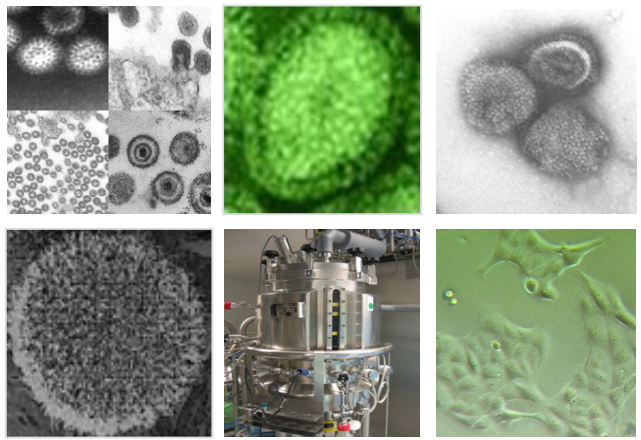
Use of MDCK Cells for Manufacture of Inactivated Influenza Virus Vaccines

VRBPAC – 16 Nov 05



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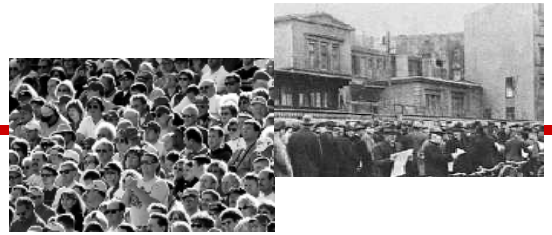
Influenza – Disease impact



- **Annual winter epidemics**
  - 10-20% of world population is infected
  - In the US:
    - 25-50 million individuals infected
    - >20,000 deaths and >110,000 hospitalizations
    - >$12 billion in direct and indirect health costs
- **Worldwide pandemics**
  - 1918-19 Spanish Flu: 20-40 million deaths
  - 1957 Asian/ 1968 Hong Kong: >1.5 million deaths

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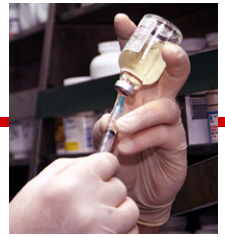
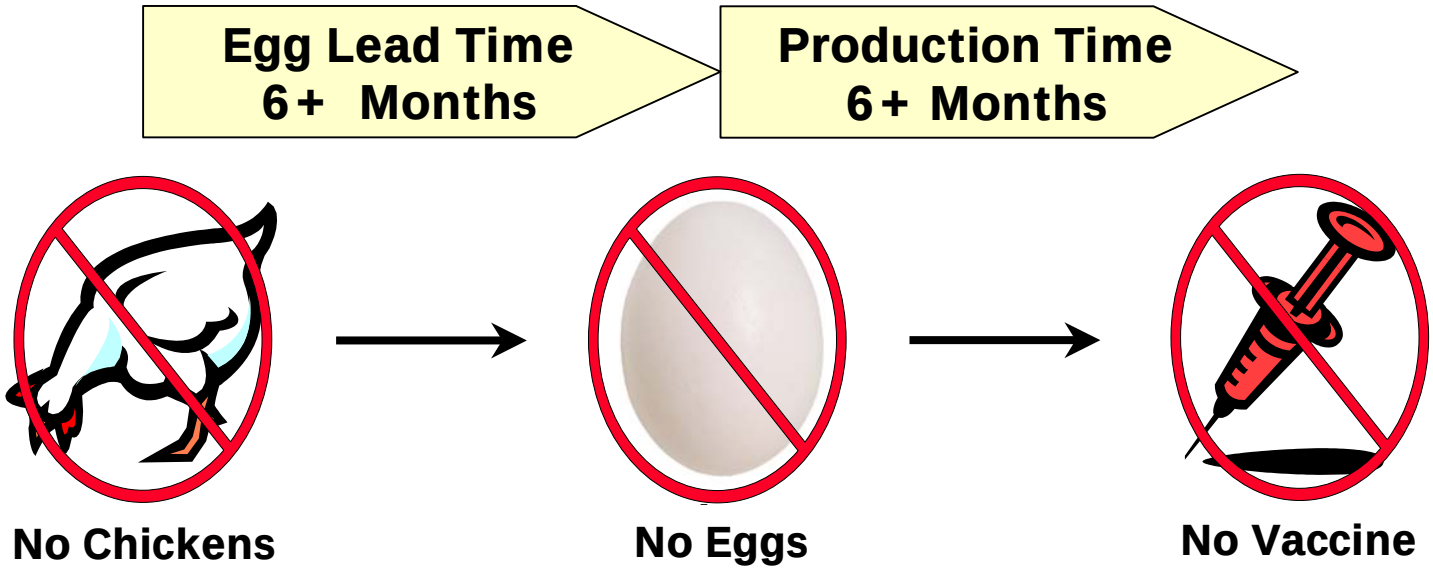
Influenza vaccine – The public need



- **Routine Immunization:**
  - Recommended for >180 million in U.S. and increasing
  - Current egg-based production for U.S. does not
    - Meet the recommendation
    - Provide flexibility to respond to fluctuating demand
- **Pandemic Immunization:**
  - Will require rapid production of vaccine for 6.5 billion worldwide, ~300 million people in the US

Influenza vaccine – Egg-based process and risk



- Embryonated eggs require 6+ months from order to delivery
- ~ 1 egg is needed per each vaccine dose
- Egg-based process limited in flexibility and reliability:
  - Chickens or embryos could be killed by virulent bird flu
  - Egg lead time hinders response to unanticipated demand, e.g. pandemic, production failures, strain changes, etc.

