Biosafety in upstream bioprocessing

Erik Kakes, Sales & Marketing director
Applikon Biotechnology
About Erik Kakes

- Studied Biochemistry
- Active in bioreactor design since 1988
  - Project manager
  - Product development
  - Marketing & Sales
- Co-owner of Applikon Biotechnology since 2008
Applikon Biotechnology

• One of the largest privately owned bioreactor companies
• Started in 1974 by Jan van Burg
• Keywords:
  – Reliable
  – New technologies
  – Long term customer relation
  – Micro scale to production scale systems
  – Local experts for sales, service and support
  – Bioreactor systems only
• Daughter companies in Netherlands, UK, USA, China
Applikon history in vaccine production

• 1970’s: Bilthoven units
  Dr. van Hemert & Ir. van Wezel

• 1989 Applikon and Contact Flow merger

• 1990’s: China Vaccine Project RIVM, DHV, Applikon

• Last 30 years Multiple large scale vaccine projects
Laboratory bioreactors
Production systems
Single Use systems

Powered by

Thermo Scientific

applikon®
BIOTECHNOLOGY

PALL Life Sciences
Vaccine Development

Discovery

Preclinical

Phase 1

Phase 2

Phase 3

Product Licensure

Phase 4

Lead Development

Process & Assay Development, Scale-up

Animal models

Clinical & Regulatory

Pilot manufacturing, QC & QA

Techtransfer

Facility development (See next detail slide)

Pharmacovigilance

QA / QP oversight including CMOs, CROs

CMC Project management
Facility Development

Phase 3
- Tech transfer
  - Plant design & engineering
- Project management, Project QA and compliance management
- Procurement
- Facility Construction
- Equipment Delivery
- Quality Systems development
- Completion and commissioning
- Recruitment & training
- Qualification
- Tech transfer and validation
- Factory licencing

Phase 4
- Product licensure

Commercial Manufacturing, QC & QA
Biosafety vs GMP

- **Biosafety**
  - Protect the operator
  - Keep them in

- **GMP**
  - Protect the consumer
  - Keep them out
Biosafety

• Responsibility of supplier & customer
  • Customer:
    – Provide safe environment for personnel
    – Inform supplier of potential risks
  • Supplier:
    – Intrinsic safety as a design criterium
    – Understand and minimize the process risks
• Communication is key
Project communication: Website

- Open information exchange
- 24/7 accessible
- Up-to-date information
- Documented communication
- Documented decisions
- Everybody works with the same documents
- No surprises
Biosafety: Single-Use or Re-Usable

- Single-Use
  - Short lead time
  - Lower initial investment
  - More flexibility
  - More manual labor so more procedures required

- Re-Usable
  - Longer lead time
  - Higher initial investment
  - Less flexibility
  - More automation
Biosafety: Single-Use bioreactors

– Report April 2016, Dutch Commission for Genetic Modification

• Integrity test of bag not standardized
• Biggest risk is during installation where manual manipulation is the highest risk
• No reliable integrity test possible after installation
• Increased risk for operator
• Continuous training programs are needed
Biosafety: Re-Usable bioreactors

- Benefits of process automation
  - Less manual manipulation
  - Automated test procedures
  - Automated documentation
  - Interlocks for increased safety
  - Verified automated transfer between units
  - Continuous feedback loops
Recap: Bioreactor Biosafety

- Heating/cooling medium: FROM JACKET
- Heating/cooling medium: TO JACKET
- Feeding pump
- Agitation system
- System monitor
- Sensors probes
- Gas Out
- Down Stream
- Sampling
- Thermal jacket
- Reactor tank
- Submerged aerator
- Risk Area

No virus to exit
No virus to exit
No virus to exit
No virus to exit
No virus to exit
No virus to exit
No virus to exit
No virus to exit
=Risk Area
uncontrolled
Recap: Bioreactor GMP

- Heating/cooling medium: FROM JACKET
- Heating/cooling medium: TO JACKET
- Feeding pump
- Agitation system
- System monitor
- Gas Out
- Sensors probes
- Thermal jacket
- Reactor tank
- Down Stream
- Sampling
- Homogeneous mixing
- Defined gas supply
- Submerged aerator
- No contaminant to enter
- No organisms to enter or exit uncontrolled
- Reproducible & Controlled conditions
- Well defined materials
- No contaminant to enter
- No organisms to enter or exit uncontrolled
Exhaust gas filter

- 0.2 micrometer pore size
- Membrane filter
- Integrity test points
- Test integrity before and after process
Exhaust gas incinerator

- Temperature measurement & Control
- Time & heat kill, continuous monitoring
- 200 °C, Up to 200 l/min
Agitator sealing

