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Importance of GMP in controlling cell substrates and production processes for biologicals — two case studies

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Importance of GMP in controlling cell substrates and production processes for biologicals: 2 case studies

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Outline

- Briefly remind ourselves of the critical manufacturing points in production of biologicals - viral vaccines and rDNA products
- Discuss the issue of viral safety of products made using mammalian cells
- Look at two case studies that illustrate importance of following cGMP
- Outcomes and lessons learned



Critical manufacturing points

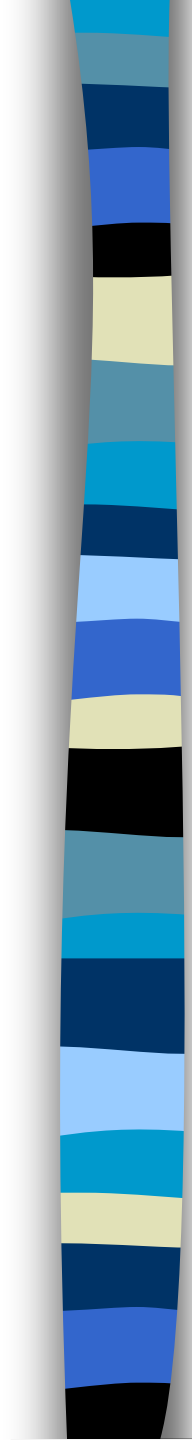
- Cell substrate - mammalian cells / bacteria / yeast / insect / plant cells or avian eggs
- **Cell banks / cell culture / fermentation - batch or continuous production systems**
- For rDNA products, DNA sequence of cloned gene / genetic stability
- Separation and purification of vaccine virus or protein product
- Characterization of resulting protein + glycosylation or other post-translational modifications or vaccine virus
- Product / host cell related impurities (including residual DNA; **Viral safety issues for mammalian cells**)
- Emphasis on consistency of production

Biologics - slight changes in process can have a major impact on clinical performance / safety of the product. Consistency of production critical.



Viral safety of biological products – critical issue

- Many biologicals produced in mammalian cells – enable glycosylation of rDNA products
- Measures put in place to ensure absence of adventitious infectious agents in product – **a SAFETY issue**
- A contaminating virus MIGHT be devastating to a recipient (patient)
- A contaminating virus MIGHT spread from recipient to contacts / community - threat to health of a country
- **Contamination of cell lines, production process intermediates and products also has considerable economic consequences for manufacturer**
- **Might lead to supply issues with significant public health impact**



Examples of biologicals produced in mammalian cell lines

□ Live virus vaccines

Polio (primary monkey kidney cells, diploid cells, Vero cells), MMR (diploid cells, MRC5), Rotavirus (continuous cells, Vero cells)

□ rDNA protein products

Growth hormone, Factor VIII, t-PA, monoclonal antibodies, cytokines, etc. etc. (continuous cells CHO, PER.C6, MDCK)



Viral safety of biological products – source of contamination?

- Cell substrate itself
- Biological materials used in production (other than the cell substrate)
- During production processes



VIRAL CONTAMINATION

- **All relevant guidelines** consider possible viral contamination **of live viral vaccines** and **rDNA products** produced in **any mammalian cell** as a **major issue** to be addressed. These cells have the capacity to propagate viral agents.
- Here see the benefits of early experience of viral vaccine production – a cell substrate issue
- Early guidance provided a framework for moving forward with production of rDNA products in mammalian cells and guidance has been updated periodically to take account of new scientific information and technologies.



Detailed Guidelines available

A belts and braces approach

- *WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological products and for the characterization of cell banks (2010):* ICH, national guidance
- Production based on cryopreserved cell bank. Master cell bank, working cell bank exhaustively screened for virus contamination, with documented history.
- **Control of raw material used in production – e.g. growth media, enzymes**
- Closed systems for growth of cell culture
- **Testing of each cell culture lot for viruses**
- **Validation of viral removal / inactivation by downstream processing** (this only possible for rDNA protein products - unlike live viral vaccines)



Does the system work?

