

# Virus Contamination of Cell Culture Manufacturing Operations

**A Question of When, Not If**

Michael E. Wiebe, Ph.D.

# Headlines You Do Not Want to See

## [GlaxoSmithKline's Rotavirus Vaccine Suspended for Pig Virus Contamination](#)

By [MELLY ALAZRAKI](#) Posted 11:30 AM 03/23/10 [Company News](#), [Health Care](#), [Merck](#), [GlaxoSmithKline](#)

## [Vaccine Shocker May 2010-Vaccine Contamination](#)

June 1, 2010 [Vaccine shocker May 2010 Vaccine contamination](#) information has just been released. This information on the Rotavirus vaccine that contains contaminated live pig viruses being injected in babies in the United States. Video shows pigs dying of this virus and now your children are in grave danger.

## [May 7, 2010: Vaccines and Related Biological Products Advisory Committee Meeting Announcement](#)

On May 7, 2010, in the morning, the committee will review and discuss available data regarding the unexpected finding of DNA originating from porcine circovirus type 1 (PCV 1) in Rotarix, a U.S. licensed vaccine manufactured by GlaxoSmithKline and ...

# More Headlines

***Virus closes Genzyme plant, holds up drugs for 8,000 ...*** Boston Globe, June 17, 2009

***Genzyme's plight leaves patients uneasy ...*** Boston Globe, June 18, 2009

***After a virus invades, Genzyme scrubs down ...*** Boston Globe, June 25, 2009

***Genzyme Plant Shutdown Could Mean up to \$300M in Lost Sales ...*** Genetic Engineering News, July 2, 2009

***Genzyme Rival, Actelion, Seeks to Fill Void Created by Cerezyme Shortage ...*** Xconomy.com, July 9, 2009

***Genzyme Corporation hit by Shareholder Class Action Lawsuit ...*** PR-inside.com, July 29, 2009

***Shire's Gaucher Drug Passes Key Trial, Putting More Heat on Genzyme During Shortage ...*** Xconomy.com, Aug 8, 2009

***As Genzyme flounders, competitors and activist investors swoop in ...*** Malorye Allison, Nature Biotechnology 28, 3-4 (2010)

***Genzyme Announces FDA Enforcement Action Regarding Allston Plant ...*** Business Wire, Mar 24, 2010

# Roadmap

- How it All Started
- BioSafety Concerns
- Redundant Control Systems
- Industry Contaminations
- Testing Barriers
- Process Barriers
- The Genzyme Story
- Making Risk Reduction Decisions
- Current State of Affairs
- On-going Initiatives
- Conclusions
- Q & A

# In the Beginning

- ***E. coli* was King**
- Advent of Recombinant Continuous Cell Lines
- BioSafety Concerns
- Regulatory Guidance
- Multifaceted Strategy

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# Use of Continuous Cell Lines

- Proteins are Glycosylated
- Proteins do not Need to be Refolded
- Proteins are Secreted out of the Cell
- Continuous Cell Lines Replicate Indefinitely
- Continuous Cell Lines can be Cultured in Large Bioreactors

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# BioSafety Concerns

- Continuous Cell Lines are Derived from Tumors
- Some Continuous Cell Lines Produce Large Quantities of Endogenous Retrovirus-like Particles
- Continuous Cell Lines Support the Replication and Amplification of Some Viruses that Can Potentially Contaminate Cell Culture Processes

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- BioSafety Concerns
- **Regulatory Guidance**
- Multifaceted Strategy

# Regulatory Guidance

- *Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals.* 1987 & 1993. FDA
- *Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses.* 1996. CPMP/BWP/268/95
- *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use.* 1997. FDA
- *Requirements for the Use of Animal Cells as in vitro Substrates for the Production of Biologicals.* 1998. WHO
- *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin.* 1999. ICH Q5A
- *Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products.* 2008. EMEA/CHMP/BWP/398498/2005
- *Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications.* 2010. FDA

