In the name of Allah, Most Gracious, Most Merciful.
Cell Cultures and Viral Diagnosis

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Some Diseases Caused by Viruses

- Rubella syndrome
- Brain inflammation
- Herpes Zoster
The laboratory diagnosis of viral infections has been considered for long time to have minimal clinical impact.

Why?

1. Some viral Diseases are too clear to be investigated e.g. Mumps, Measles and Herpes.
2. No specific antiviral agents are available.
3. Viral Diagnostic laboratories are very few.
Laboratory Diagnosis for Viral Infections

But since 1980s this concept was changed.

Why?

1. New antiviral agents became available.
2. New viral infections were recognized and

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Laboratory Diagnosis of Viral infections

1. Direct Examination of Specimen:
   Direct detection or visualization of the virus itself, its cytopathic effect in tissues, viral antigens or viral genome.

2. Indirect Examination:
   If the amount of the virus or the virus antigens are scanty in the specimen, amplification of the virus by culture is required.

3. Serology
Laboratory Diagnosis for Viral Infections

1. Direct Examination of Specimen:

A. Electron Microscopy or immune electron microscopy to detect the virus particles

B. Light microscopy to confirm histological appearance or detect inclusion bodies

C. Direct Antigen detection IF or ELISA.

D. Molecular techniques (PCR) for the direct detection of viral genomes.
EM is mainly used for the diagnosis of viral gastroenteritis by detecting viruses in faeces e.g. rotavirus, astrovirus, and for the detection of viruses in vesicles and other skin lesions, such as herpes viruses and papilloma viruses.

The sensitivity and specificity of EM may be enhanced by immune electron microscopy, whereby virus specific antibody is used to agglutinate virus particles together making them easier to recognize.
Electron Microscopy (EM)

Rota viruses
The main problem with EM is the expense involved in purchasing and maintaining the facility.

In addition, the sensitivity of EM is often poor, with at least $10^5$ to $10^6$ virus particles per ml in the sample required for visualization.

Therefore the observer must be highly skilled.
Electron Microscopy (EM)

Astroviruses
Replicating virus often produce histological changes in infected cells that may be characteristic or non-specific.

Detection of viral inclusion bodies (collections of replicating virus particles in the nucleus or cytoplasm) may also be diagnostic.

Examples of inclusion bodies include the Negri bodies and cytomegalic inclusion bodies found in rabies and CMV infections respectively.

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Light Microscopy

Cytomegalic inclusion bodies
In general, the majority of common viral infections can be diagnosed by serology. Serological diagnosis of many viral infections is hampered by the wide spread of subclinical or old infections and also by the antibodies made in response to vaccinations. A serological diagnosis of a viral infection can be made by detection of IgM, seroconversion or the detection of rising titers of antibody between acute and convalescent stages of infection.
Serological Laboratory Diagnosis

Presence of IgM:
- This offers a rapid means of diagnosis.
- However, there are many problems with IgM assays, such as
  a. interference by rheumatoid factor,
  b. re-infection by the virus, and
  c. unexplained persistence of IgM years after the primary infection.
Serological Laboratory Diagnosis

A single high titer of IgG (or total antibody):

- This is a very unreliable means of serological diagnosis since the cut-off is very difficult to define.

Seroconversion:

- This is defined as changing from a previously antibody negative state to a positive state e.g. seroconversion against HIV following a needle-stick injury, or against rubella following contact with a known case.
A significant rise in titer of IgG antibody: A significant rise is very difficult to define and depends greatly on the assay used. In the case of CFT and HAI, it is normally taken as a four-fold or greater increase in titer. The main problem is that diagnosis is usually retrospective because by the time the convalescent serum is taken, the patient had probably recovered.
Ernest William Goodpasture, together with colleagues, invented methods for growing viruses in chicken embryos and fertilized chicken eggs.