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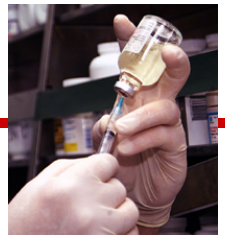
Influenza vaccine – A national priority



"Using a cell culture approach to producing influenza vaccine offers a number of benefits.

. . . help meet surge capacity needs in the event of a shortage or pandemic . . .

. . . provide security against risks associated with egg-based production . . .

. . . provide an option for people who are allergic to eggs . . ."

Department of Health and Human Services, 01 Apr 05

"I am asking Congress for $2.8 Billion to accelerate development of cell culture technology."

President George W. Bush, 01 Nov 05

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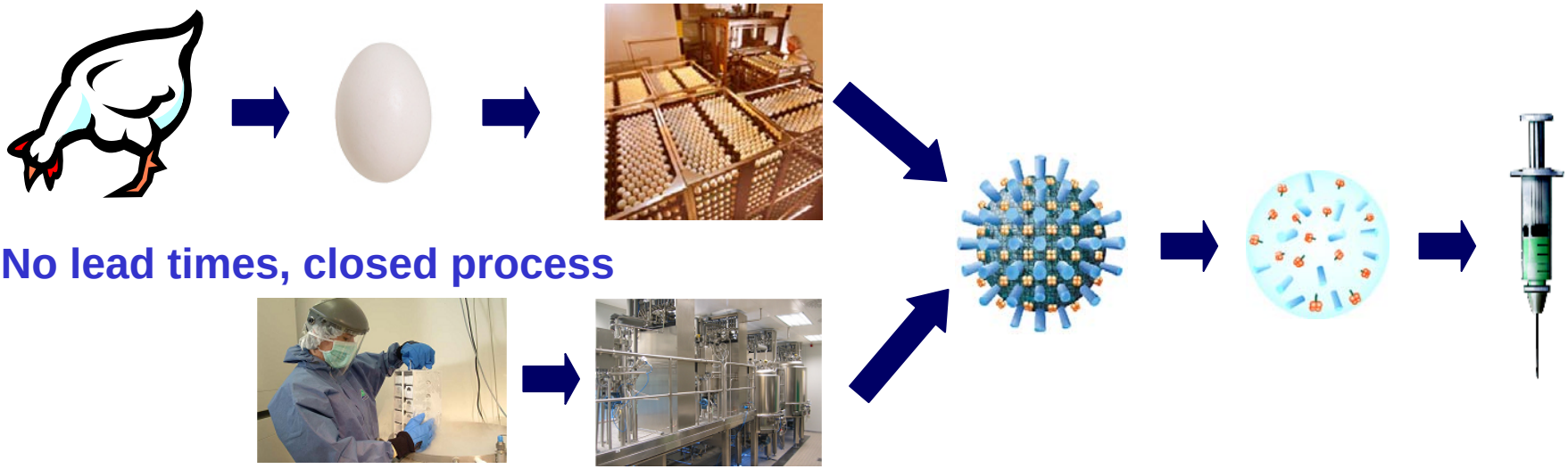
Continuous cell lines – Address limitations, utilize strengths of egg process

Long lead times, open handling steps



No lead times, closed process

- Use readily available raw materials
- Involve closed-system bioreactors in place of millions of eggs
- Allow for scalable, flexible, high volume processes
- Are characterizable, can grow without animal-derived components
- Used for ~30 US-licensed therapeutics + Inactivated Polio Vaccine

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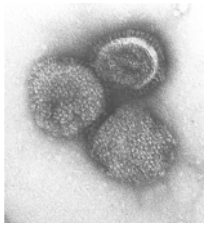
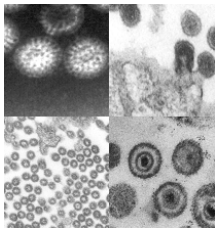
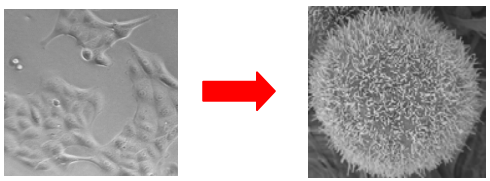
Continuous cell lines – Rationale for Chiron MDCK

