Abbott

DIAGNOSTICS DIVISION

LEVITRONIX BIOPROCESSING CONFERENCE 2022

The use of modern centrifugal pumps in Bioprocess Design

02 | June | 2022

Abbott and Levitronix

ABBOTT DIAGNOSTICS DIVISION

- Working with Levitronix pumps for approximately 5 years
  - Bioprocess Unit Operations
    - Upstream – Perfusion
    - Recovery – harvesting
    - Downstream – UF/DF
    - Cleaning - CIP
  - Multi-functional
    - Multi-, and Single - Use designs
    - Automated process control capabilities
    - Multiple sensor inputs (PendoTECH)
    - Enables simplified and safe process control

ABBOTT Covid-19 pandemic response

APRIL 2020

- Abbott was one of the first to market with EUA test for Covid-19
  - The initial tests were molecular and antibody tests
    - Molecular (PCR) – isothermal, enzyme RNA amplification
    - Serology (blood test) - Recombinant protein is used in direct immunoassay to identify IgG antibody (Ab)
  - Viral tests were developed
    - June 2020: IDNow – Rapid molecular test for viral Antigen (Ag)
    - Dec 2020: Binax Now – Rapid lateral flow test for viral Ag
- Abbott Covid test portfolio
  - In total, 12 tests are currently available for detection of either viral Ag or antibody (IgG and IgM)

Diagnostic test

BinaxNOW COVID-19 Ag Self test is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid protein from SARS-CoV-2.

LATERAL FLOW METHOD

- Based on simple Ab to Ag binding technique and is designed to be rapid (15 min) and cost effective

- Recombinant Ag is used as the control
- Patient sample (nasal swab) – may contain virus
- Recombinant Ab is used in the detection solution

- Many different Ab designs were created to ensure
  - Specificity: Detect CoV-2 vs Flu or Cold virus
  - Sensitivity: Detect at low viral level (early infection)

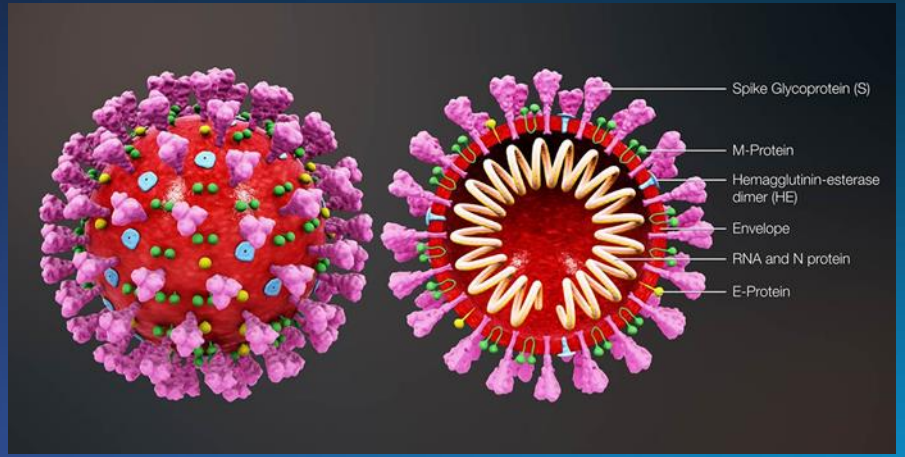
ON THE SHELF

SARS-CoV-2

The DNA sequence and viral structure of SARS-CoV-2 (COVID-19) was quickly determined and published, which enabled rapid development of diagnostic tests .