# In the Beginning

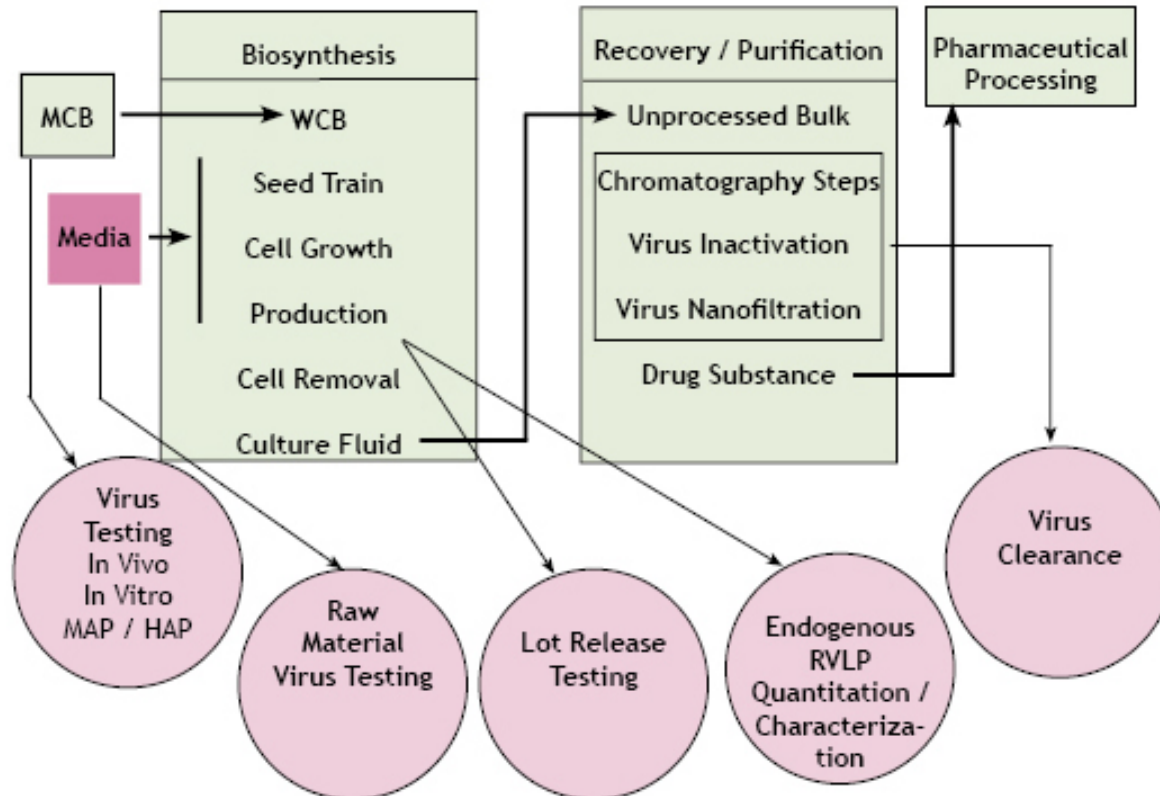
- *E. coli* was King
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- Regulatory Guidance
- **Multifaceted Strategy**

# Multifaceted Approach to Virus Safety

## “Belt and Suspenders”

- Master Cell Bank Exhaustively Characterized for Virus Contamination
- Raw Material Control
  - Limited biological raw materials to those of bovine origin which were tested for virus
  - Distillation of process water
- Engineered Closed Systems for Cell Culture
- Identification of Viruses that can Replicate in CHO cells
- Testing of Each Cell Culture Production Lot for Viruses
- Demonstration of Viral Inactivation / Removal in the Downstream Recovery Process

