# VIROLOGY

# Virus cultivation and assay 1

#### Why do we need to grow viruses?



Research

# Virus cultivation: critical issues

Viruses cannot grow outside a living cell

➤The range of cell types in which many of them replicate is limited

➤A few cannot be grown in the lab at all

# Development of host systems suitable for virus cultivation



#### Laboratory animals



Cell cultures

Embryonated chicken eggs



#### Laboratory animals in Virology



Image: State of the state

Pathogenesis studies

Vaccine development

# Laboratory animals give unique insight into virus pathogenesis



From Lembo et al., J. Virol., 78, 2004

### The first revolution in Animal Virology:

# 1932 - Introduction of methods for cultivating viruses in fertilized chicken eggs



#### E.W. Goodpasture

# Virus cultivation: Embryonated eggs



Vaccine production



Pathogenesis studies

### Embryonated eggs: inoculation





Embryonated eggs at 10 to 12 days being inoculated by automated machinery. 1st larger needle (about 1 mm diameter) punches a hole in a shell and 2nd smaller needle injects a seed into the allantoic cavity of the egg followed by incubation for 2 to 3 days. It takes less than 10 seconds to inoculate a row of eggs. Courtesy: Solvay





The most common way that flu vaccines are made is using an egg-based manufacturing process



### Embryonated eggs: CAM's pocks



-NBT

+NBT

#### The second revolution in Animal Virology:

1949 - The development of methods for cultivating viruses in *in vitro* cell cultures







J. F. Enders T. H. Weller F. C. Robbins



#### The Nobel Prize in Physiology or Medicine 1954

"for their discovery of the ability of poliomyelitis viruses to grow in cultures of various types of tissue" JOHNF. ENDERS, FREDERICKC. ROBBINS, THOMASH. WELLER

# The cultivation of the poliomyelitis viruses in tissue culture

Nobel Lecture, December 11, 1954



Fig. 1. Mouse infectivity of pools of fluids removed at four-day intervals from suspended cell cultures of human embryonic skin-muscle tissue inoculated with Lansing mouse-brain virus. (From J. Immunol., 69 (1952) 652.)

### Virus cultivation: Cell cultures



# Diagnosis of infections

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Research



#### Production of antigens

# Cell cultures in Virology



#### Primary cell cultures



Diploid cell strain





Continuous cell lines

| Culture type | Examples                  | Virus supported  |  |  |  |  |  |  |
|--------------|---------------------------|--|--|--|--|--|--|--|
| Primary      | Monkey kidney             | Influenza virus, Paramyxovirus, Enterovirus                |  |  |  |  |  |  |
|              | Rabbit kidney             | HSV  |  |  |  |  |  |  |
|              | Human embryonic<br>kidney | Adenovirus, Enterovirus                                    |  |  |  |  |  |  |
| Diploid      | HELF, MRC-5               | CMV, HSV, VZV, Adenovirus, RSV,<br>Rhinovirus, Enterovirus |  |  |  |  |  |  |
| Continuous   | Hep-2                     | RSV, HSV, Adenovirus, Paramyxovirus,<br>Enterovirus        |  |  |  |  |  |  |
|              | A459                      | HSV, Adenovirus, Enterovirus                               |  |  |  |  |  |  |
|              | MDCK                      | Influenza virus  |  |  |  |  |  |  |
|              | LLC-MK2                   | Enterovirus, Paramyxovirus                                 |  |  |  |  |  |  |
|              | RD                        | Enteroviruses, HSV   |  |  |  |  |  |  |
|              | BGMK                      | Coxsackievirus   |  |  |  |  |  |  |
|              | Vero                      | HSV, Paramyxovirus, Coxsackievirus                         |  |  |  |  |  |  |

| W.VIROMED.COM                   | Adenovirus                  | Coxsackie A               | Coxsackie B | Cytomegalovirus | Echovirus | Herpes simplex | Influenza A,B | Measles   | Mumps | Parainfluenza | Polio | Rhinovirus  | RSV | Rubella    |                         |
|---------------------------------|-----------------------------|---------------------------|-------------|-----------------|-----------|----------------|---------------|-----------|-------|---------------|-------|---|-----|------------|-------------------------|
| Rabbit kidney                   |                             |                           |             |                 |           | •              |               |           |       |               |       |   |     |            | State State State State |
| Rhesus monkey kidney            |                             | •                         | •           | 1               | •         |                | •             | •         | •     | •             | •     |   |     |            |                         |
| SERIALLY PROPAGATED CELL CULTUR | ES                          |                           |             |                 |           |                |               |           |       |               |       |   |     |            |                         |
| A549                            | •                           |                           |             |                 |           | •              |               |           |       |               |       |   |     |            | SECONDERVISE OF         |
| BGMK                            |                             |                           | •           |                 |           |                |               |           |       |               | •     |   |     |            |                         |
| H292                            |                             |                           |             |                 |           |                |               |           |       |               |       |   |     |            |                         |
| HEK293                          |                             |                           |             |                 |           |                |               |           |       |               |       |   |     |            |                         |
| HeLa                            | •                           |                           | •           |                 |           | •              |               |           |       |               | •     |   | •   |            | STOLEN STOLE            |
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| MDCK                            |                             |                           |             | 1000            |           |                | •             |           |       | 1000          |       | Sine and  |     |            | 100                     |
| Mink lung                       |                             |                           |             |                 |           | •              |               |           |       |               |       |   |     |            | Silling and and and     |
| MRC-5                           | •                           | •                         |             | •               | •         | •              |               | 1995      |       |               | •     | •   | •   |            |                         |
| RD                              |                             | •                         |             |                 |           | •              |               |           |       |               | •     |   |     |            | 30.00 M 0 2 2 2 2 2     |
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| Vero                            |                             |                           | •           | 10.97 B         |           | •              |               | 24 . J.A. | •     |               | •     |   |     |            |                         |
| WI-38                           | •                           | •                         |             |                 | ٠         | •              |               |           |       |               | •     | •   | •   | 1          |                         |



