



# Devices for Analytical Methods and Labscale Processes

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# Devices for analytical methods

## Cohn fraction characterization

- Paste aspect
- Visual observation
- Colour index
- Loss on drying (residual moisture)
- pH conductivity
- Na<sup>+</sup> content



Balances and SevenExcellence multimeter (pH-Conductivity-Sodium)

# Devices for analytical methods

## Total Protein

- Colorimetric methods
  - Biuret
  - $\mu$ Biuret (microplate)
  - Bradford
  - BCA
- Absorbance method
  - UV @ 280nm



Micro-volume UV-Vis spectrophotometer

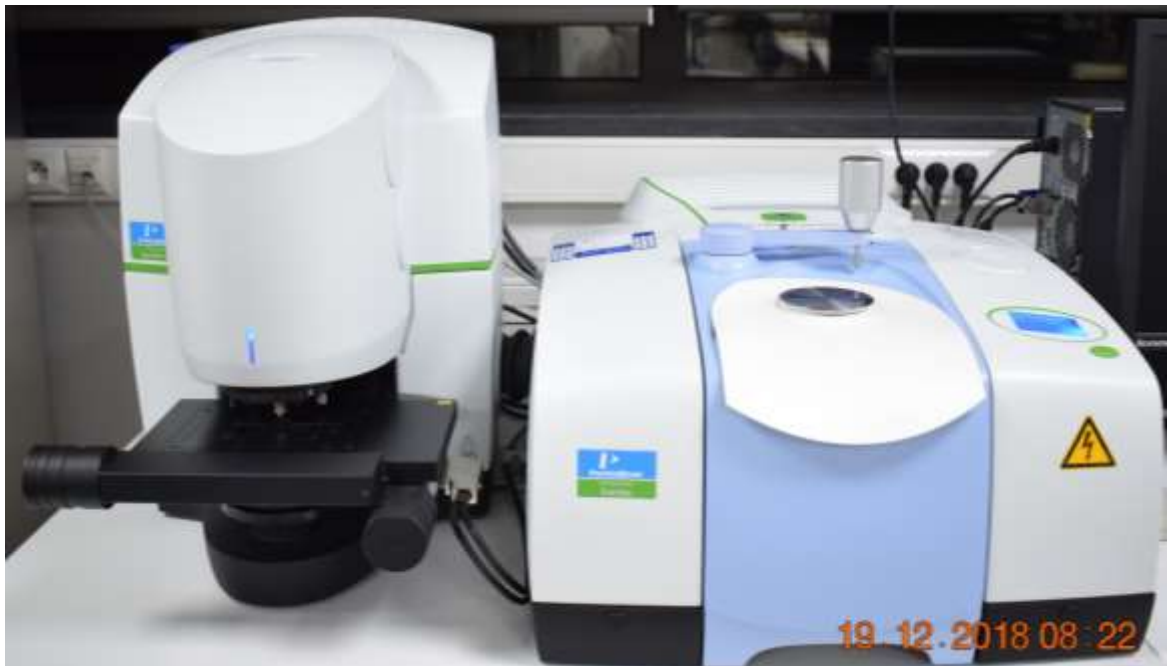


Spark 10M multimode microplate reader

# Devices for analytical methods

## Structural Integrity

- Opalescence of samples
- Intrinsic fluorescence emission
- Protein melting point (Thermal Shift Assay)
- Fourier-Transform IR spectroscopy (also polymer (chemical) identification)



FTIR spectroscopy



QuantStudio 3 Thermal cycler

# Devices for analytical methods

## Electrophoretic Protein Profile

- SDS-PAGE
- NATIVE-PAGE
- IEF gels
- Western blotting



Gel electrophoresis equipment

# Devices for analytical methods

## Nephelometry (co-purified proteins / impurities)

25 markers:

