Process development considerations for quality and safety of vaccines

Priyabrata Pattnaik, PhD
Director – Worldwide Vaccine Initiative

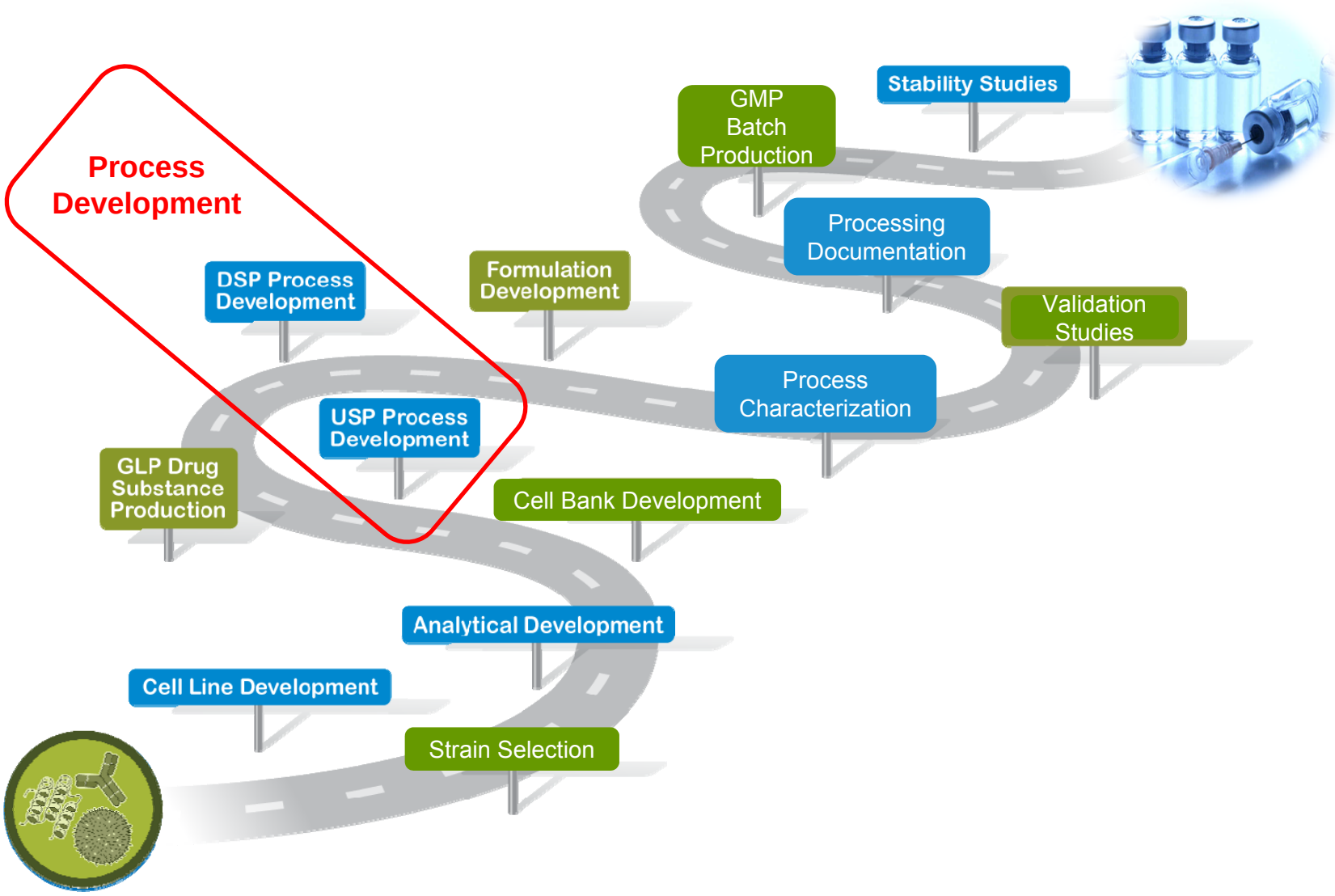
Presentation Overview

1. What can affect vaccine quality and safety
2. Bioburden control
3. TFF operating conditions
4. hcDNA removal
5. Excipient quality
6. Summary

What can affect vaccine quality and safety?

- Starting materials (seed, cell line, raw materials, etc.)
- Manufacturing practices
- Adventitious agents
- Quality control criteria and methods
- Process design and control
- Product purity
- Drug substance impurity profile
- Formulation stability
- Shipping and cold chain

The Journey of a Vaccine



Vaccine PD Strategy

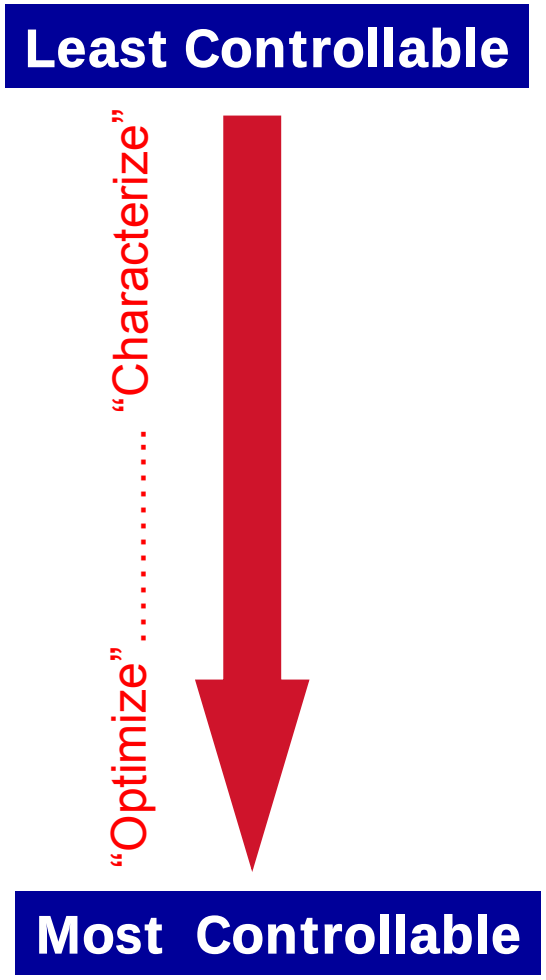
- Use un-optimized processes to generate clinical supplies (Fast-to-Efficacy) & Develop commercial process, methods, formulations during clinical development
  - Pros: Speed
  - Cons: Another PD phase is required

- Use more rigorous methods & formulation for all clinical trials (Front-Load)
  - Pros: Single development phase
  - Cons: Slower to clinic

- Use either method based on product risk profile (Hybrid)
  - Low risk → "Front Loading" (established vaccine; Pneumoccocal, HPV, etc.)
  - High risk → "Fast-to-Efficacy" (new vaccines; i.e. HepE, HFMD, Chikungunya, Staphylococcus, etc.)

