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GaBI Educational Workshops

1st ASEAN Overview Workshop on
GMP for BIOLOGICALS/BIOSIMILARS

5 August 2018, Furama Resort Da Nang, Vietnam

Fermentation: fundamentals, control of source materials and cell culture conditions

Dinesh Khokal, PhD

5 August 2018

GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

PPCI
Pro Pharma Communications International

asean

FERMENTATION: FUNDAMENTALS, CONTROL OF SOURCE MATERIALS AND CELL CULTURE CONDITIONS

First ASEAN Overview Workshop on GMP for Biologicals/Biosimilars
Generics and Biosimilars Initiative (GaBI)
5 August 2018, Da Nang, Vietnam

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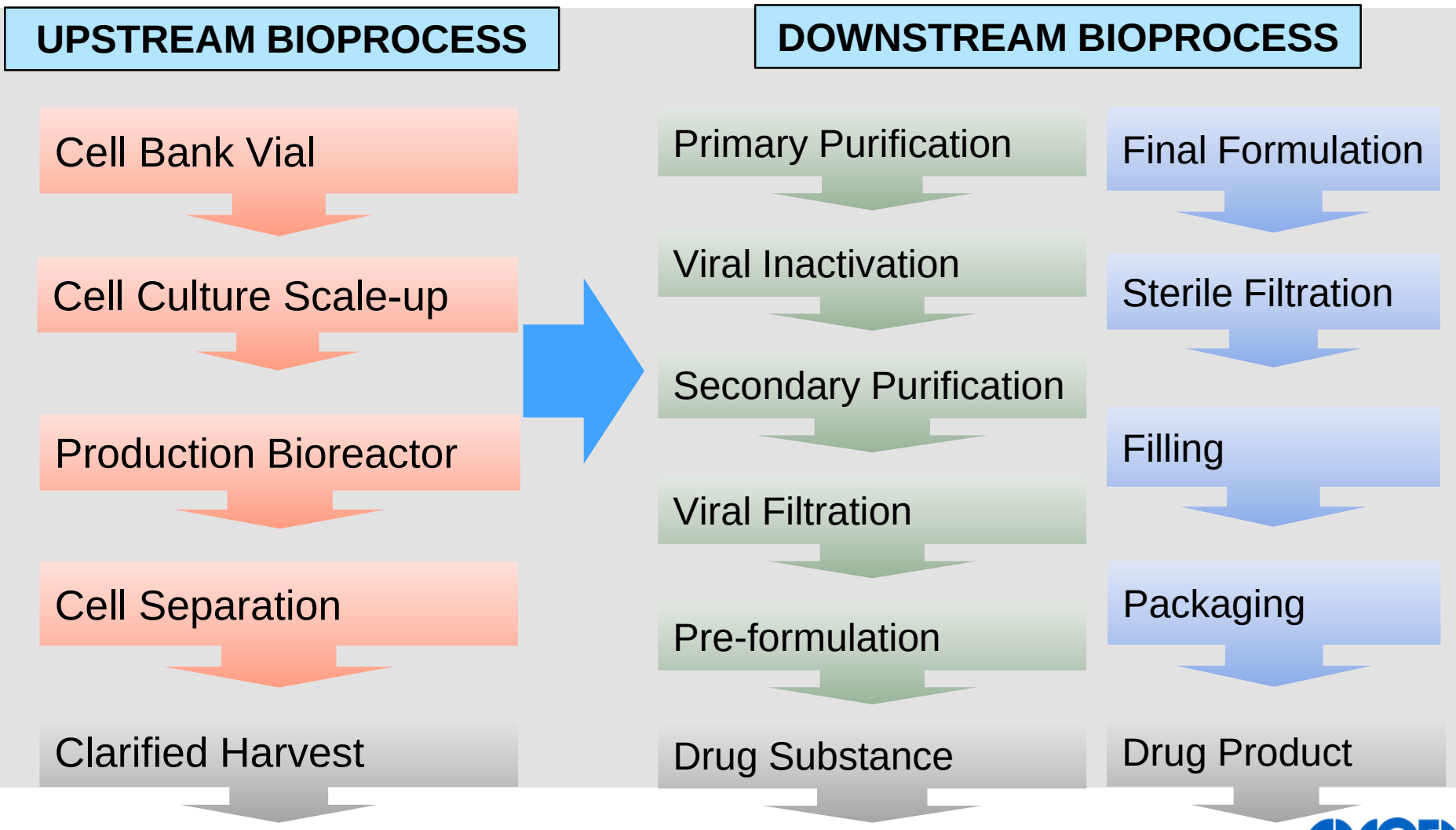
SYNOPSIS

