Cedex Analyzers for Process Control in Bio-Processing

June 28th 2018
Webinar agenda

1. Cedex bioprocess control solutions
   Roche CustomBiotech

2. Application of new Mouse IgG test
   Roche Diagnostics

3. Application of new Osmolality estimation
   Roche Pharma

4. Improved solutions for analytics workflow control and data management
   AGU Industrial IT
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   AGU Industrial IT
Roche CustomBiotech
Know-how and products to power your ideas

Customers worldwide in the pharmaceutical and diagnostics industries
Roche CustomBiotech in Penzberg, Germany
Advantage of Pharma and Diagnostics on one campus

Roche Pharma
Process development, bio-manufacturing

Roche Diagnostics
Broad expertise in analytics

Shared research facilities, technologies and discoveries
**Roche CustomBiotech**

*Solutions for all steps in bio-processing*

### Development
- **Cell isolation**
- **Cell yield & viability testing**
- **Cell expansion, media optimization**
- **Passaging & harvesting**

- **Liberase Portfolio**
- **Cedex HiRes Analyzer**
- **Cedex Bio Analyzers**
- **Rec Trypsin, GMP**

### Manufacturing
- **Scale-up**
- **QC testing**

- **Liberase blends, GMP**
- **Recombinant Trypsin, GMP**
- **Cedex Analyzers**
- **MycOTOOL kit**
Roche CustomBiotech
Instrumentation for cell culture process control

Substrate, metabolite & product monitoring

Cedex Bio Analyzer
Cedex Bio HT Analyzer

Cell density & viability

Cedex HiRes Analyzer
Cedex HiRes Analyzer
Image-based cell counter

- Cell density
- Cell viability
- Cell morphology
Cedex HiRes Analyzer
High-resolution image capturing

**Resolution**
- 0.8 μm/pixel
- Suited for cell diameters of 2 – 40 μm

**Image recognition**
- Algorithms for differentiation of
  - viable cells ⇔ dead cells
  - cell aggregates ⇔ other particles

14 μm cell diameter

17 pixels
**Cedex Bio / HT Analyzer**

*Same tests for different sample load*

<table>
<thead>
<tr>
<th></th>
<th>Cedex Bio Analyzer</th>
<th>Cedex Bio HT Analyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Throughput</strong></td>
<td>≈ 75 tests/h</td>
<td>250 – 400 tests/h</td>
</tr>
<tr>
<td><strong>Sample capacity</strong></td>
<td>8 samples at once,</td>
<td>90 samples at once,</td>
</tr>
<tr>
<td></td>
<td>- Continuous loading during operation -</td>
<td>- up to 60 tests -</td>
</tr>
<tr>
<td><strong>Parallelization</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Auto-dilution</strong></td>
<td>selectable</td>
<td>free programmable</td>
</tr>
</tbody>
</table>
Cedex Bio Analyzer

Robustness based on long-established Roche technology

Track record of a Cedex Bio Analyzer in a large-scale fermentation lab of Roche Pharma:

- in operation for 8 years now
- > 125,000 tests performed
- only 8 cases of instrument outage
- 5 of 8 failures could be fixed at once, total down time < 10 days
Cedex Bio test menu

For process control in cell culture and microbial fermentation

- Ala-Gln (GlutaMAX)
- Cholesterol
- Calcium
- Galactose
- Glucose
- Glutamate
- Glutamine
- Glycerol
- Iron
- Magnesium
- Phosphate
- Potassium
- Pyruvate
- Sodium
- Sucrose
- Formate
- Lactate
- LDH
- Osmolality, calc.
- Optical Density
- Total Protein

Substrates

Metabolites

Products
### Cedex Bio test menu

**Continuous menu extension according to the needs in bioprocessing**

<table>
<thead>
<tr>
<th>Year</th>
<th>Test Menu</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011 / 2012</td>
<td>Glucose, Glutamate, Glutamine, IgG, Lactate, LDH, NH3, Potassium, Sodium</td>
</tr>
<tr>
<td>2013</td>
<td>Iron, Phosphate, Magnesium, Calcium, Acetate, Glycerol, Improved Multi-Controls</td>
</tr>
<tr>
<td>2014</td>
<td>Cholesterol, Pyruvate</td>
</tr>
<tr>
<td>2015</td>
<td>Galactose, Sucrose, Glutamate V2, Glutamine V2, Acetate V2</td>
</tr>
<tr>
<td>2016</td>
<td>Optical Density (OD), Formate, Ethanol</td>
</tr>
<tr>
<td>2017</td>
<td>Ala-Gln (GlutaMAX)</td>
</tr>
<tr>
<td>2018 ff</td>
<td>Galactose V2, Mouse IgG, Osmolality calculated</td>
</tr>
</tbody>
</table>

**System launches Cedex Bio / Cedex Bio HT**

- Glucose
- Glutamate
- Glutamine
- IgG
- Lactate
- LDH
- NH3
- Potassium
- Sodium
- Improved Multi-Controls

