Outline

- Before the Floor
  - All the activities that happen before large scale cGMP manufacturing
- Manufacturing Process
  - Manufacturing steps
  - Equipment Requirements
  - How it all works together
- Supporting Cast
  - All the groups that make a process successful

Building a foundation for success

BEFORE THE FLOOR

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Before the Floor

- A huge amount of work occurs before the process ever enters a manufacturing facility
  - Drug Discovery
  - Expression System
  - Small Scale Process Development
    - Design Space
      - Media, Buffer, filter determination, chromatography resins and types and on and on
  - Assay Development
    - How do we measure our success?
  - Technology Transfer and Scale up
    - Does the as design process fit?
    - What can we change without impacting the product quality
- These initial steps have a huge impact on the final process!

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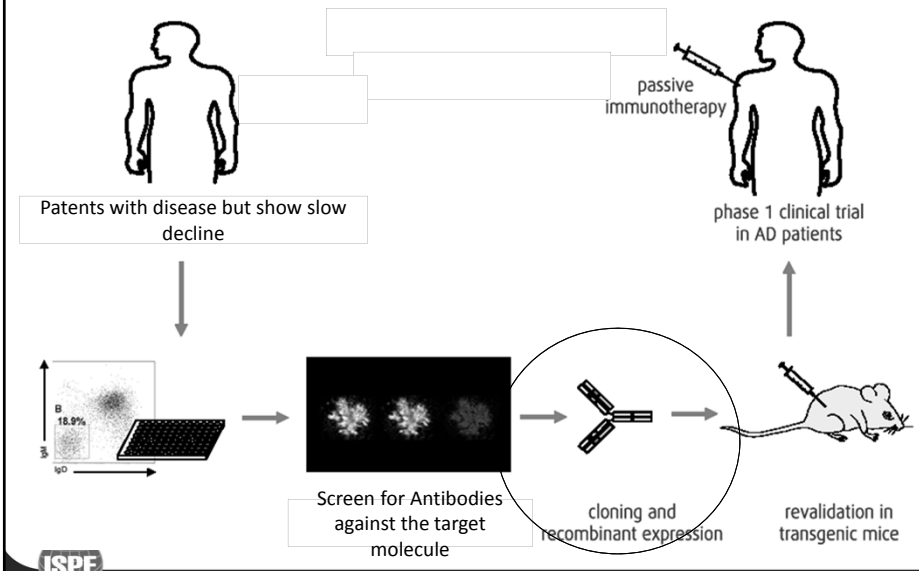
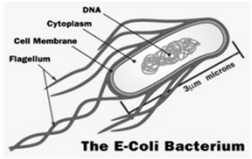
Discovery of Biologics (Antibodies)

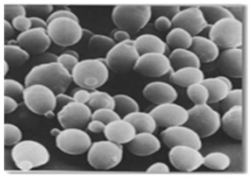


Typical Cell Types

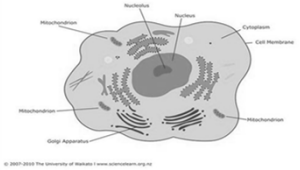
- Cell type selection based on both economic and biologic requirements
  - Considerations
    - Tertiary Structure
    - Glycosylation
    - Expression levels
    - Process Time
- Cell Types
  - Bacterial (E.Coli; B.subtilis)
  - Yeast (Saccharomyces Cerevisea, P.Pastoris)
  - Mamalian (CHO; BHK; Vero)
  - Plant
  - Insect



http://www.nature-education.org/cell-ecoli.jpg



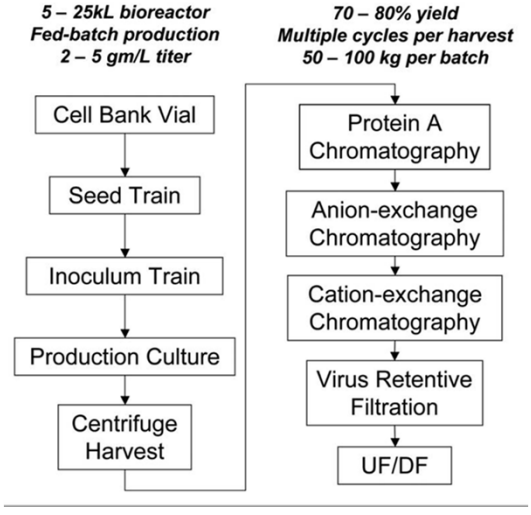
http://www.umass.edu/vetimm/img/pemap/close-