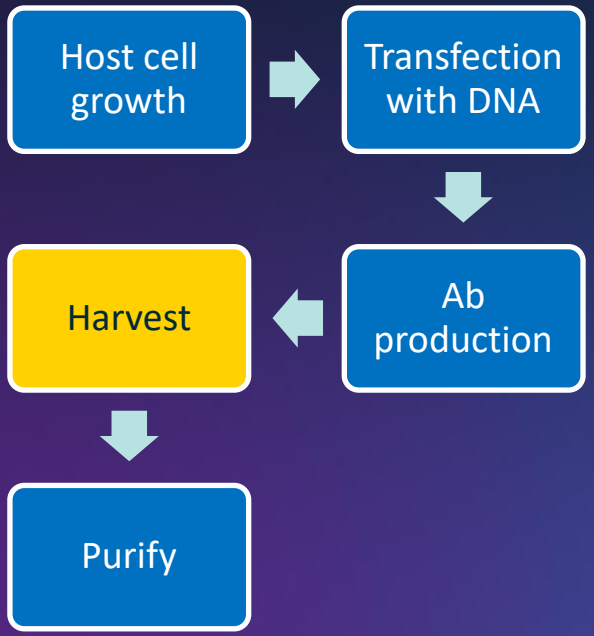
VIRUS STRUCTURE AND PROCESS DESIGN

- Comprised of multiple functional sub-units
- Diagnostic tests identify different parts of viral structure
- Viral proteins can be detected from nasal swab
- Biologic process design: *development of a method to produce large quantities of specific biologic molecules*
- Rapid process methodology: transient transfection



3D illustration of 2019 Novel Coronavirus (now named SARS-CoV-2), derived from a CDC released image.
Image: Scientific Animations Inc., CDC Organization
Worldmap of COVID-19 (2019-nCoV novel coronavirus) outbreak - Nations Online Project

Process overview of propagation and recovery of recombinant Ab



- Harvest culture
  - Relatively large volume (TT)
  - High cell density
  - Low cell viability – cell debris
- Clarification method
  - Normal flow filtration (NF)
- Concentration
  - Flat sheet UF TFF

➢ Regular batch turnover

Harvesting: Clarification and Concentration

Recombinant Ab is produced and secreted into the culture medium. Therefore, to efficiently recover and purify the product, the media must be separated from the cells and cell debris.

STANDARD CLARIFICATION PROTOCOL

- Normal flow filtration
  - Multi-stage filtration (2-step)
  - Low surface area
- Challenges
  - Slow flow rates – peristaltic pump
  - Build up of debris in filter (fouling)
- Multiple small or largest filters
  - Not efficient time or use of materials
  - Reduced volume recovery

TANGENTIAL FLOW FILTRATION

- Recirculating flow across the membrane
  - Turbulent flow clears fouling layer
  - Large surface areas (x M^2)
  - Ultra-, Micro- and Macro- filtration pore sizes
- Key control factors
  - Recirculation flow rate or Cross flow (shear)
  - Pressures (P_{in}, P_{out}, P_{perm})
- Challenges
  - High flow rates and pressure differential

Flow rate and pressure control

RECIRCULATION FLOW

- Highly fouling harvest
  - Tangential flow across the membrane helps reduce protein build-up
  - Maintain flux (through pores)
- Turbulent flow
  - Re > 3000 ~ (S^{-1})

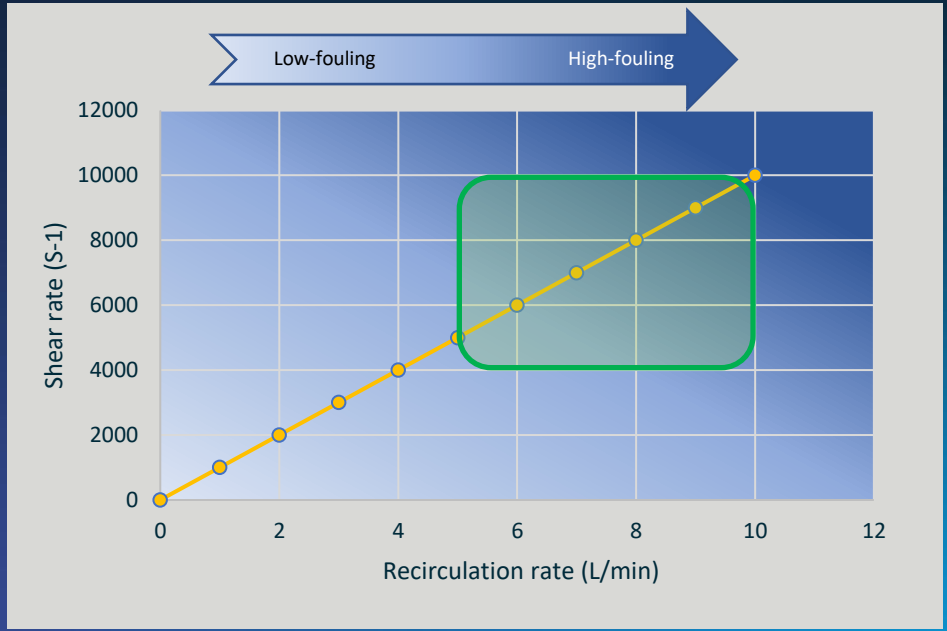
PRESSURE DIFFERENTIAL

- Transmembrane pressure (TMP)