Adopted from: *Geoffrey Slaff, Bioprocess International Meeting, Boston, 2005*

Factors Affecting Vaccine Quality



Bioburden control

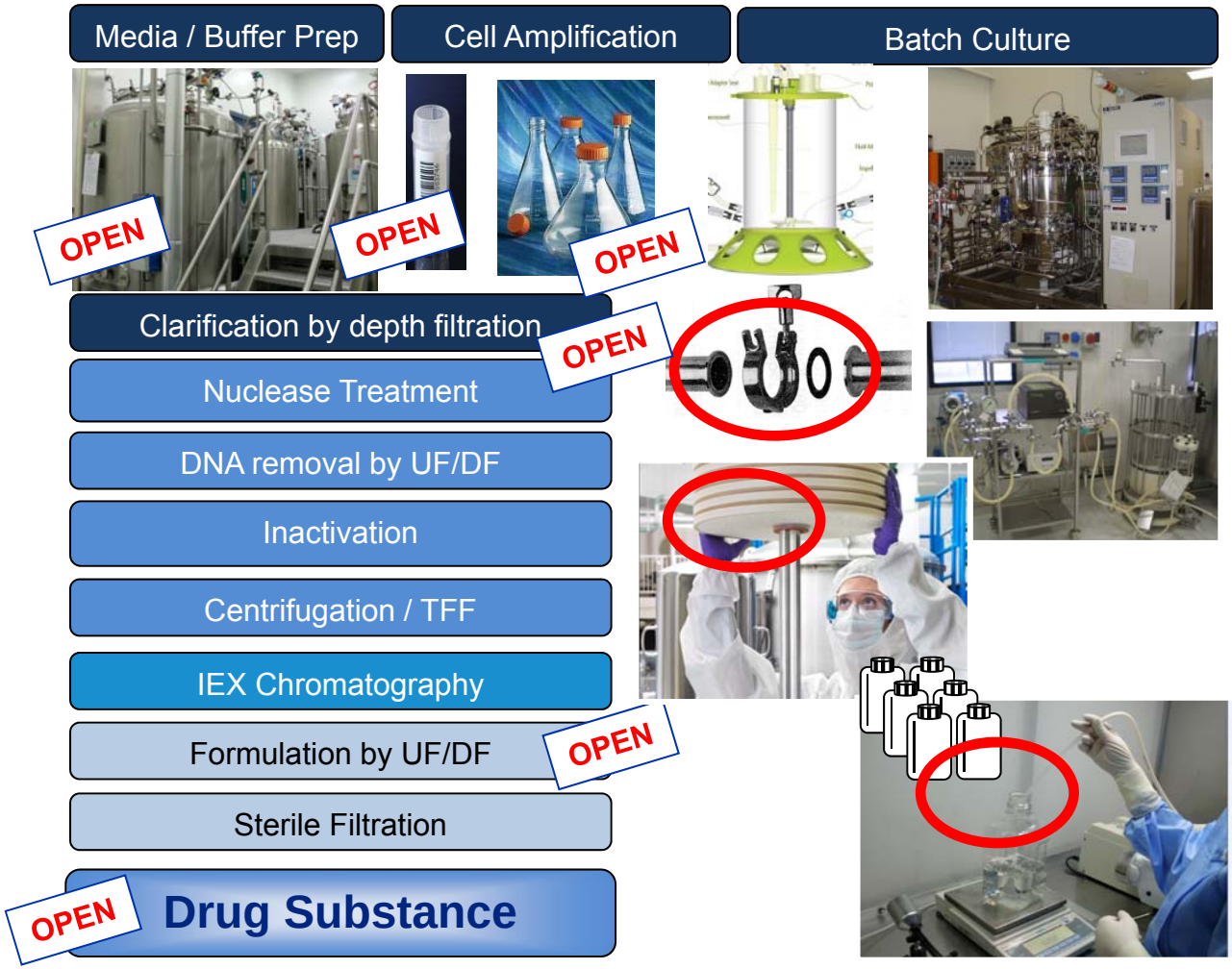
Manufacturing Process and Contraints

Biocontamination or cross-contamination can occur at different stages of the process and for various reasons:

- Raw material
- Cleaning or Sterilization procedures
- Buffer and Media Preparation
- USP / Cell culture
- DSP

Sterile ≠ Closed process

Manufacturing of Vaccine Process



Buffer and Media Preparation

- USP:
  - Cell amplification open steps under laminar flow
- DSP:
  - Direct Open steps are perfomed under Laminar Flow
  - Indirect open steps are still happening

Examples of issues due to Bioburden

FDA warning letter to ID Biomedical Corp., a subsidiary of GSK Biologicals, 12 Jun 2014

……. manufacturing processes are inadequate to control bioburden and endotoxin. Throughout 2013-2014 the process continued to generate Out of Specification (OOS) results for bioburden and endotoxin…….

Source: http://www.fda.gov/iceci/enforcementactions/warningletters/2014/ucm401719.htm; accessed 13th Aug 2015

FDA warning letter to Sanofi Pasteur's vaccine manufacturing plant in Pennsylvania, 30 Jun 2006

……..bioburden sampling size used was not representative of the lot size in determining pre-sterile filtration bioburden levels…….

Source: http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2005/ucm075964.htm; accesses 13th Aug 2015

Examples of issues due to Bioburden

FDA warning letter to MedImmune, UK**, 24 May 2007**

……. Three of the five FluMist bulk monovalent lots that exceeded the interim bioburden action limits were used in the formulation of final product (lots 600147, 600153, and 600157). We acknowledge that the subsequently filtered monovalent lots and the final vaccine product resulting from those lots met all specifications. However, based on FDA's experience, there is a high probability that the observed cGMP deviations, if not corrected, would substantially increase the risk of product failures. …….

Source: http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2006/ucm076398.htm; accessed 13th Aug 2015

Two Key Regulatory References on Bioburden

"80. The bioburden should be monitored before sterilisation. There should be working limits on contamination immediately before sterilisation, which are related to the efficiency of the method to be used. Bioburden assay should be performed on each batch for both aseptically filled product and terminally sterilised products."

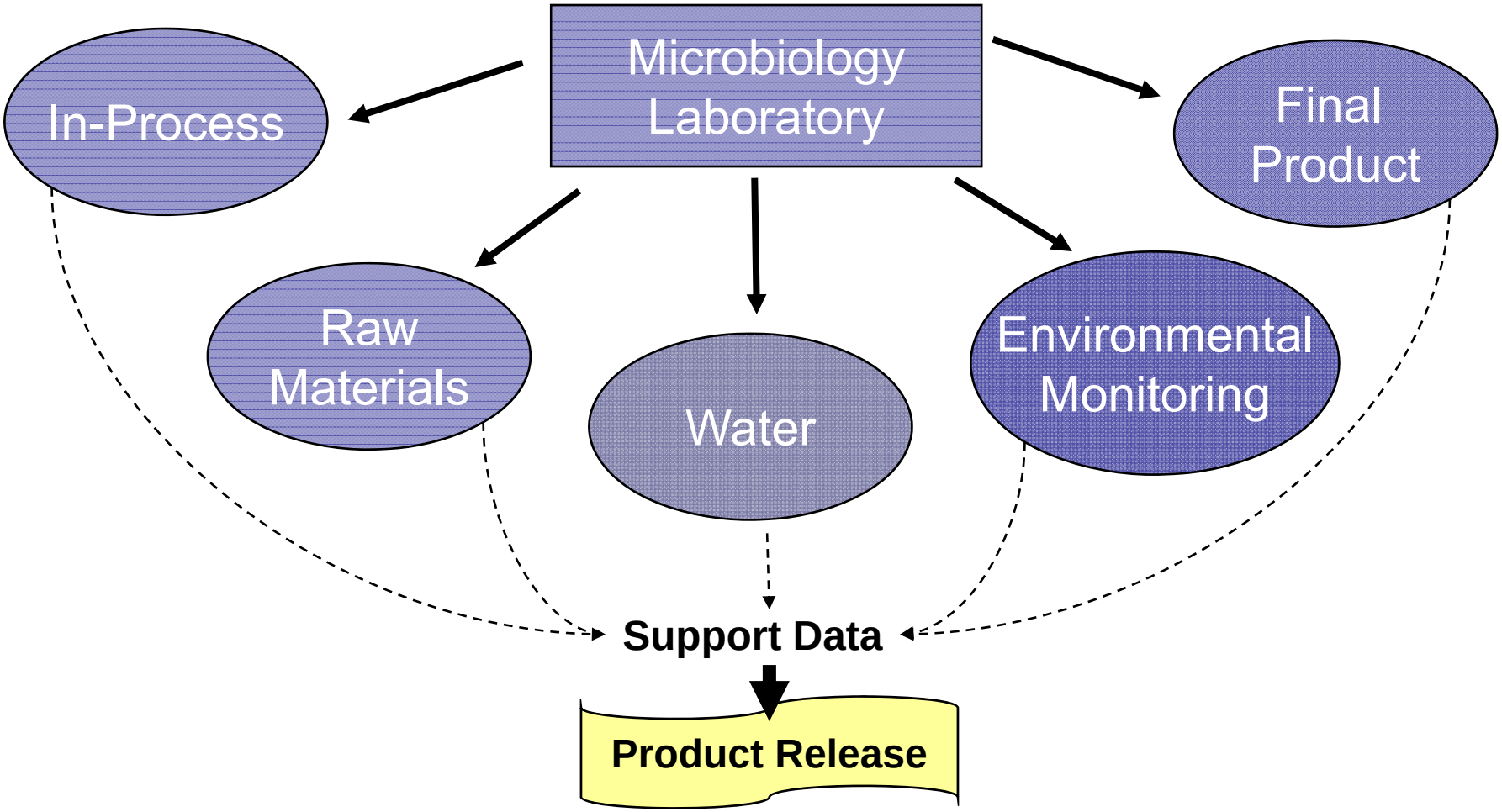
- EMA Vol 4, Annex 1 – Annex 1 Manufacture of Sterile Medicinal Products, 2008

"It is important to characterize the microbial content (e.g., bioburden, endotoxin) of each component that could be contaminated and establish appropriate acceptance limits."

"The manufacturing process controls should be designed to minimize the bioburden of the unfiltered product. Bioburden of unsterilized bulk solutions should be determined to trend the characteristics of potentially contaminating organisms."

