Methods in virus diagnosis Cell culture and virus cultivation

450MBIO

Practical lesson -4

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Indirect Examination

1. Animals	disease or death
2. Eggs	pocks on CAM haemagglutination inclusion bodies
3.Cell Culture	cytopathic effect (CPE) haemabsorption immunofluorescence

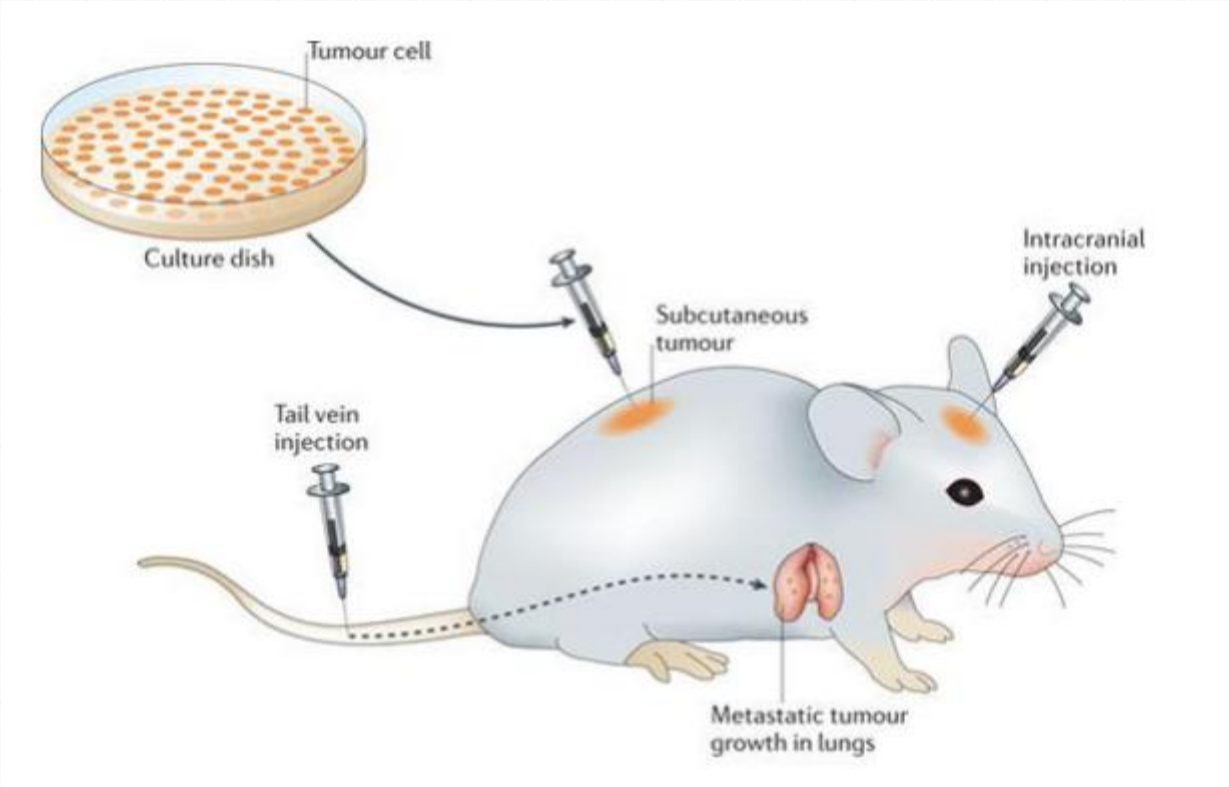
Methods for Cultivation of Virus

Since the viruses are obligate intracellular parasites, they cannot be grown on any inanimate culture medium. Viruses can be cultivated within suitable hosts, such as a living cell. Generally three methods are employed for the virus cultivation.

1. Inoculation of virus into animals.
2. Inoculation of virus into embryonated eggs.
3. Tissue culture

Inoculation of Virus in Animals

- Laboratory animals are widely used for routine cultivation of virus; they play an essential role in studies of viral pathogenesis.
- Live animals such as monkeys, mice, rabbits, guinea pigs, ferrets are widely used for cultivating virus.
- Monkeys were used for the isolation of Poliovirus. But due to their risk to handlers, monkeys find only limited applications in Virology.
- Mice are the most widely employed animals in virology. The different routes of inoculation in mice are intracerebral, subcutaneous, intraperitoneal or intranasal.
- After the animal is inoculated with the virus suspension, the animal is observed for signs of disease, visible lesions or is killed so that infected tissues can be examined for virus.


Tumour cell
Culture dish
Intracranial injection
Subcutaneous tumour
Tail vein injection
Metastatic tumour growth in lungs

Advantages

1. Animal inoculation may be used as diagnostic procedure for identifying and isolating a virus from a clinical specimen.
2. Mice provide a reliable model for studying viral replication.
3. Gives unique insight into viral pathogenesis and host virus relation.
4. Used for the study of immune responses, epidemiology and oncogenesis.

Disadvantages

- Expensive and difficulties in maintenance of animals.
- Difficulty in choosing of animals for particular virus.
- Some human viruses cannot be grown in animals,or can be grown but do not cause disease.
- Mice do not provide models for vaccine development.
- It will lead to generation of escape mutants.
- Issues related to animal welfare systems.

