LABORATORY DIAGNOSIS OF
VIRAL INFECTIONS

Methods of viral identification
## Viral (direct) diagnosis

<table>
<thead>
<tr>
<th>ISOLATION OF VIRUSES</th>
<th>Cell culture</th>
<th>Embryonated eggs</th>
<th>Laboratory animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPE</td>
<td>Pocks</td>
<td>Disease /death</td>
</tr>
<tr>
<td></td>
<td>Interference</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemadsorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIRAL IDENTIFICATION</td>
<td>Viral antigens (IF, ELISA)</td>
<td>Viral nucleic acid (hybridization, PCR)</td>
<td>Direct visualization of viral particles (EM)</td>
</tr>
</tbody>
</table>
Identification of viruses

- directly in clinical samples
- after isolation of viruses in the systems of living cells

Based on:

- viral morphology
- viral antigens
- viral nucleic acid
Viral Morphology

- Transmission electron microscopy (TEM)
  Magnification >500,000X
  2D-picture

- Scanning electron microscopy (SEM)
  Magnification 10,000-100,000X
  3D-picture
Viral Morphology

- size and shape of viral particle
- presence or no presence of viral envelope
- structure (symmetry) of capsid

VIRAL FAMILY
Viral Morphology

Adenoviridae

Ortomixoviridae

Papilomaviridae

Enteroviridae

Herpesviridae

Poxviridae
Viral Morphology

Identify the virus:

1. Shape and Size
2. Presence or absence of envelope
3. Capsid Symmetry (number of capsomers)

- Family Reoviridae (Rotavirus)
Viral Morphology

Limitation of EM - low sensitivity
- specimen need to contain at least $10^7$ virions/ml

EM in routine laboratory diagnosis of:
- viral gastroenteritis
  (Rotaviruses, Caliciviruses, Astroviruses, Adenoviruses)
- viral infections with vesicular rash
  (Herpesviruses, Poxviruses)
Viral Morphology

Caliciviruses

Astroviruses

Viral gastroenteritis
Viral antigens

Use of Antigen-Antibodies reactions:

- Immunofluorescence
- ELISA
- Latex (indirect) agglutination
- Radioimmunoassay (RIA)
- Complement fixation
Viral antigens

Immunofluorescence

Diagram:
- Direct method: Virus-specific Ab → Indicator → Viral antigen
- Indirect method: Anti-mouse Ab → Virus-specific Ab (mouse Mab) → Indicator → Viral antigen
Viral antigens

Immunofluorescence

Detection of Herpes simplex virus (HSV) antigens
Viral antigens

Example:

Rapid diagnosis of Herpes simplex virus (HSV) infection
Viral antigens

Rapid diagnosis of HSV infection

Incubation 30min at 37C

Washing

Results

Anti HSV1 Ab labeled with fluorescent color

Anti HSV2 Ab labeled with fluorescent color

- 

+
Techniques of molecular biology used in laboratory diagnosis of viral infections:

- In situ hybridization - DNA
- PCR (Polymerase chain reaction) - DNA
- RT-PCR (Reverse Transcriptase-PCR) - RNA
**Viral nucleic acid**

**In situ hybridization**

- **Denaturation** at 94°C
  - dsDNA → two ssDNA

- **Hybridization** at 37°C
  - ssDNA + labeled probe → hibrid DNA-probe
Viral nucleic acid

In situ hybridization

Detection of viral nucleic acid directly in:

- tissues
- cell smears
- histopathological preparations

Example: Diagnosis of Human papilloma virus (HPV) infection
In situ hybridization

- Cervical Cell smear
- DNA denaturation
- Labeled probe
- Hybridization
Viral nucleic acid

In situ hybridization

Negative

Positive

Diagnosis of Human papilloma virus (HPV) infection
Viral nucleic acid

PCR – Polymerase chain reaction

PCR – amplification of target sequence of DNA.
Viral nucleic acid

PCR: Polymerase Chain Reaction

30 - 40 cycles of 3 steps:

Step 1: denaturation
1 minute 94 °C

Step 2: annealing
45 seconds 54 °C
forward and reverse primers !!!

