Quality and Consistency of Cell Culture Media with a Highlight on FMDV

Dr. Serge Ohresser

4th International Conference on Vaccines & Vaccination, September 24, 2014 - Valencia

Bioproduction Process



Upstream Processing

- Most of the critical quality attributes of a final product in biological process are determined in Upstream steps
- Minor changes in cell culture media or bioreactor conditions can effect the end product

Process Improvements

- Moving to simplified and more defined composition of media
- Consistency of cell culture media minimizes risks of deviation in biological processes
- Raw material qualification and chemical expertise in media production

Risks in Upstream Processing

“Raw Materials as a Source of Contamination in Large-Scale Cell Culture”

- Particular **risk for virus & bacterial** contamination
- Arising **awareness** for upstream virus safety from high profile contaminations
- Manufacturing process bears **multiple sources** of potential adventitious virus (e.g. facility, equipment, process, materials, utilities, personnel)



Raw materials are important pillars of cell culture media formulations:

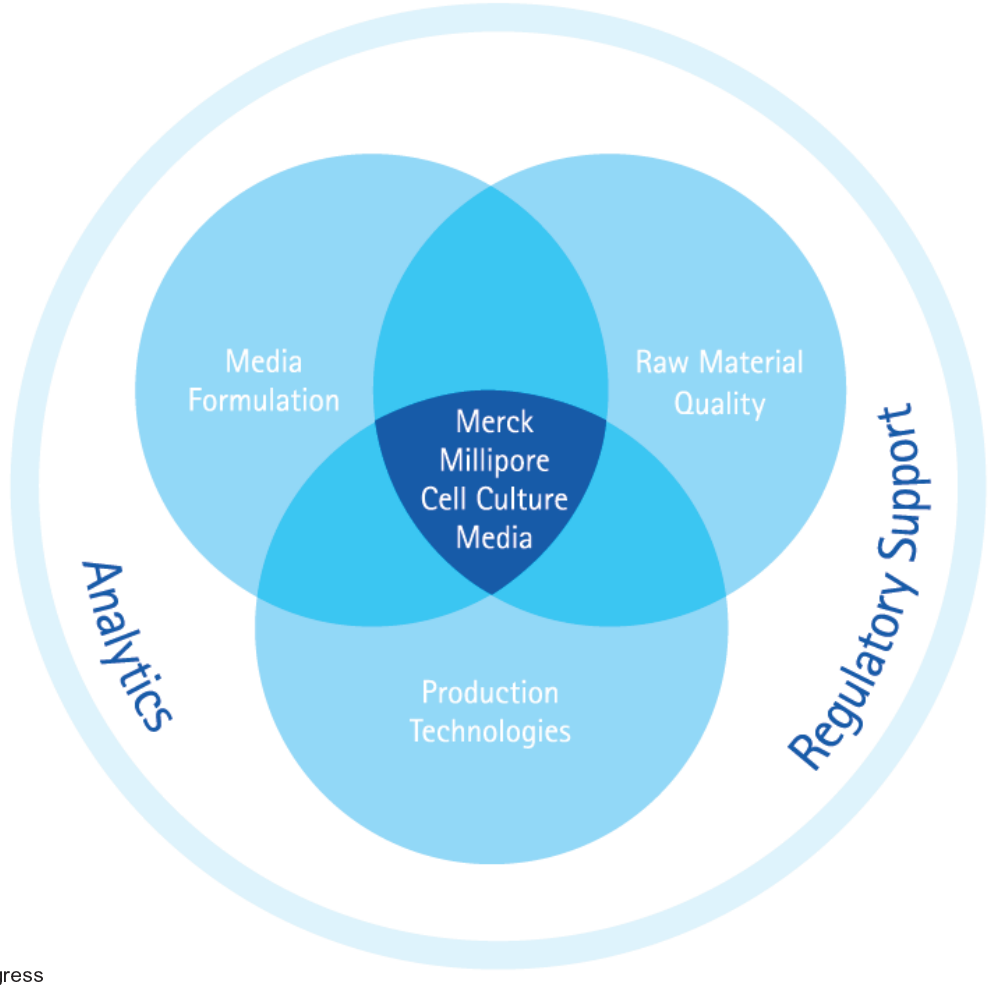
- **Origin** – important for risk assessment
- **Traceability** – qualification and documentation
- **Qualification** – suitability for cell culture media and consistency