Eggs

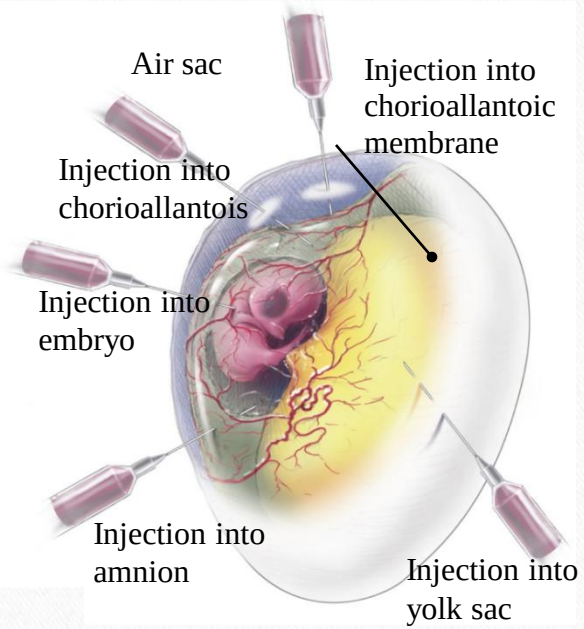
Chicken embryo technique

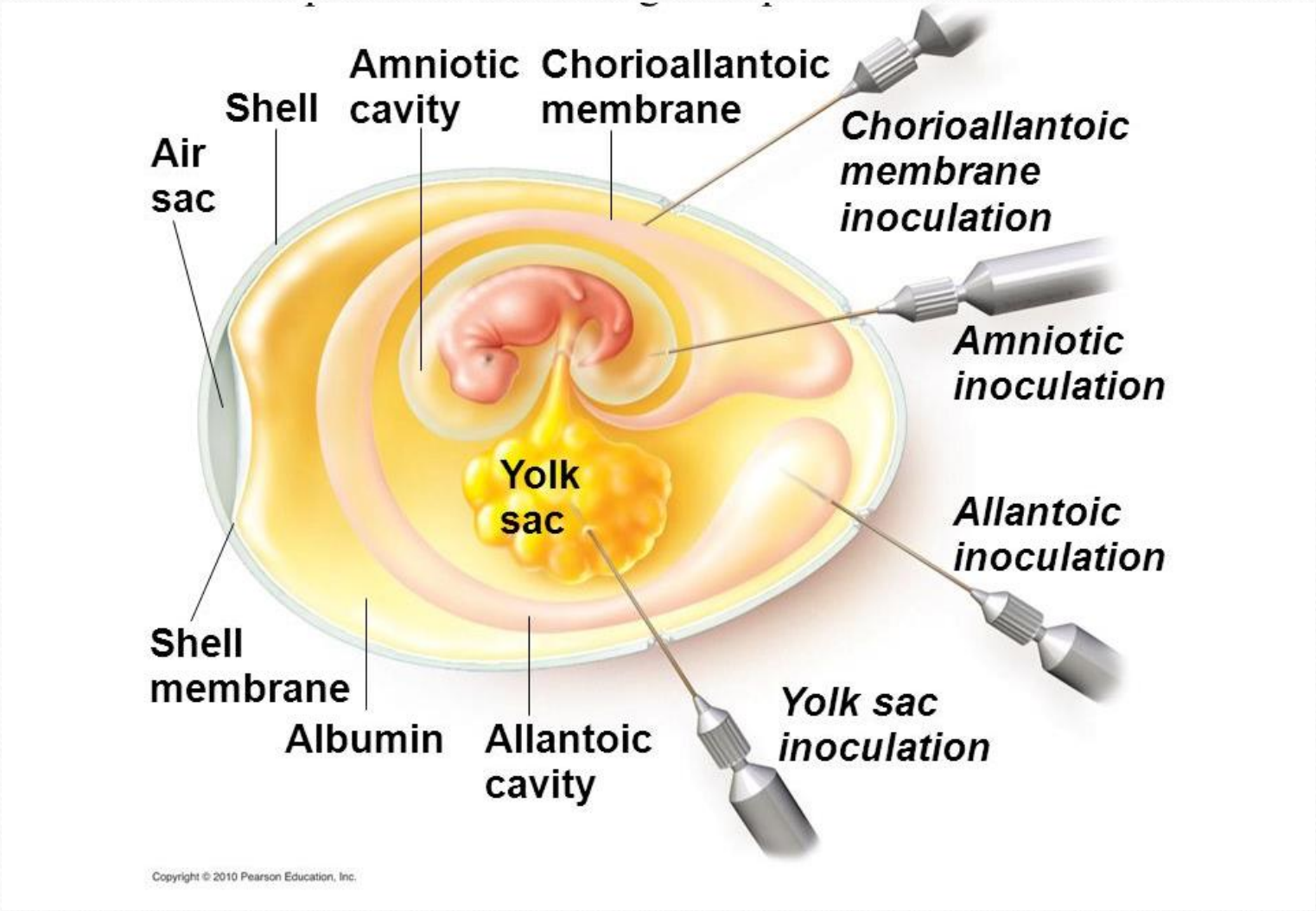
Chicken embryo technique

•Prior to the advent of cell culture, animal viruses could be propagated only on whole animals or embryonated chicken eggs.
•Good pasture in 1931first used the embryonated hen's egg for the cultivation of virus.
• The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used. The egg used for cultivation must be sterile and the shell should be intact and healthy.
•A hole is drilled in the shell of the embryonated egg, and a viral suspension or suspected virus- containing tissue is injected into the fluid of the egg. Viral growth and multiplication in the egg embryo is indicated by the death of the embryo, by embryo cell damage, or by the formation of typical pocks or lesions on the egg membranes

Chicken embryo technique

- An embryonated egg offers various sites for the cultivation of viruses. The different sites of viral inoculation in embryonated eggs are:
- Chorioallantoic membrane(CAM).
- Amniotic Cavity.
- Allantoic Cavity.
- Yolk sac.
- Embryo.
- Air sac