**Continuous menu extension according to the needs in bioprocessing**

- Glucose
- Glutamate
- Glutamine
- IgG
- Lactate
- LDH
- NH3
- Potassium
- Sodium
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New Mouse IgG test
Common applications of monoclonal mouse IgG antibodies

Research
Immuoassays (e.g. ELISA, Western blots, fluorescence microscopy)

In-vitro diagnostics
Immuoassays (e.g. Clin Chem, flow cytometry, immunohistology)

Pharma
Drug discovery phase for pharmaceutical antibodies
New Mouse IgG test

Process control in mouse IgG production

So far, determination of product titer was done by:

- **Manual methods**
  like manual ELISA, OD-280, Biuret)
  - poor reproducibility
  - excessive hands-on time

- **Centralized instrumentation**
  like HPLC or other types of analyzers
  - long time to receive results
  - dependence on other departments

Now available:

- **Cedex test**
  - automated testing
  - high reproducibility
  - results after 10 – 15 min
  - wide test range
New Mouse IgG test
Assay characteristics

- Launched in May 2018
- Specificity: Fc part of mouse IgG, subtypes IgG1, IgG2a, IgG2b
- Range: 10 – 7,500 mg/L
- Generic calibrator and controls provided, option for custom calibration
New Mouse IgG test

Assay verification in Roche Diagnostics manufacturing

Linearity of the assay:

![Graph showing the linearity of the assay with R² = 0.9989](image)
New Mouse IgG test

Assay verification in Roche Diagnostics manufacturing

Accuracy with all IgG subtypes (compared to validated Elecsys test):

IgG1

IgG2a

IgG2b
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Osmolality in bio-process control

Purpose

• Quality control of media and supplements, general check for correct water content
• Continuous monitoring of osmotic pressure in cell cultures, to avoid critical dilution or concentration of the medium

Common method

• Determination of freezing point depression using a special osmometer instrument
Cedex Osmolality
Calculation based on relevant cell culture media components

Osmolality

\[ \text{Na}^+ \quad \text{Cl}^- \quad \text{Glc} \quad \text{Gln} \quad \text{Lac}^- \]

- \text{Na}^+ > 85\%
- \text{Cl}^- \quad 3 \text{ – } 12\%
- \text{Glc} \quad 3 \text{ – } 8\%
- \text{Gln} \quad < 3\%
- \text{Lac}^- \quad < 3\%
- \text{K}^+ \quad \text{Ca}^+ \quad \text{Mg}^+ \quad \text{NH}_4^+ \quad \text{HCO}_3^- \quad \text{SO}_4^{2-}
- \text{Amino acids} \quad \text{Proteins} \quad \text{Others}

Cedex profile:
Determination of \textbf{Na}^+ + \textbf{Glc} + \textbf{Gln} only
Cedex Osmolality
Calculation based on relevant cell culture media components

Osmolality

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>&gt; 85%</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>3 – 12%</td>
</tr>
<tr>
<td>Glc, Gln, Lac</td>
<td>3 – 8%</td>
</tr>
<tr>
<td>K⁺, Ca⁺, Mg⁺, NH₄⁺, HCO³⁻, SO₄²⁻</td>
<td>&lt; 3%</td>
</tr>
<tr>
<td>Amino acids, Proteins, Others</td>
<td></td>
</tr>
</tbody>
</table>

Cedex profile:

\[
Osmo = (2 \cdot \text{Na}^+ + 1.3 \cdot \text{Glc} + \text{Gln}) \cdot 1.003
\]
**Cedex Osmolality**

*Performance evaluation*

Comparison “freezing point“ vs. Cedex calculation

- 5,800 cell culture samples,
  30 different culture media

- Accuracy: 98% of results in ± 10% range
Cedex Osmolality
Performance evaluation

Comparison “freezing point“ vs. Cedex calculation

- Examples of two CHO cell cultures with different feeding principles
- Samples were taken daily over the fermentation period
- Accuracy: < 10 % deviation
Osmolality Cedex Bio HT

Method evaluation in Pharma Operations

- 250 samples of CHO fermentation runs, 5 different clones, chemically defined media
- Freezing point depression as reference method
- Sodium, glucose and glutamine measured on Cedex Bio HT
- Adjusted empiric formula used
- Comparability and correlation of the two methods are tested
**Osmolality Cedex Bio HT**

*Comparability (Clone 1)*

**Comparability**

- General equivalence to reference method (on average 96% recovery)
- Both methods show similar course
- Calculation with Cedex Bio HT lower as reference method
Osmolality Cedex Bio HT
Comparability (Clone 2)

Comparability
• General equivalence to reference method (on average 96% recovery)
• Both methods show similar course
• Calculation with Cedex Bio HT lower as reference method
Osmolality Cedex Bio HT
Comparability (Clone 3)

Comparability

- Very good equivalence to reference method (on average 98% recovery)
- Both methods show similar course
- No offset between methods
Osmolality Cedex Bio HT

Comparability (Clone 4)

Comparability

- Very good equivalence to reference method (on average 99% recovery)
- No offset between methods
**Osmolality Cedex Bio HT**

