IMPROVING PROCESS UNDERSTANDING

Multivariate data analysis (MVDA) as a PAT tool for early bioprocess development data

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April 24th, 2012

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WAGENINGEN UR

Agenda

- Project background
- Crucell upstream processes
- Implementation of PAT on Crucell's platform process
  - Strategy
  - Exploration of historical data
- Current work

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Project background

PhD project

- Conducted at **Crucell (Johnson & Johnson)**

  Bas Diepenbroek, Dr. Ciska Dalm, Alfred Luitjens

- In collaboration with the **University of Wageningen**

  Prof. Dr. ir. Rene Wijffels, Dr. Mathieu Streefland

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Crucell's core technology: PER.C6®

20,000L Stainless Steel

Serum free
Suspension cultures

Cell growth to **150,000,000** cells/mL

- Human cell line
- Showed scalability
- Able to grow to very high cell densities

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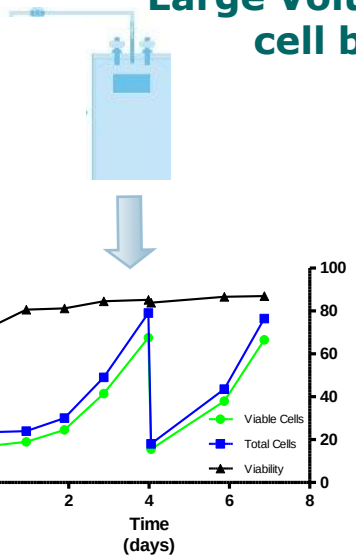
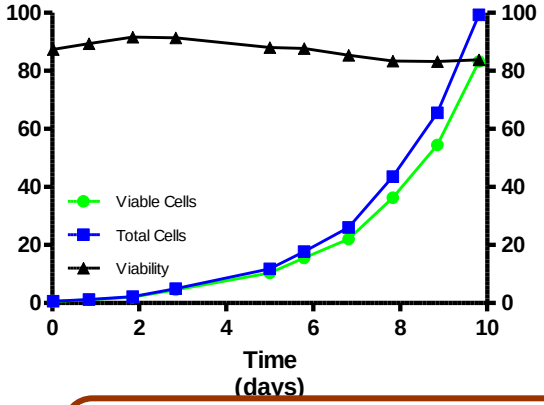
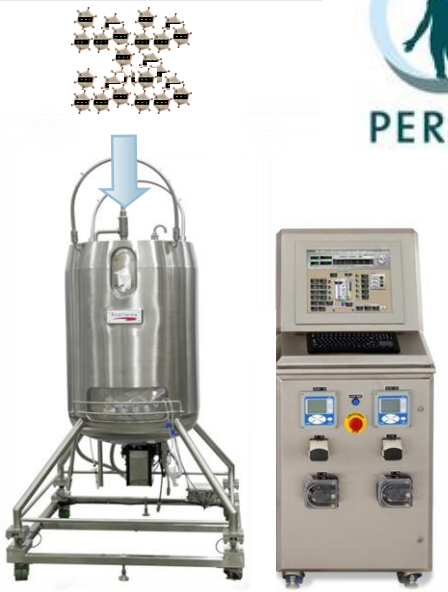
Upstream process: fully disposable

Large Volume High Density cell bank (LVHD)







WAVE culture

100L intensified Cell Production (iCeP)

500L intensified Virus Production (iViP)

PER.C6®

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Bioreactor processes in the upstream platform

Virus based vaccines

PER.C6®

iCeP - iViP

- Perfusion processes with retention of cells and viruses in the bioreactor
  - Exponential cell growth throughout run
  - High maximum viable cell density
  - High final virus concentration
- **iCeP process is used as a basis for a pilot PAT implementation**