# Belt & Suspenders



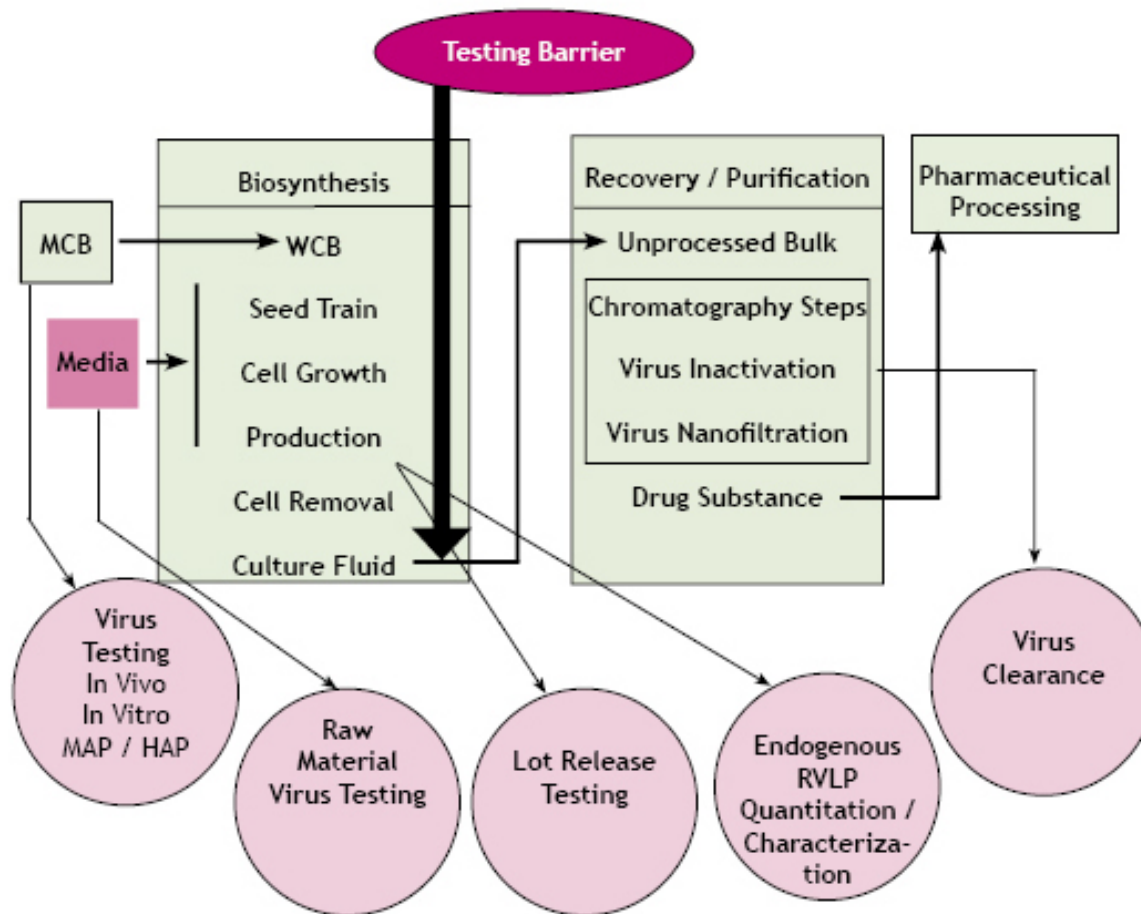
# Virus Contaminations Known in the Biopharmaceutical Industry

<b>Virus</b>	<b>Year</b>	<b>Company</b>	<b>Reported By</b>
EHDV	1988	Bioferon GmbH	Bioferon
MMV	1993	Genentech	Genentech
MMV	1994	Genentech	Genentech
Reovirus	1999	Abbott Labs	FDA
Cache Valley	2000	?	BioReliance
Vesivirus 2117	2003	Boehringer-Ingelheim	BI
Cache Valley	2004	?	BioReliance
Human Adeno	?	Eli Lilly	Eli Lilly
Vesivirus 2117	2008	Genzyme, Belgium	Genzyme
Vesivirus 2117	2008	Genzyme, USA	Genzyme
Vesivirus 2117	2009	Genzyme, USA	Genzyme
PCV 1	2010	GlaxoSmithKline	GSK
PCV 1&2	2010	Merck	Merck

# Questions that Need to be Addressed Following a Virus Contamination

- Should we be concerned about the safety of exposed personnel?
- What is the contaminating virus?
- How many product lots are involved?
- Is the contamination contained?
- What is the status of the products involved?
- Have any contaminated drug product lots been released?
- What is the status of the facility?
- What is the impact on clinic / market supply?
- What is the source of the contamination?
- When should we communicate the incident with regulatory authorities?
- What corrective and preventive actions should we take?