- US FDA Sterile Drug Products Produced by Aseptic Processing, 2004

Microbiological Monitoring Programs

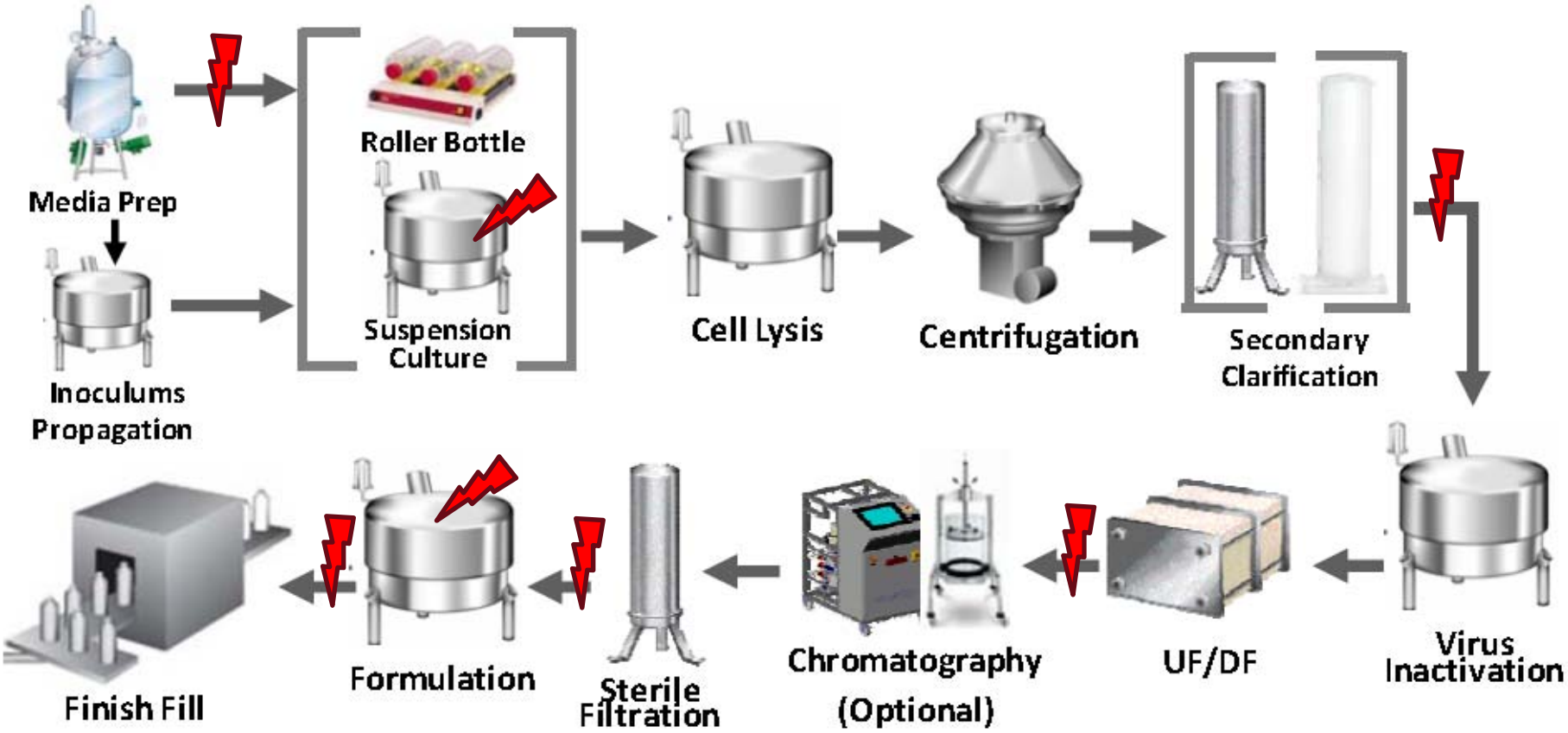


General guidelines on bioburden control

- Paying attention to handling of open process steps
- Environmental bioburden control and monitoring
- In-process checks
- Monitor and control bioburden of product, container, and closure
- Bioburden control of WFI and excipients
- Minimum possible process time and hold steps
- Introduction bioburden reduction filtration

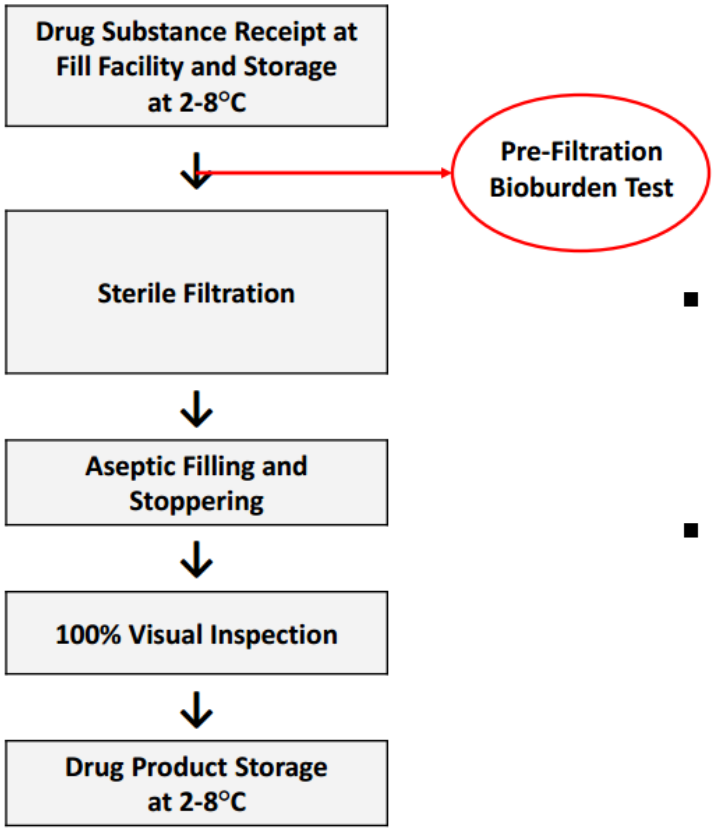
There is more to safety than meets the eye.

Where can bioburden reduction filtration be implemented in vaccine process?



Locations for bioburden control (depends on risk assessment)

Typical vaccine manufacturing flow diagram for a liquid sterile drug product (final steps)



- In-process control bioburden sample pulled immediately prior to sterile filtration step
- Validation of the sterilization process

Source: Field R, Yang H, Murphy D, Alvino J, Chang S. (2003) Bioburden Control at the Sterile Filtration Step: A risk-based approach. CASSS, CMC Europe, 06 May 2013
Yang H, Li N, Chang S. (2013) A Risk-based Approach to Setting Sterile Filtration Bioburden Limits. PDA J Pharm Sci Technol. 2013 Nov-Dec;67(6):601-9.

Why is paying attention to bioburden control at the PD stage is important?

- Performance of unit operation can be impacted (Chromatography, TFF)
- Product stability and degradation can be impacted (formulation stability)
- Analytical interference (QC testing)

Benefits:

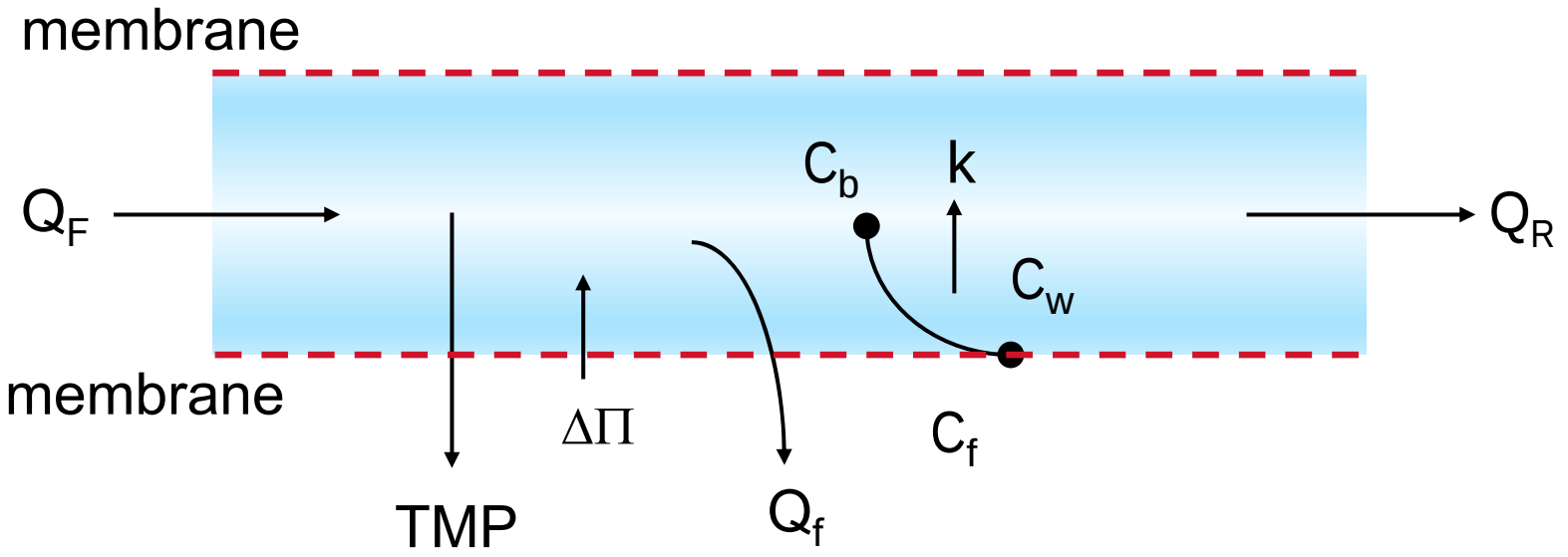
- Robust scale-up
- Easy to troubleshoot process issues

