New Cells for New Vaccines II: September 19, 2007

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

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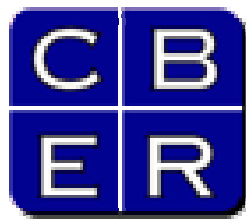


Topics Covered

- 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

Cell Substrate	Live Vaccines	Inactivated Vaccines
Mouse brain		JEV
Calf lymph	Smallpox	
Embryonated hens' eggs	Influenza Yellow Fever	Influenza
CEF	Measles Mumps	Rabies

Cell Substrates of Current U.S. Licensed Viral Vaccines: Diploid Cell Strains

Cell Substrate	Live Vaccines	Inactivated Vaccines
FRhL-2		Rabies
WI-38	Rubella Adenovirus	
MRC-5	Varicella/Zoster	Poliovirus Hepatitis A Rabies

Cell Substrates of Current U.S. Licensed Viral Vaccines: Continuous Cell Lines

Cell Substrate	Live Vaccines	Inactivated Vaccines
Vero	Rotavirus Smallpox	Poliovirus

Types of Viral Vaccines

- "TRADITIONAL"

- Live, attenuated virus
- Inactivated, whole or subunit virions

- "NEW-GENERATION"

- Live, vectored-virus
- Purified recombinant proteins
- 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**

Routine Tests for Vaccine Cell Substrates

- **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)**

Routine Tests for Vaccine Cell Substrates

- **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 μg) into < 4 day-old animals**