Selection of Raw Materials

Traceability and testing	▪ Selection of high traceable materials ▪ In-coming testing
Maximize control of animal derived products	▪ Serum bovine guideline ▪ Virus clearance at manufacturer
Moving towards alternatives to animal origin products	▪ Serum-free media ▪ Recombinant protein alternatives
Moving towards chemically defined	▪ Chemically defined media ▪ Non-animal origin



Cell Culture Media Quality and Performance… What Makes the Difference?



Cell Culture Media Quality Matters

Product Homogeneity

Consistent process by constant particle size distribution over all scales



Homogenous Merck Millipore medium vs. competitors medium showing crystals and in-homogenous blending

Gentle Milling

Oxygen or temperature sensitive raw materials handled with care by gently milling technology



White appearance of suppliers media, while Merck Millipore medium preserves the pinkish color resulting form vitamin B

Media Formulation

Enhanced component solubility and concentration to optimal formulation design



Pre-treated raw materials can enhance their solubility

Merck Millipore Cell Culture Media Products

Consistent performing cell culture media

- Formulation improvement by physico-chemical expertise
- Comprehensive raw material qualification process
- Gentle manufacturing process
- Comprehensive documentation and regulatory support

Cell Culture media products

- Customized DPM – according to customer formulations
- Cellvento™ CHO – optimized for CHO cell cultures
- Cellvento™ BHK – optimized for BHK-21 cell cultures



A Highlight on FMDV Production Improvement

Foot-and-mouth disease (FMD), an infectious and sometimes fatal viral disease that affects cloven-hoofed animals

The production process

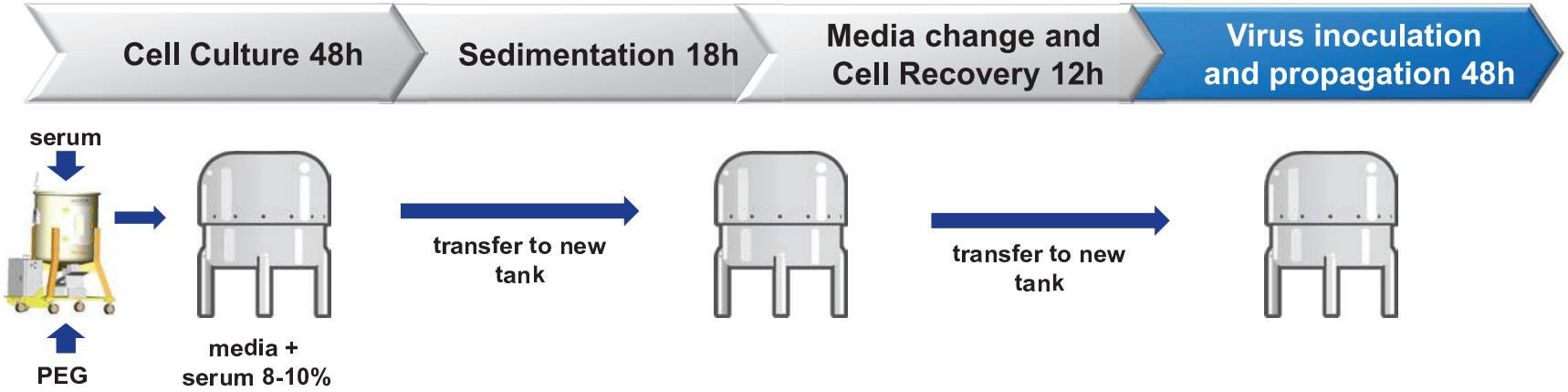
- Complex media used for cultivation of BHK-21 cells for FMDV production, e.g. GMEM + TPB + 10% adult bovine serum
- Similar GMP requirements as media for human vaccine manufacturing
- Highly potent vaccine needs to be produced at low cost