Take away message

Pay attention to bioburden control and monitoring during process development

TFF operating conditions can have an impact on product stability

Concentration Profiles in a TFF Process



Q_F = feed flow rate [L/h]

Q_f = filtrate flow rate [L/h]

P_F = feed pressure [bar]

P_R = retentate pressure[bar]

P_f = filtrate pressure [bar]

C_b = product concentration in bulk solution [g/L]

C_w = product concentration at membrane [g/L]

C_f = product concentration in filtrate [g/L]

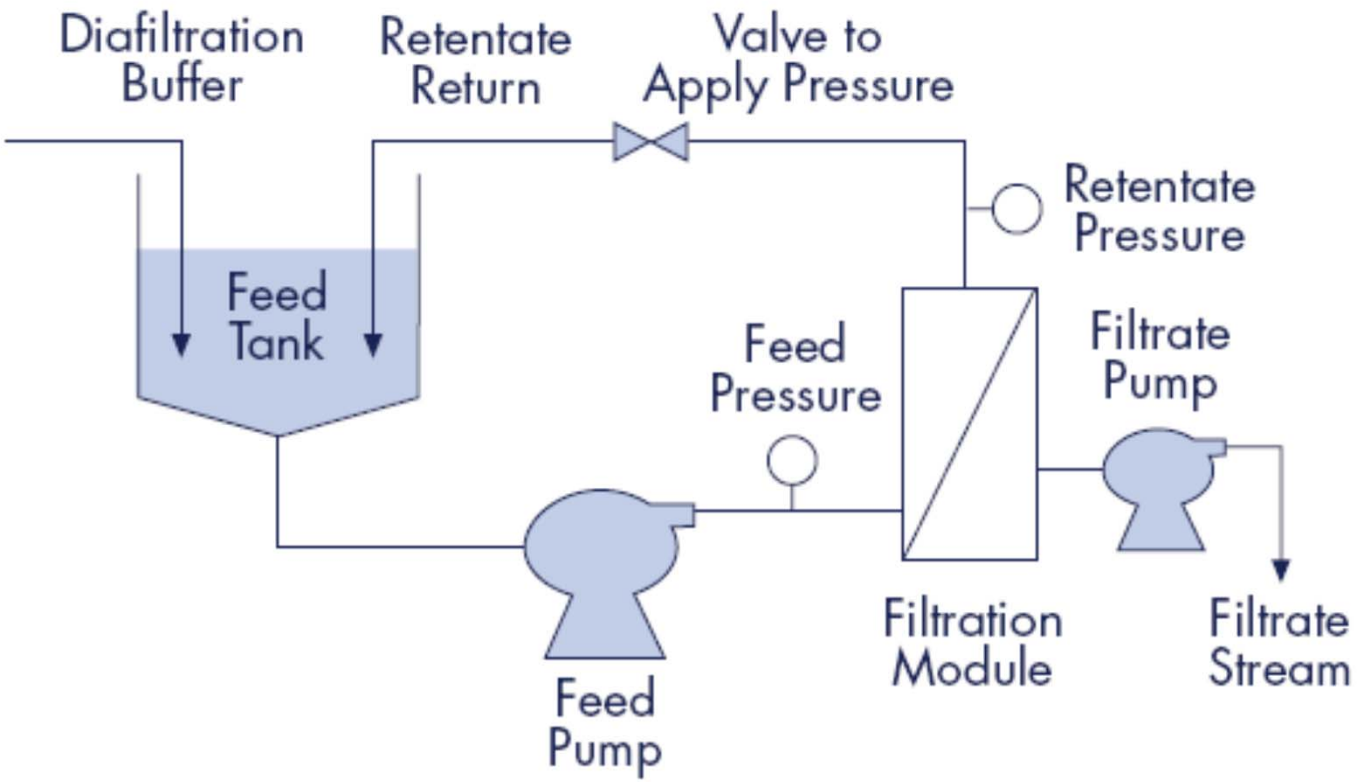
k = mass transfer coefficient [L/m^2*h]

Π = product osmotic pressure [bar]

How TFF can impact product quality

- **Aggregation**
  - Stability issues due to over concentration
  - Poor selection of buffers
  - Air-liquid interface
- **Sheering**
  - High pressure
  - Wrong pump selection
  - Poor tank and system design
- **Product degradation**
  - Air-liquid interface – poor tank design
  - High recirculation rate and process time
  - Temperature

Microfiltration: Best done by Permeate Controlled operation



Tangential Flow Filtration for the Recovery of Acellular Pertussis Vaccine Components

Membrane-based TFF technology can ease scale-up and provide a higher recovery percentage compared to conventional purification methods.

RAKESH KUMAR, S.V. KAPRE, PRIYABRATA PATTNAIK, SUBHASIS BANERJEE, M.S. MAHADEVAN

ABSTRACT

The commercial preparation of acellular pertussis vaccine depends on the effective recovery and purification of the antigens pertussis toxin (PT), filamentous haemaglutinin (FHA), and pertactin (PRN) from *Bordetella pertussis* fermentation. This study describes the recovery of the antigens using an open channel 0.45-µm tangential flow filtration module with optimization of the process parameters of transmembrane pressure, cross flow, and flux. Under the optimized conditions, greater than 98% recovery of FHA and PT was obtained.

Pertussis or whooping cough is an acute infectious disease of the respiratory tract caused primarily by *Bordetella pertussis* and less commonly by *Bordetella parapertussis*.[1] Pertussis still continues to cause significant morbidity and mortality globally. "Per" meaning intensive and "tussis" cough: describes the clinical manifestation of

RAKESH KUMAR, PhD, is a director and S.V. KAPRE, PhD, is an executive director of the Serum Institute of India, Ltd., Pune, India, PRIYABRATA PATTNAIK, PhD, is a technical manager of the biomanufacturing sciences network at Millipore Singapore Pte. Ltd., Singapore, +65.6403.5308, priyabrata_pattnaik@millipore.com, SUBHASIS BANERJEE, PhD, is a manager of process applications and M.S. MAHADEVAN is a vice president of the bioprocess division at Millipore India Pvt. Ltd., Bangalore, India.

Consideration for TFF operation to manage product quality impact

Aggregation

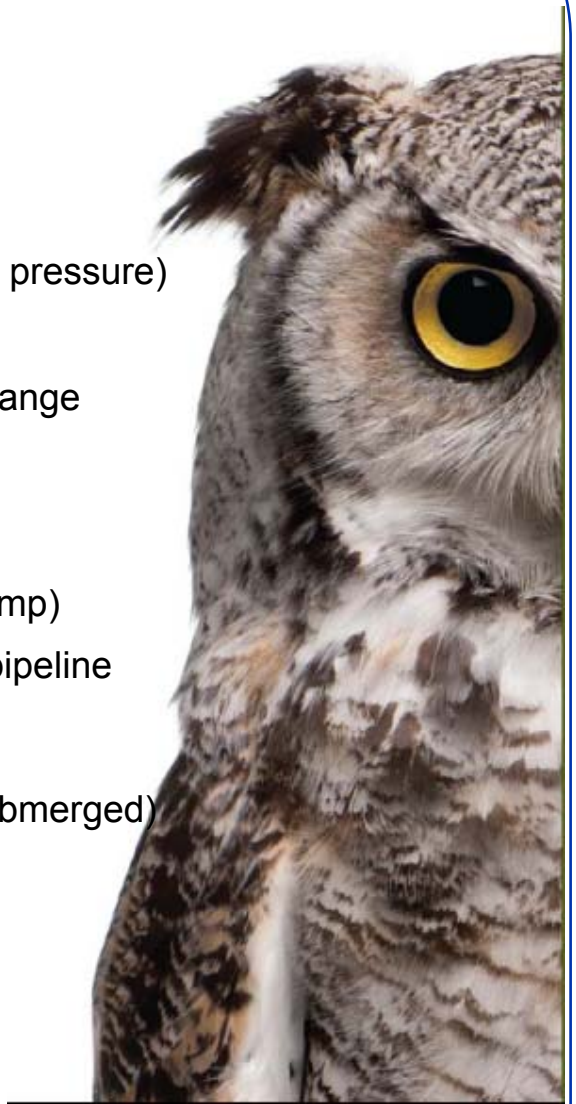
- Select low binding membrane
- Operate in controlled polarization mode (balance pump flow and pressure)
- Select buffers compatible to product
- Know the effect of product stability over required concentration range

Sheering

- Avoid high pressure operation and large pressure drops
- Select low shear pump (peristaltic or low speed sanitary lobe pump)
- Use sanitary valves and fittings. Avoid reducers in TFF system pipeline

Product degradation

- Avoid air-liquid interface (ensure retentate/diafiltrate lines are submerged)
- Maintain homogeneity (ensure feed tank is well-mixed)
- Optimize pump rate (product recirculation)



BioProcessing JOURNAL
TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

Best Practices for Optimization and Scale-Up of Microfiltration TFF Processes

By BALA RAGHUNATH*, WANG BIN, PRIYABRATA PATTNAIK, and JEROEN JANSSENS

Introduction

Tangential flow filtration (TFF) is widely used in biopharmaceutical processing for protein purification—a common application for TFF is ultrafiltration for concentration/diafiltration of proteins. In this type of application, the product protein is retained (concentrated) within the feed side of the ultrafiltration membrane, while the buffer components and other small impurities (smaller than the membrane pore size) freely pass through the membrane into the permeate side. Several scholarly articles are available in literature[1–6] which discuss the ultrafiltration application as well as its optimization strategies.

