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Regulatory Guidance on Using New Cell Substrates for Manufacture of Viral Vaccines

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Topics Covered

- Different types of cell substrates in vaccines
- Cell banking
- Cell bank testing
- Guidance documents
- Different vaccine types and testing
- Summary
- References

Types of Cell Substrates Used in Current U.S. Licensed Viral Vaccines

- **Primary Tissues or Cell Cultures:** 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 Used in Current U.S. Licensed Viral Vaccines

Cell Substrate		Vaccines	
Type	Origin	**Live**	**Inactivated**
Primary Tissues or Cell Cultures	Calf lymph, Mouse brain, Chicken eggs, CEFs	Smallpox, Influenza, YFV, Measles, Mumps	JEV, Influenza, Rabies
Diploid Cell Strains	Human (MRC-5 and WI-38)	Rubella, Varicella/Zoster	Poliovirus, HepA, Rabies,
Continuous *(Non-tumorigenic)* Cell Lines	African green monkey (Vero)	Smallpox, Rotavirus	Poliovirus

Cell Banking in Vaccines

- **Primary Tissues and Cell Cultures**
  - **No cell banks**

- **Diploid Cells and Continuous Cell Lines**
  - **Master Cell Bank (MCB)**
  - **“Manufacturer’s” Working Cell Bank (WCB)**
  - **End of Production Cell Bank (EOP)**

Cell Bank Definitions

MCB (Master Cell Bank)

An aliquot of a single pool of cells which generally has been prepared from the selected cell clone under defined conditions, dispensed into multiple containers and stored under defined conditions. The MCB is used to derive all working cell banks.

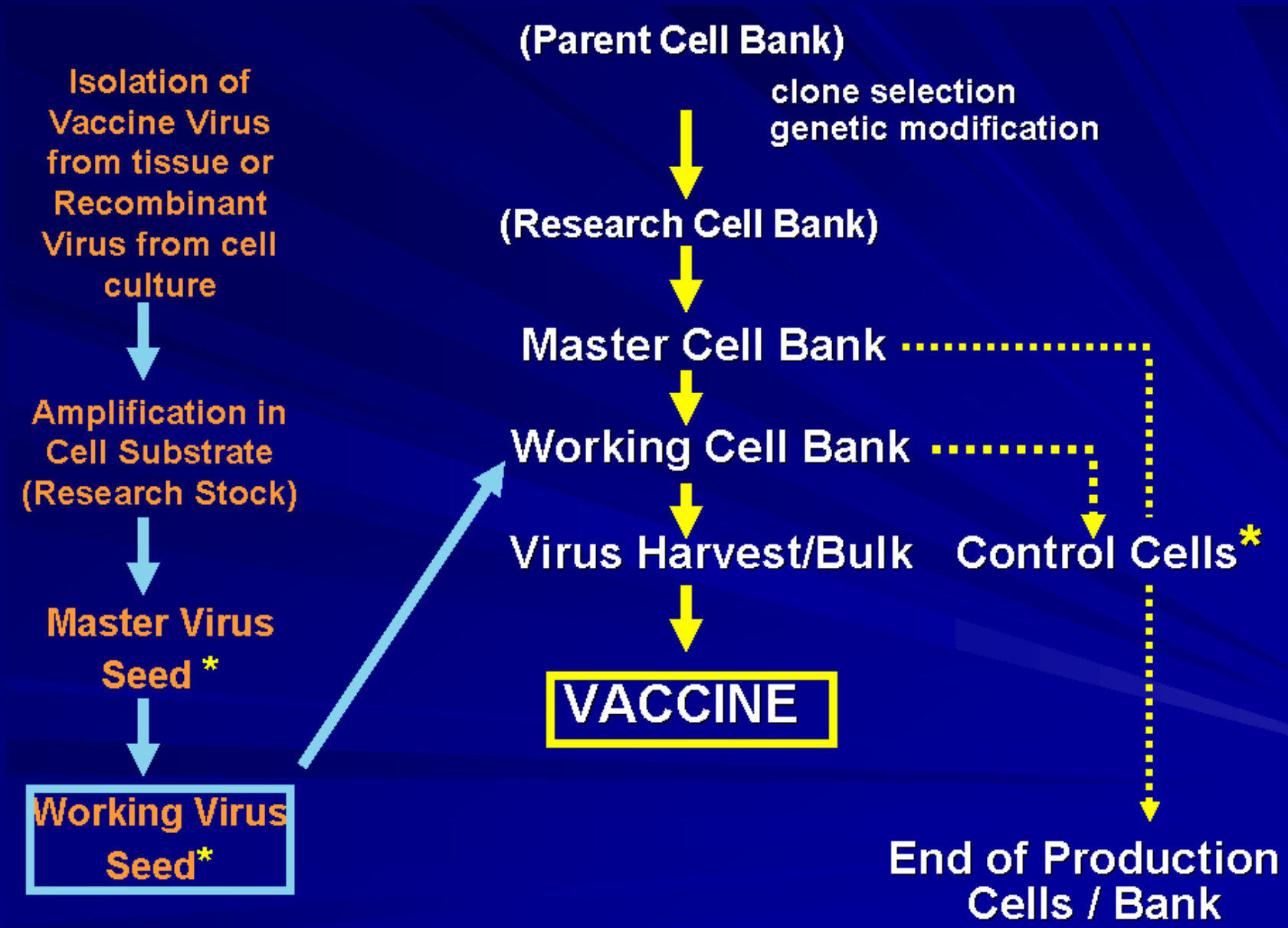
WCB (Working Cell Bank)