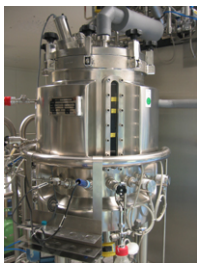
- **Inherent characteristics:**
  - **Broadly and highly permissive for a wide variety of flu strains**
  - **Restricted growth of non-flu human pathogens that may be present in the viral seed**





- **Selected characteristics:**
  - **Suspension adapted to provide scalable, high yield, high volume production**
  - **Adapted for growth in chemically defined medium (no animal-derived components)**





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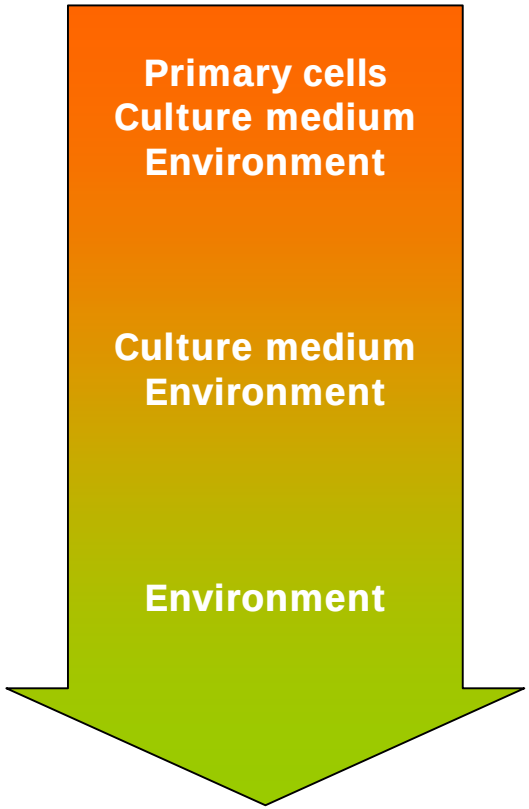
Continuous cell lines – Advantages over primary or diploid cells

Potential sources of adventitious agent contamination



Decreased risk from adventitious agents

Cell Types

1950's
Primary
(Egg-based Influenza Vaccine Measles)

1970's
Diploid
(Rubella, Hepatitis A, Varicella Rabies)

1980's
Continuous Cell Lines
(IPV)

Characterization of cell substrate

Increased characterization

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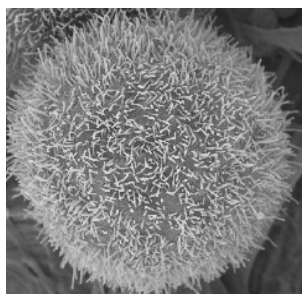
Continuous cell lines – Potential concerns

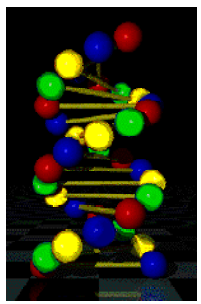
- **Continuous cell lines have the potential to be *tumorigenic* and/or *oncogenic***
  - **Tumorigenicity – growth of intact cells in a host animal**
  - **Oncogenicity – transformation of host animal cells into tumor cells**

- **The potential concerns come from three sources:**

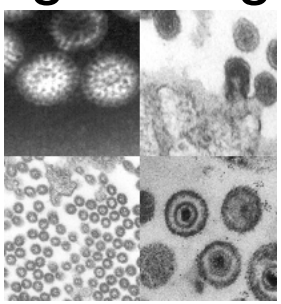
Cells



DNA



Oncogenic Agents



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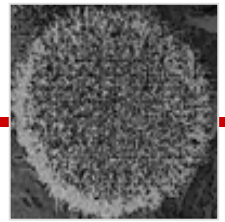
Continuous cell lines – Regulatory approaches to risk assessment



- **Testing paradigms have been defined to assess potential risk to allow safe use**
  - **CBER's Points to Consider and Defined Risks Approach Algorithm (applicable to tumorigenic and non-tumorigenic cell lines)**
  - **ICH Guidelines**
  - **CHMP Guidelines**

Chiron has applied these paradigms to safety testing of the MDCK cell line in consultation with regulatory authorities

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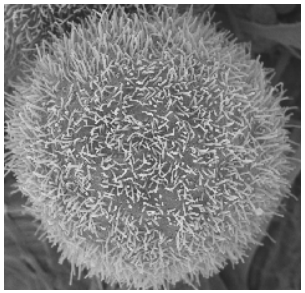
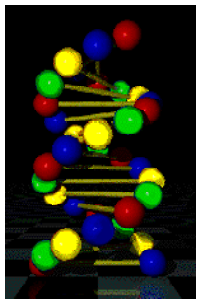
Chiron MDCK cells – Demonstrating acceptability as a cell substrate