Serum-free compositions become a must

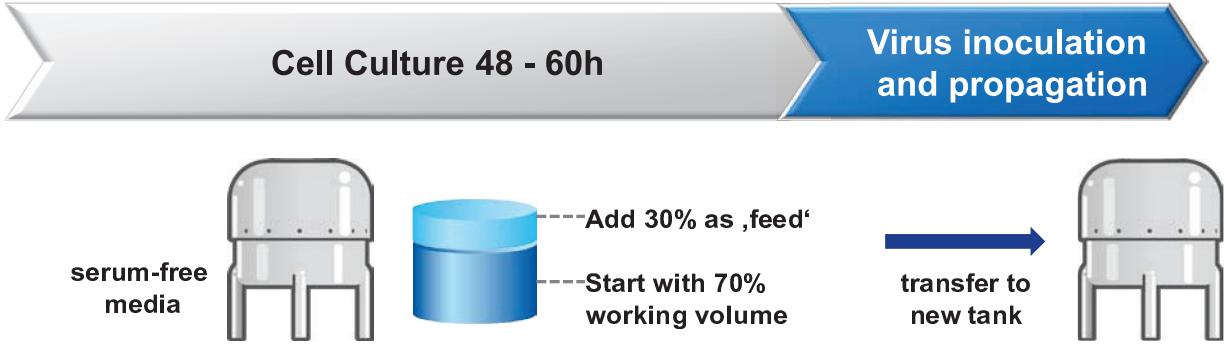
- Due to high variability of serum
- Risk of introducing adventitious agent into the process
- Serum may contain antibodies against FMDV
- Associated cost of supply & serum treatment before process entering

Comparison of Serum-containing and Serum-free Process

Serum-Containing Process



Serum-free Process

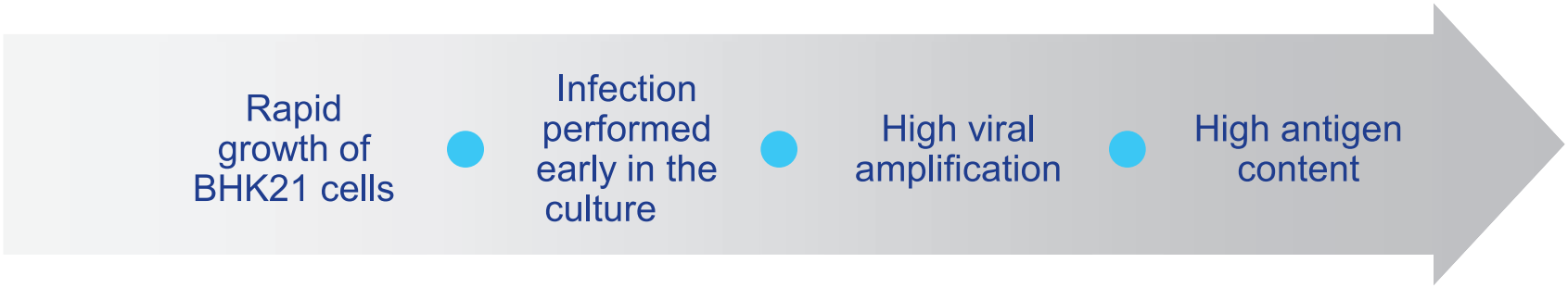


Serum-free Process for FMDV Production

Advantages

▪ No extra serum needed	➡	Less process costs
▪ No need for serum batch testing ▪ No additional depletion of anti-FMDV Abs ▪ No exchange of media before viral growth	➡	Simplified process
▪ Non-animal origin raw materials	➡	Safety/less regulatory exposure
▪ More defined composition	➡	Process consistency
▪ Simple upstream process	➡	Easier downstream purification

