US Regulatory Perspective on New Cell Substrates for Manufacture of Viral Vaccines

Arifa S. Khan, PhD
Office of Vaccines Research and Review, CBER, FDA
Topics Covered

- Cell substrates for licensed vaccines
- Novel cell substrates
- Development of new guidance document
- Cell-substrate testing
- Summary
- References
Role of Cell Substrates in Vaccines

Generation of Virus Seed Stock or Virus Vector Stock
- Isolation of virus seed
- Development of vector virus
- Virus selection
- Propagation

Development of Selected Cell Clones or Genetically Modified/Engineered Cells
- To enhance and/or support growth of vaccine virus

Vaccine Production
- Amplification of vaccine virus
Types of Cell Substrates Used in Current U.S. Licensed Viral Vaccines

- **Primary Cells or Tissues:** used without passage in tissue culture

- **Diploid Cells:** cells with a finite lifespan and passage in tissue culture

- **Continuous Cell Lines, Non-tumorigenic:** immortal, neoplastic cells with unrestricted passage in tissue culture
# Cell Substrates of Current U.S. Licensed Viral Vaccines: Primary Tissues and Cells

<table>
<thead>
<tr>
<th>Cell Substrate</th>
<th>Live Vaccines</th>
<th>Inactivated Vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf lymph</td>
<td>Smallpox</td>
<td>JEV</td>
</tr>
<tr>
<td>Embryonated hens’ eggs</td>
<td>Influenza</td>
<td>Influenza</td>
</tr>
<tr>
<td></td>
<td>Yellow Fever</td>
<td></td>
</tr>
<tr>
<td>CEF</td>
<td>Measles</td>
<td>Rabies</td>
</tr>
<tr>
<td></td>
<td>Mumps</td>
<td></td>
</tr>
<tr>
<td>Cell Substrate</td>
<td>Live Vaccines</td>
<td>Inactivated Vaccines</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>FRhL-2</td>
<td></td>
<td>Rabies</td>
</tr>
<tr>
<td>WI-38</td>
<td>Rubella</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adenovirus</td>
<td></td>
</tr>
<tr>
<td>MRC-5</td>
<td>Varicella/Zoster</td>
<td>Poliovirus</td>
</tr>
</tbody>
</table>
|                |                | Hepatitis A
|                |                | Rabies |
## Cell Substrates of Current U.S. Licensed Viral Vaccines: Continuous Cell Lines

<table>
<thead>
<tr>
<th>Cell Substrate</th>
<th>Live Vaccines</th>
<th>Inactivated Vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vero</td>
<td>Rotavirus</td>
<td>Poliovirus</td>
</tr>
<tr>
<td></td>
<td>Smallpox</td>
<td></td>
</tr>
</tbody>
</table>

- Rotavirus
- Smallpox
- Poliovirus
- Smallpox
Types of Viral Vaccines

“TRADITIONAL”
q Live, attenuated virus
q Inactivated, whole or subunit virions

“NEW-GENERATION”
q Live, vectored virus
q Purified recombinant proteins
q Synthetic antigens
Introduction of Novel Cell Substrates in Vaccine Manufacture

- **Egg based Cell lines (influenza virus)**
  - Higher virus yield
  - Easy scalability
  - Availability of cell substrate to meet production demand
  - Well characterized cell banks
  - Reduced risk of unknown agents due to the animal species of origin

- **Non-tumorigenic cells tumorigenic cells (influenza virus, HIV)**
  - Higher virus yield
  - Susceptibility of cells to viruses for novel vaccines

- **Unmodified cells genetically engineered cells (adenovirus)**
  - Requirement for complementation for some vectored virus vaccines

- **Development of new cell lines**
  - To replace depleting existing cell stock
  - For novel vector development
  - For high virus particle or protein yield
Novel Cell Substrates for Investigational Vaccines

- Genetically-engineered cells: 293, PER.C6
- Tumorigenic cell lines: MDCK
- Insect cells: Sf9, Hi-5
Discussions on Novel Cell Substrates

- **1998**: CBER engages Vaccines Advisory Committee on topic of neoplastic and tumorigenic cells for vaccine manufacture.