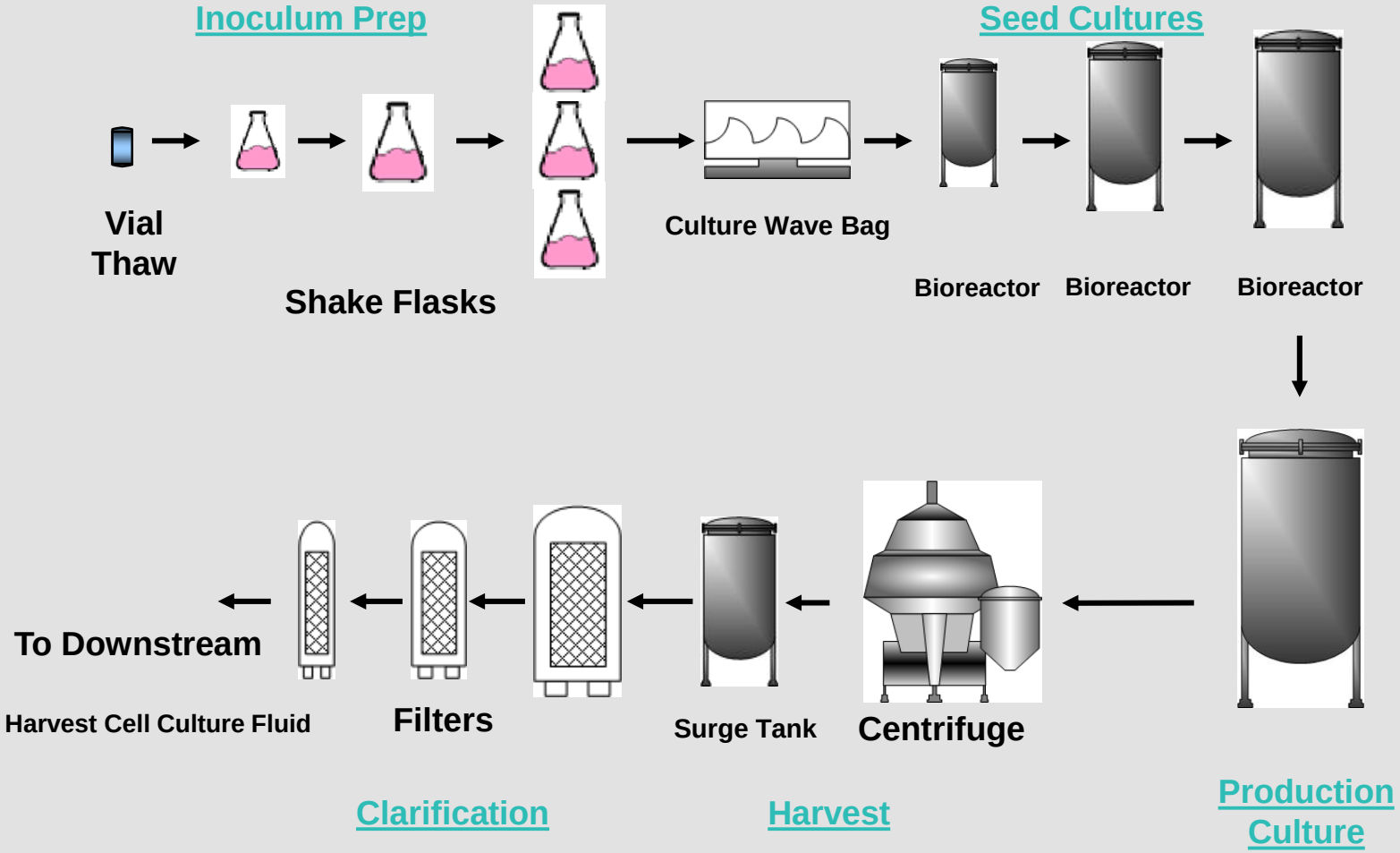
- Introduction
- Fundamentals of fermentation
- Control of source materials
- Control of cell culture conditions

BIOLOGICS MANUFACTURING FLOWCHART
UPSTREAM AND DOWNSTREAM BIOPROCESS



Courtesy: Eli Kraus, Amgen

UPSTREAM BIOPROCESS



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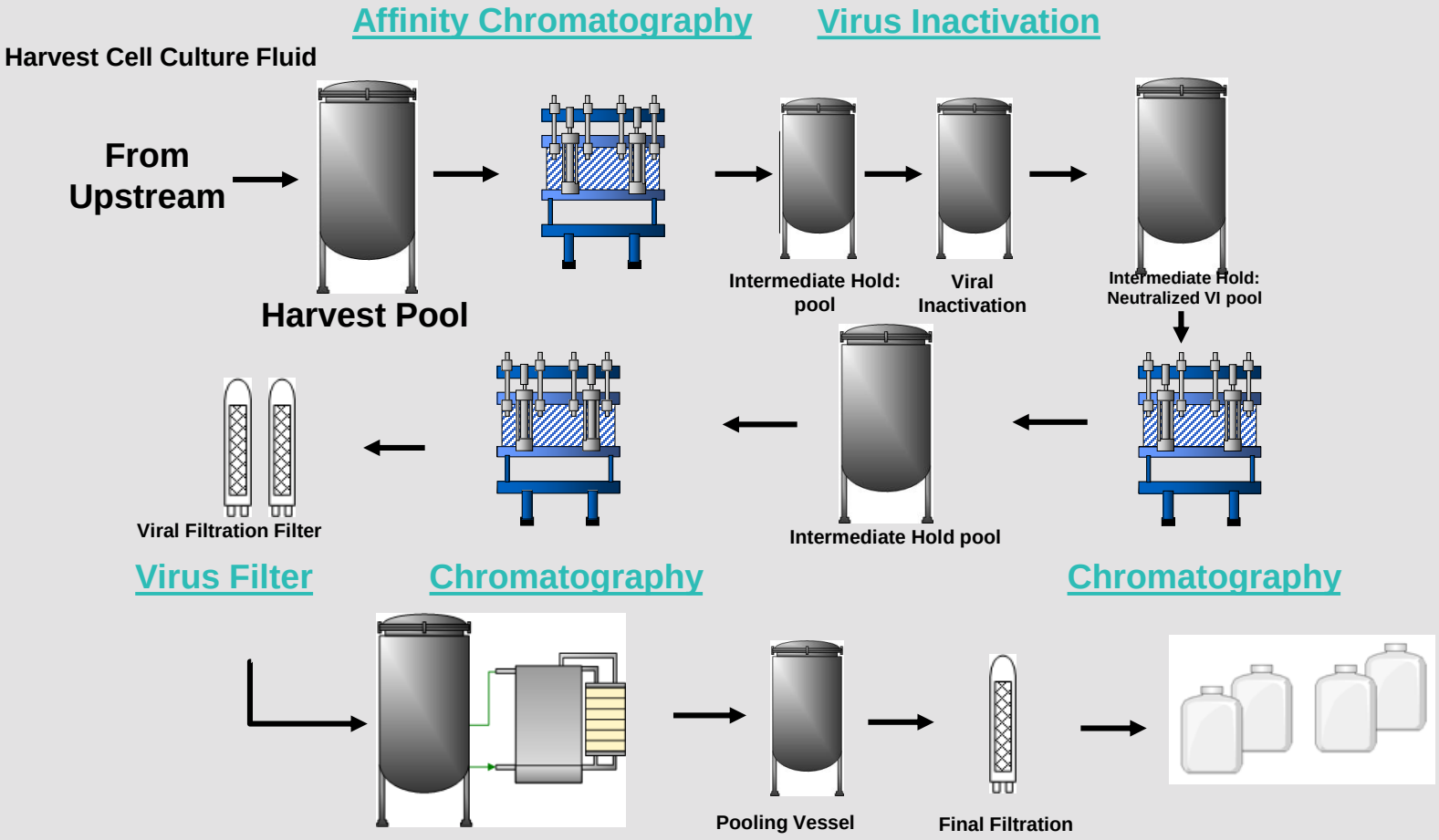
DOWNSTREAM BIOPROCESS