Another category of application where TFF finds significant use is in the clarification of cell culture bioreactor and microbial fermenter feed solutions using microfiltration membranes. In some of these microfiltration TFF applications (e.g., mammalian cell culture clarification), the product (protein) freely passes through the microfiltration membrane and is recovered on the permeate side, while the contaminating impurities (cells, cell debris, colloids) are retained on the feed side of the membrane. In certain other microfiltration TFF applications (allantoic fluid clarification in egg-based flu process), the product (flu virus) may get concentrated on the feed side of the microfiltration membrane (similar to an ultrafiltration step), while the contaminating impurities (ovalbumin, etc.) may get removed into the permeate side.

In either category of microfiltration TFF application, a noteworthy feature is the low transmembrane pressures (TMP) (in the range of 1–3 psi) required for the process operation. Secondly, a microfiltration TFF step is also limited by a maximum throughput or capacity obtainable under a given set of operating conditions, which may potentially limit or determine process sizing. The low TMP requirement, along with the potential throughput limitations, demands a special approach or methodology to develop a robust, optimized process condition for a microfiltration TFF process. While there is a fair amount of literature that describes the theory and propensity for fouling of a microfiltration TFF process[1,7–9], a practical method for developing and optimizing a microfiltration process in biopharmaceutical processing is often not clearly treated or delineated.

The current article is devoted to describing a robust methodology to develop and optimize a microfiltration TFF process.

ABOUT THE AUTHORS

Bala Raghunath, PhD, is the Director of Asia/India BioManufacturing Sciences Network, Merck Millipore, Singapore.
Wang Bin, MS, is the Manager of BioManufacturing Sciences Network, Merck Millipore, China.
Priyabrata Pattnaik, PhD, is the Technical Manager of Biomanufacturing Sciences Network, Merck Millipore, Singapore.
Jeroen Janssens, BE, is a Biomanufacturing Engineer, Merck Millipore, Belgium.

**Dr. Raghunath is the corresponding author:*
Biomanufacturing Sciences and Training Centre
1 Science Park Road, #02-10/11 The Capricorn, Singapore 117528
Phone: +65-6403-5314 | Fax: +65-6403-5322 | Email: bala.raghunath@merckgroup.com

Take away message

Pay attention to impact of technology or tools on antigen aggregation and degradation during process development

Nucleic acid removal

Vaccine and hcDNA

- Viral vaccines and biological products contain contaminating residual DNA from cell substrate

- Primary and diploid cell based vaccines is not of concern, but continuous cell line is a concern due to potential oncogenicity

- WHO Expert Committee on Biological Standardization says.....
  "DNA considered as cellular contaminant rather than risk factor which requires removal to extremely low levels"

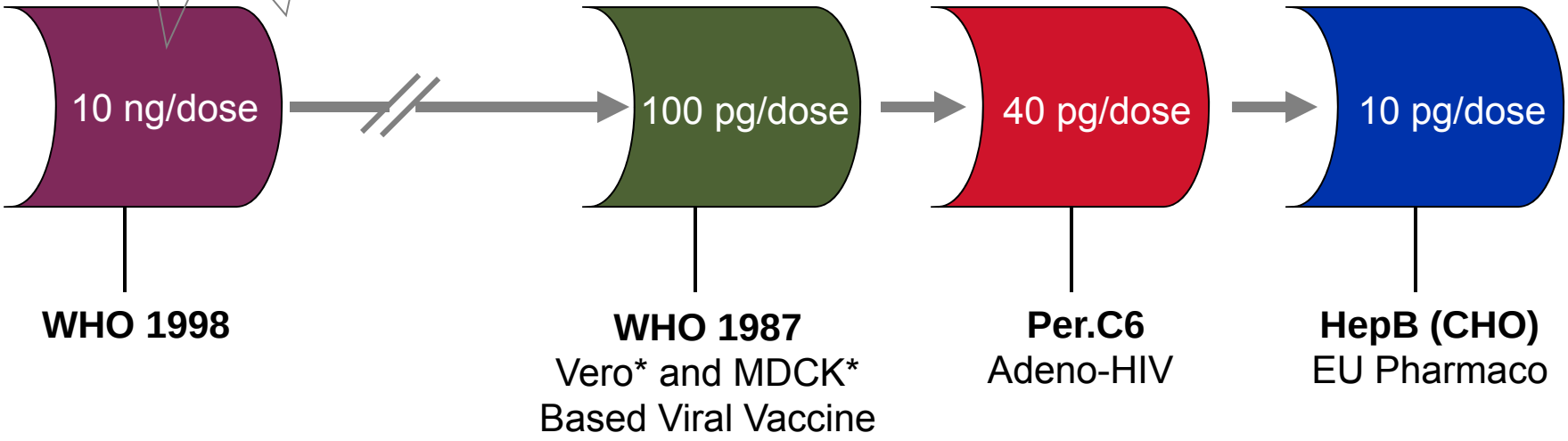
- The amount of residual cell-substrate DNA in a vaccine will depend on the vaccine and the manufacturing process
  - protein/subunit (*e.g.*, HBV)
  - inactivated virus (*e.g.*, IPV, influenza virus)
  - live, attenuated virus (*e.g.*, OPV, MMR, varicella)

Regulatory requirement on Purity and Safety

- *Residual DNA content*

FDA: Case by case
Reduce size (<200bp)
and amount (<10ng/dose)

EMEA position on tumerigenic cells of human origin
DNA as low as possible with risk assessment study



WHO 1998

WHO 1987
Vero* and MDCK*
Based Viral Vaccine

Per.C6
Adeno-HIV

HepB (CHO)
EU Pharmaco

* Non tumerigenic at the passage of production.

*** DNA <10 ng/dose commonly accepted**

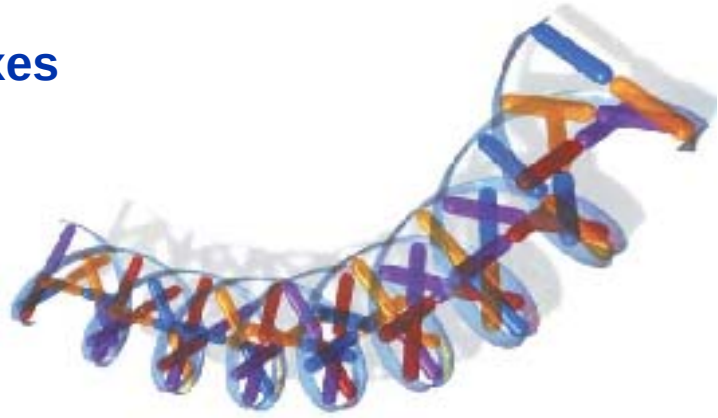
Interference caused by nucleic acids

Increase of viscosity

- Impedes liquid handling
- Extended risk of proteolytic attack
- Reduced efficiency of separation methods (filtration, centrifugation, chromatography)

Formation of protein/nucleic acid complexes

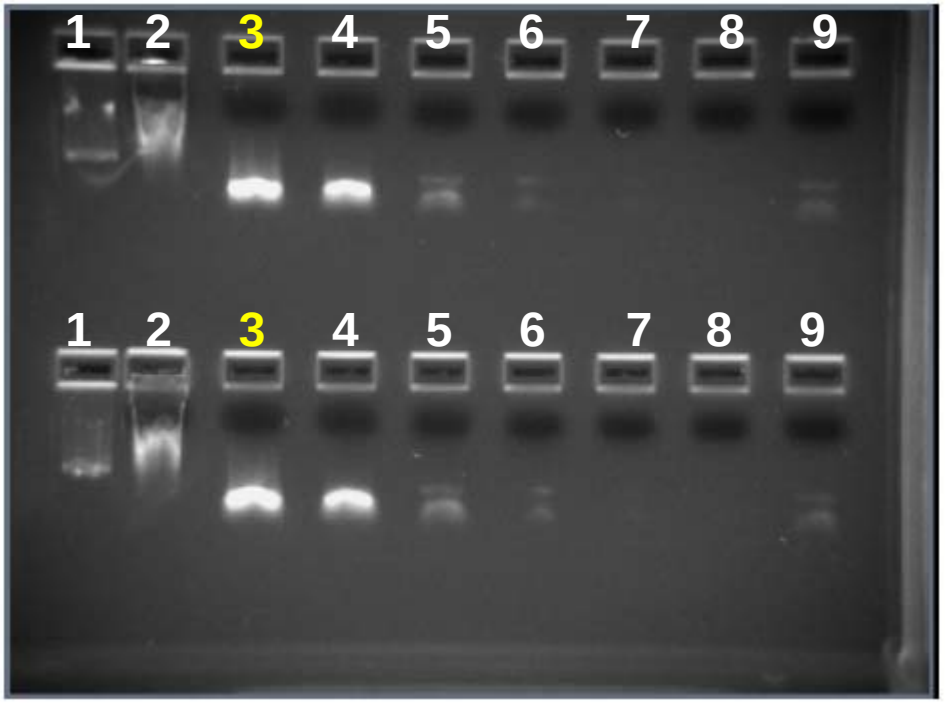
- Changes in virus / protein characteristics
- Unpredictable purification (shift in pI, retention times etc.)