> The aim of the experiment

Limitations in the in vitro host range

Ease of alternative possibile procedures

#### How to chose the appropriate cell culture system?



How to study the viruses that have proved difficult to propagate in cell culture?

An example: the organotypic culture system approach to study in vitro the HPV replicative cycle

### Replication cycle of papillomavirus



Nature Reviews | Cancer

# Organotypic epithelial raft cultures



Preparation of organotypic epithelial raft cultures from dispersed cells or tissue biopsy explants.



# HPV growth in raft cultures



#### Morphology of HPV45 cell line grown in raft culture

From McLaughlin-Drubin et al., Virology 312, 2003

#### HPV growth in raft cultures



Positive HPV16 L1 staining in a fully stratified and differentiated epithelial raft culture tissue



Co-cultures of primary human keratinocytes and HPV-positive cells to evaluate the selectivity of anti-HPV agents

# Recognition of Viral Growth in Culture



Cytopathic effect



**Inclusion** bodies



Hemadsorption

# Virus cultivation: The Cytopathic Effect

# The simplest and most widely used criterion for infection

# Examples of CPE

| Cytopathic effect(s)                                    | Virus(es)   |  |  |  |  |  |
|---|---|--|--|--|--|--|
| Morphological alterations                               |   |  |  |  |  |  |
| Nuclear shrinking (pyknosis), proliferation of membrane | Picomaviruses   |  |  |  |  |  |
| Proliferation of nuclear membrane                       | Alphaviruses, herpesviruses                                   |  |  |  |  |  |
| Vacuoles in cytoplasm                                   | Papovaviruses   |  |  |  |  |  |
| Syncytia (cell fusion)                                  | Paramyxoviruses, coronaviruses                                |  |  |  |  |  |
| Margination and breaking of<br>chromosomes              | Herpesviruses   |  |  |  |  |  |
| Rounding up and detachment<br>of tissue culture cells   | Herpesviruses, rhabdoviruses,<br>adenoviruses, picornaviruses |  |  |  |  |  |

#### Viral CPE: cell rounding, detachment and lysis



# Herpes Simplex Virus CPE in Vero cells



#### Measle Virus CPE in B95a cells



# CPE: cell rounding and size increase



HCMV cytopathic effect on a fibroblast monolayer

#### HCMV cytopathic effect on a fibroblast monolayer



# Human metapneumovirus CPE



# Virus CPE: syncythium formation



В





### Virus CPE: syncythium formation



Formation of giant multinuclear cells (syncytium) by measles virus infection

# Virus CPE: syncythium formation



Formation of giant multinuclear cells (syncytium) by RSV infection

# Inclusion bodies

| Inclusion bodies                                |              |
|---|--------------|
| Virions in nucleus                              | Adenoviruses |
| Virions in the cytoplasm<br>(Negri bodies)      | Rabies virus |
| "Factories" in the cytoplasm (Guarnieri bodies) | Poxviruses   |
| Clumps of ribosomes in virions                  | Arenaviruses |
| Clumps of chromatin in nucleus                  | Herpesvir    |

### Inclusion bodies: Pox and Rhabdo



#### Purkinje cell with Negri body in the cytoplasm

#### Monkey kidney cell with Guarnieri body in the cytoplasm



#### Inclusion bodies: HCMV



Human Cytomegalovirus infection of a lung pneumocyte, showing owl's eye appearance of a large cell at center.



The photomicrograph shows a section of kidney taken at autopsy from a three-month-old boy who died of disseminated HCMV infection contracted in utero. A single periglomerular renal tubule contains large, intranuclear viral inclusion bodies typical of those found in cells infected with cytomegalovirus. Such inclusion bodies are commonly seen at autopsy or in biopsy specimens from the kidneys, lungs, and other organs in cases of congenital or acquired cytomegalovirus infection.

Herriot R, Gray ES. N Engl J Med 1994;331:649-649.

# Hemadsorption



Red blood cells attach specifically to virus-infected cells