Cells



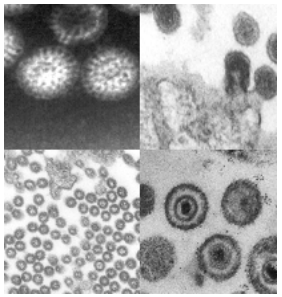
- Demonstrate removal of intact cells
- Demonstrate no capacity for transformation (oncogenicity)

DNA



- Demonstrate lack of oncogenicity
- Demonstrate acceptable DNA removal and/or inactivation

Adventitious Agents



- Demonstrate lack of inherent agents
  - Infectious
  - Oncogenic
- Demonstrate removal and/or inactivation of potential agents

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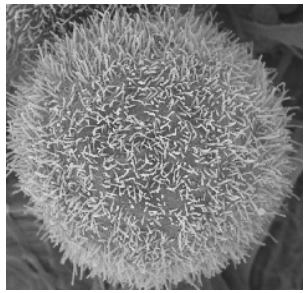
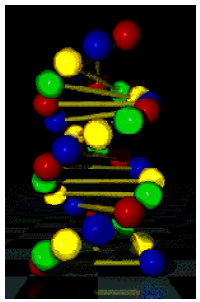
Chiron MDCK cells – Demonstrating acceptability as a cell substrate

Cells



- **Demonstrate removal of intact cells**
- Demonstrate no capacity for transformation of host cells (oncogenicity)

DNA



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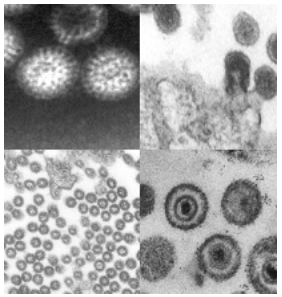
Adventitious Agents



- Demonstrate lack of inherent agents
  - Infectious
  - Oncogenic
- Demonstrate removal and/or inactivation of potential agents

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Demonstrating acceptability as a cell substrate – Tumorigenicity Study

- As expected, MDCK cells were tumorigenic in nude mice
- As few as 10 cells formed tumors

Group	Animals examined (N)	Histologically confirmed tumors (N)
10^1 MDCK	24	3
10^3 MDCK	25	3
10^5 MDCK	24	10
10^7 MDCK	24	11

Therefore, assurance of cell removal during manufacturing is important

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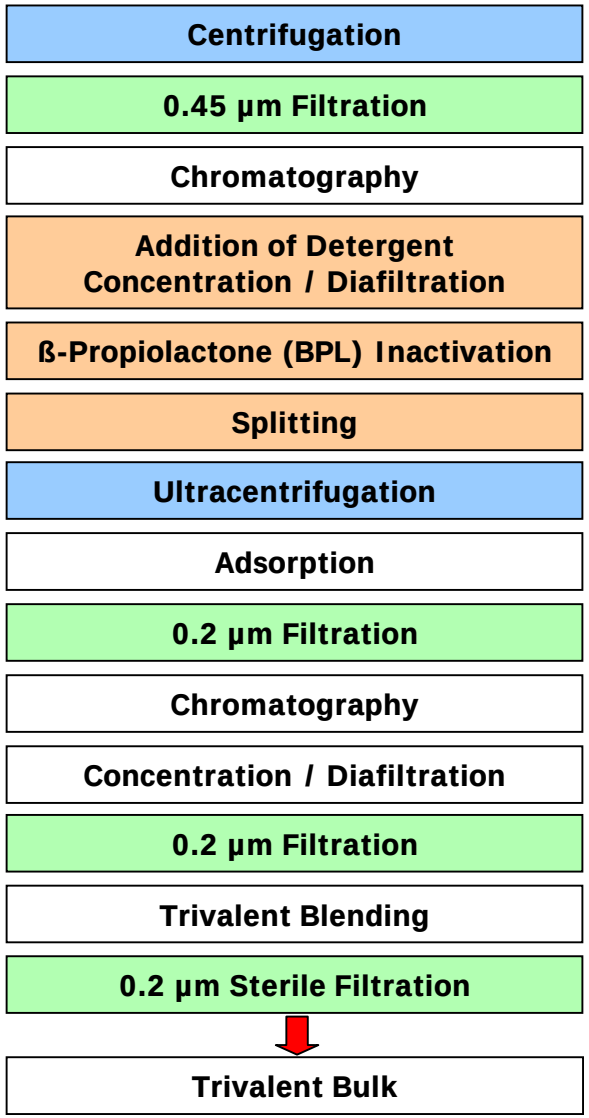
Demonstrating acceptability as a cell substrate – Removal of intact cells



- **Most cells are lysed by influenza virus growth**
- **Multiple, redundant processes designed to remove cells**

Centrifugation
Filtration
Chemical inactivation / disruption

- **Cells would be also removed by chromatography**

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Demonstrating acceptability as a cell substrate – Cell reduction by centrifugation