Amniotic cavity
Chorioallantoic membrane
Shell
Air sac
Chorioallantoic membrane inoculation
Amniotic inoculation
Yolk sac
Allantoic inoculation
Shell membrane
Albumin
Allantoic cavity
Yolk sac inoculation
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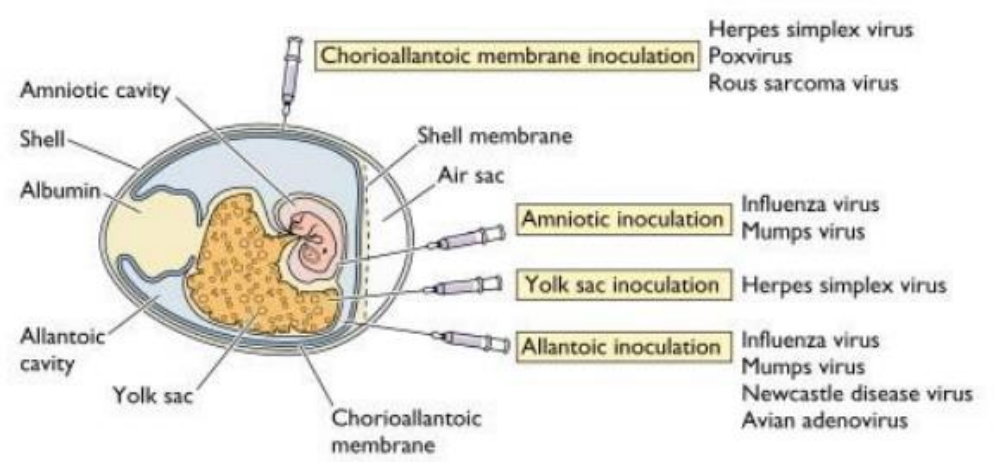
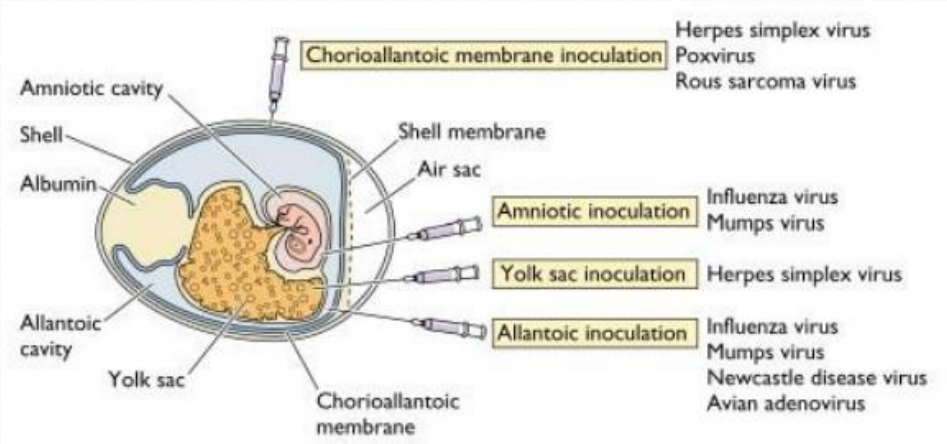
Chorioallantoic Membrane(CAM):

Is mainly employed in the growth of poxvirus. Virus growth and replication in the CAM is indicated by visible lesions (pocks); grey white area in transparent CAM. Herpes simplex virus is also grown. Each pock is derived from a single virion. The morphology of the pocks may vary depending on the nature of the virus. Under optimal conditions, each infectious virus particle can form one pock. Hence this method is suitable for plaque studies. Herpes simplex virus can also be inoculated via CAM.



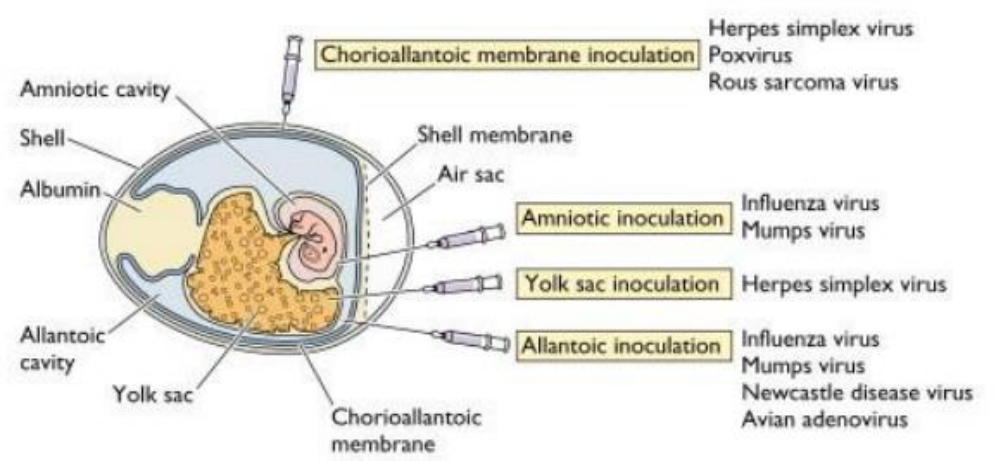
Allantoic Cavity

Is the most popular and simple method for viral inoculation. Allantoic inoculation is employed for the growth and replication of the influenza virus for vaccine production. This will provide a rich yield of influenza and some paramyxoviruses. Other allantoic vaccines include Yellow fever and rabies vaccines. Duck eggs provide a better yield of rabies virus and were used for the preparation of the inactivated non-neural rabies vaccines. But they need a longer incubation period than embryonated hen's egg. Most of avian viruses can be isolated using this method.



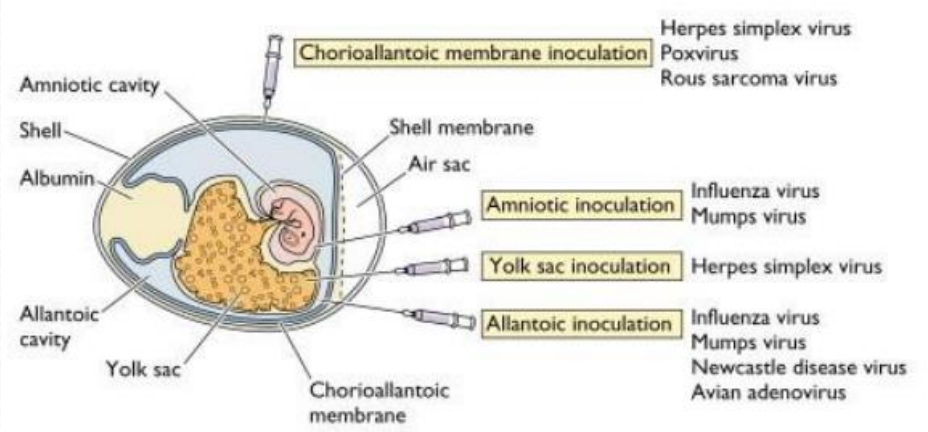
Amniotic Cavity:

The amniotic sac is employed inoculated for primary isolation of influenza a virus and the mumps virus. Growth and replication of virus in egg embryo can be detected by haemagglutination assay.