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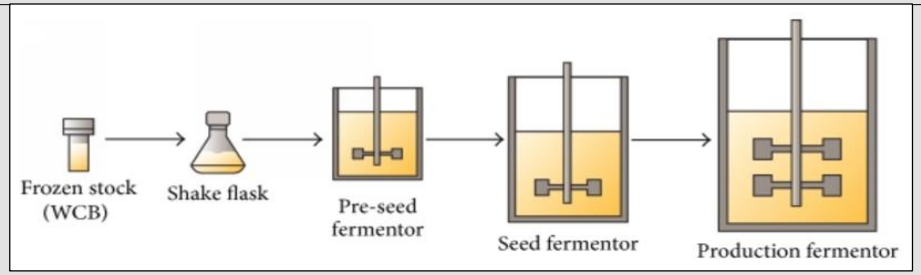
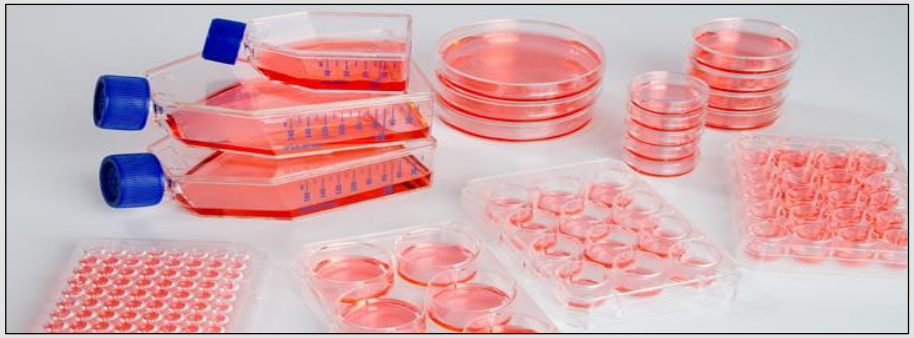
FERMENTATION VS CELL CUTLTURE?

➢Fermentation:



Microbes obtain energy by breaking down glucose and other molecules

➢Cell Culture:

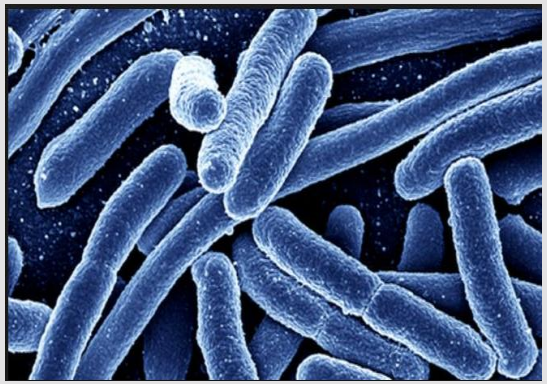


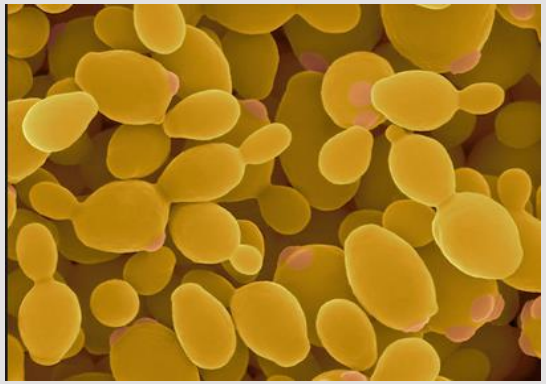
Cells taken from living organisms and grown under controlled conditions in a laboratory or manufacturing system

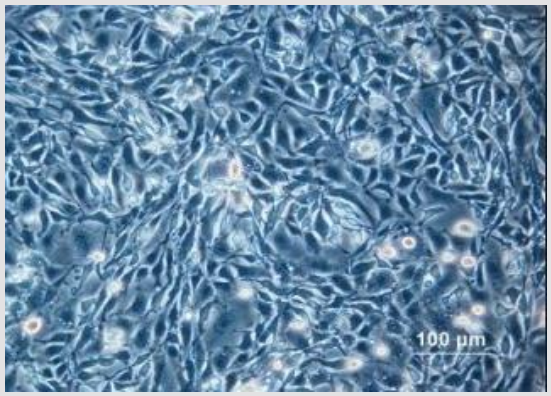
Fermentation and cell culture are essentially the same thing

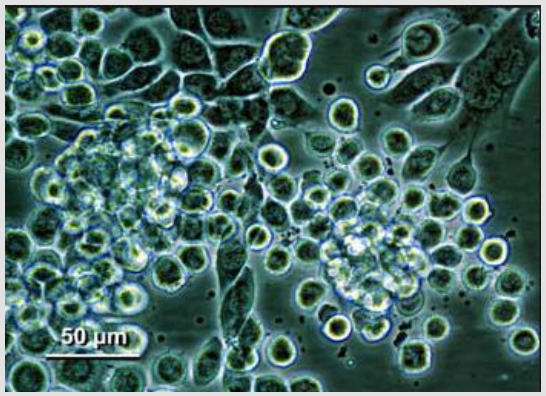
COMMONLY USED ORGANISMS

- **Bacteria**
  - *Escherichia coli (E. coli)*
  - *Bacillus subtilus*
- **Yeast**
  - *Pichia pastoris*
  - Saccharomyces cervisiae,
  - Schizosaccromyces pompe
- **Mammalian Cell**
  - Chinese hamster ovary (CHO) cells
  - African green monkey kidney cells
  - Baby hamster kidney (BHK) cells
  - NSO murine myeloma cells
  - PER.C6 Human cells
- **Insect cells: Sf9, Sf21**