- HSA
- IgG
- IgG<sub>1</sub>
- IgG<sub>2</sub>
- IgG<sub>3</sub>
- IgG<sub>4</sub>
- IgA
- IgM
- C3
- C4
- TRF
- A2M
- HPT
- PLS
- AAG
- HPX
- CER
- RBP
- FNC
- C1I
- PRE
- Apo-A1
- Apo-B
- FIB
- ATIII



BNII nephelometer

# Devices for analytical methods Human Coagulation Factor Quantification

- Non Activated Partial Thromboplastin Time (NAPTT)
- FVIII one-stage assay and chromogenic assay
- FIX one-stage assay
- FXI one-stage assay
- FXIa one-stage and chromogenic assay
- FII, FVII and FX chromogenic assays
- Thrombin Generation Assay (CBER methods) FXI, FXII and FVIII
- Specific and aspecific chromogenic substrate (i.e. S2288 etc...)
- PKA



Sysmex CS-2100i  
coagulation analyzer

# Devices for analytical methods

## Immunodetection

- Antigen detection:
  - Chemiluminescence
  - Fluorescence
  - Colorimetric
  - Time resolve fluorescence
- Example of ELISA available :
  - vWF and FVIII
  - Collagen
  - FXI and PK
  - Human Clusterin  
etc.....



Spark 20M  
multimode  
microplate reader



Imaging system  
LAS 4000



# Devices for analytical methods

## Assessment of aggregates

- Size Exclusion Chromatography (SEC) aiming to measure Molecular weight, and Hydrodynamic Radius of proteins using refractive index, viscosimetry and Right/Low Angle Light Scattering.
- Photo Diode Array (PDA) also available
- Range from million to 5000 daltons.



OMNISEC RESOLVE and REVEAL modules

# Analytical methods

## Endotoxin and Chemical quantification

- Bilirubin
  - Carbohydrate
  - Phosphate
- 
- Endotoxin per chromogenic substrate

# Labscale processing capability

## Chromatography

- 2 NGC chromatography system with mix valve and maximum flow until 200 ml/min + UV detector
- Chromatography media in the laboratory
- Protein A/G (IgG, IgA and IgM)
- Isotypes IgG1, IgG2, IgG3, IgG4
- Albumin
- Synthetic peptides coupled on sepharose gel
- Chemical coupling of proteins on sepharose (BrCN activated)
- Any chromatography media (IEX, Hydrophobic, SEC and Mix mode)

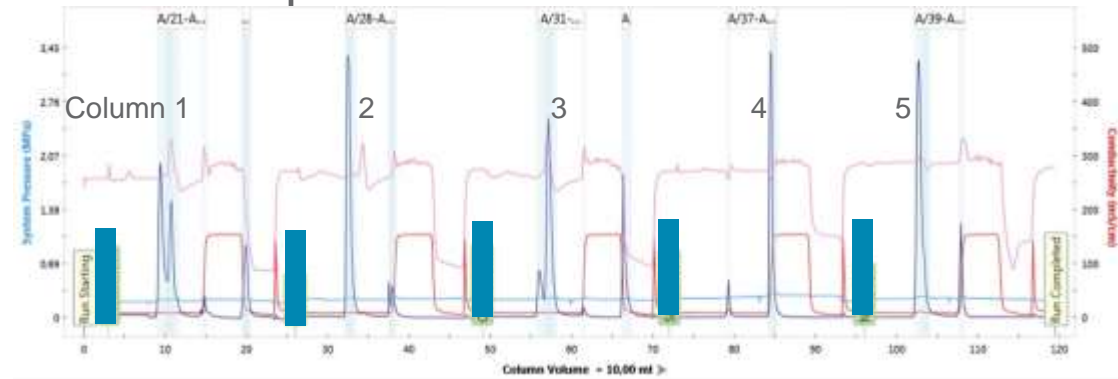
Column from 5ml until 5 liters



NGC BioRad

# Labscale processing capability Chromatography

- Intern methodology for chromatography media screening  
5 chromatography media per device screened per test in one condition



# Labscale processing capability

- e.g. Cohn fractionation from
- 25 ml to 100-200 L from Cryo Poor Plasma until intermediates