Yolk Sac:

- It is also a simplest method for growth and multiplication of virus. Mostly mammalian viruses are isolated using this method. Immune interference mechanism can be detected in most of avian viruses. This method is also used for the cultivation of some bacteria like Chlamydiae and Rickettsiae.



Advantages & Disadvantages for Chicken embryo technique

Advantages:

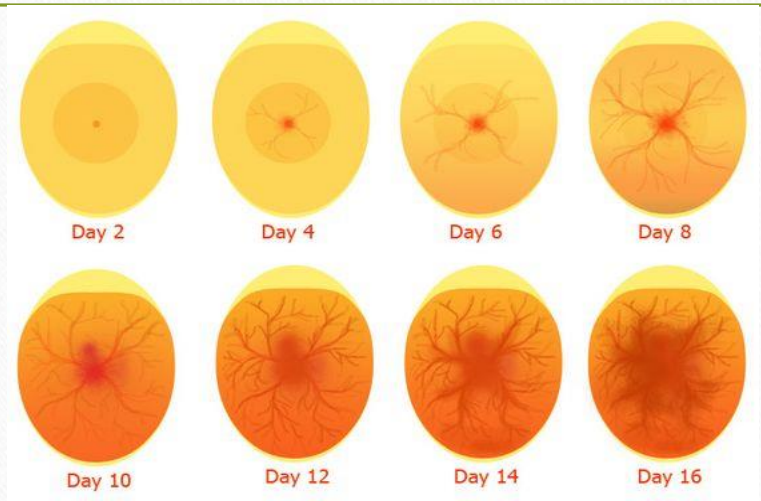
- Widely used method for the isolation of virus and growth (grow virus for some vaccine production)
- Ideal substrate for the viral growth and replication.
- Isolation and cultivation of many avian and few mammalian viruses.
- Cost effective and maintenance is much easier.
- Less labor is needed.
- The embryonated eggs are readily available.
- Sterile and wide range of tissues and fluids
- They are free from contaminating bacteria and many latent viruses.
- Specific and non specific factors of defense are not involved in embryonated eggs.

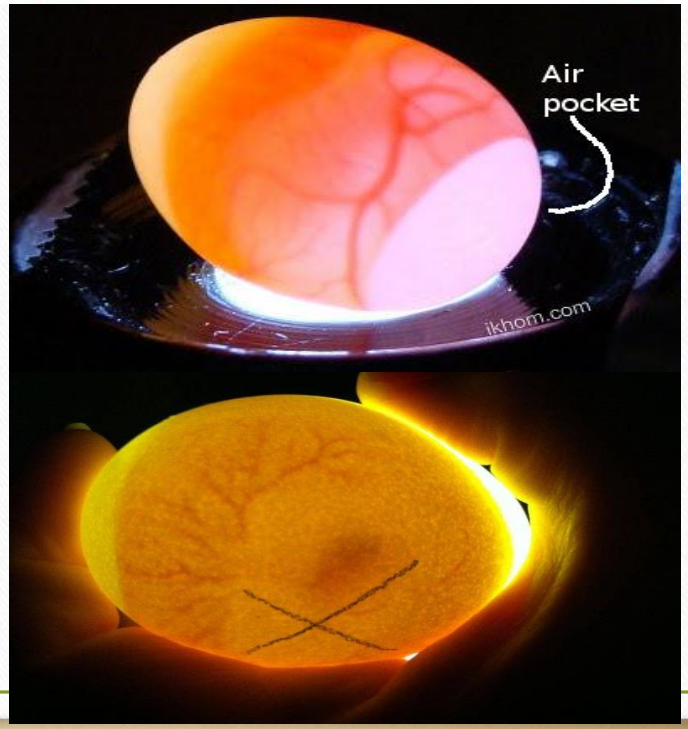
Disadvantages:

The site of inoculation for varies with different virus. That is, each virus have different sites for their growth and replication.

procedure :

For propagation of influenza virus, pathogen-free eggs are used 11-12 days after fertilization.The egg is placed in front of a light source to locate a non-veined area of the allantoic cavity just below the air sac. This is marked with a pencil. After all the eggs have been ‘candled’ in this way, a small nick is made in the shell at this position using a jeweler’s scribe.



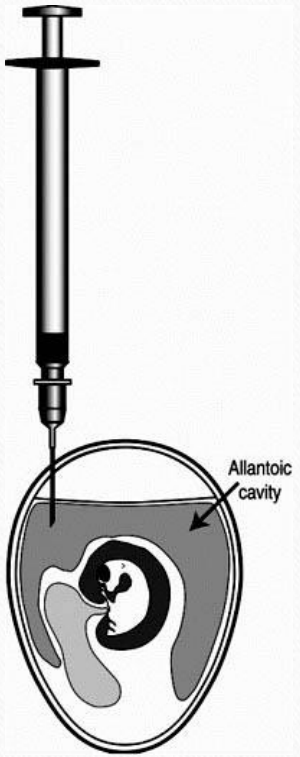


procedure :

Next, a hole is drilled at the top of the egg with a Dremel motorized tool. If this is not done, when virus is injected, the pressure in the air sac will simply force out the inoculum.

procedure :

After all the eggs have been nicked and drilled, they are inoculated with virus using a tuberculin syringe – a 1 ml syringe fitted with a 1/2 inch, 27 gauge needle. The needle passes through the hole in the shell, through the chorioallantoic membrane, and the virus is placed in the allantoic cavity, which is filled with allantoic fluid. The two holes in the shell are sealed with melted paraffin, and the eggs are placed at 37 degrees C for 48 hours



Cell Cultures

Virus Isolation

Cell Cultures are most widely used for virus isolation, there are 3 types of cell cultures:

1. Primary cells - Monkey Kidney
2. Semi-continuous cells - Human embryonic kidney and skin fibroblasts
3. Continuous cells - HeLa, Vero, Hep2, LLC-MK2, MDCK

Primary cell culture are widely acknowledged as the best cell culture systems available since they support the widest range of viruses. However, they are very expensive and it is often difficult to obtain a reliable supply. Continuous cells are the most easy to handle but the range of viruses supported is often limited.