**Comparability (Clone 5)**

**Comparability**
- Very good equivalence to reference method (on average 101% recovery)
- Both methods show same course
- No offset, almost same results
Osmolality Cedex Bio HT
Correlation

- Good correlation between two methods
- Small differences among clones (adjustment of factors possible)
- Reference method tends to result in higher osmolality
**Osmolality Cedex Bio HT**

**Summary**

- More efficiency: Osmolality without extra sample, measurement, additional analyzer and time
- Good comparability with reference method (98% within ±10%, 76% within ±5%)
- Formula can be adjusted if needed (e.g. different matrix, or units in mg/L)
  
  \[
  Osmo = (2 \times [Na^+] + 1.3 \times [Glc] + [Gln]) \times 1.003
  \]

- Perfect for osmolality monitoring during fermentation process
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Sm@rtline Data Cockpit – a middleware for data integration
Transfer of analytical data between devices and IT Systems like LIMS, ELN, MES.
Main Goals

Sm@rtline Data Cockpit (SDC)

**Goals:**
- Increase quality and reliability of laboratory data
- Increase the throughput of your laboratory hardware
- Reduction of the time for manually collecting data
- Support to full fill the GMP requirements
- Reduction off data integration costs
general situation in labs today

LIMS/MES/ELN/DCS

lost time
less accuracy
and wasted resources
Data Integration - direct

GMP validation costs for each analyzer
Development costs for each analyzer type

LIMS/MES/ELN/DCS

Each analyzer is integrated separately
- missing support for long barcodes
- missing entry fields like batch no
- missing support for multiple measurements
- missing electronic signature
- missing a common user management
- missing bidirectional communication

your analyzer
Data Integration – SDC Middleware

Data integration with SDC
- Reduce validation costs
- Reduce implementation time
- Keep the upper systems simple

SDC makes data integration easy
- support for long barcodes
- entry fields like batch no
- support for multiple measurements
- electronic signature (one/double)
- common user management

Smartline Data Cockpit®
21 CFR Part 11 compliance

Instrument layer
SDC is responsible for the interfaces!

network

- validated
- validated
- validated
- validated
- validated
- validated
- validated
- validated

your analyzer

SDC makes data integration easy
Comparison of the SDC operation modes (workflows)

LIMS/MES/ELN/DCS

Smartline Data Cockpit ® - IS
Interface System

Black-Box

archiving of results

creation of measurements with the analyzer software

Possible registration of samples

Smartline Data Cockpit ® - LS
Laboratory System

select or create samples

validation, repetition, approval, and release of results

create measurements

Smartline Data Cockpit ® - LS
Laboratory System

transfer measurements or control the analyzer

archiving of results

Templates

Templates

SDC
Sm@rtline Data Cockpit ®
Analyzer overview – Cedex HiRes

- SDC can start and monitor the measuring process.
  - SDC considers samples with priority.
  - Interaction with the Cedex UI isn’t required.
  - SDC transfers additional manual measurements.
- SDC can start the maintenance functions.
- Visualization of “runs left counter”.
- Visualization of liquid status.
- Scheduled execution of the database archive.
Analyzer overview – Cedex HiRes

Cedex Bio / Bio HT

- SDC can send multiple measurement orders to the analyzer
  - Bio: Operator has to select the order in the display
  - Bio HT: no further operator input required
  - SDC gets results with detailed flag information

Bio HT v3.4:
- SDC can start the maintenance functions.
- Visualization of “runs left counter” and test status.
- Processing of detailed calibration and liquid data
Device overview

pH-Meter
- Seven Excellence
- Portavo 907
- 3420
- 3310

Osmometer
- 3320
- 2020
- OsmoPro
- A2O

coming soon

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Device overview

miscellaneous

Climet CI-153  
DR 2800, DR 3900 photo meter
Elecsys, cobas e 411
Palltronic Flowstar Filter integrity tester
Octet® RED96

miscellaneous

LAMBDA 25/35/45
UV/Vis Spectrophotometer

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Device overview

- **Process Control**
  - Advanced integration
  - ambr 250
  - ambr 15
  - sartorius

- **Liquid Handler**
  - TECAN Fluent
  - Microlab STAR Line
  - coming soon

- **Plate Reader**
  - TECAN Sunrise

- **Automated Sampling**
  - Flownamics

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IT system overview

LIMS/ELN
- ChemLMS
- LABVANTAGE
- idbs
- LABWARE
- Generic SQL

Process Control Systems
- Finesse
- DAS Group
- MFCS/win
- sartorius

MES
- Emerson
- SIEMENS

Historian
- OSIsoft
ambr data integration

- Full tracking of liquid handling operations
- Creation of samplings and measurement orders with just a few mouse clicks.
- Sending offline analytical results back to the ambr process control system
- Prepared for auto working with automated sampling systems like

SDC

48 Batches

48 samples in one sampling

e.g. 142 measurements per sampling

ELISA reader

Osmometer

Roche Bio HT

Closing the feedback loop
Thank you.

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