Centrifugation

0.45 µm Filtration

Chromatography

Addition of Detergent Concentration / Diafiltration

ß-Propiolactone (BPL) Inactivation

Splitting

Ultracentrifugation

Adsorption

0.2 µm Filtration

Chromatography

Concentration / Diafiltration

0.2 µm Filtration

Trivalent Blending

0.2 µm Sterile Filtration

↓

Trivalent Bulk

> 2 $\log_{10}$ reduction (99 %)

Disk-stack centrifuge

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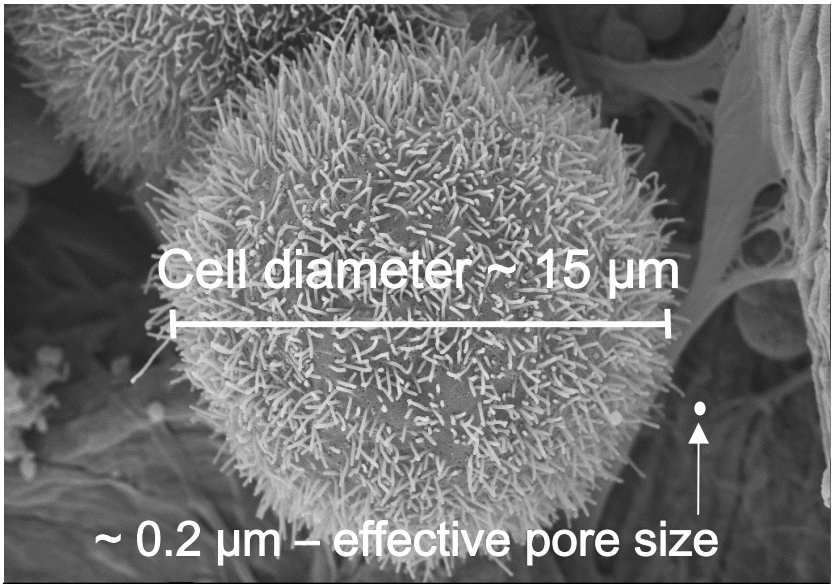
Demonstrating acceptability as a cell substrate – Cell reduction by filtration

Centrifugation	
0.45 µm Filtration	> 6.5 $\log_{10}$ reduction (99.9999 %)
Chromatography	
Addition of Detergent Concentration / Diafiltration	
ß-Propiolactone (BPL) Inactivation	
Splitting	
Ultracentrifugation	
Adsorption	
0.2 µm Filtration	> 8.8 $\log_{10}$ reduction (>99.999999 %)
Chromatography	
Concentration / Diafiltration	
0.2 µm Filtration	> 8.8 $\log_{10}$ reduction (>99.999999 %)
Trivalent Blending	
0.2 µm Sterile Filtration	> 11.5 $\log_{10}$ reduction (>99.999999999 %)
↓	
Trivalent Bulk	



MDCK cell and 0.2 µm pore

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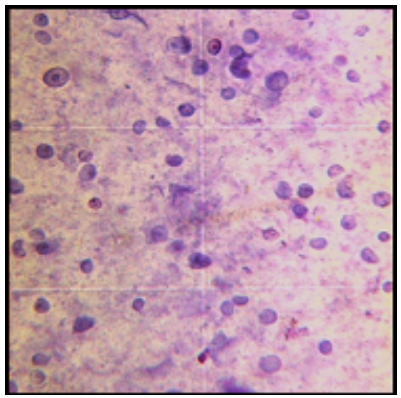
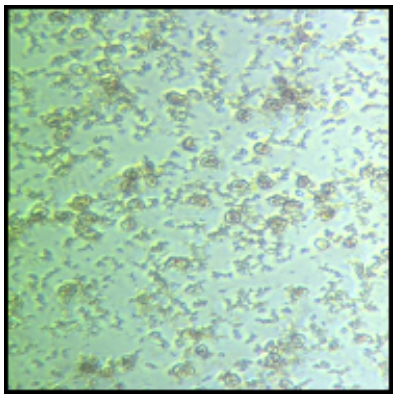
Demonstrating acceptability as a cell substrate – Cell reduction by chemical inactivation

- Centrifugation
- 0.45 µm Filtration
- Chromatography
- **Addition of Detergent Concentration / Diafiltration** — > 1 $\log_{10}$ reduction (90 %)
- **ß-Propiolactone (BPL) Inactivation** — > 1 $\log_{10}$ reduction (90 %)
- **Splitting** — > 4 $\log_{10}$ reduction (99.99 %)
- Ultracentrifugation
- Adsorption
- 0.2 µm Filtration
- Chromatography
- Concentration / Diafiltration
- 0.2 µm Filtration
- Trivalent Blending
- 0.2 µm Sterile Filtration
- ↓
- Trivalent Bulk

Cells treated with splitting agent



Trypan blue-stained cells, dead after treatment



No cell growth in medium

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Demonstrating acceptability as a cell substrate – Cumulative removal of intact MDCK cells