Cell Cultures

Growing virus may produce

1. Cytopathic Effect (CPE) - such as the ballooning of cells or syncytia formation, may be specific or non-specific.

2. Haemadsorption - cells acquire the ability to stick to mammalian red blood cells.

Cultivation of virus used to vaccine production, also for study there infectious cycle.

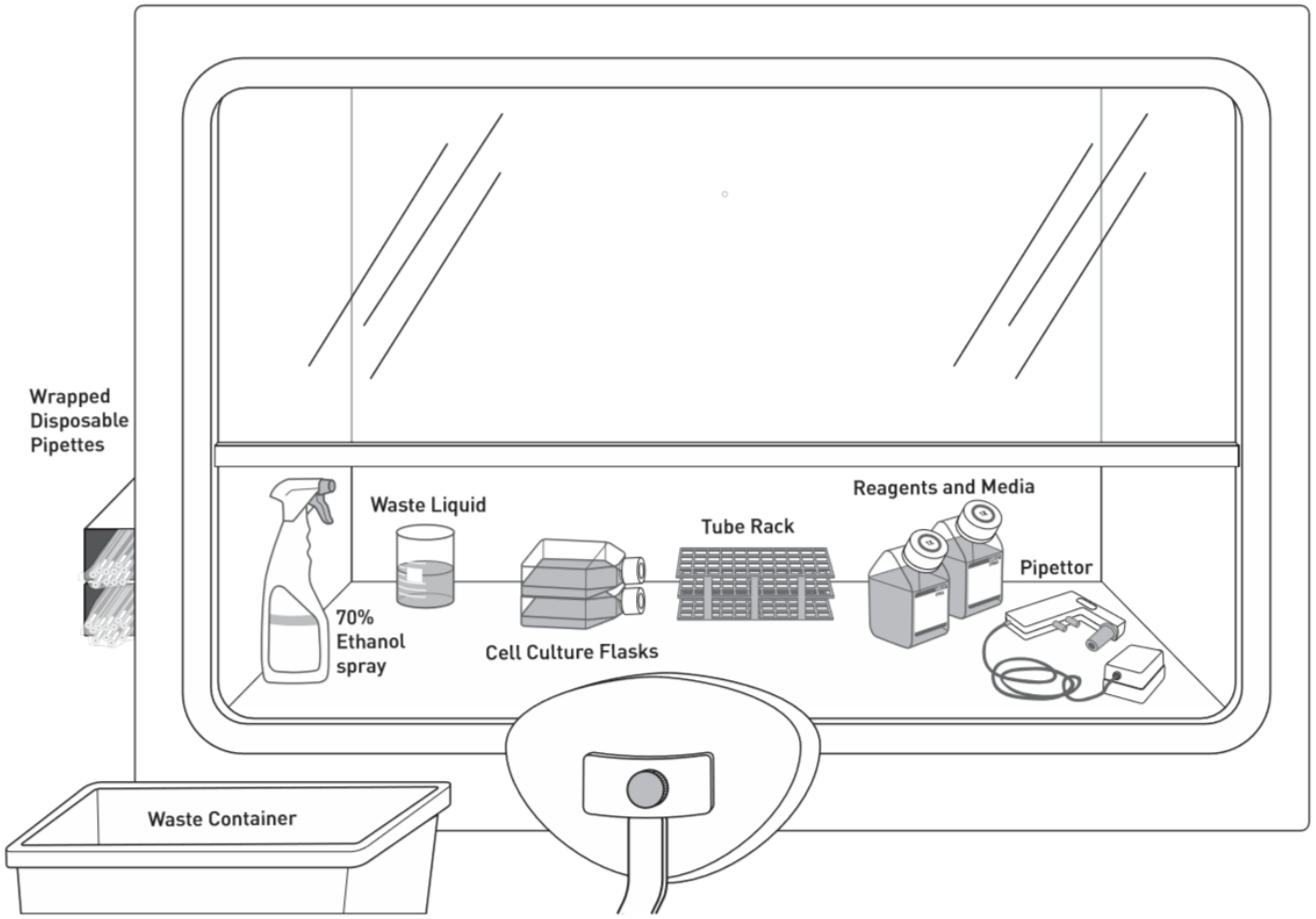
Methods is cell culture

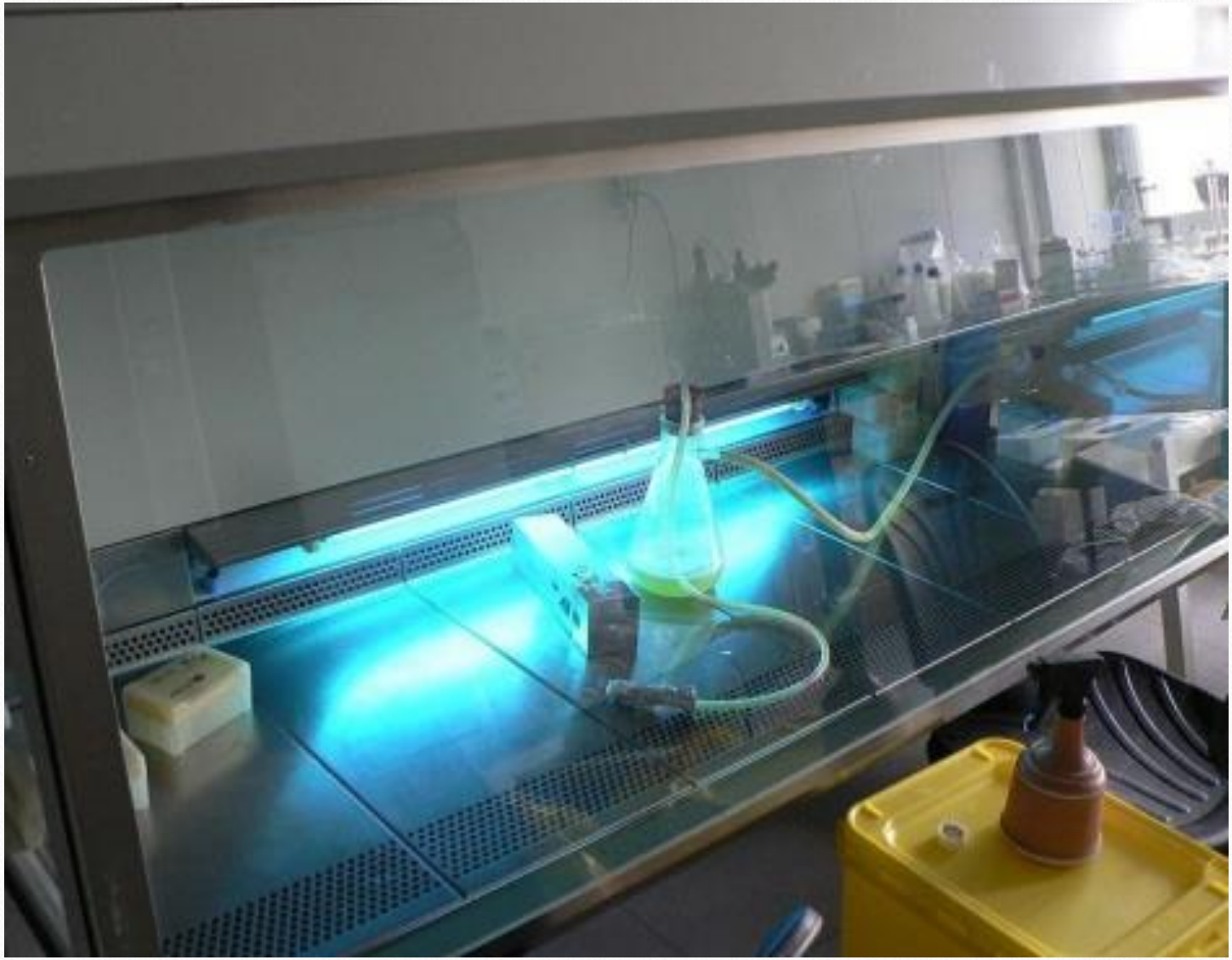
Basic Equipment

Cell culture hood (i.e., laminar-flow hood or biosafety cabinet)

- Incubator (humid CO2 incubator recommended)
- Water bath
- Centrifuge
- Refrigerator and freezer (–20°C)
- Cell counter
- Inverted microscope
- Liquid nitrogen (N2) freezer or cryostorage container
- Sterilizer (i.e., autoclave)
- Cell culture vessels (e.g., flasks, Petri dishes, roller bottles, multi-well plates)
- Pipettes and pipettors
- Media, and reagents
- Cells

Methods is cell culture

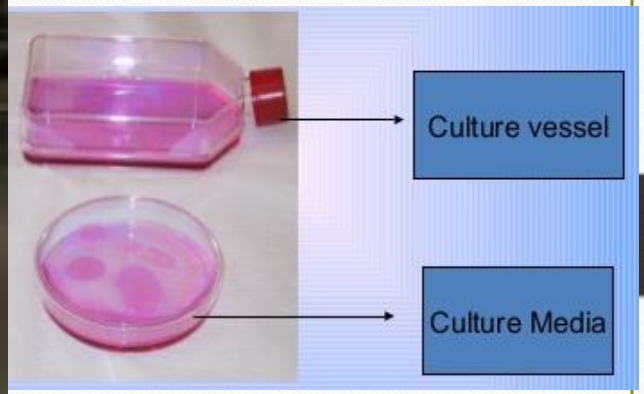




laminar-flow hood

Microscope Large stage so plates and flasks can be used. Magnification; 5X, 10X, 20X, 40X

Liquid nitrogen (N2)



Primary Cultures

- Prepared from cells obtained directly from the tissues.
- Cut into single cells (by enzymatic digestion or mechanical dispersion)
- The first subculture of a primary cell culture called secondary cell culture
- This procedure is particularly convenient for the preparation of monkey kidney cell cultures.

Primary Cultures

Advantages:

- usually retain many different characteristics of the cell in vivo

Disadvantages

- initially heterogeneous but later become dominated by fibroblasts.
- the preparation of primary cultures is labor intensive
- can be maintained in vitro only for a limited period of time.

Continuous Cultures

- It is derived from a subculture of primary culture •
- There are two types of continuous cultures:
  - Cell lines
  - Continuous cell lines

A- Cell lines:

- Short time life, die after approximately thirty cycles of division

Continuous Cultures

B- Continuous cell lines:

- Most cell lines grow for a limited number of generations after which they ceases
- Cell lines which either occur spontaneously or induced or chemically transformed into Continuous cell lines
- The established cell lines most frequently employed are Hela cell culture which was derived originally from a human cervical carcinoma, and the HEp-2 cell derived from a carcinoma of the larynx.