The Working Cell Bank is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the MCB under defined culture conditions.

EOP (End of Production Cells)

End of Production cells are derived by expansion from the MCB or WCB and are:
i) acquired at the end of an actual production run using full scale manufacturing conditions or ii) cultured from the MCB or WCB to a population doubling level comparable to or beyond cells at the end of production.

Generic Vaccine Production Scheme



Role of Cell Banking in Vaccines

A qualified cell bank can assure

- **Product safety**
- **High quality**
- **Lot-to-lot consistency**

Introduction of Novel Cell Substrates in Vaccine Manufacture

- **Egg based → Cell lines (influenza virus)**
  - Well characterized cell banks
  - Easy scalability
  - Availability of cell substrate to meet production demand
  - Reduced risk of unknown agents due to the animal species of origin
  - Higher virus yield
- **Non-tumorigenic cells →tumorigenic cells (influenza virus, HIV)**
  - Susceptibility of cells to viruses for novel vaccines
  - Higher virus yield
- **Unmodified cells → genetically engineered cells (adenovirus)**
  - Requirement for complementation for some vectored virus vaccines
- **Development of new cell lines**
  - For new-generation vaccines
    - live-vectored virus
    - Purified recombinant proteins
    - Synthetic antigens

Novel Cell Substrates for Investigational Vaccines

- Insect Cell Lines
  - Sf9, Hi-5
- Tumorigenic Cell Lines
  - *Genetically-engineered cells*
    - Human 293, PER.C6
  - *Naturally-occurring*
    - Canine MDCK cells

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 (HEK-293, PER.C6) for defective adenovirus-vectored vaccines
- **2005:** Advisory committee discussion: Tumorigenic MDCK cells for inactivated influenza virus vaccine

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

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

Cell Bank Qualification

- Identity (karyotype, species)
- Safety (freedom from adventitious agents)
- Stability (of cell phenotype and any transgenes)

Testing Considerations for 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 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[1] for Vaccine Cell Substrates

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

[1] ***1993*** **Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals**

Routine Tests[1] 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)**

[1] ***1993*** **Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals**

Routine Tests[1] 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

[1]***1993*** **Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals**

Additional Assays[2] 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**

[2] ***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” Tumorigenicity 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**