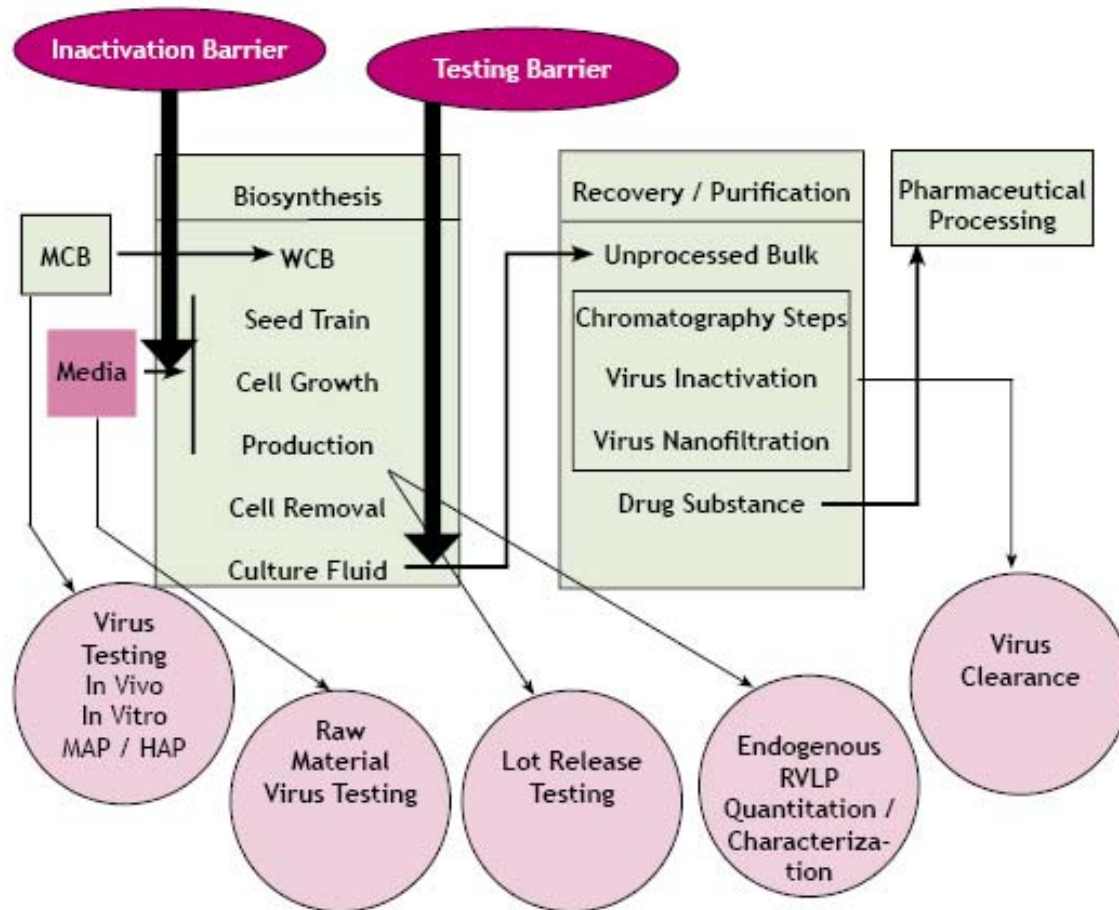
# Testing Barrier



# The 1994 Genentech Incident

- MMV PCR Detected Virus in a Single Production Bioreactor
- PCR Retest Confirmed the Positive Result
- Virus was Inactivated in the Bioreactor Before Breaking Containment
- No Downstream Recovery Processes were Contaminated
- QC *In Vitro* Virus Test Confirmed MMV Contamination
- Contaminating MMV Virus was Sufficiently Different in DNA Sequence to Rule Out Plant Recontamination with Residual 1993 Virus
- The Investigation Again Concluded that the Source was Highly Likely to be a Contaminated Raw Material Component of Cell Culture Media Although this Could Not be Shown Directly