- Magnetic coupling
- No direct contact between inside and outside of reactor
- Minimal maintenance
- Up to 40 Nm torque
- Cell culture up to 3000 liter volume
- Microbial up to 500 liter volume

\[ P_s = N_p \cdot \rho_f \cdot N^3 \cdot D_i^5 \]
Equipment risk management

• Focus on the interfaces!!!
  – Different suppliers
    • Building, upstream and downstream equipment
  – Different equipment
    • Liquid flow path, connection types, temperatures, flows
  – Different software solutions
    • Handshakes between devices, communication and data integration, validation, unified operator interfaces

• Use as many standard building blocks as possible
  – Proven performance
Hazop study

• What is Hazop?
  – Hazard and operability study
  – a structured and systematic examination of a complex planned or existing process or operation in order to identify and evaluate problems that may represent risks to personnel or equipment
## Deviation

<table>
<thead>
<tr>
<th>Cause</th>
<th>Consequence</th>
<th>Risk Category</th>
<th>before risk reduction</th>
<th>Effective Safe guards</th>
<th>after risk reduction</th>
<th>Re</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocked offgas: V-11.X2/V-11.X6 or V-9.11 fail closed</td>
<td>Elevated P due to gas supply without venting, leading to pressurize up to 2 barg</td>
<td>Safety</td>
<td>C1 F2 P1 W2</td>
<td>PSE-13.02 @ 2.43 barg</td>
<td>PSE-13.02 @ 2.43 barg</td>
<td></td>
</tr>
<tr>
<td>Blocked filter: F-11.X1</td>
<td>Continued cultivation at high P; extra CO2 production; possibility to pressurize beyond design P=3.1 barg</td>
<td>Safety</td>
<td>C2 F2 P1 W2</td>
<td>PAH-9.11</td>
<td>PAH-9.11</td>
<td></td>
</tr>
<tr>
<td>Increased P beyond 3.1 barg by continued cultivation</td>
<td>Possible operator exposure due to rupture of silicone tubing of additions.</td>
<td>Safety</td>
<td>C2 F2 P1 W2</td>
<td>BSL2 (max), no permanent injury expected</td>
<td>BSL2 (max), no permanent injury expected</td>
<td></td>
</tr>
</tbody>
</table>

### Notes:
- Temperature: 30°C
- Pressure: 1-3 barg
- Vent: 1-3 barg
- Gas Supply: 1-3 barg
Hazop rating

For risk reduction, both standards IEC 61508 and IEC 61511 basically define the following steps:
- Risk definition and assessment according to detailed probabilities of failure from sensor over controller to actuator for the overall component life time.
- Specification and implementation of measures for risk reduction.
- Use of suitable instrumentation (evaluated or certified).
- Periodic test for correct operation of the safety functions.

Risk graph according to IEC 61508/61511

<table>
<thead>
<tr>
<th>W3</th>
<th>W2</th>
<th>W1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</table>

Consequences
- C1 minor injury
- C2 serious permanent injury to one or more persons; death of one person.
- C3 death of several persons
- C4 very many people killed

Exposure time
- F1 rare to more often
- F2 frequent to permanent

Avoidance of hazard
- P1 possible under certain circumstances
- P2 almost impossible

Probability of unwanted occurrence
- W1 very slight
- W2 slight
- W3 relatively high
<p>| | | | |</p>
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</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.11.1.1.</td>
<td>Do not use chlorides in the vessel if T &gt; 50°C. In case chlorides are required, do not perform full sterilization with chlorides present, sterilize this feed in a separate vessel. Describe appropriate use in SOP.</td>
<td>Customer</td>
</tr>
<tr>
<td>4</td>
<td>1.12.1.1.</td>
<td>Unable to rank risk due to lack of knowledge on microcarrier filling procedure. Effect of breathing this dust is unknown to Applikon. Scope of equipment for Applikon ends at filling port. Recommended to be addressed by user prior to use the equipment.</td>
<td>Customer</td>
</tr>
<tr>
<td>5</td>
<td>1.35.1.1.1</td>
<td>SOP must be defined for operation of sterilization routine.</td>
<td>Customer</td>
</tr>
<tr>
<td>6</td>
<td>1.39.1.1.1</td>
<td>Confirm CIP pump specs and maximum CIP supply P</td>
<td>Customer</td>
</tr>
<tr>
<td>7</td>
<td>1.39.1.1.1</td>
<td>Consider opening other route to drain during clean offgas to bioreactor flowpath (i.e. V-14.43&amp;44)</td>
<td>Applikon</td>
</tr>
</tbody>
</table>
Resuming

- Biosafety is a shared responsibility
- Advanced automation improves safety
- Build intrinsic safety into design
- Hazop analysis identifies problems and solutions
- Hazop can be done on old and new installations
Thank you!