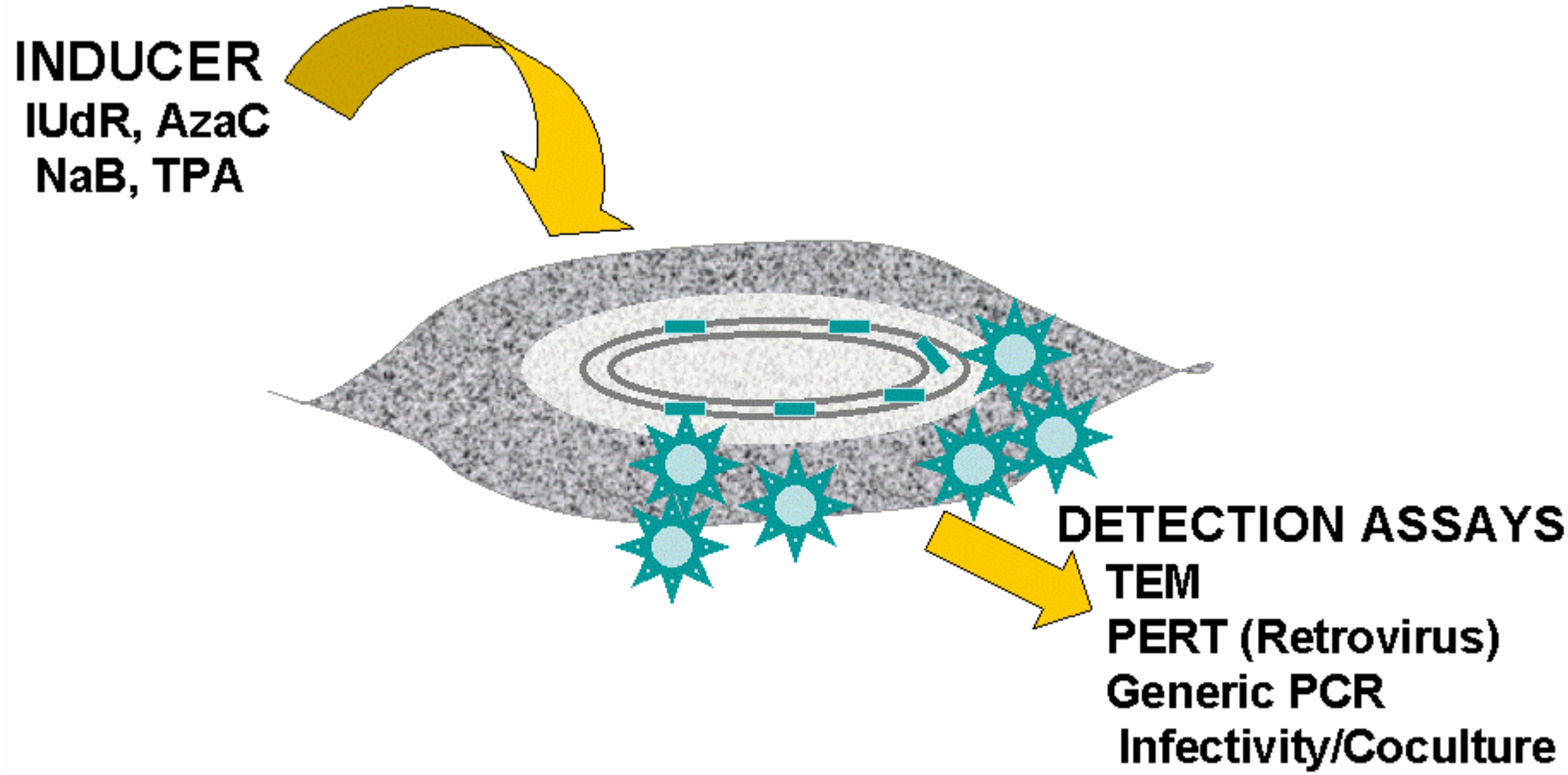
    ↓

    - **newborn hamster**
    - **newborn nude mice**
    - **newborn rats**

  - **Observation Period: 4 – 7 months**

In Vitro Induction Assays

- **Detection of Endogenous and Latent Viruses**



Chemical Inducers are Potent Virus Activators

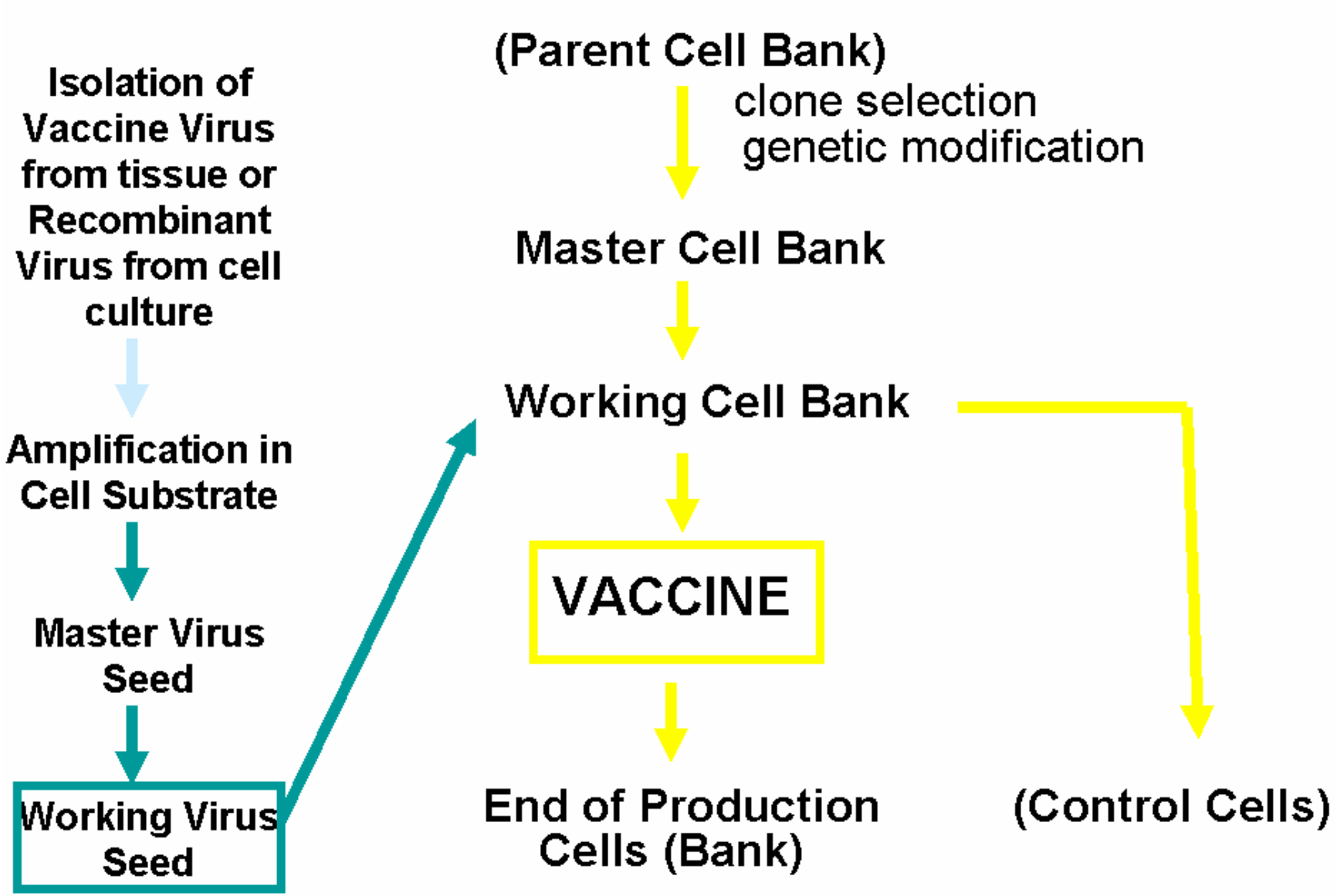
- **5’-iodo-2’-deoxyuridine (IUdR) and 5-azacytidine (AzaC) are known inducers of endogenous retroviruses from cells of different species including avian and mammalian**

- **12-*O*-tetradecanoly phorbol-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



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)**

- **PTC in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1997)**

- **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**

Relevant International Regulatory Documents

- **ICH**
- **Q5D Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products**
- **Q5A Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin**

- **WHO**
- **Requirements for Continuous Cell Lines Used for Biological Production, WHO Technical Report Series 745, Annex 3, 1987**