• Characteristics of continuous cell lines:

Smaller, More Rounded, Less adherent with a higher nucleus /Cytoplasm ratio

-Fast growth

-Reduced serum and anchorage dependence and grow more in suspension conditions

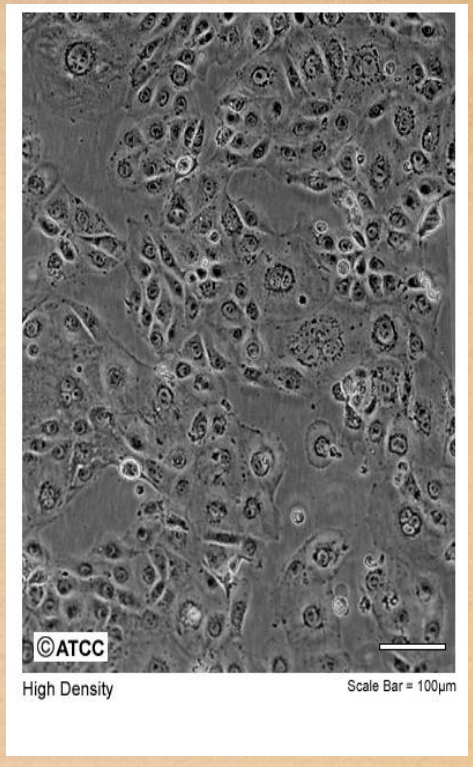
Cytopathic Effect

1- Enterovirus 71 (EV71)

- It is one of a large family of viruses which multiply in the human gastrointestinal tract (gut). EV71 infection can cause illness ranging from mild through to serious with life-threatening complications, and cause hand, foot and mouth disease.
- Symptoms:
- Fever, rash, tiredness, loss of appetite, ulcers or blisters in the mouth, and on the hands and feet
- Serious infection may involve the brain (encephalitis) and the meninges

1- Enterovirus 71 (EV71)

Fig. 1, Cytopathic effects of enterovirus 71 in rhesus monkey kidney cells



Cytopathic effect of enterovirus 71 cell culture: note the ballooning of cells, the right figure for control cells

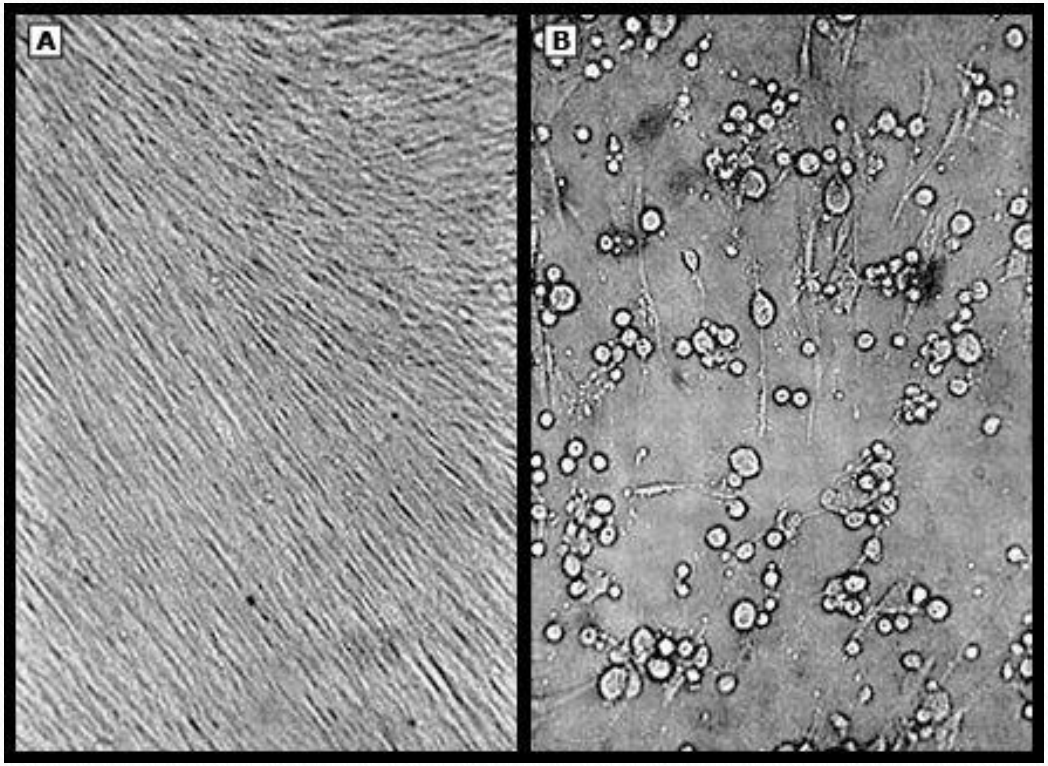
Cytopathic Effect

2- Herpes simplex virus

Herpes can appear in various parts of the body, most commonly on the genitals or mouth. There are two types of the herpes simplex virus. HSV-1, also known as oral herpes, can cause cold sores and fever blisters around the mouth and on the face. HSV-2 is generally responsible for genital herpes outbreaks.