Cellvento™ BHK-200 is an Optimal Serum-free Medium for a Batch Culture Vaccine Process

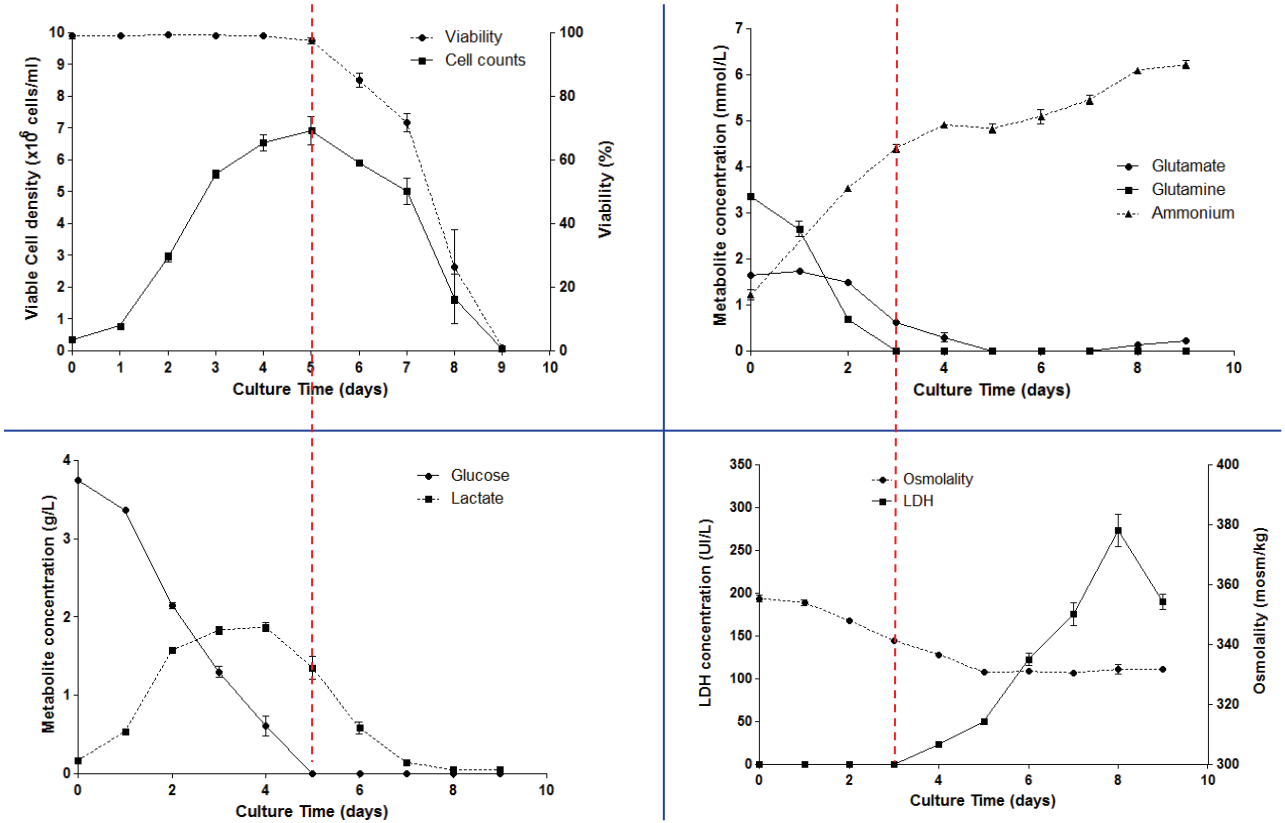


Cellvento™ BHK-200 medium along the overall vaccine process chain:

- Establishment of master and working cell bank
- Adaptation from serum-containing to serum-free conditions
- Scale-up from lab to production scale
- High-density cell propagation
- Amplification of FMD virus for industrial production