SYNOPSIS

- Introduction
- **Fundamentals of fermentation**
- Controls of source materials
- Control of cell culture conditions

DIFFERENT TYPES OF FERMENTATION TECHNIQUES

Batch Culture

- **closed system**
- **Grown to a maximum density & harvested as a batch**
- **After the start, nothing is added except aeration**
- **Volume of culture remains same**
- **Concentration of nutrition decreases continuously**
- **Toxic metabolites accumulate**
- **Characteristics growth curve – lag, log phase, stationary and decline phases**

Advantage	Disadvantage
Chance of contamination of culture is minimum	Low product yield and not economic

DIFFERENT TYPES OF FERMENTATION TECHNIQUES

Fed-batch culture

- **Semi-closed system**
- **During incubation a particular nutrient is added at intervals**
- **No removal the used up media**
- **Volume of culture increases continuously**
- **Nutrients inhibiting growth at high concentration are kept in lower concentration initially, added slowly and continuously during the course of fermentation.**

Advantage	Disadvantage
Greater product yields	Chance of contamination of culture is higher

DIFFERENT TYPES OF FERMENTATION TECHNIQUES

Continuous culture

- Open system
- Fresh sterile medium is added continuously
- Used up media is removed continuously
- The volume and bacterial density remain same in the cultivation vessel
- Bacteria grow in their log phase - steady state growth
- Cell density remains constant
- Achieved by maintaining constant dilution and flow rate.
- Secreted protein products continuously harvested by filtration

Advantage	Disadvantage
greater product yields	Chance of contamination of culture is higher

CELL CULTURING TECHNIQUES

Two kinds of systems for animal cell culture

- **Substrate or Anchored systems**
  - cells attached to the surface of the culture vessel or other solid support
- **Suspension Systems**
  - Cells suspended in a liquid medium

MEDIUM FOR GROWTH

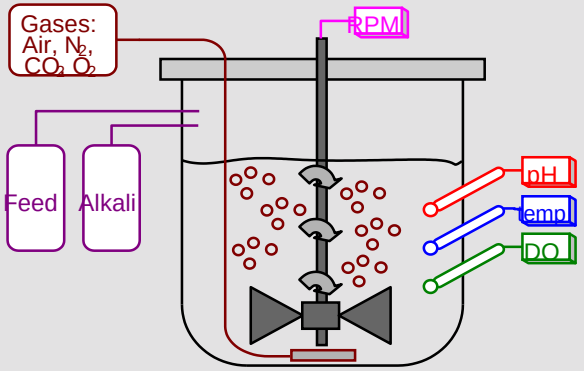
Cells	• Deteriorate and die when getting too few nutrients
Nutrients	• Provided in the form of a medium
Bovine serum	• Has long been preferred in mammalian cell culture
Serum-free and Protein-free media	• Reduces cost and increases safety
Media formulation	• Ensures consistency in production and performance of large lots

FERMENTORS AND BIOREACTORS

Parameters		Purpose
Mechanical equipment	→	Designed for cultivation of cells
Fomenters	→	To cultivate microbes
Bioreactors	→	To cultivate animal cells
Thermodynamics	→	Solubility of oxygen in the medium
Microkinetics	→	Cell growth, product formation and, transport of materials to and from cells
Optimal mixing	→	To ensures effective oxygen transfer, heat transfer and dispersal of materials.
Minor deficiencies in media	→	Have major effects on cell growth and protein production
Stirring the medium	→	Prevents cells from settling to the bottom; Ensures homogenous environment and improves oxygen transfer
Mechanism to maintain circulation	→	Motor-driven shaft impeller, can cause shear-force damage to cells.
Shear effects in bioreactors	→	Depends on the type of cells used
Larger bioreactors	→	Providing adequate oxygen is hampered by cell fragility
Animal cells	→	More fragile than microorganisms because of their large size and lack of rigid cell wall
Transport of nutrients	→	Governed by flow and diffusion; directly related to shear, mixing, mass transfer, heat transfer and macrokinetics
Scale-up problems	→	Arise due to imbalance of heat, mass, or momentum in a system. All these factors affect product yield.

PROCESS CONTROL AND AUTOMATION

- Cell growth depends on physiochemical environment.
- Must control:
  - pH, DO_2, pressure sparging, temperature,
  - foaming and concentration of nutrients
  - waste products
- Sterile probe devices used for process monitoring and control
- Process sensors are calibrated regularly
- Sophisticated monitoring and control software are used
- Cell growth is monitored
  - Sampling, Cell density, Viability
- Product concentration - HPLC and ELISA



APPROXIMATE TIMEFRAME FROM INTRODUCED GENE TO PROTEIN PRODUCTION AT USABLE LEVELS

Microbes & Cells

- *Escherichia coli* (Bacteria) **5 DAYS**
- *Pichia pastoris* (yeast) **14 DAYS**
- Insect cell **25 DAYS**
- Mammalian cell **4-5 MONTHS**

Transgenic Plants & Animals

- Tobacco **6 MONTHS**
- Corn **10 MONTHS**
- Sheep **18 MONTHS**
- Cattle **33 MONTHS**

BIOLOGICAL CONTAMINANTS

Contamination of cell cultures is the most common problem

- **Chemical contaminants**
  - impurities - source materials
- **Biological contaminants**
  - Bacteria
  - Moulds and yeasts
  - Viruses
  - Mycoplasma
  - Cross contamination

by other cell lines

Impossible to eliminate contamination entirely

Possible to reduce its frequency and seriousness

- Understand source of contamination
- Following good aseptic technique

ASEPTIC PROCESSING

cGMPs	• Controlling bioburden and sterility
Low bioburden, Sterility	• Don’t have sterility, Low bioburden, don’t have a culture
Batch and fed-batch process	• Allow downtime for taking out parts for cleaning • Thorough cleaning of the equipment
Cleaned in place (CIP)	• Cleaning using chemicals and steam
Steam in place or sterilize-in-place (SIP)	• Cleaning and Sterilizing using clean steam
Material of Choice	• High-grade stainless steel (Grade 316L)
Use of Disposables	• Piping, fittings, plastic bags • Single-use bioreactors

