

New Cells for New Vaccines IV: October 14, 2009

Cell Substrates for Viral Vaccines: A Regulatory Update

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Topics Covered

- ❑ Cell substrates in US licensed vaccines
- ❑ Novel cell substrates
- ❑ Issues related to use of tumorigenic cells, insect cells, plants and embryonic stem cells
- ❑ Approaches for safety testing of novel cell substrates
- ❑ Reference guidance documents

Animal Cell Substrates Used in Current U.S. Licensed Viral Vaccines

TYPE	SUBSTRATE	VACCINES	
		Live	Inactivated
Animal Tissues	Calf skin (lymph)	Smallpox	
	Mouse brain		JEV
	Chicken eggs	Influenza, YFV	Influenza
Primary Cell Cultures	CEFs	Measles, mumps	Rabies
Diploid cells	Human MRC-5 cells		Hepatitis A, rabies, poliovirus
	Human WI-38 cells	Rubella, varicella-zoster	
Continuous Cell Lines <i>(Non-tumorigenic)</i>	African green monkey cells (Vero)	Smallpox, rotavirus	Poliovirus

Additional Cell Substrates Used in U.S. Licensed Viral Vaccines

□ Current

- Yeast cells
 - Hepatitis B (subunit/protein)
 - HPV (subunit/protein)

□ Past

- Primary African green monkey kidney cells
 - OPV (live)
- Diploid fetal rhesus monkey lung FRhL-2 cells
 - Rotavirus (live)
 - Rabies (inactivated)

Cell Banking

Primary Cells and Tissues

- ❑ **No Cell Banks**
 - Donor history
 - Donor testing
 - Use of SPF donors (when possible)
 - Extensive testing of control cells

Diploid Cells and Continuous Cell Lines

- ❑ **Master Cell Bank (MCB)**
 - Extensive characterization and testing (usually)
- ❑ **“Manufacturer’s” Working Cell Bank (MWCB)**
 - Limited testing (usually)
- ❑ **End of Production Cell Bank (EOP)**
 - Limited testing usually (“additional tests”)

Introduction of Novel Cell Substrates in Vaccine Manufacture

- ❑ **Replacement of Eggs with Cell Lines to meet high production demands and reduce risk of unknown agents**
 - Influenza virus vaccines
- ❑ **Use of tumorigenic cells due to susceptibility to viruses for novel vaccines and for higher virus yield**
 - Influenza virus vaccines
 - Adeno-associated virus-vectored HIV-1 vaccines
- ❑ **Generation of genetically-engineered cells for complementation for some vectored virus vaccines**
 - Defective adenovirus-vectored vaccines
- ❑ **Development of well-characterized cell lines**
 - For novel vector development
 - To remove adventitious agent concerns
 - For high virus particle or protein yield

Novel Cell Substrates in Vaccines

Investigational products

Insect Cell Lines

- Sf9, Hi-5

Tumorigenic Cell Lines

- 293, PER.C6 (human)

- MDCK (canine)

Potential / emerging products

Genetically-engineered cell lines (mammalian, avian)

Tumor-derived cells (mammalian)

Embryonic stem cells (avian)

Plants and plant cells

Bacteria

Adventitious Agents are a Major Safety Concern in Cell Substrates

❑ Exogenously-introduced viruses

- Passage history

❑ Latent or occult viruses

- Species of cell origin

- Retroviruses (Endogenous and Exogenous)
- RNA viruses
 - Arenaviruses: LCMV
- DNA viruses
 - Adeno-associated viruses, Adenoviruses, Hepadnaviruses, Herpesviruses, Papillomaviruses, Polyomaviruses

Additional Safety Concerns in Novel Cell Substrates

□ **Tumorigenic cells**

- Tumorigenicity of intact cells
- Oncogenicity of cell DNA

□ **Genetically-engineered cells**

- Stability of phenotype
- Transgene stability
- Absence of novel replication-competent viruses (RCVs)

Tumorigenic Cell Substrates

FDA Experience with Tumorigenic Cells

THERAPEUTIC PRODUCTS (highly purified)

- ❑ **Namalwa cells** for interferon
 - *EBV genome*
- ❑ **BHK** for recombinant Factor VIIa
- ❑ **CHO cells** for many products including recombinant Factor VIII
- ❑ **Murine Hybridoma cells** (myeloma, NS0, SP2/0) for monoclonal antibodies
 - *endogenous retroviruses*
 - *murine viruses: MVM, LCMV, EDIM, LDH*
- ❑ **293 cells** for Drotrecogin alfa (activated) [Xigris]
 - *Adenovirus 5 DNA sequences*