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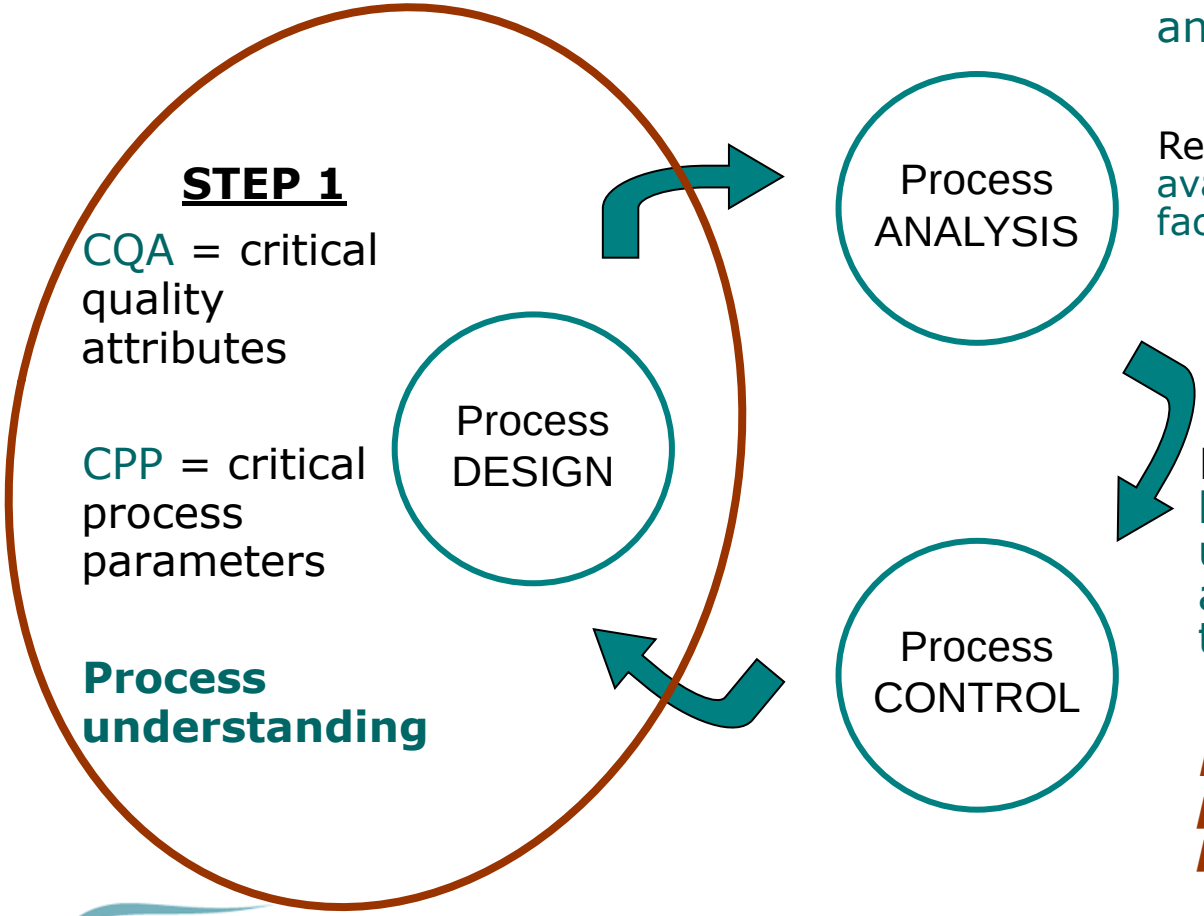
Strategy for PAT implementation



STEP 2

Implementation of (online) analyzers to capture CQA and monitor CPP

Results from analytics must be available in time-frame that facilitates decision making

STEP 3

Design of process control based on process understanding and using analyzers for making real-time process decisions