The boots on the floor

MANUFACTURING PROCESS

process flowsheet for mAb Bulk Drug Substance



Adapted from Brian Kelley, 2009, MAbs

Types of Upstream Process Equipment

- Cell Culture MFG:
  - Incubators
  - Bioreactors
  - Filter transfer stations
  - Centrifuges
  - Depth filters
  - Microfiltration systems
  - Hold tanks
- Dispensary/Support MFG:
  - Media prep tanks
  - CIP systems
  - Carboy washers
  - Glass washers
  - Autoclaves

Typical Process – Cell Cultivation

WCB vial

wave bag

N-3 reactor (seed)

N-1 reactor (seed)

N reactor (production)

http://www.firstenbergequip.com

Human Body Process Control

- Breathing
  - Lungs transfer oxygen
- Temperature
  - Shiver/sweat
- pH
  - Food
- Sterility
  - Skin and immune system
- Mixing
  - Circulation of Nutrients

Bioreactor Process Control

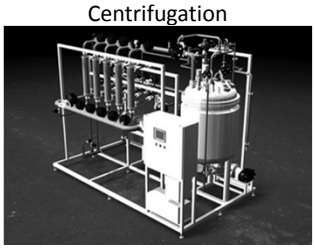
- Breathing
  - Lungs transfer oxygen
- Temperature
  - Shiver/Sweat
- pH
  - Food
- Sterility
  - Skin and immune system
- Mixing
  - Circulation of Nutrients

- Dissolved Oxygen
  - Agitation
  - Air/Oxygen Flow
- Temperature
  - Glycol Supply
  - Steam
  - Heat Exchangers
- pH
  - CO2
  - Chemical Addition (NaOH)
- Sterility
  - Steaming
  - Positive system pressure
- Mixing
  - Agitation

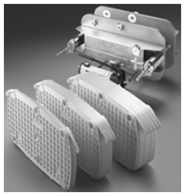
Typical Process – Clarification

Highly product-specific

Centrifugation



Microfiltration



Depth Filtration

Sterile Filtration

Hold Tank

Centrifugation

Purification

- Start with “Clarified Conditioned Media”
- Contains target protein and impurities
  - Host cell proteins, DNA, metabolic byproducts, media components, “theoretical contaminants”
- Need to separate the target protein from the impurities
- Need to get target protein into a buffer that is close to physiological conditions

Types of Purification

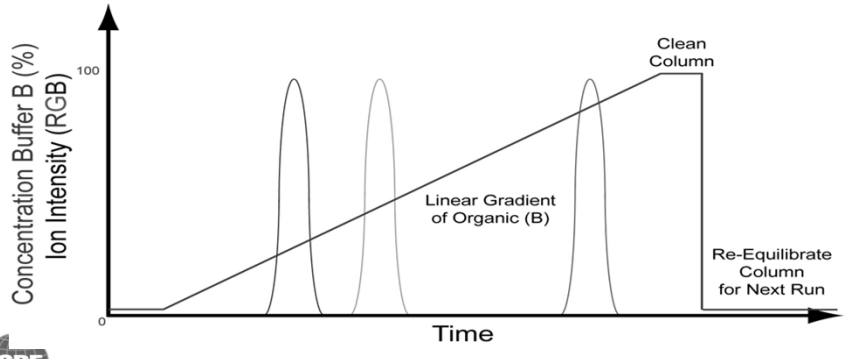
- Chromatography
  - Can remove non-target proteins, aggregated or partial target protein, HCP, DNA, viruses
- Filtration
  - Depth Filtration: Removal of precipitates and other impurities from CCM
  - Viral Filtration: Ensures removal of virus
  - Ultrafiltration/Diafiltration: Concentrates and performs buffer exchange
- Membrane Adsorbers
  - Filters that act a as a chromatography step removing residual impurities
- Other
  - Chemical viral inactivation