FDA Experience with Tumorigenic Cells- *cont.*

Accepted for human use based upon:

- ❑ Risk-benefit assessment
- ❑ Extensive testing regimens for testing different stages of production: cell banks, raw materials and lots
- ❑ Development of sensitive assays for specific agents of concerns;
- e.g., in rodent CHO and BHK
 - 324K cell-based infectivity assay for Minute Virus of Mice
 - PERT assay for retroviruses
- ❑ Viral validation studies to evaluate the effectiveness of the manufacturing process in clearing virus that may potentially be present in Master Cell Bank

Discussions on Tumorigenic 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
- ❑ **2001:** Advisory committee discussion: *In vitro* transformed human cells (HEK-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

Safety Issues Associated with Tumorigenic Cell Substrates

❑ Intact cells

- Tumorigenicity

❑ Residual cellular DNA

- Infectivity
- Oncogenicity

❑ Latent/Occult viruses

- Retroviruses
- RNA viruses
- DNA viruses

❑ TSE

Use of Tumorigenic Cells in Vaccines

SUBUNIT / PROTEIN VACCINES (highly purified)

- ❑ **CHO cells** for investigational protein vaccines
- ❑ **MDCK cells** for investigational subunit influenza vaccines

Insect Cell Substrates

Insects and Insect Cell Substrates

- ❑ Mosquitoes
- ❑ Caterpillars: *Bombyx mori* (silkworm)

CELL LINES

- ❑ Drosophila
- ❑ Mosquito
- ❑ Caterpillar
 - *Spodoptera frugiperda* (fall armyworm)
 - Sf-9 and Sf21
 - *Trichoplusia ni* (cabbage looper moth)
 - Hi-5

Potential Safety Issues in Insect Cell Substrates

- ❑ Adventitious Agents
- ❑ RT activity
- ❑ Baculovirus (vector)
- ❑ DNA (baculovirus, insect retrotransposons)
- ❑ Post-transcriptional modification e.g., glycosylation may lead to unintended immunogenicity, allergenicity or, immune tolerance

Vaccines Produced in Insect Cells

- ❑ Human papillomavirus vaccine (Licensed in several countries)
- ❑ Influenza virus vaccines (Investigational)

Plants and Plant Cells

Plant Substrates

- ❑ Fungi
- ❑ Algae
- ❑ Carrot
- ❑ Mushrooms
- ❑ Tobacco

Safety Considerations in Plant Cells

- ❑ Allergenicity
 - Host cell proteins
 - Mold, animal dander, animal excrement, or dust mite due to field or storage conditions
- ❑ Immunogenicity
 - post-transcriptional modification e.g., glycosylation may contribute to unintended immunogenicity or immune tolerance or to allergenic responses
- ❑ Toxicants (protease inhibitors, hemolytic agents, neurotoxins, heavy metals)
- ❑ Pesticides, herbicides and fungicide levels
- ❑ Adventitious agents

Experience with Plants

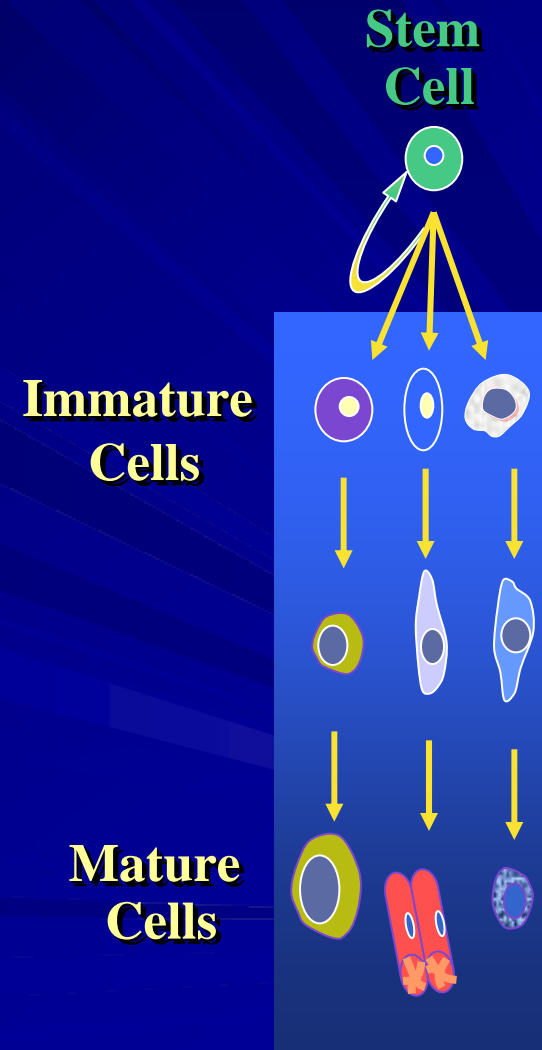
- ❑ **FDA Draft Guidance for Industry: Drugs, Biologics, and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals. Sept. 2002**
- ❑ **European Food Safety Authority: Scientific Opinion on Guidance for the risk assessment of genetically modified plants used for non-food or non-feed purposes. EFSA Journal 2009; 1164: 1-42**