  $= ((P_{in} + P_{out}) / 2) - P_{perm}$

  Limitation: $P_{in} < 25$ psi

HF TFF FLOW RATE GRAPH



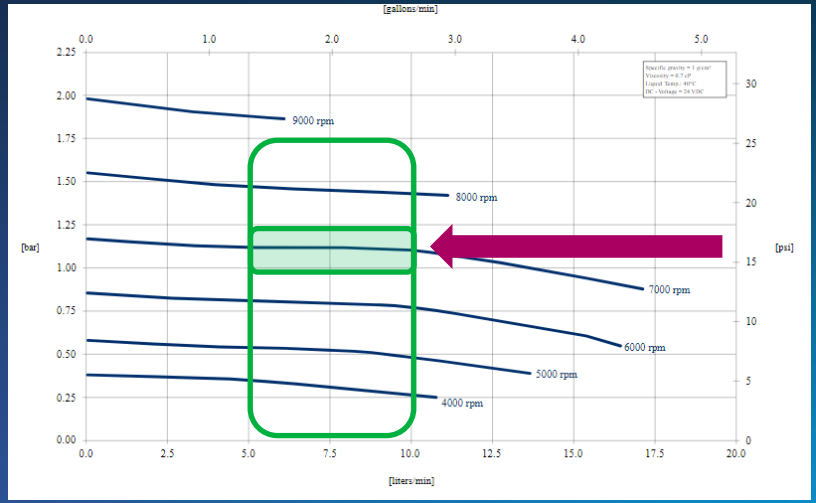
Graph recreated from vendor data @Repligen.com

Flow rate and pressure control

LEVITRONIX CONSOLE

- Centrifugal pump head
  - Single-use
  - Sanitary couplings or hose barb
- Pressure monitoring
  - 3 inputs: P_{in}, P_{out} and P_{perm}
- Flow meter (not used)
- Pressure control application
  - Maintain flow rate based on pressure decay curve

PRESSURE FLOW CURVE



PuraLev® i100SU – Levitronix
Image from Levitronix.com

BIOPROCESS DESIGN

HF TFF set-up

SEMI-DISPOSABLE BENCH TOP DESIGN

PUMP

- Feed flow priming
  - Gravity, pump or over-pressure

PRESSURE CONTROL

- Input pressure
  - Feedback control
  - Automated pump speed (rpm) adjustment

Rapid turnover enabled us to meet demands for biologics needed for assay development during early pandemic response

Process summary

The Levitronix centrifugal pump system when used with hollow fiber TFF was able to:

- ✓Complete process within short timeframe
  - ➢ Total run time (~2h)
- ✓Improve process safety
  - ➢ Automated control of input pressure ensured system integrity – no over-pressured tubing
- ✓Improve process throughput
  - ➢ Automated control freed up scientist time – improves hands-on capacity

HF clarified harvest is fed directly into TFF Concentration – reducing process time (parallel process)

Multi-functional

The Levitronix centrifugal pump system is multi-functional across a range of bioprocess applications

- Fermentation
  - Filling –media transfer
  - Biomass Concentration/cell wash
  - Clean-in-place
- Cell culture
  - Filling bioreactors (sterile set-up)
  - Perfused culture
  - Harvest clarification
  - Concentration/diafiltration (UF/DF)
- Purification
  - Chromatography
  - UF/DF
  - Filtration

Capability:
Process method used with large-scale Single-Use Microbial fermenter to recover biomass.

LCO600, ~50 lpm, 3.9 to 7.8 M^2 filter area

Concluding remarks

Covid-19 (SARS-CoV-2) pandemic global impact

- Biologics at forefront of defense: tests, therapeutics and vaccines
- Rapid response from industries
- Innovative bioprocessing strategies needed
- Innovative tools required to support

Thank you

Levitronix

Aris Legorreta

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