Double jackets for lab scale resuspensions + circulating water baths

# Labscale processing capability

## UF/DF Labscale

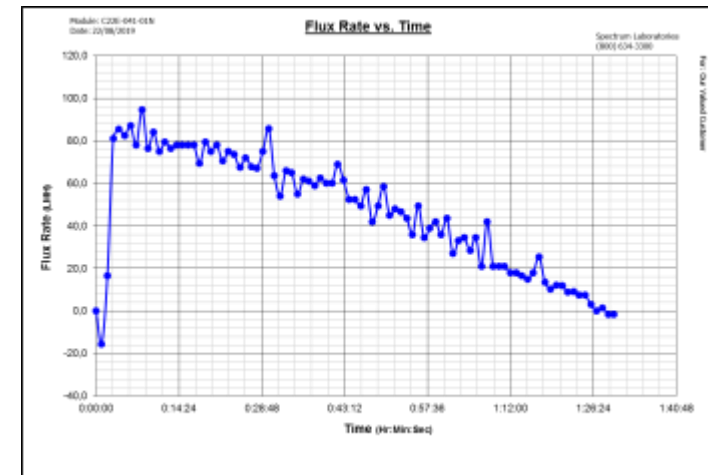
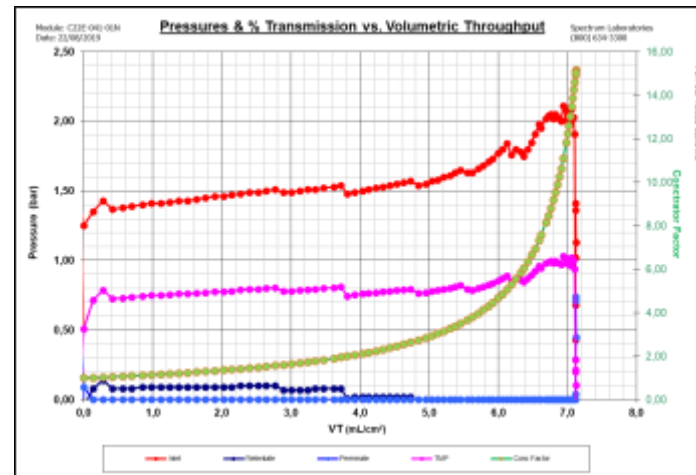
- From 0,5ml – max 400L
- UF/DF develop 'pment including constant feed



Kr2i max 30L 4 units



KMPi max 400L 1 unit



# Labscale processing capability separation Centrifuge

From 1ml – 1,6L

## Filtration

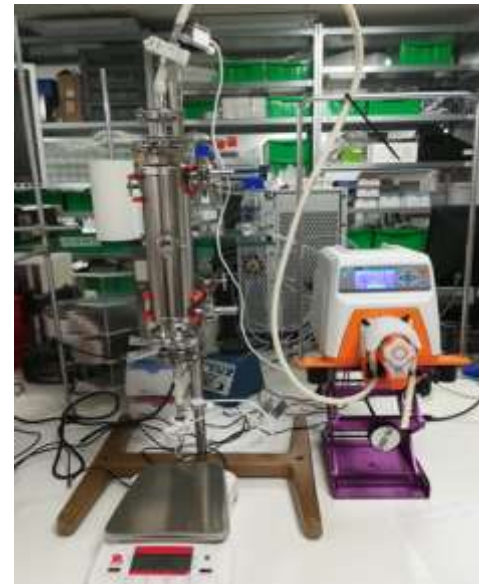
- From 1ml – 200L
- Filter press capability from 0,1 m<sup>2</sup> until 7 m<sup>2</sup>