Embryonic Stem Cells (Avian)

- No experience with ESCs in vaccines**
- Human stem cells as cellular therapies regulated in Office of Cellular, Tissue and Gene Therapies (OCTGT)**

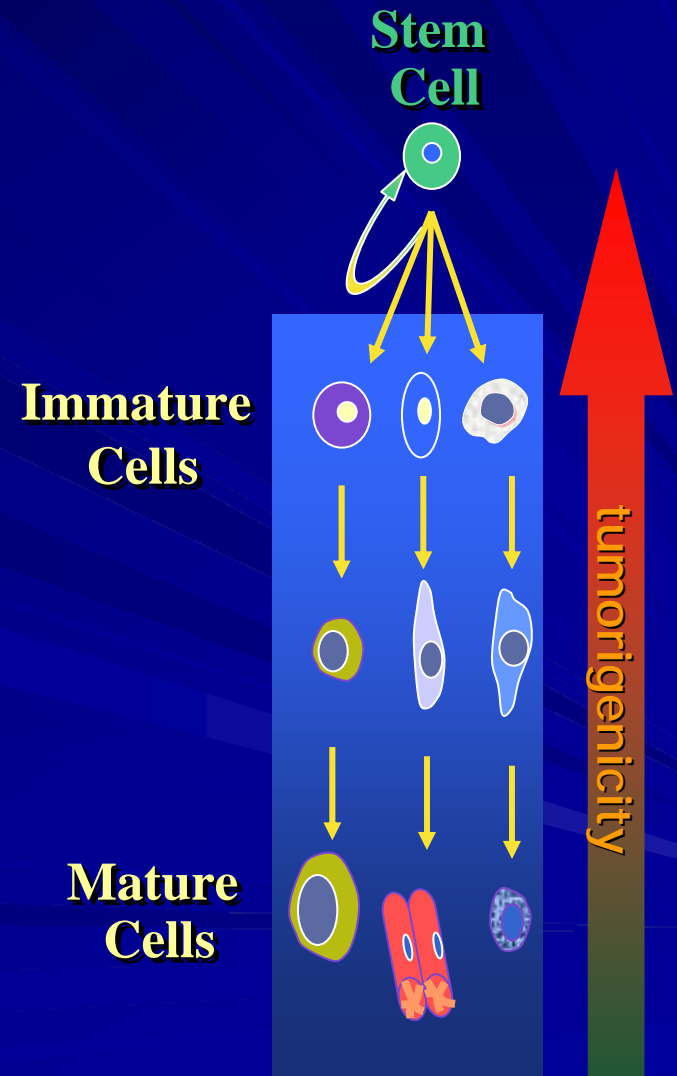
Scientific Considerations Related to ESCs

- ❑ Self-renewal
- ❑ Pluripotency
- ❑ Differentiation in culture
- ❑ Genetic stability



Potential Safety Issues in ESCs

- ❑ Adventitious agents
- ❑ Tumorigenicity
- ❑ Host cell DNA



Safety Concerns in Novel Cell Substrates

ALL CELL SUBSTRATES

❑ **Adventitious Agents**

- **Acquired (Exogenous)**

- donor species of source materials (virus seed, cell substrate, biological raw materials used in manufacture)
- accidental introduction during cell culture passage history
- due to handling or equipment

- **Genetically-inherited sequences in the cell substrate (Endogenous)**

INSECT AND PLANT CELLS

❑ **Host cell proteins**

❑ **Toxicants**

EMBRYONIC STEM CELLS

❑ **Tumorigenicity**

Designing Safety in Vaccines

- **Comprehensive testing regimens for detection of known and unknown adventitious viruses in novel cell substrates to minimize the risk of virus contamination**
- **Steps in the manufacturing process to inactivate and/or remove contaminating viruses to maximize virus clearance**

General Considerations for Development of a Comprehensive Testing Scheme for Cell Substrate Safety

