VIROLOGY LECTURE

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Laboratory Diagnosis Of Viral Infection

1. Detection of IgM-Ab in the patient serum, using EIA or IF, commercially diagnostic kits are available.

- IgM is a marker of recent infection.
- IgM is the first immunoglobulin that appears in circulation.
- It appears and peaks in the acute phase of the disease, persists for about 4-6 weeks then starts to decline and finally replaced by IgG.
- IgM is a pentamer-does not cross the placenta, therefore, it is not responsible for maternal immunity.
- IgG is a manomer, crosses the placenta and gives maternal immunity.
- IgG is a marker of immunity.
- IgG appears in circulation after IgM and persists for several years.
Laboratory Diagnosis Of Viral Infection (Continued)

2. Detection of viral-Ag in the patient specimen, using EIA or IF, commercially diagnostic kits are available.

Specimens:

- **Serum**
  - Example: HBsAg, HIV-Ag

- **Nasopharyngeal aspirate (NPA)**
  - Infants with LRT-infections
  - Viruses: RSV, parainfluenza, Influenza and adenoviruses

- **Stool**
  - Viral diarrhea
  - Rota, adenotypes 40 & 41, astroviruses
Laboratory Diagnosis Of Viral Infection (Continued)

- Scrapping from the base of the vesicles:
  - Viral vesicular rashes
  - HSV-1, HSV-2, Varicella-Zoster virus

- Conjunctival scrapping:
  - Viral conjunctivitis
  - HSV, Adenoviruses, Varicella-Zoster virus.
3. Detection of the viral-DNA in the patient specimen, using PCR.

- **PCR**
  - Automated technique, for amplification of specific sequence of DNA (target DNA), followed by detection of the amplified material.

- **Requirements for PCR:**
  - Extraction of the target DNA from the patient specimen.
  - Amplification (replication) of the target-DNA using thermal cycler.
  - All the nucleotides that form the newly strand.
  - Primers to initiate DNA-replication
  - Taq DNA polymerase (Heat stable), this enzyme is extracted from a bacterium known as Thermus aquaticus.
  - Buffer
Laboratory Diagnosis Of Viral Infection (Continued)

PCR Steps:

1. Denaturation, separation of the two strands, by heating at 91°C
2. Annealing of primers to initiate DNA-replication
4. Usually 32- cycles are required.

Results of PCR:

- Qualitative – PCR, results are expressed as positive or negative.
- Quantitative – PCR, results are expressed in the form of either.
  - Number of DNA-molecules/ml
  - International units/ml
RNA Viruses:

- Must be reverse transcribed to a complementary DNA strand, using the enzyme reverse transcriptase.

4. Isolation of the virus in tissue culture, followed by identification of the isolated virus.