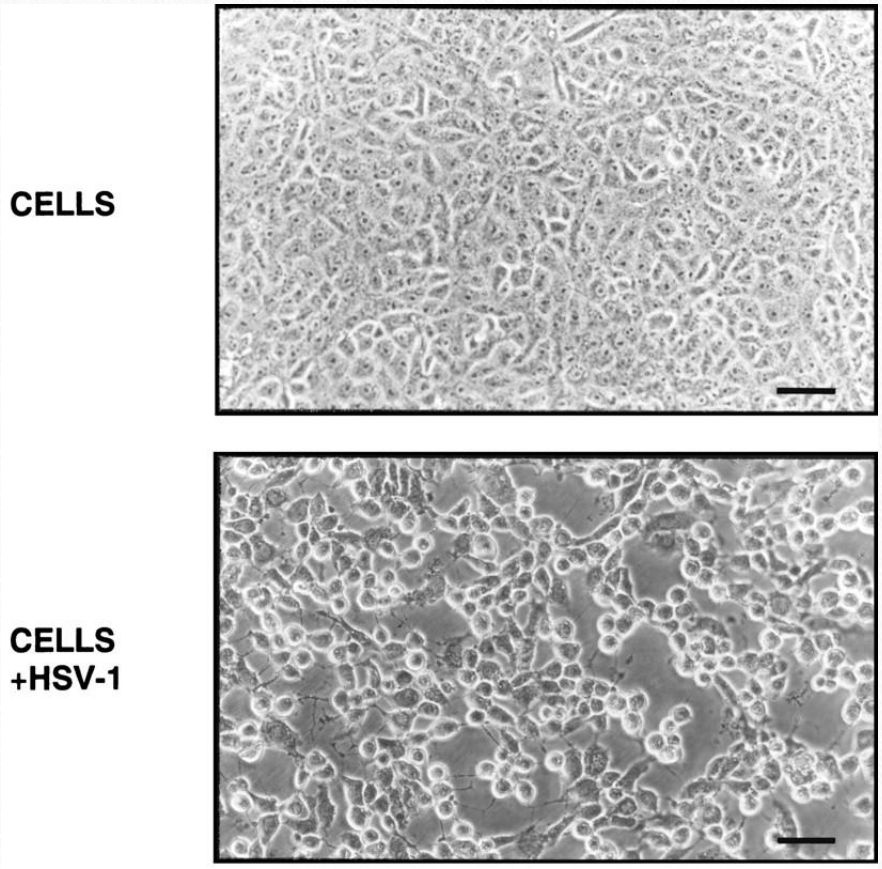
- Symptoms:
- blistering sores (in the mouth or on the genitals)
- pain during urination (genital herpes)
- itching

2- Herpes simplex virus



The microscopic appearance of a monolayer of uninfected human fibroblasts grown in cell culture (A) and the same cells after infection with herpes simplex virus (B), demonstrating the cytopathic effect caused by viral replication and concomitant cell lysis.

2- Herpes simplex virus



Cytopathic effect of HSV-1 on Vero cells

3- Poliovirus & Herpes simplex virus

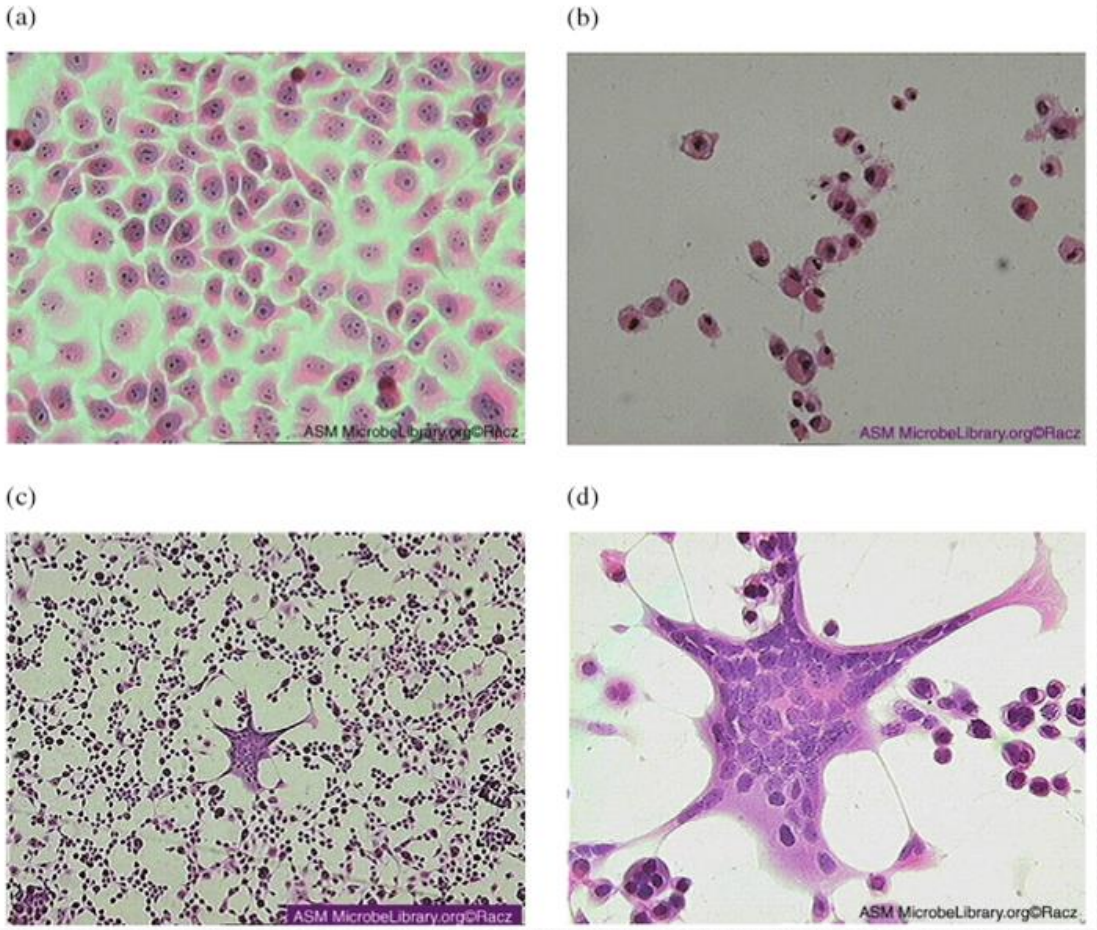


Figure 2.11 *Cytopathic effects caused by replication of poliovirus and herpes simplex virus in cultures of Vero (monkey kidney) cells.* (a) Uninfected Vero cells. (b) Infected with poliovirus. (c), (d) Infected with herpes simplex virus. (a), (b) and (d) were viewed at × 400 magnification; (c) was viewed at × 100 magnification. The cells were stained with haematoxylin and eosin. Reproduced by permission of Dr. Maria-Lucia Rácz (University of Sao Paulo) and the American Society for Microbiology MicrobeLibrary.