Must achieve consistent process performance and product quality

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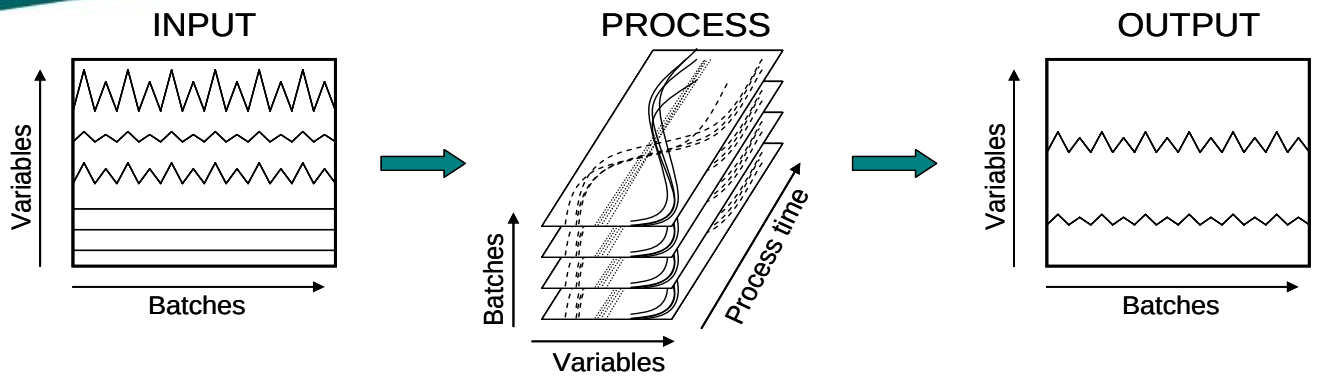
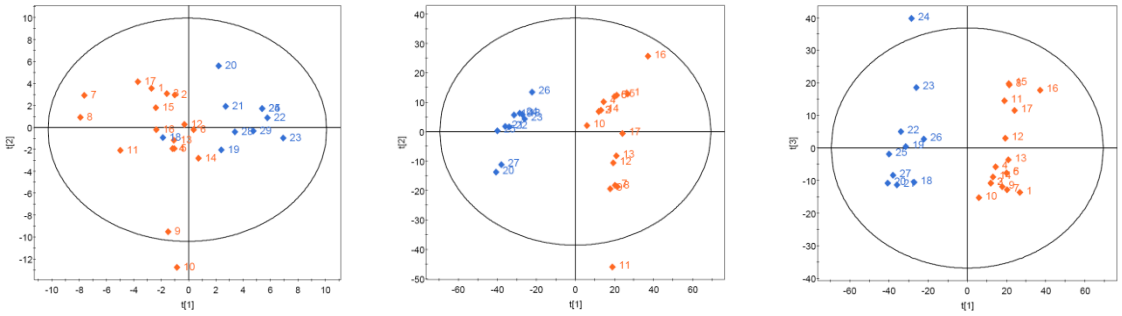
Why Multivariate data analysis?





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Multivariate techniques used

- Structure of dataset and underlying trends
  - **PCA**: Principal Component Analysis
  - Provides a summary or overview of a data set

- Relations among process variables
  - **PLS**: Partial Least Square
  - Explains relations between data set and process response