Step 3: extension
2 minutes 72 °C
only dNTP's

(Andy Viensrteis 1991)
Viral nucleic acid

PCR

The first 4 cycles of PCR in detail

- wanted gene
- template DNA

<table>
<thead>
<tr>
<th>cycle</th>
<th>1st cycle</th>
<th>2nd cycle</th>
<th>3rd cycle</th>
<th>4th cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of double strands with the right length:</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

(Andy Vierstraete 2001)
PCR method

First step:

DNA or RNA extraction from clinical samples
PCR method

Second step: PCR mix

PCR Reaction Components

• Water
• Buffer
• DNA template
• Primers
• Nucleotides
• Mg++ ions
• DNA Polymerase
Second step: PCR mix

<table>
<thead>
<tr>
<th>Component</th>
<th>1X</th>
<th>20X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile Water</td>
<td>38.0 ul</td>
<td>760 ul</td>
</tr>
<tr>
<td>10X PCR Buffer</td>
<td>5.0 ul</td>
<td>100 ul</td>
</tr>
<tr>
<td>MgCl2 (50mM)</td>
<td>2.5 ul</td>
<td>50 ul</td>
</tr>
<tr>
<td>dNTP’s (10mM each)</td>
<td>1.0 ul</td>
<td>20 ul</td>
</tr>
<tr>
<td>PrimerFWD (25 pmol/ul)</td>
<td>1.0 ul</td>
<td>20 ul</td>
</tr>
<tr>
<td>PrimerREV</td>
<td>1.0 ul</td>
<td>20 ul</td>
</tr>
<tr>
<td>DNA Polymerase</td>
<td>0.5 ul</td>
<td>10 ul</td>
</tr>
<tr>
<td>DNA Template</td>
<td>1.0 ul</td>
<td>--</td>
</tr>
<tr>
<td>Total Volume</td>
<td>50.0 ul</td>
<td>980 ul</td>
</tr>
</tbody>
</table>

Aliquot 49 ul

Add DNA as last step
Typical PCR protocol

1. Initial Denaturation  95 C  4 min
2. DNA Denaturation      95 C  1 min
3. Primer Annealing      65 C  1 min
4. Primer Extension      72 C  1 min
5. Go to step #2, repeat 29 more times
6. Hold at 4 C
7. End
PCR method

Third step: Gel electrophoresis

Detection and identification of amplified target DNA fragments based on molecular size
Viral nucleic acid

PCR

373 bp
Techniques of molecular biology are method of choice in laboratory diagnosis of:

- Viral infections of CNS
- Congenital viral infections
- Viral infections of immunocompromised patients (HIV, transplantation...)

Viral nucleic acid
Viral quantitation in vivo

• In peripheral blood

  “viral load”

* Number of copies of viral nucleic acid /ml

Molecular method used for viral quantitation

Ø Real time PCR
Real time PCR
Importance of quantitation of viral nucleic acid

Ø After transplantation (CMV, HSV, EBV, VZV, HHV-6...)

Ø HIV positive patients, before and during therapy

Ø HCV, HBV positive patients, during and after therapy
Problem no. 1:

A young man is admitted to the hospital with headaches, fever and vomiting. Physical examination showed neck rigidity and positive meningeal signs.

Infection of CNS?
Problem no. 1:

<table>
<thead>
<tr>
<th>CSF</th>
<th>Viral infection</th>
<th>Bacterial infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le</td>
<td>500-1000/ml</td>
<td>&lt; 60 000/ml</td>
</tr>
<tr>
<td></td>
<td>Mononuclear cells (Ly)</td>
<td>(usually 10 000) Neutrophils</td>
</tr>
<tr>
<td>Glucose</td>
<td>Normal</td>
<td>Low</td>
</tr>
<tr>
<td>Proteins</td>
<td>Normal</td>
<td>High</td>
</tr>
</tbody>
</table>

→ Viral infections of CNS
Viral nucleic acid

Problem no. 1:

Sample: CSF

Method of choice: PCR

Differencial diagnosis: Mumps, Enteroviruses (Echo, Polio), HSV, Rubella, CMV, rabies
Problem br. 1:

PCR products: Mumps ~ 250bp, CMV ~ 500bp

L - 100bp
1 - Negative control for Mumps
2 - Positive control for Mumps
3 - Sample (CSF)
4 - Negative control for CMV
5 - Positive control for CMV
6 - Sample (CSF)
Problem no. 2: Child 11 months old, with high temperature (higher than 39°C), vomitus, diarrhea, lassitude, dehydration and crying on abdominal palpation.

Infection of gastrointestinal tract?
Problem no. 2:

Sample: CSF

Method of choice: PCR

Differencial diagnosis: E. coli, Rotaviruses, Norwalk viruses, V. cholerae, G. Lamblia,........
Problem no. 2:

PCR products: Norwalk virus ~ 342bp, Rotavirus ~ 445bp

L - 100bp
1 - Negative control for Norwalk
2 - Positive control for Norwalk
3 - Sample (CSF) for Norwalk
4 - Negative control for Rotavirus
5 - Positive control for Rotavirus
6 - Sample (CSF) for Rotavirus