How to remove residual DNA

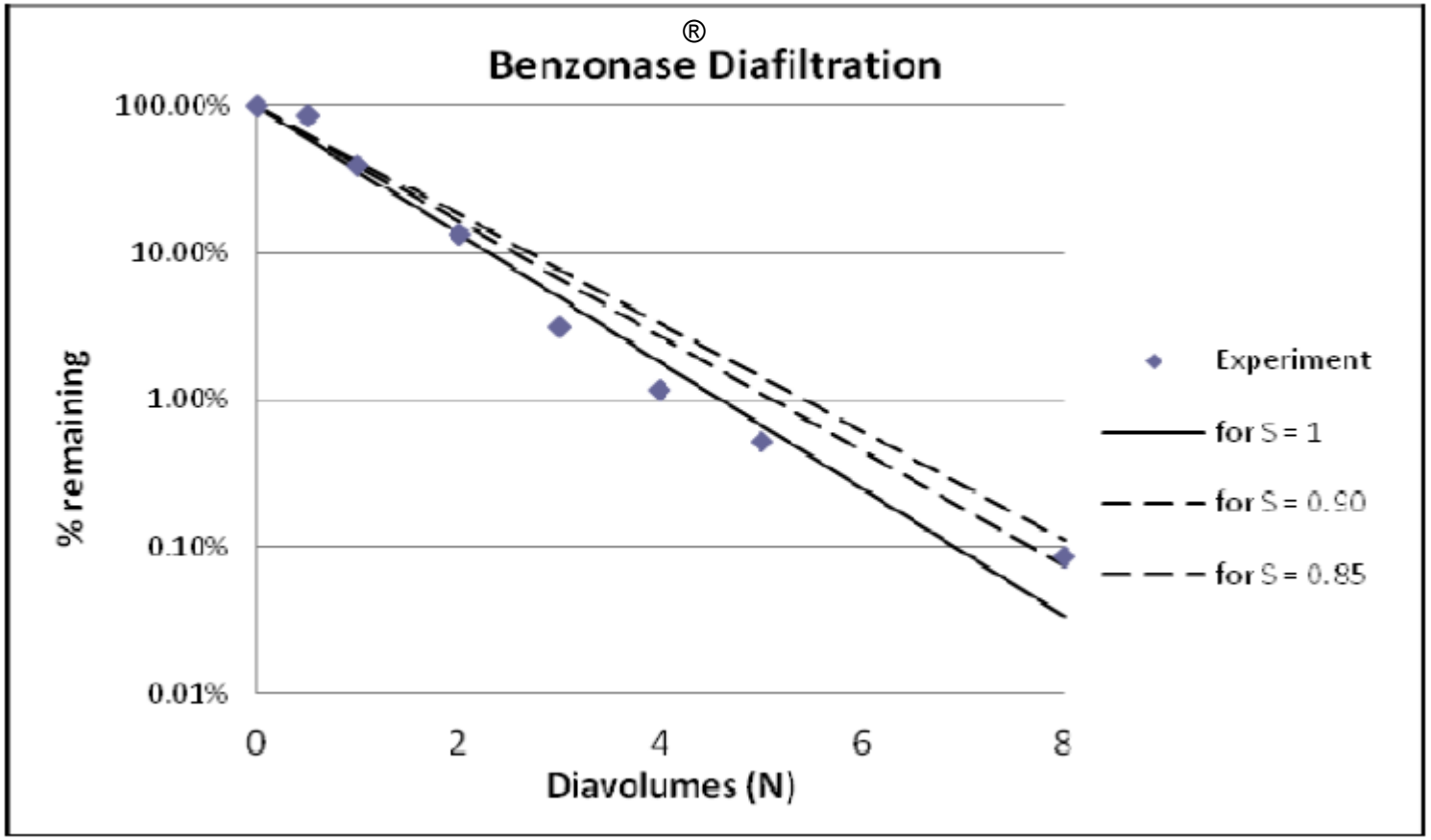
- Precipitation (Acid/base treatment, organic solvent)
  - Ex. Conjugated polysaccharide vaccine
- Treatment by β-propiolactone (BPL)
  - Ex. Killed viral vaccine
- Adsorptive Depth Filters
  - Inactivated Polio
- Chromatographic methods
  - Bind and elute (chromatography media)
  - Flow Through (membrane absorber)
- **Nuclease treatment –** regulatory authorities recommend
  - HepA, LAIV, Rabies, HPV
- Tangential Flow Filtration (TFF)
  - Several vaccines

Clearance of Benzonase® digested DNA from Influenza vaccine (Pellicon® 2, Biomax® 300kDa)



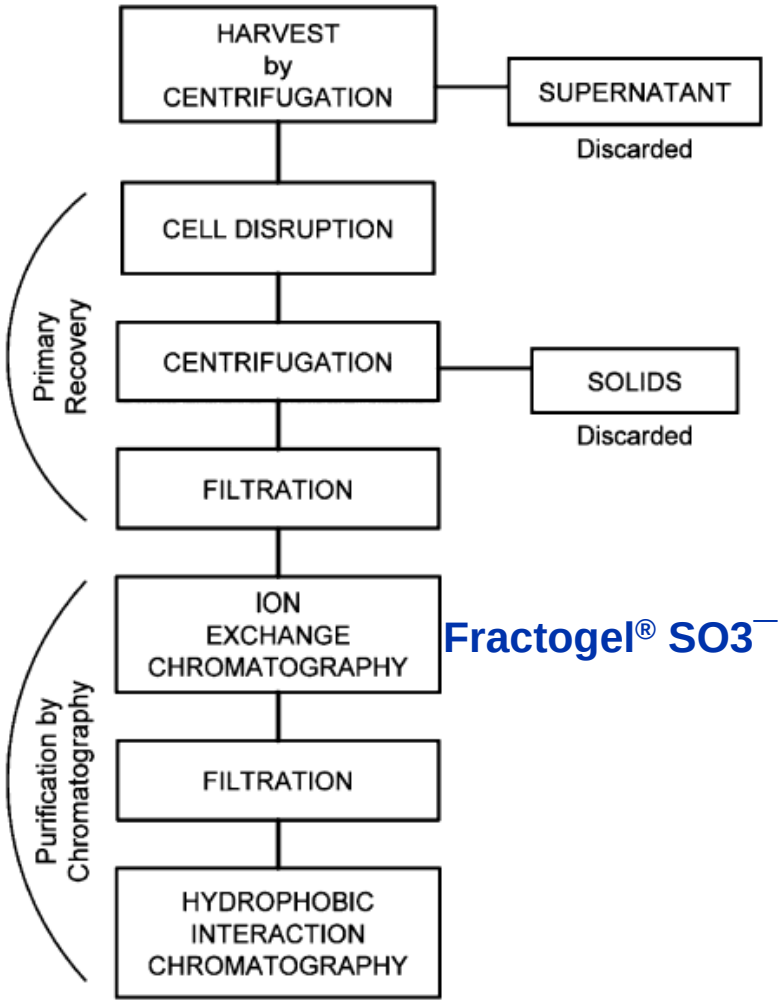
- Lane 1 – Marker (100 BP)
- Lane 2 – Undigested DNA in Feed
- Lane 3 – After Benzonase® digestion
- Lane 4 – Post Recirc retentate
- Lanes 5, 6, 7, 8 – Retentate samples after 1, 3, 5, 8 DV
- Lane 9 – Permeate at 5DV

Diafiltration of Residual Benzonase®



99.5% clearance at 5 diavolumes and > 99.9% (3 log) clearance after 8 diavolumes across the UF/DF step

Fractogel® SO3 for removal of spent Benzonase from AAV vaccine process



- Adeno-assocuated virus (AAV) and DNA can form aggregates, since there is a net positive charge on AAV at pH 7.5 and negative on DNA

- Digesting cellular DNA in by adding Benzonase® in lysis buffer prevents binding of DNA to AAV during and after the cell rupture step

- Lysis Buffer: 50mM Tris, 2mM $MgCl_2$ and 5U of Benzonase®/million cells at pH 7.5

SOURCE: Chahal et al., Journal of Virological Methods 139 (2007) 61–70.