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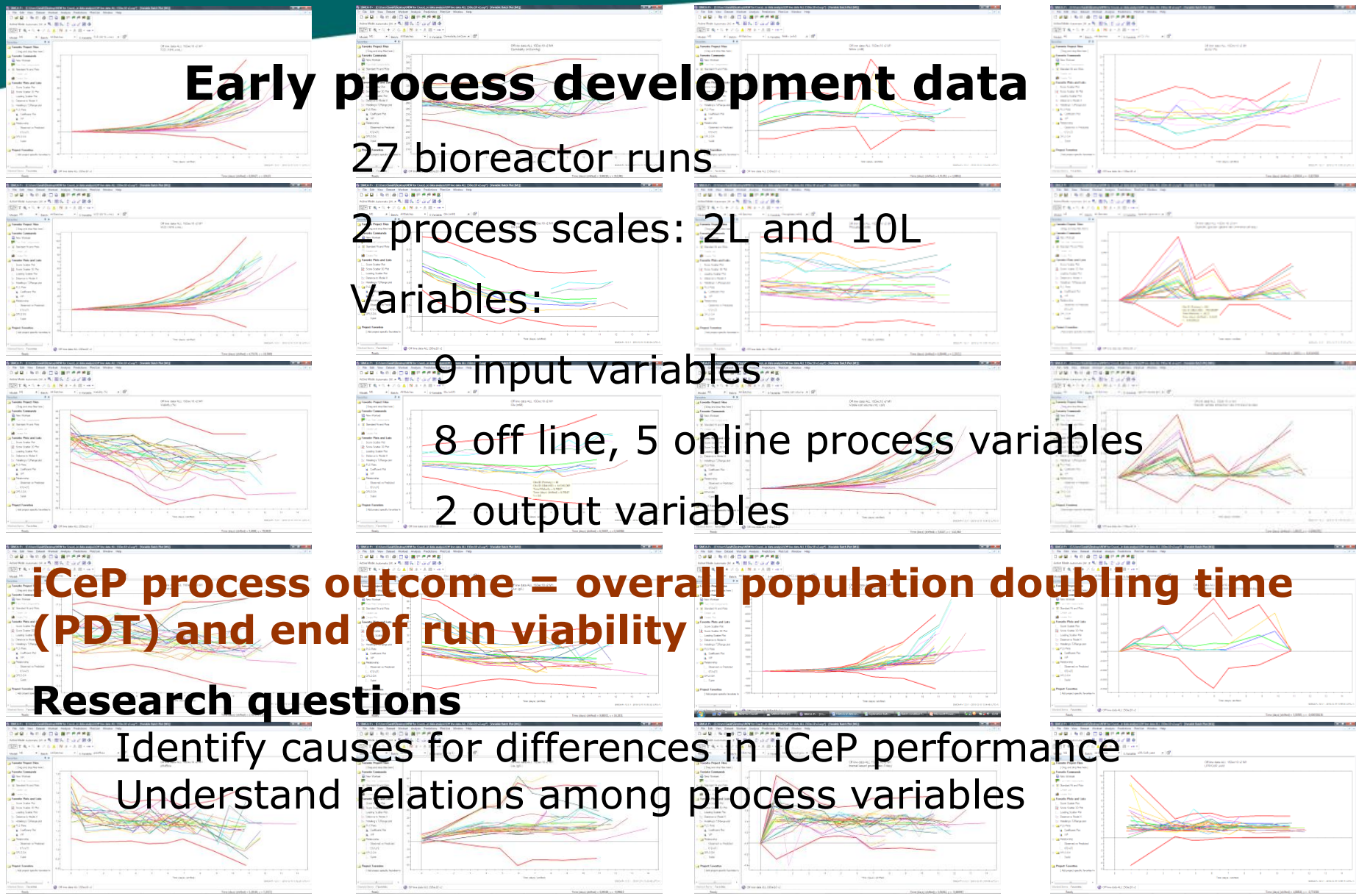
Multivariate analysis of available iCeP data



Early process development data

27 bioreactor runs

2 process scales: 2L and 10L

Variables:

9 input variables

8 off line, 5 online process variables

2 output variables

iCeP process outcome = overall population doubling time (PDT) and end of run viability