- **1999**: International Meeting: Evolving Scientific and Regulatory Perspectives on Cell Substrates for Vaccine Development.

- **2000**: Advisory committee discussion: Vero cells (non-tumorigenic passage) for live-attenuated vaccines.

- **2001**: Advisory committee discussion: In vitro transformed human cells (293, PER.C6) for defective adenovirus-vectored vaccines.

- **2004**: IABS/NIAID meeting: Vaccine cell substrates.

- **2005**: Advisory committee discussion: Tumorigenic MDCK cells for inactivated influenza virus vaccine.
New Draft Cell-Substrate Guidance

- Provides guidance to develop comprehensive testing regimens for detection of known and unknown adventitious viruses in novel vaccine cell substrates.

- Provides more details of many testing procedures and includes specific tests originally promulgated in 21 CFR part 630.

- Provides updates of testing procedures.

- Includes more detail and scientific rationale for recommendations to allow manufacturer’s additional flexibility.

- Fosters early discussions between regulators and manufacturers regarding development of specific assays for novel cell substrates.
2006 DRAFT Guidance for Industry

Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases

http://www.fda.gov/cber/gdlns/vaccsubstrates.pdf
Definitions

- **Tumorigenicity** is the property of a cell to form a tumor in an immune compromised animal.

- **Oncogenicity** is the activity of an agent (such as a virus) or a cellular component (such as DNA) to induce a tumor in an animal.

- **Endogenous retroviruses** are stable, genetically inherited viral sequences in the host cell DNA:
  - dead (defective for virus production)
  - latent (with the potential for virus induction)
  - active (produce non-infectious or infectious virus)

- **Latent viruses** are quiescent in the cell with the potential to reactivate.
Testing Considerations for Novel Cell Substrates

- **Health/Medical history of the donor**

- **Viruses in Donor Species**
  - Naturally occurring
    - Genetically Transmitted: Endogenous retroviruses
    - Horizontally Transmitted: Exogenous retroviruses; RNA viruses; DNA viruses
  - Specific exposure to other infectious agents

- **In case of diploid cells**
  - Karyotype

- **In case of genetically engineered cells**
  - Stability, expression, and copy number of transgene
Testing Considerations for Novel Cell Substrates

**Cell growth:** highly proliferative cells may have:
- Increased susceptibility for virus infection and replication
- Broader host range to different families of viruses

**Cell line and passage history**
- Propagation in different labs and facilities
- Biological reagents used (serum, trypsin, others)
- Other cell lines or viruses grown at same time

**Cell phenotype (transformed or tumor-derived):**
- Tumorigenicity may be associated with:
  - Oncogenic viruses
  - DNA oncogenicity
Routine Tests for Vaccine Cell Substrates

- **IDENTITY**
- **STERILITY**
- **NON-VIRAL AGENT TESTING**
  - Mycoplasma
  - Bacteria and Fungi
  - Mycobacteria
- **TUMORIGENCITY**
  - Nude mice
  - ATG-treated or irradiated newborn rats or newborn mice

**1993 Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals**
ADVENTITIOUS VIRUS TESTING: General

- In vitro cell culture tests
  - same species and tissue type as that used in production
  - human diploid cells
  - monkey kidney cells

- In vivo assays
  - adult mice
  - suckling mice
  - embryonated hens’ eggs
  - (guinea pigs, rabbits)

- Transmission electron microscopy (TEM)

- Reverse transcriptase assay for retroviruses (PERT)
ADVENTITIOUS VIRUS TESTING: *Species specific*

- **Tests for animal viruses** due to raw materials such as trypsin, serum (9CFR113.47 and 113.53)

- **Antibody production assays** for rodent viruses (MAP, RAP, HAP)

- **Assays for known viruses** based upon species
  - PCR amplification
  - DNA hybridization
  - Infectivity
  - Antibody detection

1993 Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals
Additional Assays for Testing Novel Cell Substrates

- **EXTENDED TUMORIGENICITY ASSAY**
  - Whole cell tumorigenicity

- **ONCOGENICITY ASSAY**
  - Oncogenicity of DNA
  - Oncogenic viruses

- **INDUCTION ASSAYS**
  - Endogenous retroviruses
  - Latent DNA viruses

- **TSE**

*2006 DRAFT Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases*
“Extended” Tumorigencity Assay