Centrifugation	> 2.0 $\log_{10}$ reduction
0.45 µm Filtration	> 6.5 $\log_{10}$ reduction
Chromatography	
Addition of Detergent Concentration / Diafiltration	> 1 $\log_{10}$ reduction
ß-Propiolactone (BPL) Inactivation	> 1 $\log_{10}$ reduction
Splitting	> 4 $\log_{10}$ reduction
Ultracentrifugation	
Adsorption	
0.2 µm Filtration	> 8.8 $\log_{10}$ reduction
Chromatography	
Concentration / Diafiltration	
0.2 µm Filtration	> 8.8 $\log_{10}$ reduction
Trivalent Blending	
0.2 µm Sterile Filtration	> 11.5 $\log_{10}$ reduction
↓	
Trivalent Bulk	> 41 $\log_{10}$ reduction = cumulative cell removal

Theoretical starting cells / dose	10^{7}
Cumulative cell removal	10^{-41}
Probability a single cell could be in a dose	10^{-34}

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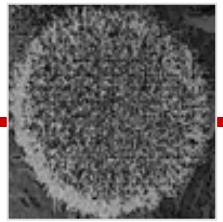
Demonstrating acceptability as a cell substrate – What does the risk of 1 cell in 10^{34} doses mean?



If every person who has ever lived or will live received the vaccine each year for 100 years…

Then the probability of even *one* person receiving *one* MDCK cell is *less than one in one trillion* (1 in 10^{12})!

People living	~ 6.5×10^{9} (6.5 billion)
. . . plus people who have ever lived	~ 1×10^{10} (10 billion)
. . . plus people who will live in next 5 billion years *(the expected time before the sun burns out)*	~ 10^{20}

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Demonstrating acceptability as a cell substrate – Summary of *in vivo* testing

Program includes *in vivo* rodent studies designed in consultation with CBER

	Assay	
Test Material (species)	**Tumorigenicity**	**Oncogenicity**
Intact cells (nude mice)	**Yes** (Canine Tumors)	**No** N=104
Cell lysates (neonatal nude mice, rats, and hamsters)	N/A	**No** N=139
Cellular DNA (neonatal nude mice, rats, and hamsters)	N/A	**No** N=224

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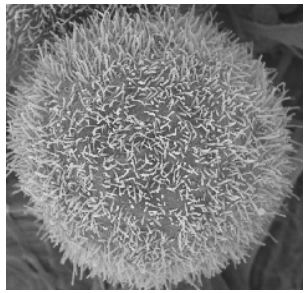
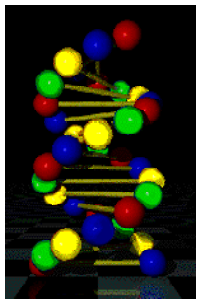
Demonstrating acceptability as a cell substrate – Oncogenicity

Cells



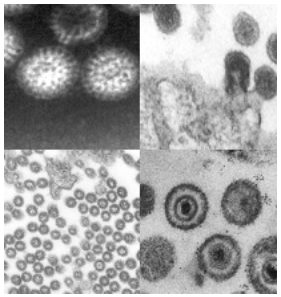
- Demonstrate removal of intact cells
- **Demonstrate no capacity for transformation of host cells (oncogenicity)**

DNA



- **Demonstrate lack of oncogenicity**
- Demonstrate acceptable DNA removal and/or inactivation

Adventitious Agents



- **Demonstrate lack of inherent agents**
  - Infectious
  - **Oncogenic**
- Demonstrate removal and/or inactivation of potential agents

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Demonstrating acceptability as a cell substrate – Oncogenicity

- **Studies for oncogenicity – Cells**
  - **Up to 1 x 10^7 intact MDCK cells tested in adult nude mice**
  - **No murine tumors observed**

Conclusion: no oncogenicity observed

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Demonstrating acceptability as a cell substrate – Oncogenicity

- **Studies for oncogenicity – Lysates**
  - Cell lysates from 5 x 10^6 – 1 x 10^7 cells in neonatal nude mice, rats and hamsters
  - No tumors observed

Treatment	Mouse (N)	Rat (N)	Hamster (N)	Total (N)
MDCK	11	30	28	69
BPL-Flu-MDCK	12	28	30	70
Total	23	58	58	139

Conclusion: no oncogenicity observed

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Demonstrating acceptability as a cell substrate – Oncogenicity

- **Studies for oncogenicity – DNA**
  - **> 2800 times the dose limit of purified, high molecular weight DNA in neonatal nude mice, rats and hamsters**
  - **No tumors observed**

Treatment	Mouse (N)	Rat (N)	Hamster (N)	Total (N)
MDCK	4	29	30	63
Flu-MDCK	16	28	27	71
BPL-Flu-MDCK	30	30	30	90
Total	50	87	87	224