Alexis Carrel (Nobel Prize in 1912) began growing bits of chick heart in drops of horse plasma. He placed tissue cultured from an embryonic chicken heart in a stoppered Pyrex flask of his own design, with regular supplies of nutrient.
The Embryonated egg

- Shell membrane
- Air sac
- Egg shell
- Allantoic fluid
- Amniotic fluid
- Embryo
- Yolk
- Albumen
Eggs with embryo aged 10-days are usually used.
Viability should be checked before and after injection.
Injection should be away from the major blood vessels.
After 3 days incubation the egg is frozen before dissection to avoid living moving embryo.
The cells and extraembryonic membranes of the chicken embryo provide varied substrates that allow the growth of many viruses.
The Embryonated egg

Growth of virus on embryonated eggs

Chorioallantoic membrane inoculation
(chorionic epithelium)

Amniotic cavity
Shell
Albumin
Shell membrane
Air sac
Amniotic inoculation
(amniotic epithelium)
Yolk sac inoculation
(yolk sac lining)
Allantoic inoculation
(allantoic epithelium)

Yolk sac
Allantoic cavity
Chorioallantoic membrane

Davis, Duylbeoco, Eisen, Ginsberg "Microbiology" 4th ed, J.B. Lippincott 1990, Fig. 48-1
Cell Cultures
Cell Cultures for viral diagnosis

A variety of cell lines can be used for virus isolation.

The choice of the cell line depends on:

1. The sensitivity of the cells to a particular virus
2. Distinctiveness of the CPE produced in these cells
3. Cell growth characteristics and
4. The relative cost to grow and maintain the cells in culture.
Specimen collection is the foundation upon which all other procedures are built.

Collection of (the correct specimen, by an appropriate method, at the proper time during infection) will enhance and improve the clinical relevance of the laboratory results.

Without good specimen collection, even the best virus isolation procedure is worthless.
Collecting samples for Virology

1- Body Fluids:

- CSF, pleural fluids, pericardial fluids and other body fluids should be collected in a sterile container.

- Most body fluids contain enough protein to stabilize viruses, therefore no need for viral transport medium.

- Specimens should be sent on wet ice (do not freeze at -20 C). It may be stored at 2-8 C for up to 24 hours. If longer delay is anticipated, the specimen must be frozen at -70 C.

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Collecting samples for Virology

2. Swabs:
   a. Nasopharyngeal swabs: for URTI
   b. Throat swabs: for URTI and other viruses
   c. Conjunctival swabs: for ocular infections
   d. Rectal swabs.

- Dacron or cotton swabs (sometimes pre-wet) are used.

- Place the swab in viral transport medium and cut the shaft so that the swab fits into the tube.

- Send to the laboratory as soon as you can on wet ice (do not freeze).
Collecting samples for Virology

3- Others

a. Sputum or BAL
b. Semen
c. Vesicular fluid
d. Urine
e. Stool
f. Tissue

Can be collected on Viral transport medium (1:5 dilution when applicable).

Send as soon as you can on wet ice.
Specimens should be placed in secure plastic bags and labeled.

Request forms must include:

1. Patient details (hospital number, date of birth)
2. Date of onset of illness
3. Date of specimen collection
4. Suspected clinical diagnosis
5. Tests required.
After arrival to the Laboratory register the sample and give it a code number.

Vortex swabs or tubes containing samples vigorously to disrupt cells.

Centrifuge at 1000 – 1500 x g to sediment cells.

Supernatants may contain the cell-free viruses and can be taken in sterile tubes and used or frozen to – 70.

Cells in the deposit may be used for direct IF.
Cell Cultures for viral diagnosis
Cell Cultures for viral diagnosis

General considerations:

1. Decide the type of the cells to be used for any specimen.
2. Be ready by your cells (enough amounts in the suitable flasks, tubes or plates and in the suitable stage).
3. Frozen cells can not be used immediately after thawing, they need at least one cycle of fresh replication.
4. Never use unhealthy cells.
5. Add the specimen when cells are in the best growing conditions.

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If you are expecting long incubation time use flasks or tubes.

If you are expecting short incubation or you are comparing factors (e.g. effect of some agent) use plates with the number of wells enough to make all the experiment in one plate.

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Cell Cultures for viral diagnosis

Use cells in the semi confluent condition (log phase of the growth curve).
Cell Cultures for viral diagnosis

1. Frozen samples are brought to the room temp.
2. Examine your cells and use the most suitable one.
3. Label the tubes or the wells of the plate correctly.
4. Remove the growth medium and add 100 – 200 μl of the sample under aseptic conditions.
5. Leave 1 – 2 hr to allow virus adsorption at 37°C.
6. You may (or may not) remove the sample after that time and add the suitable amount of the growth medium.
7. Incubate at 37°C in humidified CO2 adjusted incubators.

