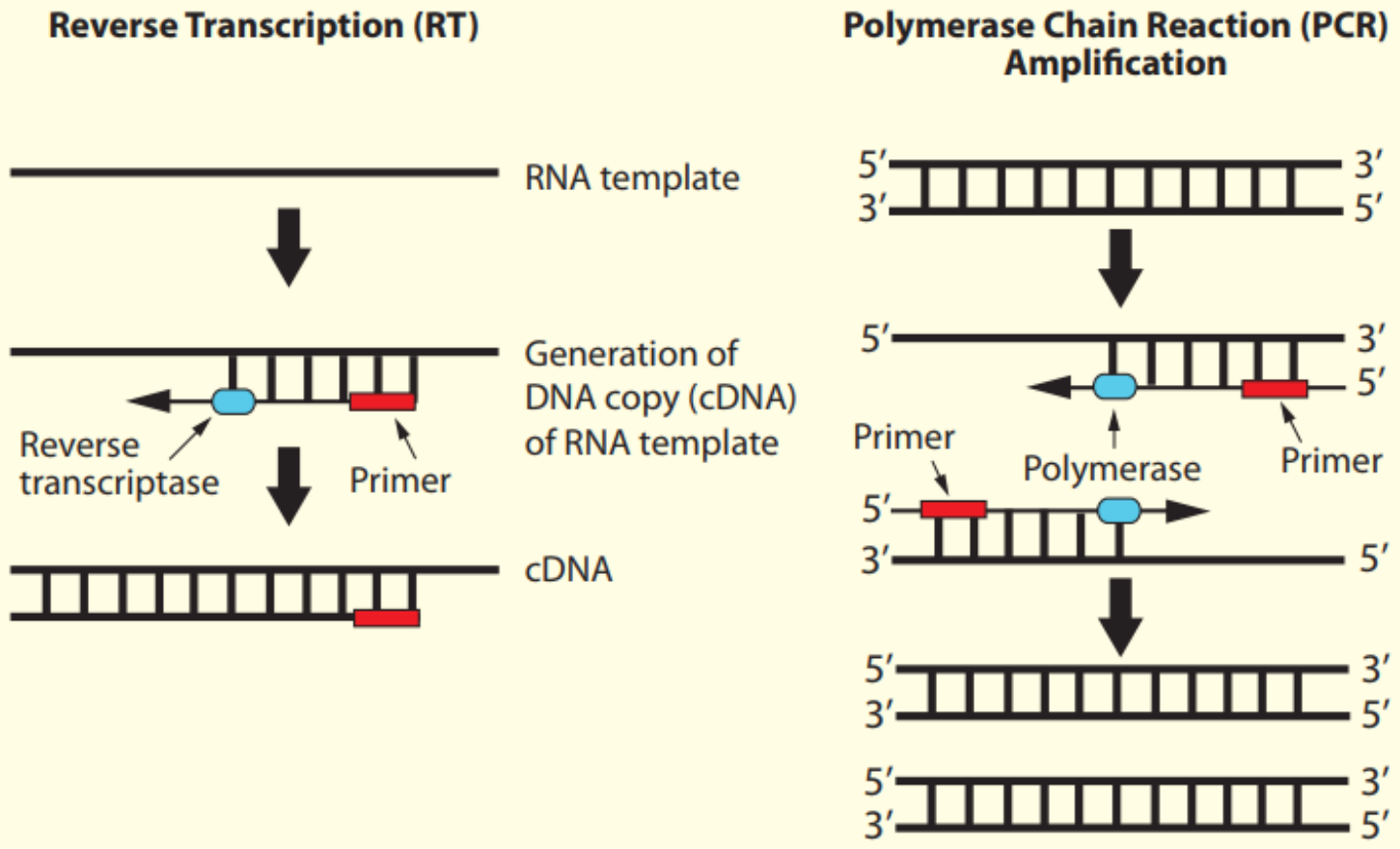
RT-PCR

- 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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