# Barrier to Process



# Methods of Virus Inactivation / Removal in Cell Culture Media

- Gamma Radiation
  - Ultraviolet
  - Heat (HTST)
  - Nanofiltration
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- pH Extremes
  - Chemical Treatment
    - Solvent/Detergent (TNBP / Tween 80)
    - Chaotropes (Urea; Guanidine HCl)

# HTST Development Results

- Heat Treatment of Medium for 10 Seconds at 102 C Achieved > 7 logs of MMV Inactivation
- Heat Treatment of Medium for 10, 30 and 60 Seconds Showed No Differences from Control in:
  - Cell Growth
  - Viability
  - Product Titer
- All Amino Acids were Stable Except for Glutamine which had a  $t_{1/2}$  of 50 minutes
- Product Quality was Unchanged

# HTST Implementation

- Large Scale HTST Skid was Designed, Fabricated, Installed and Validated
- This Process Change was Approved by Regulatory Authorities
- HTST was Implemented Sequentially and Over Time for all Genentech Cell Culture Products
- HTST was Installed into all New and Acquired Cell Culture Manufacturing Facilities
- No Viral Contaminations have Occurred in Genentech Cell Culture Manufacturing Operations Since 1994
- Genentech has Shared this Story through Publications and in Talks at Open Industry Meetings

# Genzyme Story

Tuesday, June 16, 2009

## Virus halts production at Genzyme facility

By Julie M. Donnelly

### Video Gallery



MHT's Galen Moore: New 'hyperlocal' web trend



MHT's Marc Songini: Technology to keep seniors healthy

Genzyme Corp. has temporarily halted production at its Allston-based manufacturing facility after the company detected a virus that impairs cell growth in one of the site's six bioreactors.

Investors turned on the company's stock shortly after the news was confirmed Tuesday, pushing Genzyme's (Nasdaq: GENZ) stock down roughly 7 percent to \$51.80 a share.

Genzyme is currently working to sanitize the facility and is collaborating with regulatory agencies as it works to resume production. But the company does not expect the plant to be fully operational until the end of July.

The virus is called Vesivirus 2117, and company officials say it has not been shown to cause human infection. However, the virus is known to interfere with the growth of cells used to produce biologic drugs. Genzyme says the virus was likely introduced through a nutrient used in the manufacturing process.

# Chronology of Genzyme Events

- Sept 2008 4,000L bioreactor in Belgium producing Myozyme is terminated because of suspected virus contamination.
- Nov 2008 2,000L bioreactor in Allston (U.S.) is terminated because of suspected virus contamination.
- Feb 2009 Genzyme receives FDA Warning Letter related to observations during 2008 inspection.
- May 2009 2,000L bioreactor in Allston (U.S.) is terminated because of suspected virus contamination.
- May 2009 FDA re-inspection in Allston
- June 2009 Company identifies virus that contaminated all 3 bioreactors as Vesivirus 2117 by PCR analysis

# Chronology of Genzyme Events (2)

- June 16, 2009      Genzyme Press Release: 1) Virus contaminates Allston manufacturing facility, 2) plant is shutdown to clean up, 3) inventories of Cerezyme and Fabrazyme will not meet global demand and will need to be rationed, 4) company notified FDA and EMEA on June 15.
- June 25, 2009      Allston plant is being sanitized to inactivate virus. Company expects period of product constraint to last 6 - 8 weeks.
- July 31, 2009      FDA informs Genzyme that it will re-inspect its Allston facility as a follow-up to May inspection, and review remediation efforts related to the virus contamination.
- Aug 10, 2009      Genzyme announces that it will discard 80% of work in-process material. That will result in a \$22.6M write-off. Company now projects a longer supply shortfall lasting through the end of the year. Company implemented a dose conservation program to ensure most vulnerable patients receive Cerezyme.