□ Risk assessment of cell substrate contamination

- **Host species of origin**
 - Health of donor
 - Naturally-occurring exogenous and endogenous viruses
- **Cell passage history**
 - Exogenously introduced adventitious agents due to handling, raw materials, other cell lines
- **Cell characteristics**
 - Genotype (diploid vs. neoplastic/continuous cell line)
 - Phenotype (tumorigenic vs. non-tumorigenic)

□ Evaluation for risk reduction

- **Susceptibility to known viruses**
- **Inactivation or clearance due to manufacturing process**
- **Use of genetically-modified cell lines that minimize generation of RCVs**
- **Use of qualified biological starting materials (virus seed, vectored virus stock) and reagents (serum, trypsin)**

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
- ❑ As guidance, the document does not establish requirements, but provides guidance on how regulatory requirements can be addressed
- ❑ 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 manufacturers additional flexibility
- ❑ Fosters early discussions between regulators and manufacturers regarding development of specific assays for novel cell substrates

Routine Tests for Vaccine Cell Substrates

□ IDENTITY

□ STERILITY

□ NON-VIRAL AGENT TESTING

- Mycoplasma
- Bacteria and Fungi
- Mycobacteria

□ TUMORIGENCITY

- Adult nude mice
- ATG-treated or irradiated newborn rats or newborn mice

Routine Tests for Vaccine Cell Substrates

□ ADVENTITIOUS VIRUS TESTING

□ General

- *In vitro* cell culture tests (cells from 3 species including human)
- *In vivo* assays (adult mice, suckling mice, embryonated hens' eggs)
- Transmission electron microscopy (TEM)
- Reverse transcriptase assay for retroviruses (PERT)

□ Species-specific

- Tests for animal viruses (9CFR 113.47 and 113.53)
- Antibody production assays for rodent viruses (MAP, HAP, RAP)
- Assays for known viruses (PCR, DNA hybridization, Infectivity, Antibody detection)

Additional Assays for Consideration in Testing Novel Cell Substrates

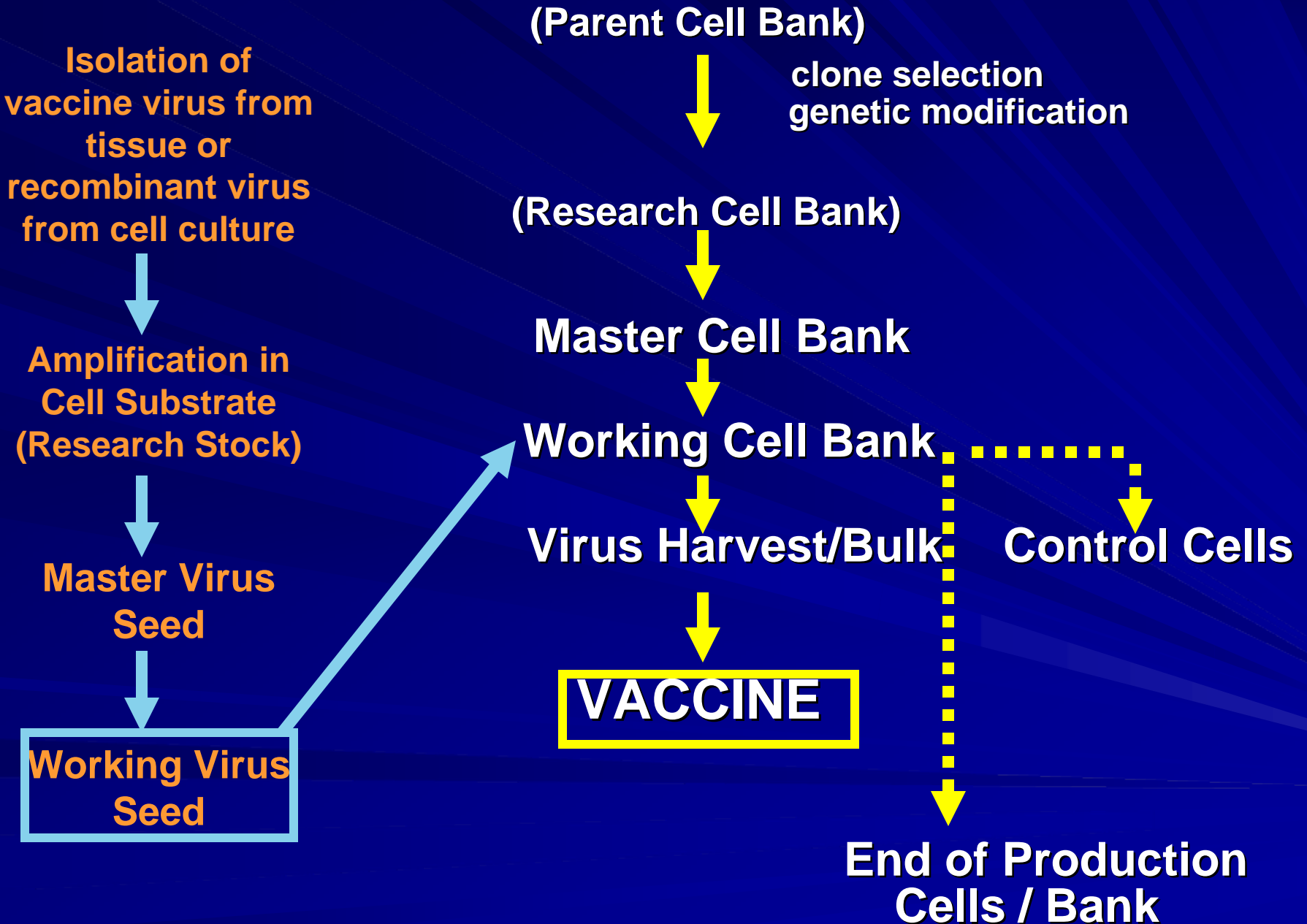
- **EXTENDED TUMORIGENICITY ASSAY (INTACT CELL)**
 - Characterization of tumorigenic phenotype of neoplastic cells
 - Using the most sensitive animal model (newborn in some cases)
 - Extended observation period: 4 – 7 months in some cases

