



Vaccine Cell Substrates 2004

Adventitious Virus Tests

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June 30, 2004





Cell Substrate Characterization

- **Each manufacturer characterizes the cell substrates banked and used in production in their facility**
 - **history of isolation and banking**
 - **growth characteristics**
 - **karyology, identity, and tumorigenicity**
 - **freedom from adventitious agents**



Tests to Characterize Cell Banks

- **Karyology**
- **Isoenzyme analysis**
- **Tumorigenicity**
 - **tumor formation (progressing nodules and/or metastases) in immunosuppressed rodents**
 - **colony formation in soft agar**
 - **not necessary for cells of rodent origin (as all continuous cell lines of such are tumorigenic by in vivo assay)**
 - **other cells expected to pass tumorigenicity testing**



Adventitious Agent Tests

- **Bacterial and fungal sterility (610.12)**
- **Mycoplasma (& spiroplasma)**
- **Mycobacteria (animals/culture-650.13)**
- **Viruses (*in vitro*, *in vivo*)**
 - Acute (Lytic, Hemadsorbing/Hemagglutinating)
 - Latent (e.g., retroviruses or other oncogenic viruses)
- **Retroviruses**
- **Specific tests (PCR)**
- **Animal-derived raw materials**
 - 9 CFR 113 tests
 - from BSE-free countries





Adventitious Virus Tests

➤ *In Vitro* tests

- monolayers of at least 3 cell types
 - same species, tissue as substrate
 - human diploid cells
 - monkey kidney cells
- tests for hemadsorption and hemagglutination at end of culture period

➤ Animal-derived raw materials

- 9 CFR 113 tests
- from BSE-free countries





Adventitious virus tests (2)

➤ *In Vivo* tests

- adult and suckling mice
- embryonated hens' eggs
- when appropriate
 - guinea pigs
 - rabbits
 - monkeys



Adventitious Virus Tests (3)

➤ For rodent substrates

- MAP, RAP, HAP - antibody production tests
- Lymphocytic choriomeningitis virus

➤ For human substrates

- EBV, CMV
- HBV, HCV
- *in vitro* techniques
- tissue source and donor medical history



Adventitious Virus Tests (4)

➤ If appropriate

- papillomaviruses
- adenoviruses
- Herpes Virus Type 6 (HHV-6), others

➤ Retroviruses

- Transmission Electron Microscopy
- Reverse Transcriptase assays
- Infectivity assays





Where did it start?

- One of the earliest class of cell-culture derived vaccines was poliovirus vaccines
- March 16, 17, 1961
 - Hearing before a subcommittee on interstate and foreign commerce (committee of the House of Representatives)
 - Initial proposed regulations for the manufacture of OPV
 - These regs ultimately became part of 21 CFR 630's



Tests for polioviruses harvests

- In 1961, poliovirus harvests were intended to be tested for “adventitious and other infectious agents including polioviruses of other types or strains, simian agents, *Mycobacterium tuberculosis*, pox viruses, lymphocytic choriomeningitis virus, Echoviruses, coxsackieviruses, and B-virus.”



Specific Tests Required

- Rabbits
- Adult mice
- Suckling mice
- Guinea Pigs
- Monkey Kidney Tissue Cultures
- Human Tissue Cultures
- Rabbit Kidney Tissue Cultures



What were these tests for?

- **Rabbits – B-virus**
- **Rabbit Kidney Tissue Cultures – B-virus**
- **These tests are not routinely (although sometimes) included for most vaccines, so methods won't be elaborated**



Adult Mice

- LCMV or other viruses
- ≥ 20 adult mice
- i.p. with 0.5 mL, i.c., with 0.03 mL
- Mice must survive 21 days
 - $\geq 80\%$ survival
 - No sign of viral infection
- Capable to detect LCMV, coxsackieviruses, flaviviruses, rabies



Suckling Mice

- **Coxsackieviruses (particularly type A)**
- **≥20 mice less than 24 hours old**
- **i.c., 0.01 mL, i.p., 0.1 mL**
- **14 days**
- **Subinoculation into additional mice for 14 days**
- **Mice must survive**
 - **≥80% survival both inoculations**
 - **No signs of viral infection**
- **Capable of detecting coxsackieviruses, other picornaviruses (polioviruses, echoviruses), alphaviruses, herpesviruses (HSV), flaviviruses, rabies, many murine agents, others**





Guinea Pigs

- LCMV, M. Tb
- ≥ 5 guinea pigs
- 0.1 mL i.c., 5 mL i.p.
- 42 days
- $\geq 80\%$ survival
 - No signs of LCMV or M. Tb infection
- Capable of detecting paramyxoviruses, reoviruses, filoviruses



Tissue Cultures

- **Monkey Kidney Cells (simian agents)**
- **Human Diploid Cells (originally to detect measles)**
- **Same species, tissue as production**
- **14 days, subculture for additional 14 days**
- **CPE, hemadsorption/hemagglutination (RBCs of guinea pigs, chickens, and human or rhesus monkey – 2 temperatures, 1/2 each)**
- **Capable of detecting a wide array of viruses**



Egg tests

➤ 0.5 mL allantoic route

- Orthomyxoviruses (influenzaviruses), paramyxoviruses (mumps, measles, parainfluenzaviruses), alphaviruses,

➤ 0.5 mL yolk sac route

- Herpesviruses (HSV), poxviruses, rhabdoviruses, rickettsiae, mycoplasmas, bacteria