# Chronology of Genzyme Events (3)

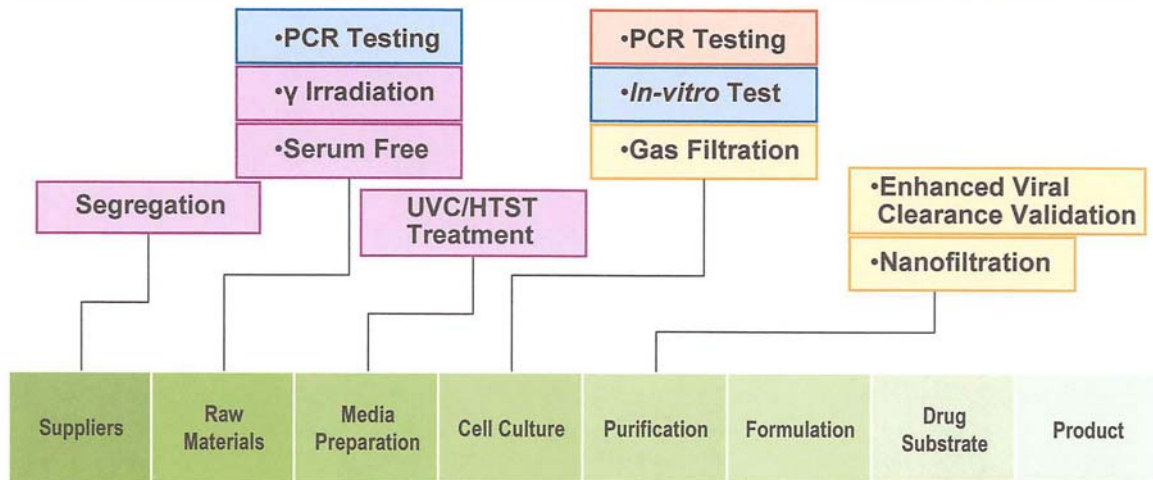
- Aug 17-21, 2009 European Medicines Agency inspect Allston plant and identify one major observation.
- Aug 26, 2009 Protalix BioTherapeutics Inc., a potential competitor for Cerezyme, announced that the FDA had granted its pipeline candidate prGCD a fast-track status.
- Sep 23, 2009 Genzyme announces that it is now half-way through the anticipated shortage period for Cerezyme and Fabrazyme. The restart of the Allston facility is complete with all 6 bioreactors on-line. Genzyme expects it can meet anticipated patient demand for both products during Q1, 2010.  
Company expects 2009 Cerezyme revenue of ~\$800M (\$1.2B in 2008), and Fabrazyme to be ~\$450M (\$494M in 2008).

# Chronology of Genzyme Events (4)

- Nov. 13, 2009 Stainless steel, foreign particles found in popular Genzyme drugs like Cerezyme, Fabrazyme, others
- Dec. 1, 2009 Genzyme begins shipping newly produced Cerezyme from Allston plant
- Feb. 22, 2010 Carl Icahn is taking the fight to Genzyme in a bid to wrest control of the biotech company from embattled Chairman and CEO Henri Termeer
- Mar. 24, 2010 Genzyme announced that the FDA intends to take enforcement action which will likely result in a consent decree
- May 24, 2010 Terms of the consent decree were finalized: \$175 million up-front disgorgement fee; fill/finish operations to move out of Allston plant; remediation plan to be overseen by third-party consultant, Quantic, for 2-3 years

# Genzyme Virus Risk Mitigation

## Overlapping and Redundant Controls



Prevent Forward Flow of Contaminants

■ = Prevention   ■ = Removal  
■ = Detection   ■ = Containment

genzyme

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# The Cost

- Allston Manufacturing Facility Shut Down from June – Sept 2009
- Inventories of Cerezyme and Fabrazyme did not meet global demand and were rationed through remainder of 2009
- Company discarded more than 80% of work in progress and wrote off \$22.6M
- FDA granted “fast-track” status to competing product
- Cerezyme revenue dropped to \$793M in 2009 from \$1.2B in 2008; Fabrazyme dropped to \$431M from \$494M
- FDA conducted comprehensive, in-depth GMP inspection from Nov 8 to Dec 13, 2009; Resulted in 49 observations (22 pgs)
- Additionally, Shareholder Class Action Lawsuits were filed
- Company receives a FDA consent decree including an up-front disgorgement fee of \$175 million

# Why are Decisions to Prevent Expensive Problems So Difficult?

- Human Nature
- Sometimes Difficult to Identify Risk
- Difficult to Quantify Actual Risk
- Difficult to Determine How Much Money to Allocate to Risk Reduction
- Risk Assessment is Often After the Fact (or Random at Best) When it Should Really be Systematic.