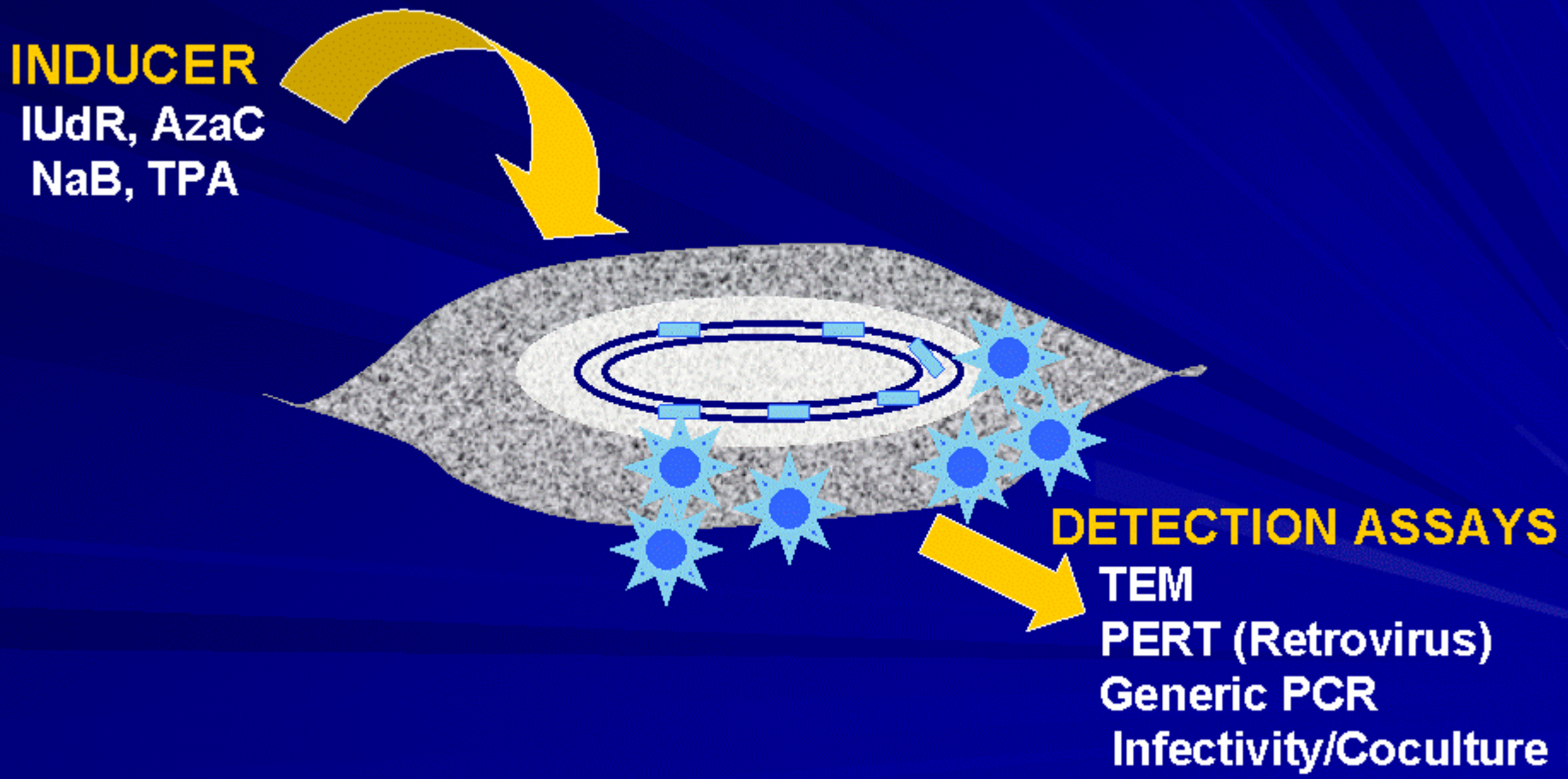
    ↓

    - **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 herpes viruses and some retroviruses (HIV-1)

General Considerations for Cell Bank Testing

- Potential sources of contamination
  - Endogenous or exogenous
- Cell Substrate
  - Traditional or novel
- Production scheme
  - Derivation of seed virus
  - Virus inactivation/clearance steps
  - Reagents used for enhancing cell growth or virus amplification
  - Overall production time
- Vaccine type
  - Live, Killed or Subunit

Testing to Assure Product Safety

- Testing regimen may need to be customized based upon the manufacturer's production scheme and vaccine type

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**

  - Test for adventitious agents
  - - Virus seed
    - - Biological raw materials
    - - Cell substrate(s)
    - - In process
  - Demonstrate
    - - Removal of whole cells
    - - Reduction of host cell DNA and proteins

Killed Vaccines

- **Moderately processed with some reduction in levels of cellular materials**
  - **Demonstrate**
    - **- Removal of whole cells**
    - **- Virus inactivation**
    - **- Reduction of cellular DNA and protein**
    - **- Process validation**

Subunit Vaccines

- **Highly processed with minimal levels of contaminating cellular materials**
  - **Demonstrate**
  - **- 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