ASEPTIC PROCESSING

- Use of other equipment to provide
  - ultrapure oxygen
  - carbon dioxide
  - Air
  - water-for-injection (WFI) and
  - various ingredients for the fermentation medium
- Pumps move fluid
- Filters guard against impurities
- Inlet gas is sterile filtered
- Exhaust gas goes through condensers and sterilizing filters
- Valves direct fluid and gases
- Culture medium is filtered
- Serum many irradiated or heat inactivated

PERSONNEL TRAINING AND MONITORING

- Minimize personal intervention
  - well-designed facility
  - well maintained
  - well operated aseptic processes
- As operator activities increase, risk to finished product sterility also increases
- Critical for operators involved in aseptic activities to use aseptic technique at all times.
- Appropriate training is critical

PERSONNEL TRAINING & MONITORING

- Fundamental training topics
  - aseptic technique
  - cleanroom behaviour
  - microbiology
  - Hygiene
  - Gowning
  - patient safety hazards
  - aseptic manufacturing operations
- Ongoing training program
- Supervision on conformance to aseptic operations
- Quality control oversight

PERSONNEL TRAINING AND MONITORING

- **Techniques aimed at maintaining sterility**
  - Contact sterile materials only with sterile instruments
  - Move slowly and deliberately
  - Keep the entire body out of the path of unidirectional airflow
  - Maintain Proper Gown Control
- **Laboratory Personnel**
  - Basic training in aseptic technique
  - personnel qualification in aseptic manufacturing processes and systems

MONITORING PROGRAM

- Personnel can significantly affect the environment
- Vigilant and responsive personnel monitoring program
- Monitoring surface samples of each operator's gloves
  - daily basis
  - in association with each lot
- Appropriate sampling frequency from strategically selected locations of the gown.
- Comprehensive monitoring program for operators by QC

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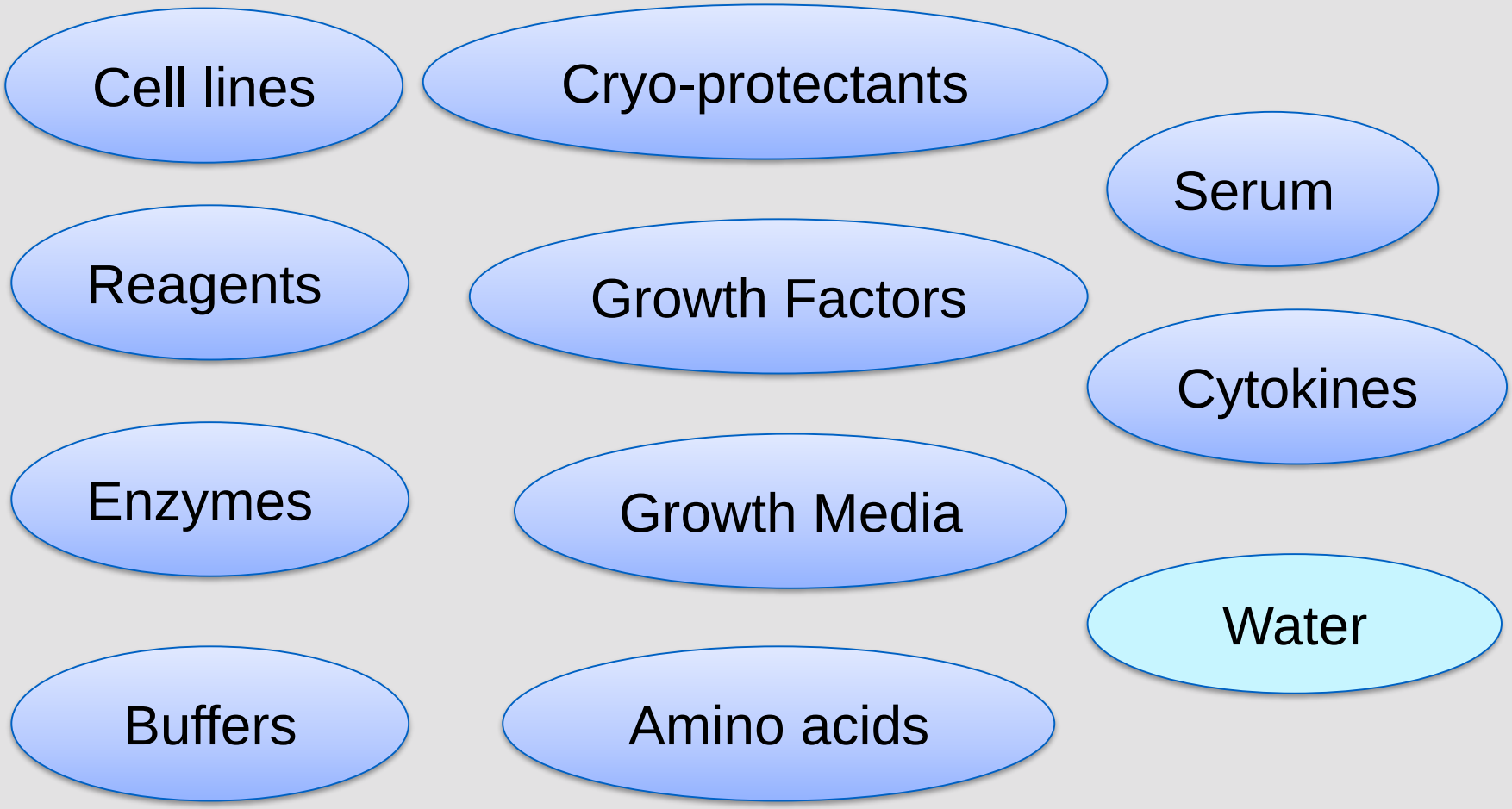
CONTROL OF SOURCE MATERIALS



CONTROL OF SOURCE MATERIALS – IMPORTANCE?