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The next day (never cultivate before week ends) examine cells for contamination.

If cells are doing well, keep examining daily or as frequent as you can for the expected CPE.

Rapid cell death without characteristic CPE is usually nonspecific and may be due to contamination or toxicity.
Cytopathic Effect (CPE)

CPE: vaccinia on monkey kidney (BSC40)

Low multiplicity of infection (moi) single plaque

High moi, 48 hr
<table>
<thead>
<tr>
<th></th>
<th>CI #10</th>
<th>CI #14</th>
<th>CI #17</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(R5/X4 strain)</td>
<td>(R5 strain)</td>
<td>(X4 strain)</td>
</tr>
<tr>
<td>Negative Control</td>
<td><img src="negative-control_10.png" alt="Image" /></td>
<td><img src="negative-control_14.png" alt="Image" /></td>
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<tr>
<td>AMD3100 1 μg/ml</td>
<td><img src="amd3100_10.png" alt="Image" /></td>
<td><img src="amd3100_14.png" alt="Image" /></td>
<td><img src="amd3100_17.png" alt="Image" /></td>
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<td><img src="sch-c_17.png" alt="Image" /></td>
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<tr>
<td>AMD3100 + SCH-C 1 μg/ml / 1 μg/ml</td>
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<td><img src="amd3100+sch-c_14.png" alt="Image" /></td>
<td><img src="amd3100+sch-c_17.png" alt="Image" /></td>
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Figure 1. Measles cytopathic effect (CPE) in B95a cells. Top photo shows uninfected B95a
Cytopathic Effect (CPE) may be specific or non-specific e.g. CMV, HIV produces a specific CPE (syncytium formation and giant cells), whereas enteroviruses do not.

Confirmation of the identity of the virus may be carried out using neutralization, hemadsorption, IF, or molecular tests.
Hemadsorption

[Diagram showing the process of hemadsorption, with an initial interaction, addition of red blood cells, and resulting adsorption event]
# Neutralization Test

<table>
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<tr>
<th>Patient serum (dilution)</th>
<th>0</th>
<th>0</th>
<th>1/1000</th>
<th>1/100</th>
<th>1/10</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus concentration</td>
<td>0</td>
<td>5000 pfu</td>
<td>5000 pfu</td>
<td>5000 pfu</td>
<td>5000 pfu</td>
<td>5000 pfu</td>
</tr>
</tbody>
</table>

**Virus concentration**

<table>
<thead>
<tr>
<th>CELL CULTURE serum/virus mixture</th>
<th>No virus</th>
<th>CPE</th>
<th>CPE</th>
<th>No CPE</th>
<th>No CPE</th>
<th>No CPE</th>
</tr>
</thead>
</table>

**Infection**

- Hemagglutination

**Neutralization**

- Hemagglutination inhibition
Immunofluorescence
Polymerase Chain Reaction (PCR)
The main problem with cell culture is the long period (up to 4 weeks) required for a result to be available. Also, the sensitivity is often poor and depends on many factors, such as the condition of the specimen, and the condition of the cell sheet. Cell cultures are also susceptible to toxic and bacterial contamination from the specimen. Lastly, many viruses will not grow in cell culture at all e.g. HBV, HCV and parvovirus.
Rapid culture techniques are available whereby viral antigens are detected 2 to 4 days after inoculation. Examples of rapid culture techniques include shell vial cultures.

The shell vial technique is a variation on standard tissue culture in that it takes advantage of using a living cell system and enhances viral recovery by centrifuging the clinical sample onto the monolayer.
In this technique a small bottle (vial) with a removable round glass cover slip is used to grow the cells as a monolayer on the cover slip.
Although the vials can be kept until CPE occurs the CPE can not be seen unless the cover slip is removed from the vial.

Usually it is possible, using this technique, to identify the presence of a virus before CPE occurs.

The cover slip can be removed, fixed and used for IF without trypsinization which is usually destroying outer protein antigens.
The shell vial

Advantages of shell vials:
- They are fast, easy to do, no skill in looking for CPE necessary.
- It is also quite economical.

Disadvantages of shell vials:
- Some viruses may require longer incubation to adapt to the particular cell lines.
- Once used, the monolayer can not be re-incubated or re-inoculated as the fixed cells and viruses are dead.

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Thank You
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2010