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Nucleic Acid Impurity Reduction in Viral Vaccine Manufacturing

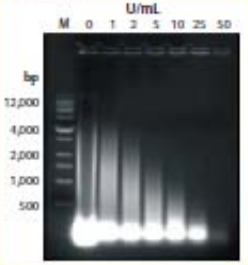
Elina Gousseinov, Willem Kools, and Priyabrata Pattnaik

Commercial-scale viral vaccine manufacturing requires production of large quantities of virus as an antigenic source. To deliver those quantities, a number of systems are used for viral replication based on mammalian, avian, or insect cells. To overcome the inherent limitations in production outputs with serial propagation of cells, mammalian cells can be immortalized, which increases the number of times they can divide in culture. Modifications that immortalize cells are typically accomplished through mechanisms similar to those converting normal cells to cancer cells. Thus, the presence of residual host-cell nucleic acids in final vaccine products would create significant concerns about the potential for transfer and integration into a patient's genetic material.

Host-cell nucleic acids in feed material depends on cell/virus type and on methods and techniques used in harvesting. The presence of DNA can contribute to process fluid viscosity and fouling of separation media, reduce useable capacity, cause coprecipitation, and threaten product safety. The risk of oncogenicity and infectivity of host-cell nucleic acid can be minimized by suppressing its biological activity. That can be achieved by decreasing the amount of residual DNA and RNA and reducing their size (with enzymatic/nuclease or chemical treatment) to below the functional gene length of ~100 base pairs.

Health authorities and regulatory bodies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have set limits for acceptable amounts of residual DNA in final biological products. According to requirements published by the FDA, a parentally administered dose is limited to 100 pg of residual DNA. The EMA and the World Health Organization (WHO) allow 10 ng per parenteral dose and 100 µg/dose for an orally administered vaccine (1). Orally administered DNA is taken up about

Figure 1: Benzonase nucleic acid digestion demonstrating size reduction of DNA after nucleic-acid digest with various Benzonase concentrations



DNA Removal

Precipitation with
- Cationic detergents — e.g., cetyltrimethyl ammonium bromide (CTAB) or domiphen bromide (DB)
- Short-chain fatty acids (e.g., caprylic acid)
- Charged polymers — e.g., polyethyleneimine (PEI) and polyacrylic acid (PAA)
- Polyethylene glycol (PEG)
- Ammonium sulfate
- Tri(n-butyl)phosphate (TNBP) with Triton X-100 detergent solution

Filtration:
- Normal-flow filtration (NFF) with depth-charged or diatomaceous-earth–containing media
- Tangential-flow filtration (TFF)
- Ultrafiltration/diafiltration (UF/DF)

Chromatography and membrane adsorbers:
- Anion-exchange chromatography (AEX)
- Gel-filtration (size-exclusion) chromatography
- Hydrophobic charge-induction chromatography (HCIC)

Degradation with
- Enzymes
- Physical forces (shearing)
- Alkylating agents (e.g., β-propylactone)

Product Focus: Vaccines

Process Focus: Downstream Processing

Who Should Read: Process engineers, QA/QC, analytical

Keywords: Chromatography, biocatalysis, tangential-flow filtration, nucleic acid detection assays

Level: Intermediate

Take away message

Integrate nucleic acid removal as one of the essential product quality and safety criteria during process development phase

Quality of Formulation Excipients impacts drug product safety

Typical Composition of Vaccine Formulations

...this is a very generalized overview, other compositions are possible

3%
- Buffers/ pH adjustments
- Tonicity adjustments
- Sweetening agents
- Preservatives/ Antioxidants
- Surfactants
- Stabilizers
- Thickeners

95% Solvents

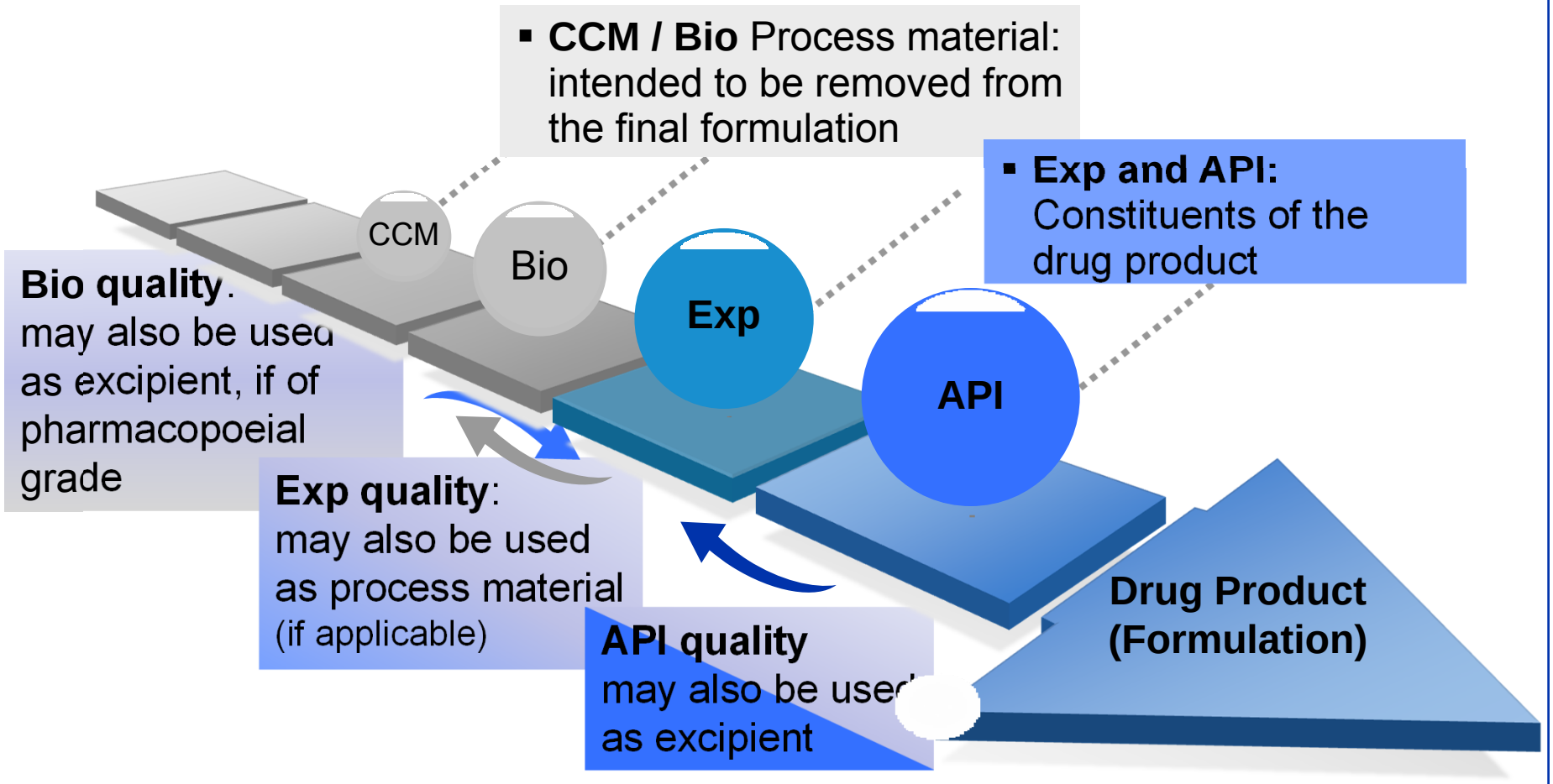
Excipients

1-2% **Antigen**

Some considerations for chemicals (ICH Q6)

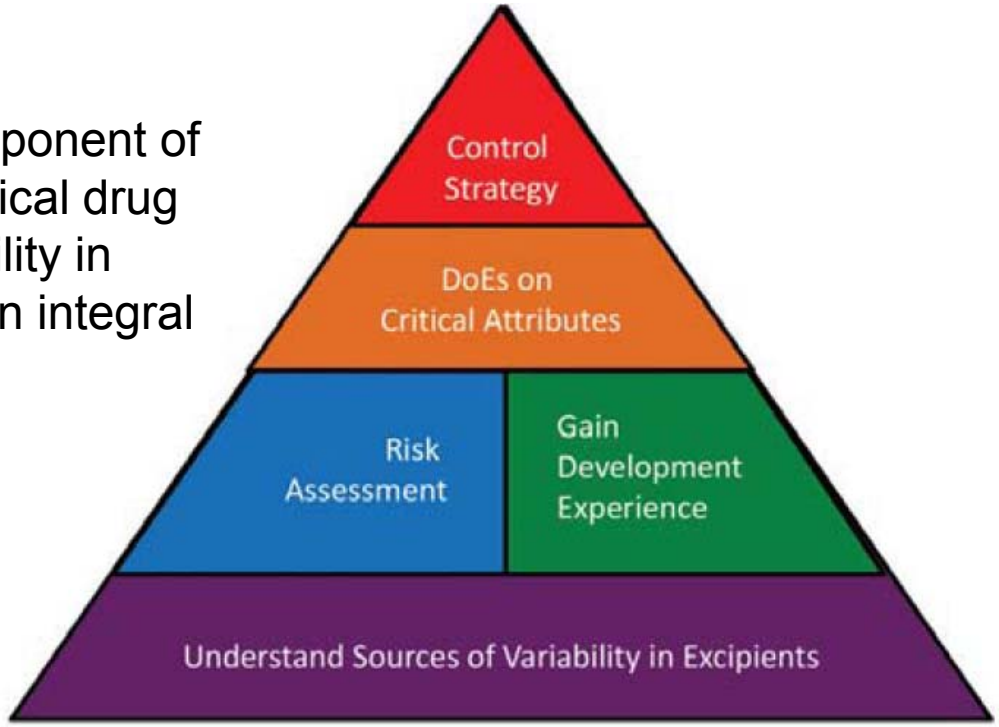
Critical impurities	Measures of control
Potential process-related impurities	▪ Impurity profiling: covers manufacturing process and raw material ▪ Residual solvents and catalysts ▪ Non-animal origin
Bioburden, endotoxines, enzymatic activity	▪ Production designed to eliminate such impurities or to prevent from potential contamination ▪ GMP zone concept in manufacturing and downfilling
Leachables and extractables from transport containers	▪ Rigid qualification principles for primary and secondary packaging material ▪ Stability studies for solids ▪ Leachables/extractables studies for liquids