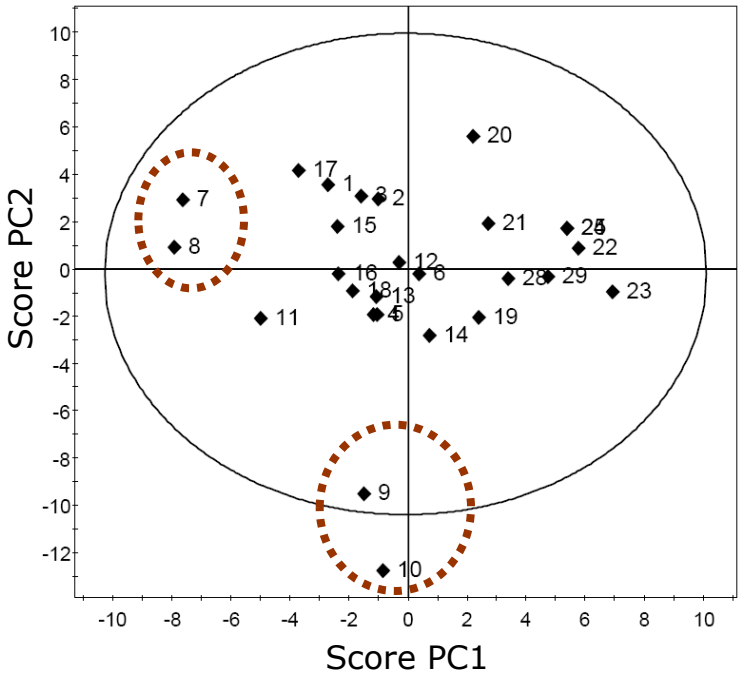
Research questions

Identify causes for differences in iCeP performance

Understand relations among process variables

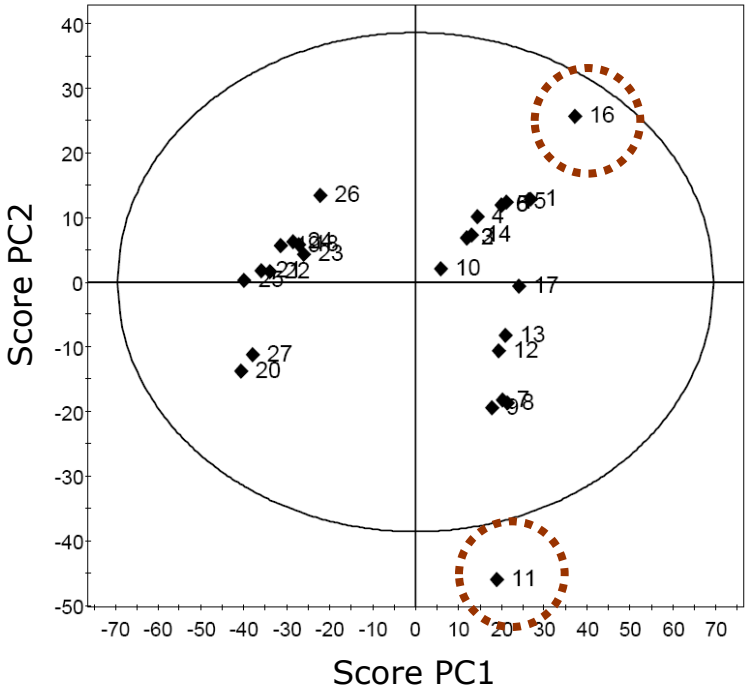
PCA score plots and batch diagnosis

Offline variables model



Offline variables model: 4 PC, capturing 66% of the variation contained in the dataset

Online variables model



Online variables model: 5 PC, capturing 89% of the variation contained in the dataset

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Batch diagnosis using PCA

Model	Deviations in multivariate models	Process deviations
Offline variables	Scores on PC1; contribution of **cell diameter and osmolality during second half of process**	**Altered concentration of additives in feed medium**
	Scores on PC2; contribution of **viable cell density throughout process**	**Inoculation cell density twice the target**
Online variables	Score on PC1; contribution of **pH and CO_2 demand throughout process**	No deviation identified
	Score on PC2; contribution of **pH throughout process**	**Powder medium hydrated in-house**
	Score on PC3; contribution of **dissolved oxygen throughout process**	**Deviation in calibration of dissolved oxygen probe**
	Outlier in residuals	No deviation identified