Conclusion: no oncogenicity observed

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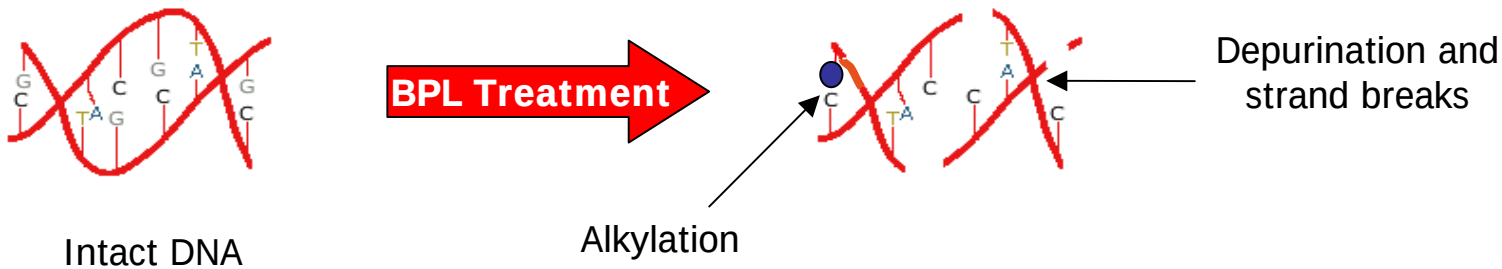
Demonstrating acceptability as a cell substrate – Production process removes and degrades DNA

- < 10ng DNA/dose (as recommended by WHO for continuous cell lines)
- Remaining DNA is
  - Degraded to < 200 base pairs primarily by ß-propiolactone treatment (typical oncogenes are >1000 base pairs)
  - Inactivated by ß-propiolactone treatment



- Analysis for canine genes by PCR at the end of production – none found

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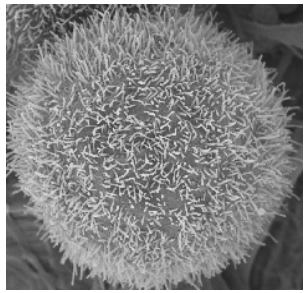
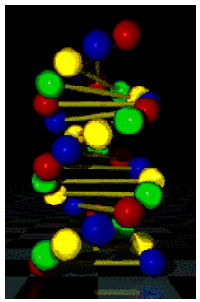
Demonstrating acceptability as a cell substrate – Adventitious agents

Cells



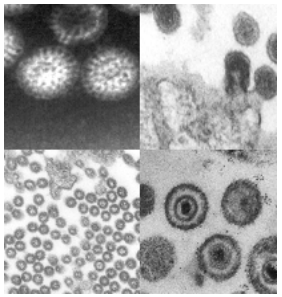
- Demonstrate removal of intact cells
- Demonstrate no capacity for transformation (oncogenicity)

DNA



- Demonstrate lack of oncogenicity
- Demonstrate acceptable DNA removal and/or inactivation

Adventitious Agents



- **Demonstrate lack of inherent agents**
  - **Infectious**
  - **Oncogenic**
- **Demonstrate removal and/or inactivation of potential agents**

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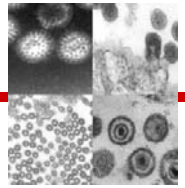
Demonstrating acceptability as a cell substrate – Viral testing of MDCK cells



- Viruses could be introduced from multiple sources during cell line development

- Testing was performed in
  - Pre-cell bank
  - Master cell bank
  - Working cell bank
  - End of production cells

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Demonstrating acceptability as a cell substrate – Viral testing of MDCK cells

- **Broad screening assays used for virus families**
  - **Electron microscopy**
  - ***In vitro* infectivity using indicator cell lines**
  - ***In vivo* assays**
  - **Reverse transcriptase for retroviruses**

- **Specific and non-specific assays used for individual viruses**
  - **Animal viruses (canine, bovine, porcine, equine, murine)**
  - **Human viruses**

All tests negative

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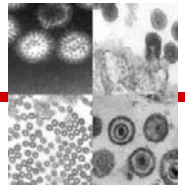
Demonstrating acceptability as a cell substrate – Testing for latent adventitious agents



- **Redundant PCR assays for herpesviruses and polyomaviruses conducted**
  - **Negative (report not yet submitted to CBER)**
- **Induction assays for latent viruses**
  - **Protocol in development**