- **Controlling the quality of source materials - challenging and critical task**
- **Risk of adventitious agent contamination**
- **Risk of other serious quality deviation**
- **Potential to disrupt manufacturing process**
- **Potential to impact product:**
  - **Quality/ safety/Efficacy**
  - **Lot-to-lot consistency**
  - **Specification failure**
  - **Comparability**
  - **PK/PD**
  - **Adventitious agents**
  - **Chemical contaminants**
  - **Immunogenicity**

CONTROL OF SOURCE MATERIALS

- Qualify suppliers
- Manage source materials quality
  - identification
  - meeting appropriate standard
  - intended use
  - variability
  - clearance and control of adventitious agents of biologically-sourced materials
- Define and control the source, origin and suitability according to GMP principles
- Retain information on the source and quality of the biological materials
- Sampling, testing and monitoring program
- Qualify alternative source

CONTROL OF SOURCE MATERIALS

- **Biologic reagents**
  - bovine serum albumin
  - transferrin
  - insulin
  - growth factors
  - TSE Risk Evaluated Certificate of Suitability (CEP)
- **Source materials derived from cell lines**
  - Origin, Source and History of Host Cell Line
  - Passage number or the number of generation during cell growth history and characterization
  - Information on genetic modification for the cell line, cloning vectors, plasmid maps and construction of the intermediate cloning vectors
  - Procedures on cell transfection, screening and sub-cloning should be provided.

CONTROL OF SOURCE MATERIALS

- **Cell lines**
  - Source, history, and generation of cell line
  - Analysis of expression construct used to genetically modify cells
  - Cell banking system, characterisation and testing:
    - Genetic, phenotypic & immunological markers of the cell
    - Cell viability, genetic, phenotypic stability
- **Specifications on MCB and WCB include tests**
  - Sterility
  - mycoplasma
  - virus associated with the cell line
  - other adventitious viruses
- **Water**
  - meet appropriate quality standard for PW and WFI

CONTROL OF SOURCE MATERIALS

Risk of Contamination	• Assessment of source materials during their passage along the supply chain • Emphasis on adventitious agents
Control Measures	• Ensure the quality of sterile source materials • Aseptic manufacturing process based on cGMP principles
Transportation	• Control transportation of critical materials - reference materials, active substances and cells to the manufacturing sites

SYNOPSIS

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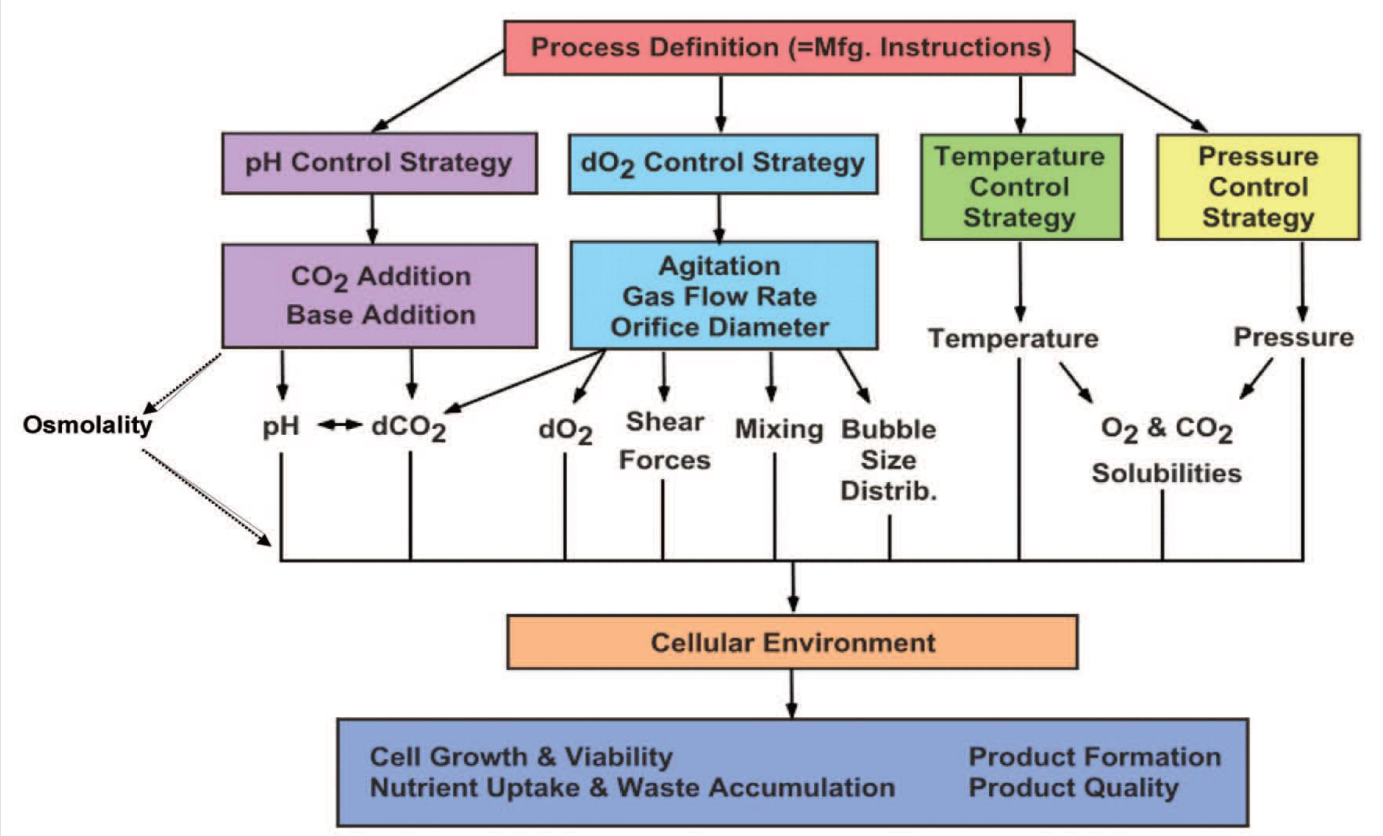
CONTROL OF CULTURE CONDITIONS



Cell culture operating parameters affect process performance and product quality.