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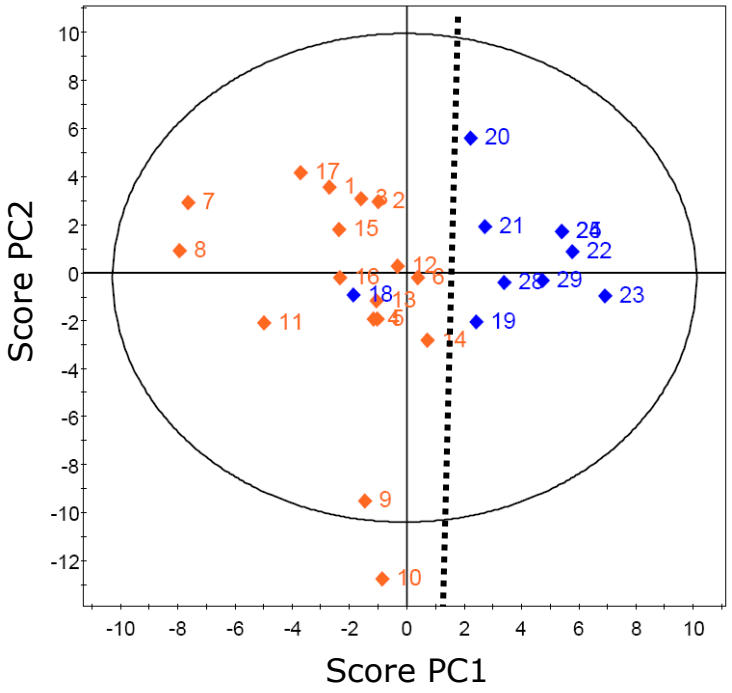
PCA score plots and scale effect

Offline variables

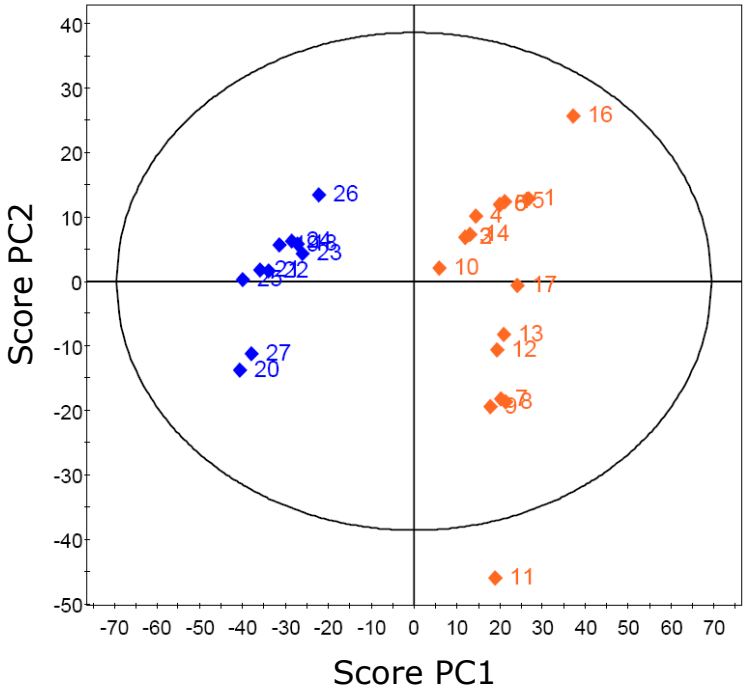


Offline variables model: 4 PC, capturing 66% of the variation contained in the dataset

2L

10L

Online variables



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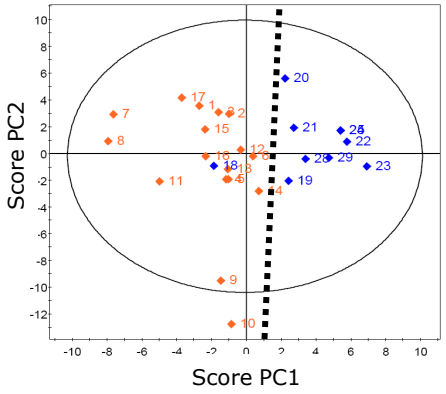
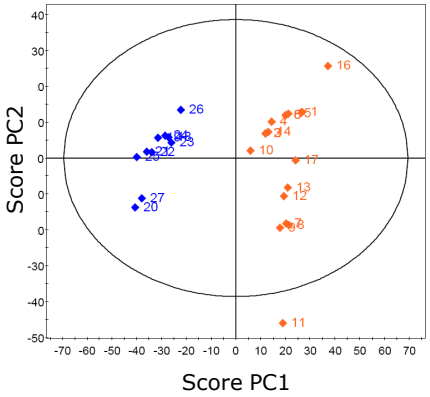
Sensitivity of iCeP to process scale