- Generally yes: Testing evolved and updated with time - now includes range of traditional and molecular methods (PCR, PERT assay for reverse transcriptase depending on circumstances)
- However, viral contamination has occasionally occurred but contained and usually prevented from getting into product on the market
- **Seems that not all contaminations are reported publicly**; manufacturers concerned about bad publicity in media (see Nature 472, (2011) 389-390)
- Some manufacturers have reported contaminations - MVM, Genentech 1993, 1994: Vesivirus 2117 , Boehringer-Ingelheim 2003: Vesivirus 2117, Genzyme (Belgium and USA), 2008: PCV 1 & 2, GSK and Merck, 2010.



2 case studies illustrating two very different outcomes

- Genentech experience
- Genzyme experience



Genentech experience with contamination in cell culture 1993

- Contamination of large scale cell culture by Minute Virus of Mouse (MVM) detected during routine production control process
- Testing takes time and product already well on way through downstream purification processes by time detected
- Lot production promptly stopped, reported to US FDA and clean up started
- Investigation of source instigated



Genentech experience with contamination in cell culture 1993

- No definitive source of contamination identified; consistent with media used in production as source. Feral mice from land surrounding plant examined but no MVM found
- Clean up process expensive
- **At no time was a contaminated product let through the system and the regulator was aware of all developments**
- New PCR and infectivity assay developed to speed up early testing and introduced routinely



Genentech experience with contamination in cell culture **1994**

- New PCR and infectivity assays used and nothing found for 12 months
- Then another MVM positive signal but this time contamination detected before any downstream processing started. **Downstream protected**
- Source again highly likely to be contaminated cell culture media but not shown directly
- New heat treatment of medium developed, approved by FDA and installed
- **No viral contamination detected since 1994**



The Genzyme Experience

- Several bioreactor runs (Belgium and USA, 2008–2009) terminated early due to poor growth of cells – **suspected contamination**. Seem not to have dealt with problem promptly. Eventually informed FDA.
- US FDA warning letter and re-inspection
- Virus identified as Vesivirus 2117 using PCR in 2009: not known to be a human health risk but interferes with growth of CHO cells.
- Likely introduced by contaminated media
- USA plant shut down for major clean up and re-organization. Virus had spread into manufacturing facility – bioreactors and expensive chromatography columns. Clean up very costly.



The Genzyme Experience

- Global supply of two rDNA derived orphan drugs, Cerezyme (Gaucher's disease) and Fabrazyme (Fabry's disease), were seriously compromised and the products rationed. No alternative to Fabryzyme
- Cause of concern to regulators (e.g. Health Canada) as to how to handle the situation.
- Overall Genzyme needed lot of GMP actions, stock prices dived and together with sales shortfall left the company vulnerable to takeover - acquired by Sanofi in 2011



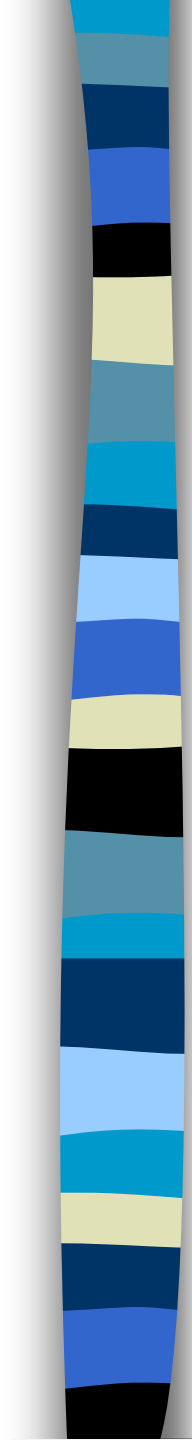
Outcomes and lesson learned

- **Virus contamination is a serious business.**
- Manufacturers need to deal **promptly** with contamination or suspected contamination (compare Genentech and Genzyme)
- As new inexperienced manufacturers come into operation it is essential that they understand the need for great care and attention regarding development and production of biological products.
- Role of NRA in overseeing these developments is critical
- **Continued vigilance essential. Don't be complacent.**



References - viral contamination

- Zhan D, et al (2002) Detection of minute virus of mice using real time quantitative PCR in assessment of virus clearance during the purification of mammalian cell substrate derived biotherapeutics, *Biologicals* 4, 259-270
- Garnick RL, (1996) Experience with viral contamination in cell cultures *Dev Biol Stand* 88 49-56
- June 2009 Press Release from Genzyme
- Nature Editorial (2011) Pharmaceutical firms should come clean to tackle drug contamination, *Nature* 472, 389-390



**THANK YOU FOR YOUR
ATTENTION**