Courtesy: Feng Li *et.al.*, mAbs 2:5, 2010

CONTROL OF CULTURE CONDITIONS

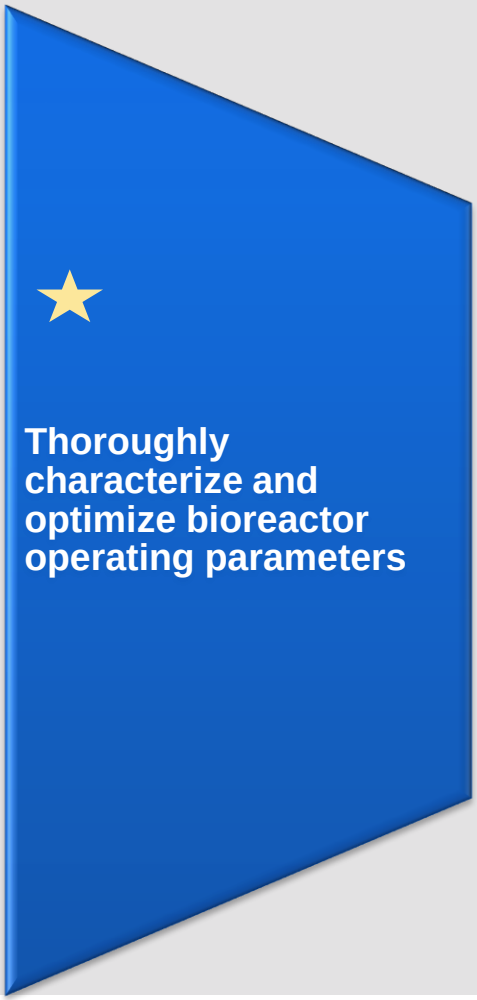
- **Operating parameter optimization**
  - to achieve high expression of product
  - acceptable product quality profiles
- **Parameters**
  - Physical
  - Chemical
  - Biological

- **Physical parameters**
  - Temperature, gas flow rate, agitation speed
- **Chemical parameters**
  - dissolved O_2
  - CO_2, pH, osmolality, redox potential, metabolite levels, substrate, amino acids and waste by-products
- **Biological parameters**
  - cell concentration
  - viability
  - intracellular and extra-cellular measurements such as NADH, LDH levels, mitochondrial activity and cell cycle analysis

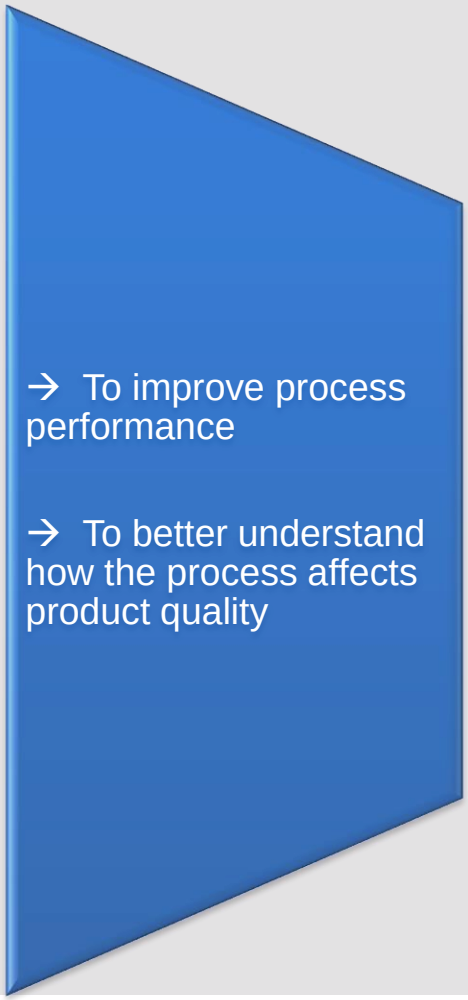
CONTROL OF CULTURE CONDITIONS

- **Variations parameters from optimal levels can impact**
  - culture performance
  - Productivity
  - product quality
- **A typical stirred tank bioreactor is equipped with temperature, pressure, agitation, pH and dissolved oxygen controls**
- **Operating strategies and parameters effect**
  - dissolved oxygen (DO) and CO_2
  - pH
  - Osmolality
  - mixing,
  - hydrodynamic shear
- **Influences measures of process performances**
  - cell growth, metabolite concentrations, product titer and product quality