Offline variables model

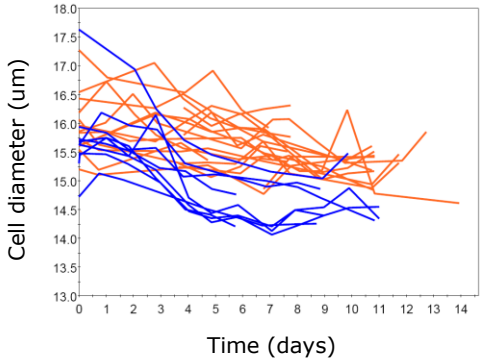


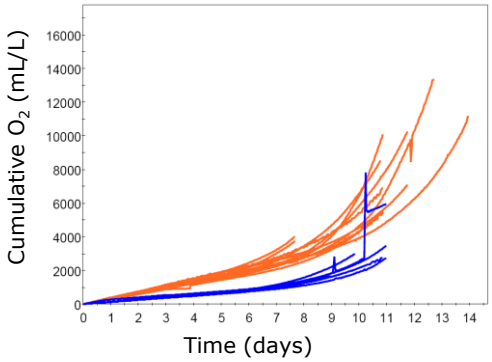
Online variables model

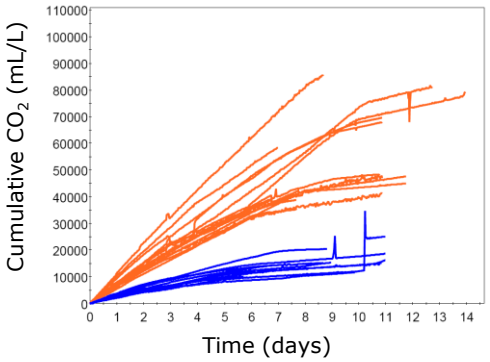


2L

10L







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PLS modeling

- What are the relations among process parameters?
  - Overall NO or very weak models were obtained

  ⇨ **There is no or very limited relations between process variables and process outcome as currently defined**

- What can be concluded?

1. ~~The iCeP process is very robust and process responses are not affected by variations in process variables~~
2. The chosen process responses (PDT and viability) do not reflect the "real" CQAs of the process
3. Process variables were not varied in broad enough ranges

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Why can't we identify relations among variables?

2. Nature of variables monitored
   - Many variables currently monitored do not strongly correlate with process outcome, therefore some **relevant process information is not monitored**

3. Structure of experimental designs
   - **Early development dataset**, based on trial-and-error experiment sets
   - MVDA is used ideally with **DoE** type of data, where variation is **introduced** and **structured**

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Current work

- **Are we measuring the right outcome of iCeP process?**
  - Historically: overall PDT and end of run viability
  - iCeP cells are a seed for virus infection ⇨ **biological and cellular features** of the culture can be CQAs, which would define the **best physiological conditions** for the cells to be infected

- **How to identify the CPPs?**
  - Need to measure appropriate process response and to vary process variables

QbD / PAT implementation

1. Identify "real" CQAs
2. Identify CPPs and their relation to CQAs
3. Define design space in which CPPs are operated to ensure proper CQAs

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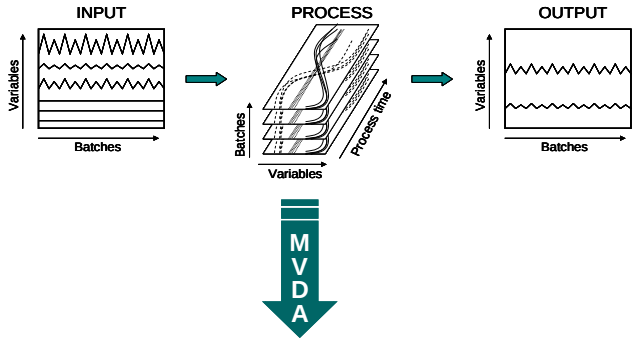
Take home message



- **MVDA on early process development** data yielded **process understanding**

- Leads for further development and investigation of **innovative process responses** were identified

Application of MVDA on early development data is a first step in QbD

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