# Current State of Affairs

- Revised WHO Cell Substrate Guidance Document is in Preparation
- More Companies are Implementing Viral Process Barriers
- Recognition that Emergency Preparedness is Important
- New Innovative Virus Detection and Identification Technologies are being Developed and Implemented
- Regulatory Authorities Seem More Open to Accepting Molecular Detection Methods
- Companies are Starting to be More Open about Their Virus Contamination Experience than in the Past
- Discussions are Ongoing for Ways to Build and Share a Database of BioPharmaceutical Industry Contamination Experience While Still Maintaining Corporate Confidentiality

# PDA Cell Substrate Task Force

- **Mission** – To address issues that impact cell substrate safety and/or quality that have arisen as a result of scientific and technical advances within the industry over the past decade.
- **Members** – Experts from industry, CROs and regulatory agencies
- **Workshop** – PDA Cell Substrate Workshop – July 29-30, 2009, Bethesda, MD
  - Session 1 – New Cell Lines
  - Session 2 – Raw Materials
  - Session 3 – Virus Testing
- **Proceedings** – Oct 2010, PDA Journal Supplement
- **Technical Documents**
  - Emerging Technologies for Detection of Viral Contamination
  - Viral Contamination During Drug Manufacture

# New Virus Detection / Identification Methods

- PCR and Q-PCR
- Degenerate PCR
- PCR / Mass Spec (Ibis)
- Virus Microarrays (ViroChip)
- Massively Parallel Sequencing (Roche)
- Molecular Bar Coding (NanoString)

# Virus Contamination During Drug Manufacture: Detection, Response and Prevention

- **Detection**
  - Assays
  - Extent of Contamination
- **Response**
  - Emergency Response Team
  - Quarantine
  - Patient Impact
  - Product Supply Implications
  - Facility and Process Investigation
  - Personnel Safety
  - Source Identification
  - Decontamination
  - Corrective Actions
  - Manufacturing Restart
- **Prevention**
  - Risk Management
  - Personnel Training
  - Facility Modifications
  - Implementing Barriers to Contamination

# Upcoming Meetings of Interest

- PDA / FDA Workshop on Adventitious Viruses in Biologics: Detection and Mitigation Strategies, Dec 1-3, 2010, Bethesda, MD
- IABS Meeting on Adventitious Agents, New Technology, and Risk Assessment, May 19-20, 2011, Baltimore, MD (Satellite meeting of the National Foundation of Infectious Diseases Annual Vaccine Research Meeting)

# Industry Consortium

- Purpose – To confidentially collect and analyze relevant information related to virus contaminations in the biotech industry; and to make the information available to the industry without revealing specific sources.
- Status - Now being organized
- Project Management – Industry partnership with major academic institution
- Participation – Contact me if interested in participating:  
[QuantumCo@comcast.net](mailto:QuantumCo@comcast.net)

# Conclusions

- Making BioPharmaceuticals in Continuous Cell Lines Makes Sense
- However, Some Viruses Can Infect and Replicate in Cell Substrates
- Viruses Are a Product Safety Concern
- Industry has Taken a Multifaceted Approach to the Prevention and Control of Virus Contamination
- Never-the-less, Many Virus Contaminations Have Occurred
- Some of These Contaminations Have Been Reported and Many Have Not.

# Conclusions (2)

- No Drug Product Contamination Has Occurred
- Virus Contaminations are Disruptive and Can be Very Expensive
- Repeat Virus Contaminations Can Result in Long and Thorough Regulatory GMP Inspections
- Methods for Better Control and Risk Reduction are Available and Should be Implemented
- Manufacturers Should be Prepared for a Virus Contamination and have a “WHEN”, not “IF” Perspective

# THE END



# Q & A

**Michael E. Wiebe, Ph.D.**  
**Quantum Consulting, LLC**

**Contact Information:**

Email: [QuantumCo@comcast.net](mailto:QuantumCo@comcast.net)

Phone: (650) 365-7022