CONTROL OF CULTURE CONDITIONS

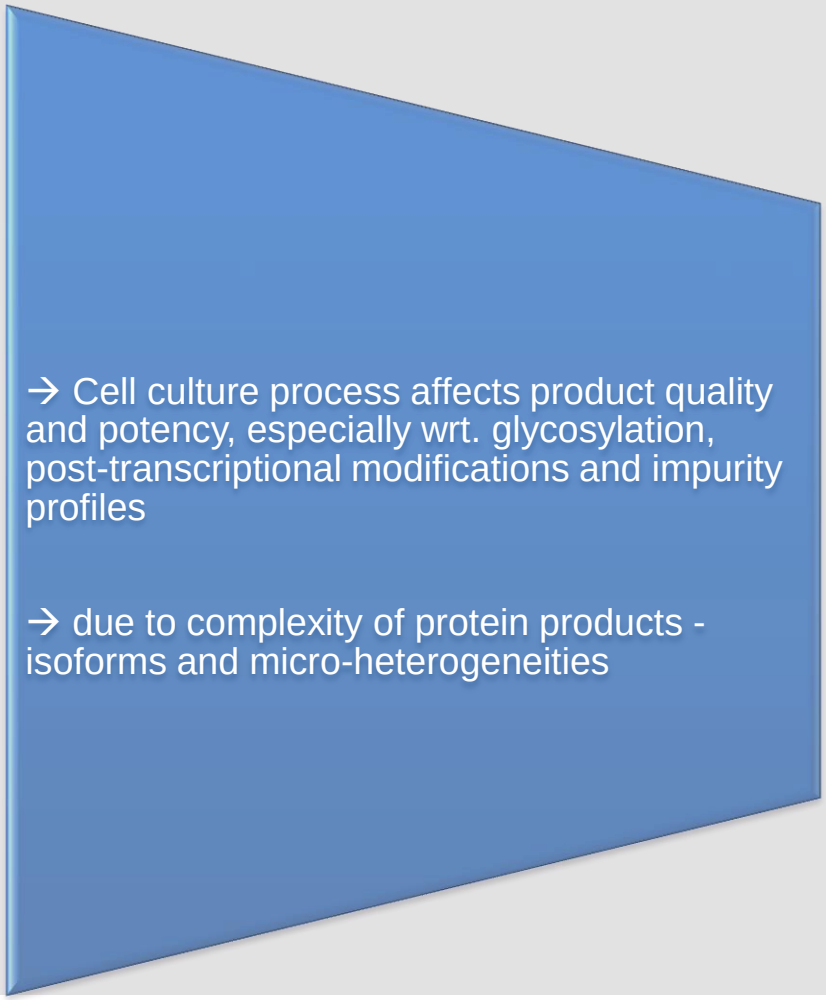


Thoroughly characterize and optimize bioreactor operating parameters



→ To improve process performance

→ To better understand how the process affects product quality

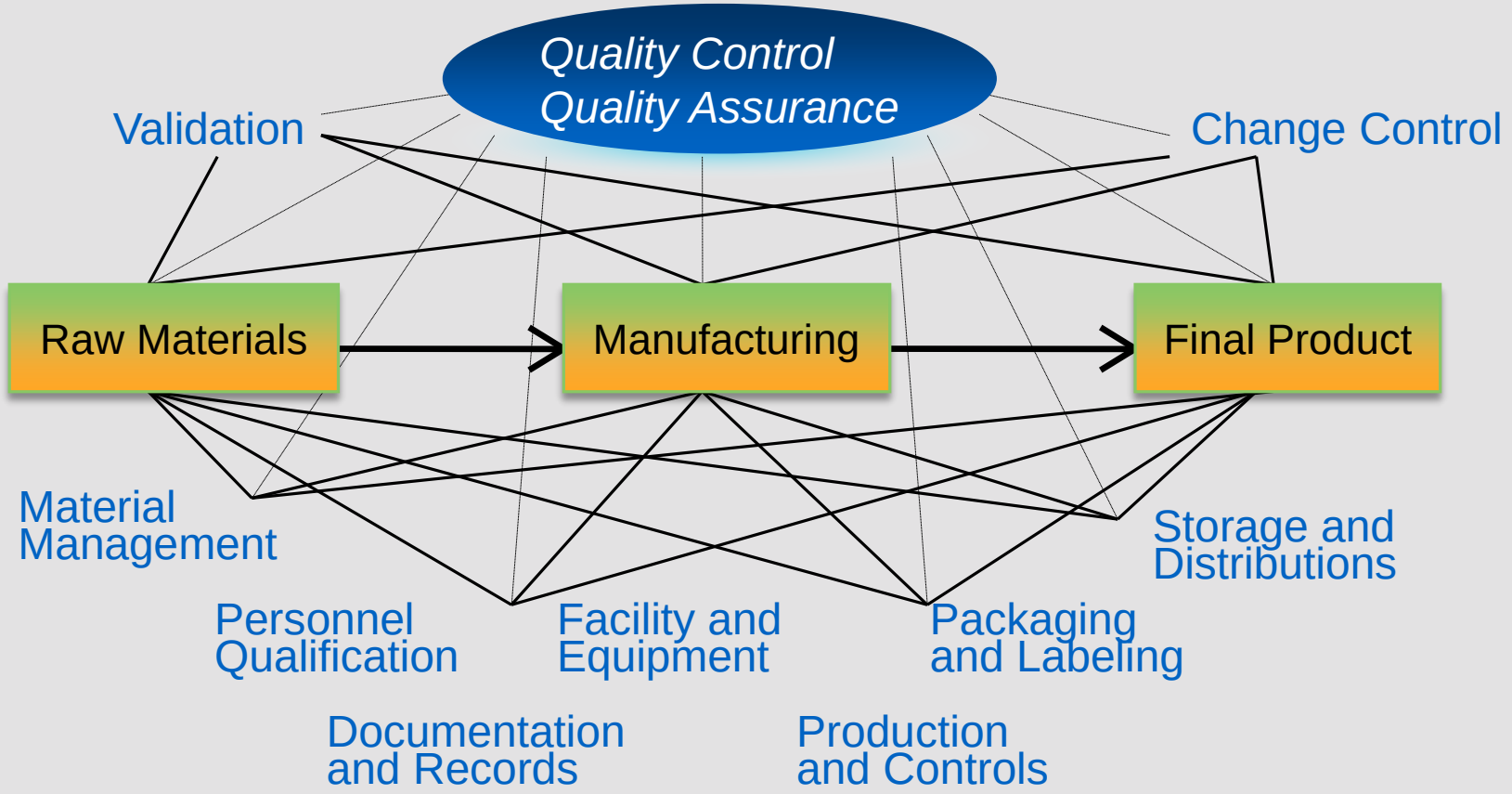


→ Cell culture process affects product quality and potency, especially wrt. glycosylation, post-transcriptional modifications and impurity profiles

→ due to complexity of protein products - isoforms and micro-heterogeneities

MANUFACTURING OCCURS UNDER CGMP TO ENSURE PRODUCT QUALITY AND SAFETY

cGMP Interrelationship Web



Courtesy: Eli Kraus, Amgen

CONCLUSION

- **Fermentation processes, sterile practices, control of bioburden, control of source materials and control of cell culture conditions should be:**
  - **risk-based**
  - **science-based**
  - **in accordance to WHO good manufacturing practices for biological products**

THANK YOU FOR YOUR ATTENTION