BHK-21 Cell Growth in Cellvento™ BHK-200 Medium

Growth of BHK-21 cells in a batch culture experiment

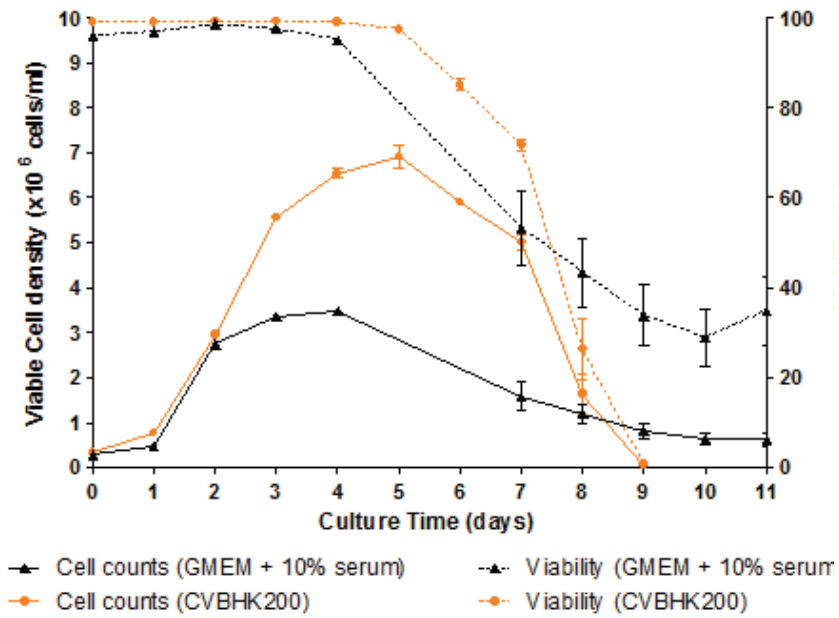


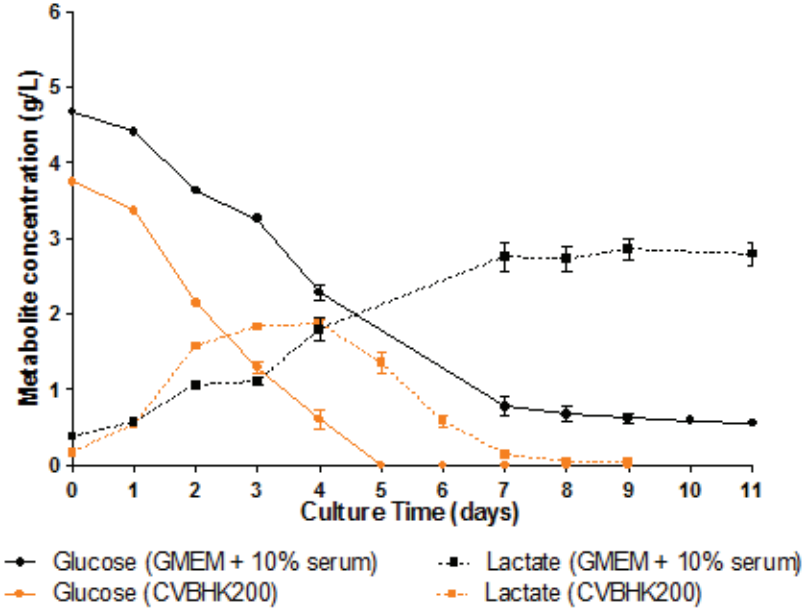
Metabolic profile was analyzed during growth