Chromatography

- Separation by Size
  - SEC or Gel Filtration
- Separation by Charge
  - Ion Exchange Chromatography
- Separation by Hydrophobicity
  - HIC or Reverse Phase
- Separation by Specific Interaction (Affinity)
  - IMAC – immobilized metal affinity chromatography
  - Protein A

Chromatography Example

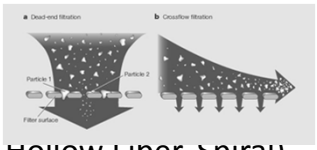
- Reverse Phase
- Change in fluid conditions effect binding of a given species (in this case organic solvent)



http://www.lamondlab.com/MSResource/LCMS/NanoflowHPLC/hplc.php

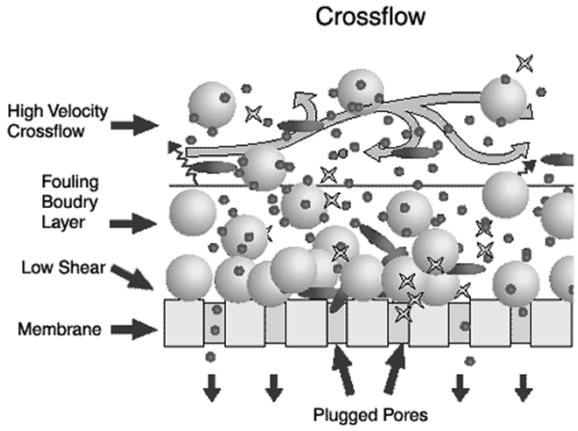
Tangential Flow Filtration

- Separation of components by size
- Pore size determines type of TFF
  - Microfiltration
  - Ultrafiltration
  - Nanofiltration
  - Reverse Osmosis



- Different configurations (Flat Sheet, Hollow Fiber, Spiral)
- Uses cross flow to mitigate fouling of filters
  - Cleaning filter surface using turbulence
- Useful for:
  - Concentration of product
  - Removal a media components

Tangential Flow Filtration

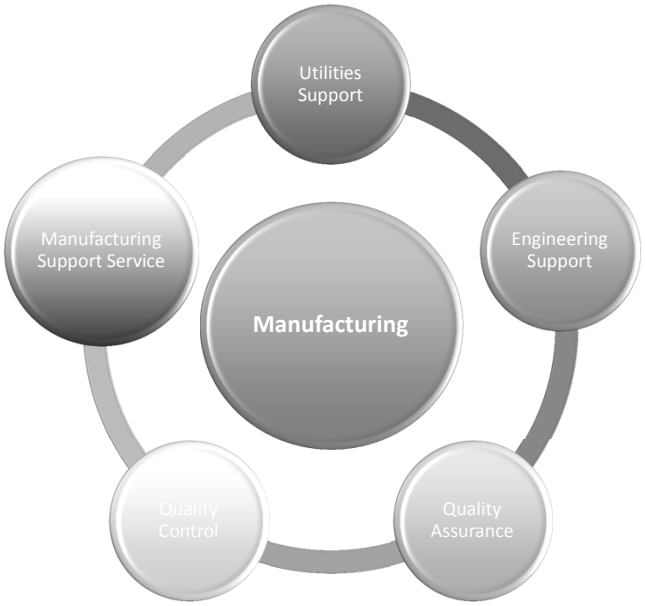


http://individual.utoronto.ca/babak_haghiri/my%20reasearch.html

A shoulder to lean on

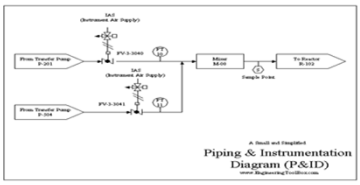
SUPPORTING CAST

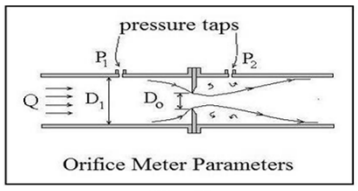
What Makes A Facility Tick?