- **ONCOGENICITY ASSAY (DNA ONCOGENICITY and ONCOGENIC VIRUSES)**
 - Inoculation of cell lysates (from 10^7 cell equivalent) or cell DNA ($\geq 100 \mu\text{g}$) into < 4 day-old animals (newborn hamster, newborn nude mice, newborn rats)
 - Observation Period: 4-7 months

- **CHEMICAL-INDUCTION ASSAYS (LATENT ENDOGENOUS RETROVIRUSES, and OCCULT RNA and DNA VIRUSES)**
 - Chemical inducers (IdU, AzaC, NaB, TPA)
 - Sensitive and broad assays for detection of induced virus (PERT, PCR, Infectivity, TEM)

- **TSE**

Generic Vaccine Production Scheme



Different Vaccine Types

- ❑ **Live, attenuated or recombinant vector virus**
- ❑ **Inactivated, Whole virus**
- ❑ **Subunit / Protein**

Live Vaccines

□ Effectiveness

- A single dose usually confers protection

□ Minimally processed and minimally purified

- Added safety concerns

□ Product Safety assured by:

- Extensive testing
 - 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

Inactivated Vaccines

❑ Effectiveness

- May need multiple doses for a protective immune response

❑ Moderately processed with some purification to reduced levels of cellular materials

- Reduced adventitious agent concerns

❑ Product safety assured by:

- Virus inactivation
- Process validation
- Reduction of cellular DNA and protein

Subunit Vaccines

□ Effectiveness

- May need multiple doses for a protective immune response

□ Highly processed with minimal levels of cellular materials

- Minimal adventitious agent and residual cell DNA concerns

□ Product safety assured by:

- Virus clearance
- Process validation
- Reduction of cellular DNA and protein

SUMMARY: General Approaches for Evaluation of Safety in Viral Vaccines

- ❑ **Characterization of cell substrate**
 - Cell phenotype: tumorigenicity may be associated with oncogenic viruses or DNA oncogenicity
- ❑ **Qualification of cell banks, virus seed, and biological raw materials**
 - Extensive testing of vaccine virus seed and cell substrates
 - 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 virus contaminants
- ❑ **Process validation**
 - Design an efficient process
 - to avoid risk of contamination
 - eliminate or reduce potential adventitious virus load
 - inactivate potentially contaminating virus
- ❑ **Reduction of residual host cell material in final product**
 - Whole cells
 - Cellular DNA and protein

Some Relevant Regulatory Documents and Guidances

U.S. FDA

- ❑ Code of Federal Regulations (CFR):
 - 21 Part 610
 - 21 Part 211
 - 21 Part 680
 - 21 Part 640 sections 3 and 63
 - 9 Part 113 sections 53, 46, and 47

- ❑ 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 for 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

Some Relevant 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 the use of animal cells as in vitro substrates for the production of biologicals, WHO Technical Report Series 878, Annex 1, 1998.