Glucose consumed at day 5 with maximum cell density of 6.5×10^6 cells/mL

Classical Media vs. Cellvento™ BHK-200 Medium

Comparison Cellvento™ BHK-200 medium vs. GMEM + TPB + 10% serum

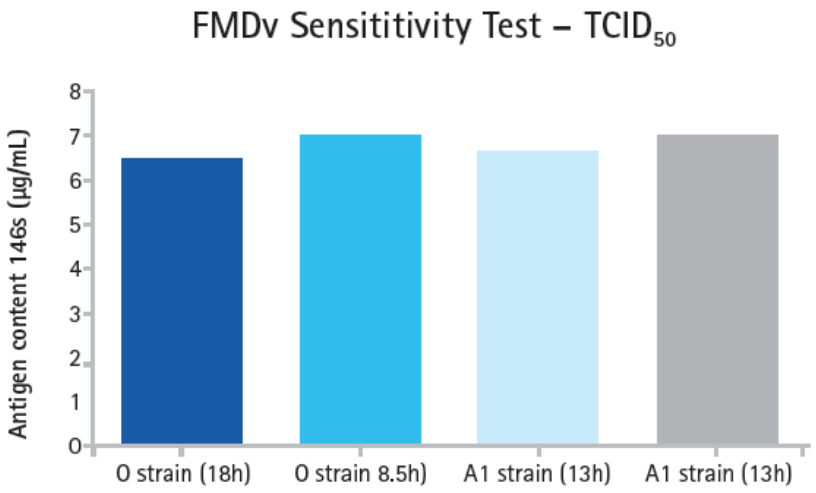


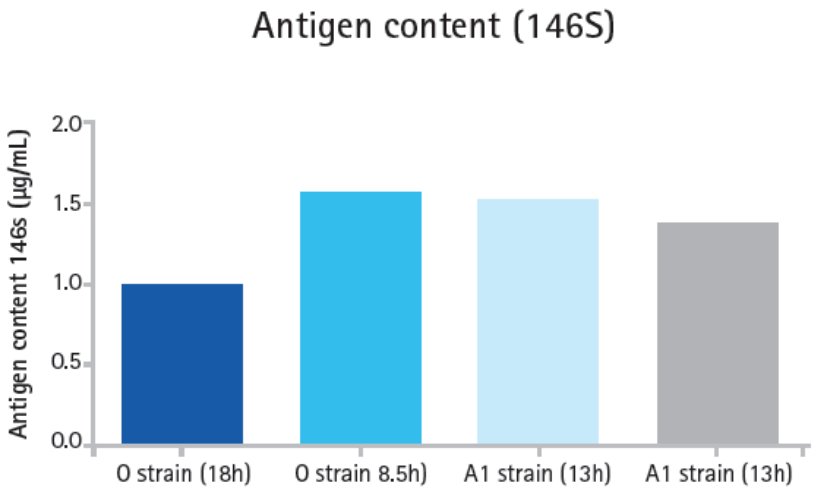


Double maximum Viable Cell Density with Cellvento™ BHK-200 medium.
With GMEM, growth stops after day 3 while Glucose is not a limiting factor.

Performance of Cellvento™ BHK-200 Medium

Infection of BHK21 cells with 3 FMDV strains at a concentration of 1.5 x10^6 cells/ml in 250 ml shaker flasks (37 °C, pH 7.4)





Antigen produced was evaluated using $TCID_{50}$ and 146S measurement methods: Comparable antigen mass produced similar to classical serum-containing conditions.

Concluding Remarks

- Serum-containing processes are complex, costly, difficult to standardize, regulatory non-friendly and bear the risk of adventitious agents in the upstream process
- Cell culture media performance is directly linked to the media formulation, quality of raw materials and the manufacturing process
- Avoidance of serum requires media optimization and translation into a manufacturable recipe
- Serum-free media can be used to grow BHK21 cells and subsequently infect them to produce the FMD antigen
- Cellvento™ BHK-200 medium is a suitable formulation for serum-free FMDV manufacturing processes

Thank you!

Dr. Serge Ohresser
Upstream Sales Development, International
Pharm Chemicals Solutions
Merck Millipore
Email: **serge.ohresser@merckgroup.com**