Choose the right type of quality –
partly interchangeable



Understanding source of variability in excipient is critical for product quality

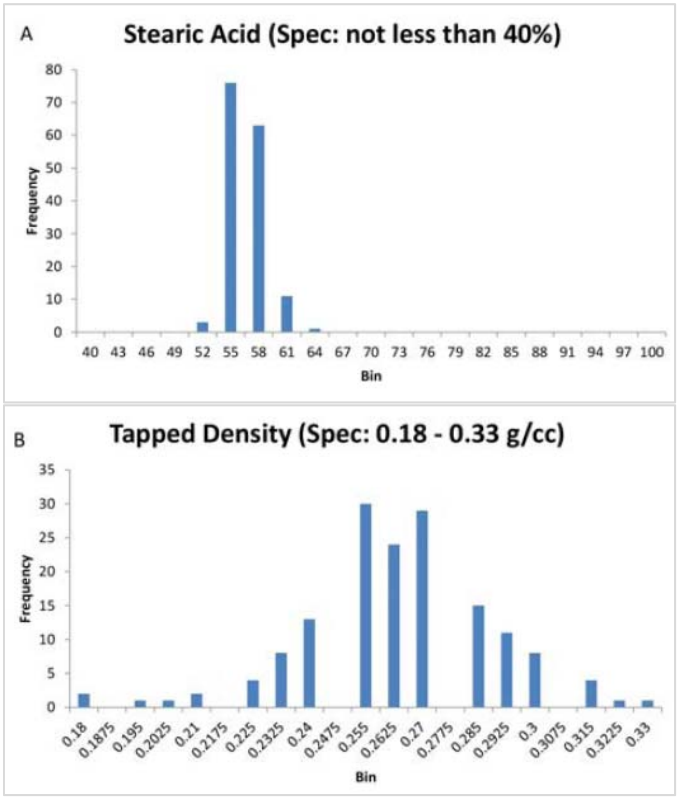
Excipients are an essential component of robust, commercial pharmaceutical drug products. Understanding variability in excipient material attributes is an integral part of drug product design and development.



Source: Joseph Kushner (2015) Incorporating QbD Excipient Variability into Pharmaceutical Drug Product Design and Development: Perspective from an Excipient User, *BioPharma Asia,* May/June 2015, 42-48.

Case Study: Typhoid vaccine (Vivotif®) has 3.6-4.4mg Magnesium stearate as stabilizer per dose

Extent of variability and specification range of two material attributes for 154 lots of magnesium stearate



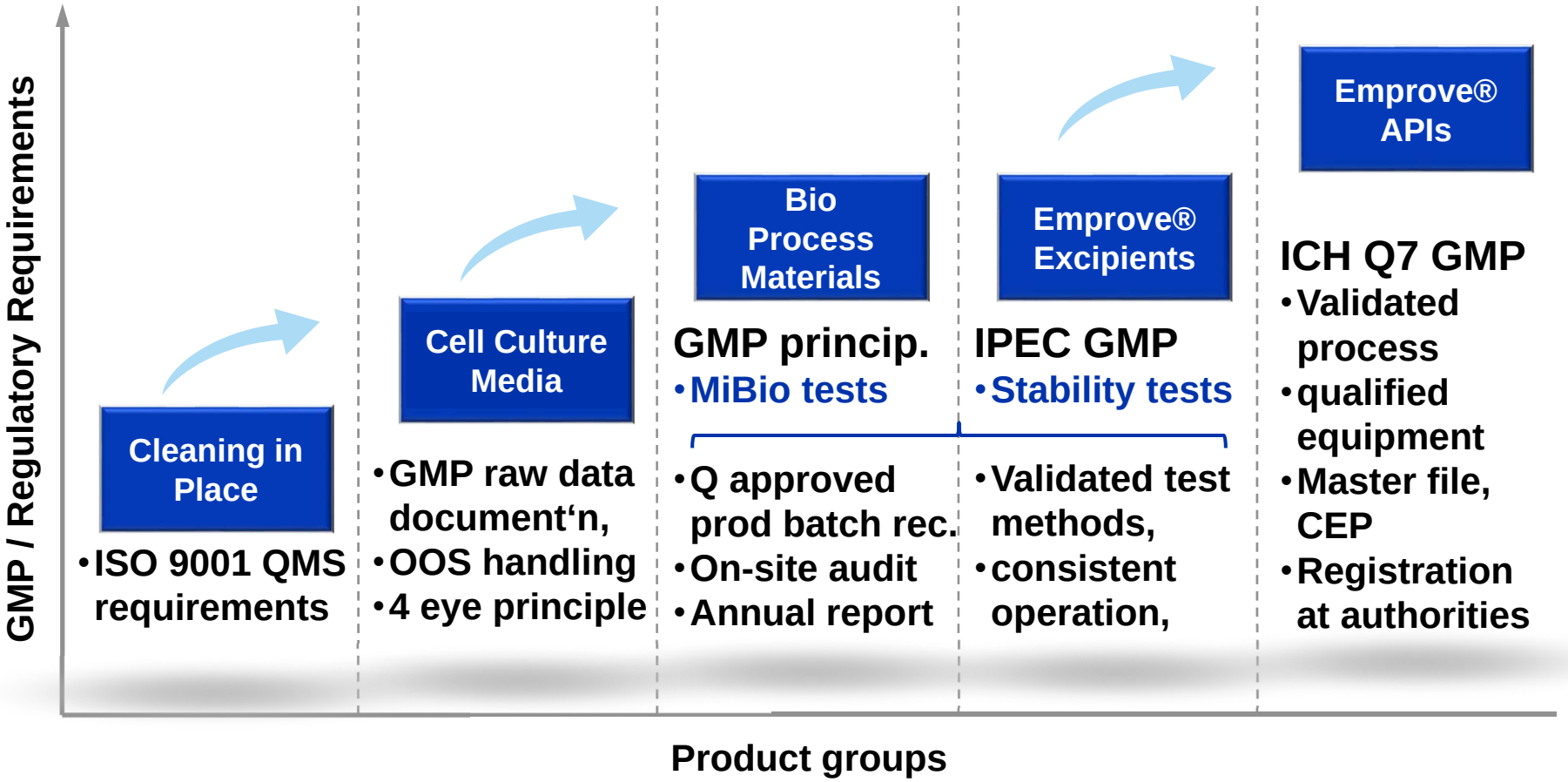
List of the Quantitative Material Attributes on the Certificate on Analysis for the Excipients

- Loss on Drying
- Specific Surface Area
- Stearic Acid Content
- Stearic + Palmitic Acid Content
- Mg Assay
- % through 325-mesh
- Bulk density
- Tapped density
- Acid Value
- D50
- D90
- Polymorph

Source: Theresa M. Finn and William Egan. Vaccine additives and manufacturing residuals in the United States: licensed vaccines. In. Vaccines, ed. Plotkin SA, Orenstein WA, Offit PA. 6th Edition, Elsevier. pp71-79.

Increasing GMP requirements* for different product groups

* examples, non-exhaustive



Take away message

Select appropriate excipient early in the process, preferably at PD or process characterization phase to ensure final product quality and safety

Summary

- Pay attention to bioburden control and monitoring during process development
- Select technology or tools used in vaccine processing with clear understanding of it's impact on vaccine aggregation, antigen sheering and degradation
- Integrate nucleic acid removal as one of the essential product quality and safety criteria during PD phase
- Select appropriate excipient early in the process, preferably at PD or process characterization phase to ensure final product quality and safety

Priyabrata Pattnaik, PhD
priyabrata.pattnaik@merckgroup.com
twitter @pattnaik_p
LinkedIn
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