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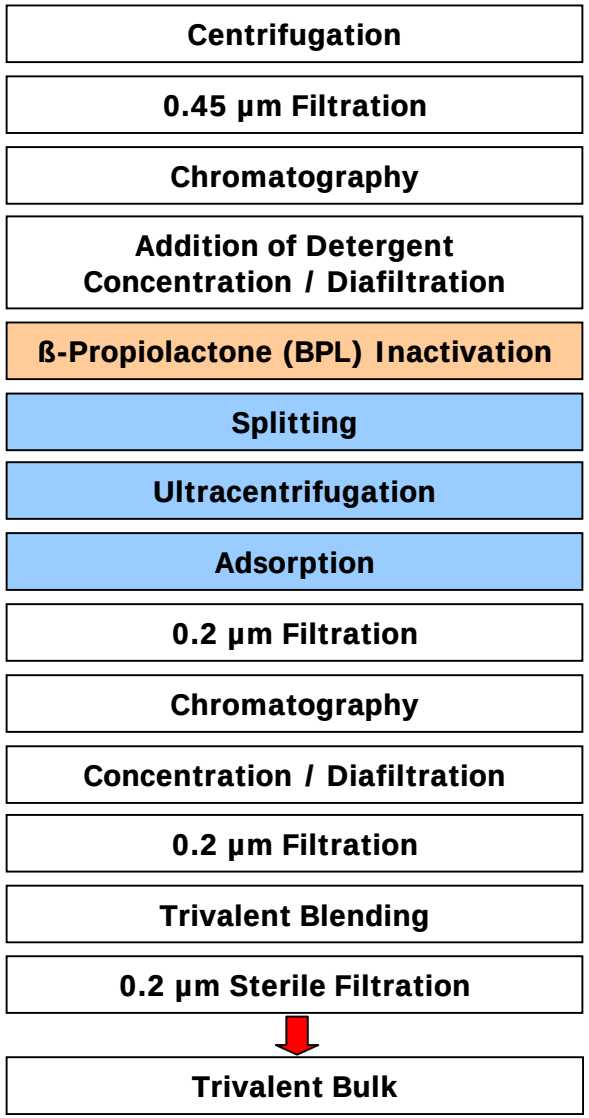
Demonstrating acceptability as a cell substrate – Removal of potential contaminating viruses



- **Viruses may be introduced during processing – from virus seed, environment, etc.**
- **Multiple processes designed to remove these viral agents, thus providing an additional margin of safety**

Inactivation by ß-propiolactone

Splitting
Ultracentrifugation
Adsorption

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Demonstrating acceptability as a cell substrate – Viral reduction by process

Centrifugation

0.45 µm Filtration

Chromatography

Addition of Detergent Concentration / Diafiltration

ß-Propiolactone (BPL) Inactivation

Splitting

Ultracentrifugation

Adsorption

0.2 µm Filtration

Chromatography

Concentration / Diafiltration

0.2 µm Filtration

Trivalent Blending

0.2 µm Sterile Filtration

Trivalent Bulk

Process material spiked with model viruses and processed

(Selection criteria: enveloped, non-enveloped, RNA, DNA, single-stranded, double stranded, BPL-resistant)

	Herpes simplex virus (ds-DNA, enveloped)	Reovirus 3 (ds-RNA, non-enveloped)	Murine Retrovirus (ss-RNA, enveloped)
BPL inactivation	4.5 $\log_{10}$	2.3 $\log_{10}$	≥ 4.5 $\log_{10}$
Splitting Ultra-centrifugation Adsorption	≥ 5.5 $\log_{10}$	≥ 7.6 $\log_{10}$	≥ 7.6 $\log_{10}$
Combined virus reduction	≥ 10 $\log_{10}$	≥ 9.9 $\log_{10}$	≥ 12.1 $\log_{10}$

Virus removal was >9.9 $\log_{10}$ for all challenges

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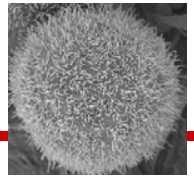
MDCK cell line and manufacturing summary



- **MDCK Cell Line**
  - Intact MDCK cells are tumorigenic
  - No oncogenicity observed in cell, lysate and DNA studies
  - No adventitious agents detected
- **Process**
  - Removes intact cells
  - DNA reduced to <10ng/dose
  - Residual DNA inactivated
  - Potential adventitious agents removed and/or inactivated

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Status of clinical development of cell-derived influenza vaccine

- **European Union activities**
  - Phase 1, 2 and 3 studies carried out in Europe
  - > 3000 subjects received vaccine since 2002
  - Tolerability and immunogenicity comparable to a licensed egg-derived subunit vaccine
- **US activities**
  - Phase 1/2 US study underway
  - Enrollment complete

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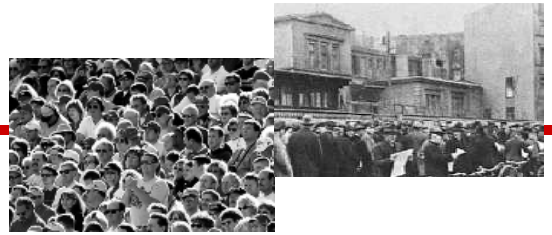
Overall summary



There is an unmet public need for a readily available and reliable supply of flu vaccine.

Chiron has developed a robust, scalable and safe manufacturing process, which utilizes MDCK cells to meet this need.

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Influenza vaccine – A national priority

In reference to the influenza vaccine:

“The Cell-based technology . . . will change the world of vaccine production forever”

Michael Leavitt, Secretary, Health and Human Services, 27 Oct 05

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