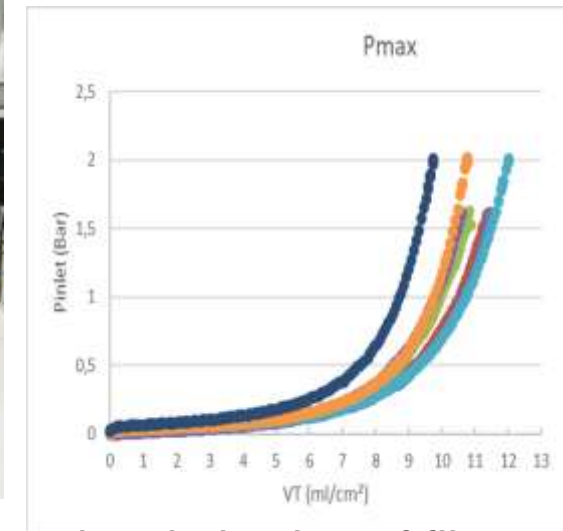
modified seitz  
20X 20 1 unit



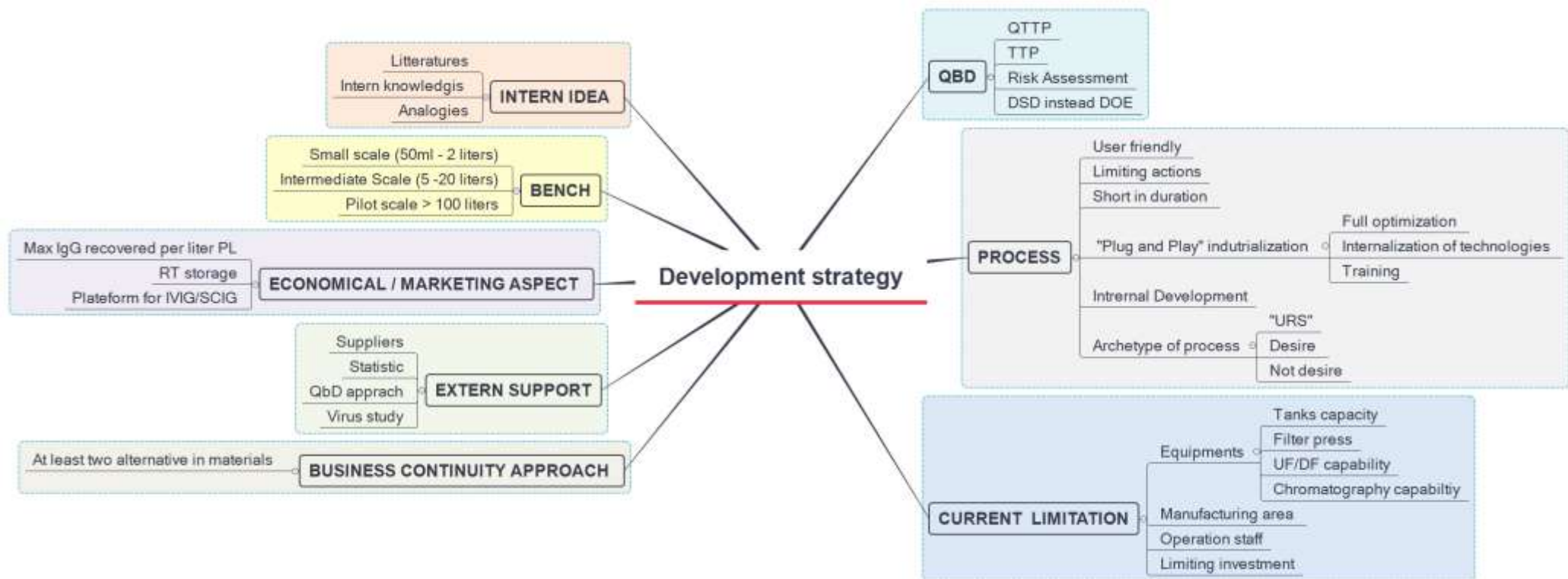
Begerow 40x40 1 unit



Vmax/Pmax screening and optimization of filters



# Labscale processing via QbD approach and Definitive screening design for process modeling







PLASMA

INDUSTRIES

BELGIUM