- Characterization of cell line
  - Determination of $TPD_{50}$ in adult nude mice
  - Using the most sensitive animal model (newborn in some cases)
  - Extended observation period: 4 – 7 months in some cases
In Vivo Oncogenicity Assay

- Detection of Oncogenic Viruses
- Evaluation of DNA oncogenicity

Inoculation of cell lysates from $10^7$ cell equivalent or cell DNA ($\geq 100 \mu g$) into $< 4$ day-old animals

- newborn hamster
- newborn nude mice
- newborn rats

Observation Period: 4 – 7 months
In Vitro Induction Assays

Detection of Endogenous and Latent Viruses

**INDUCER**
- I UdR, AzaC
- NaB, TPA

**DETECTION ASSAYS**
- TEM
- PERT (Retrovirus)
- Generic PCR
- Infectivity/Coculture
Chemical Inducers are Potent Virus Activators

5’-iodo-2’-deoxyuridine (IUdR) and 5-azacytidine (AzaC) are known inducers of endogenous retroviruses from cells of different species including avian and mammalian.

12-O-tetradecanoylphorbol-13-acetate (TPA) and sodium butyrate (NaB) can induce various latent DNA viruses including herpesviruses and some retroviruses (HIV-1).
Testing to Assure Product Safety

- Testing regimen may need to be customized based upon the manufacturer’s production scheme and vaccine type.
Generic Vaccine Production Scheme

1. Isolation of Vaccine Virus from tissue or Recombinant Virus from cell culture
2. Amplification in Cell Substrate
3. Master Virus Seed
4. Working Virus Seed
5. (Parent Cell Bank) clone selection genetic modification
6. Master Cell Bank
7. Working Cell Bank
8. VACCINE
9. End of Production Cells (Bank)
10. (Control Cells)
Different Vaccine Types

- **Live, attenuated or recombinant vector virus**
  - Minimally processed
  - Minimally purified
  - Contain residual host cell DNA and proteins

- **Killed, Whole virus**
  - Moderately processed
  - Partially purified
  - Reduced levels of host cell DNA and proteins

- **Subunit**
  - Highly processed and purified
  - Minimal extraneous host cell material
Live Vaccines

- Minimally processed and purified
  - long term protection
  - added safety concerns

- Extensive testing needed
  - virus seed
  - biological raw materials
  - cell substrate(s)
  - in process

- Removal of whole cells

- Reduction of host cell DNA (size and amount)

- Reduction of host cell protein
Killed Vaccines

Moderately processed with some reduced levels of cellular materials

- need repeated boosts for continued protection
- reduced adventitious agent concerns

- Removal of whole cells
- Virus inactivation
- Reduction of cellular DNA and protein
- Process validation
Subunit Vaccines

- Highly processed: minimal levels of cellular materials
  - Need repeated boosts for continued protection
  - Minimum adventitious agent concerns

- Removal of whole cells
- Virus inactivation
- Virus removal
- Reduction of cellular DNA and protein
- Process validation
SUMMARY: General Approaches for Evaluation of Viral Safety in Viral Vaccines

- Qualification of cell banks, virus seed and biological raw materials
  - Extensive testing of vaccine virus seed and cell substrate
  - Use of raw materials certified or tested to be free of detectable virus

- In-process testing
  - Develop a comprehensive testing plan to evaluate bulk/production lots for known and novel viruses

- Process validation
  - Design an efficient process
    - to avoid risk of contamination
    - eliminate or reduce potential virus load
    - inactivate potentially contaminating virus

- Reduction of residual host cell material in final product
  - Whole cell removal
  - Cellular DNA and protein reduction
Relevant Regulatory Documents and Guidances

U.S. Regulations
- Code of Federal Regulations (CFR)
  - 21 Part 610
  - 21 Part 630 (removed in 1996)
  - 9 Part 113

FDA Guidances and Points to Consider
- PTC Characterization of Cell Lines Used to Produce Biologicals (1993)
- Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases (2006- Draft)

www.fda.gov/cber/guidelines.htm
ICH
q Q5D Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products
q Q5A Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

WHO