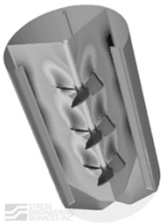


Engineering: Process

- Equipment Design
  - Process Scale Up
  - Connectivity
  - From the Largest Reactor to a orifice sizing
  - CIP/SIP design
- Equipment Troubleshooting
  - Data review
  - Hands on test runs
- Process/Equipment Interaction
  - Process Scale Up
  - Assessing root cause of process inconsistencies
- Preventative Maintenance
  - Interval Determination
  - FMEA

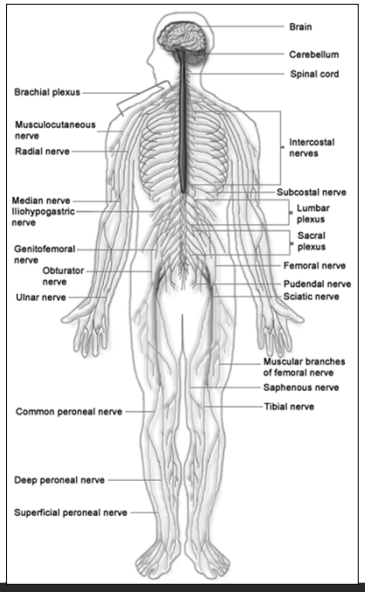






Engineering: Automation

- The Central Nervous System of the Plant
- Records and analyzes inputs from the plant
- Provides dynamic response to plant feedback
- Displays and records real-time data
- Central store for system alarms
- Can be used to improve / streamline process.



Quality Assurance

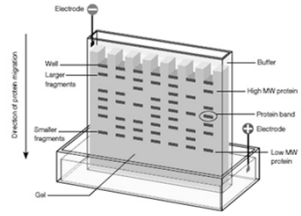
- Ensure that the Code of Federal Regulations are always followed
- SISPQ
  - Safety
  - Integrity
  - Strength
  - Purity
  - Quality
- Review all documentation
- Review all investigations
- Review all deviations
- QUAILTY IS EVERYONES RESPONSIBILTY

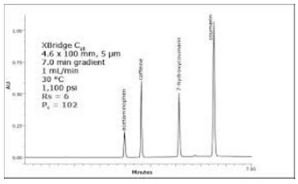
Validation

- Main goal is to ensure consistency and Repeatablity of the process and process equipment
  - Equipment
    - Cleaning
    - Sterility
    - Control of Temperature, Pressure, Humidity, etc.
- Process
  - Verify the process is robust and repeatable
    - KPA Key Process Attributes
    - CQA Critical Quality Attributes
- Statistically proven result

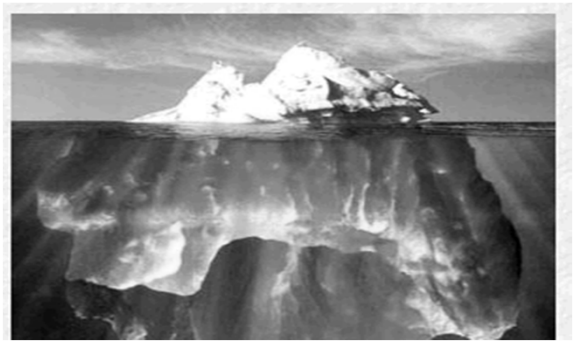
Quality Control

- Run assays and compare results to expected ranges
  - Determine if product meets the expect Quality Attributes
- Assay Examples
  - Gels (SDS, Western Blot etc.)
  - HPLC
  - Plates





Facilities Operations



Biotechnology Manufacturing Requires A Tremendous Infrastructure Of Support Systems!

These Systems Play A Vital But Often Hidden Role!

Facilities Operations

Utility Systems In Play

- Water For Injection
- Purified Water
- Pure Steam
- Plant Steam
- Process Glycol
- Compressed Air
- HVAC Systems
- Domestic Water
- Natural Gas
- Diesel Fuel Systems
- Medium Voltage (13.8KV) Electricity
- Low Voltage (120-480V) Electricity
- Liquid &Gaseous Nitrogen
- Carbon Dioxide
- Argon
- Chilled Water
- Condenser Water
- Process Waste Systems
- Biological Waste Systems

These systems spread across nearly all engineering disciplines!

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