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

- Largest privately owned bioreactor company in the world
- 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
About Applikon Biotechnology

"providing reliable solutions for the bioprocess market that will enable an improved quality of life“

Erik Kakes, Arthur Oudshoorn, Jaap Oostra, directors Applikon Biotechnology
Applikon Biotechnology

- Innovative Dutch Company (10% turnover goes to R&D)
- Fast growing (annual 25% in last 5 years)
- Enthusiastic team
- Long term vision
- Hightech & high-end products
- Part of Dutch knowledge economy
Laboratory bioreactors
Production systems
Single Use systems

Powered by

Thermo Scientific

Applikon Biotechnology

PALL Life Sciences

Merck Millipore
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 (supply 3 complete vaccine plants)

- Last 30 years Multiple large scale vaccine projects
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 Licensing
- Commercial Manufacturing, QC & QA

Phase 4
- Product Licensure
BioSafety & GMP

- Protect the operator
- Protect the consumer
- Reproducible results
- Controlled processes
- Documented processes
Biosafety vs GMP

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

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

- Enforced by law (CFR and Eudralex)
- Customer:
  - Provide safe products
  - GMP and Eudralex
- Supplier:
  - Eudralex - Volume 4 for equipment
    - Annex 2 and
    - Annex 11 (computer systems)
  - 21CFR210 for equipment
    - Part 11 electronic signatures
  - ASME BPE design guidelines
Biosafety

• Responsibility of supplier & customer
  • Customer:
    – Provide safe environment for personnel
    – Inform supplier of potential risks
  • Supplier:
    – Intrinsic safety as a design criterium
    – Understand the process risks
Recap: Bioreactor

- Heating/cooling medium: FROM JACKET
- Heating/cooling medium: TO JACKET
- Gas Out
- Sampling
- Down Stream
Single-Use or Re-Usable

• Single-Use
  – Short lead time
  – Lower initial investment
  – More flexibility
  – Higher running costs
  – More manual labor so more procedures required
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
  - Continuous training programs are needed
  - No reliable integrity test possible after installation
  - Increased risk for operator

Single-Use or Re-Usable

- Re-Usable
  - Longer lead time
  - Higher initial investment
  - Less flexibility
  - Lower running costs
  - Advanced automation
Re-Usable bioreactors

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

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

No virus to exit

=Risk Area

uncontrolled
Recap: Bioreactor GMP

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

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

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

- Double mechanical seal
- Steam condensate lubrication
- Condensate pressure > reactor pressure
- Monitor pressure of condensate
- Preventive replacement of seal

Fig 4.4.6 Cartridge DN type
Agitator sealing

- Magnetic coupling: absolute sealing
- 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

Tipspeed: \( \pi N D_i \)

Power: \( P_s = N_p \rho_f N^3 D_i^5 \)
Liquid addition

Manual single shot addition

Manual resterilizable addition

Automated resterilizable addition
Vaccine productionscale systems

• Projects
  – One time process
  – Custom design
  – Fixed time
  – Fixed budget

• Communication is key!!
Project risk management:

- Clear Project Execution Vision provides:
  - Risk management during the project
  - An integrated project approach
  - A joint approach to achieve regulatory compliance
  - Up front specification before implementation
  - Continuous communication about the project status
  - Effective project control measures
New vaccine production risk management

• Fast track vaccine manufacturing solutions
  – Proven and documented processes
  – Proven and documented procedures
  – Proven and documented engineering solutions
  – Proven and documented partnerships
Case Study:
Manufacturing a cGMP production plant by Applikon Biotechnology

- Fully integrated production upstream facility
- Full cGMP and Biosafety
- Scalable systems and transfer lines
- Integration of 3rd party systems
- Fully automated operation
# User Requirement Specifications

## URS FERMENTOR 1000 L
(GENERAL REQUIREMENTS)

<table>
<thead>
<tr>
<th>No.</th>
<th>Item</th>
<th>URS</th>
</tr>
</thead>
</table>
| 1   | Type           | - Double jacketed  
|      |                | - Integrated : fermentor 1000 L, continuous centrifuge, harvest tank.  
|      |                | - CIP, SIP                                                           |
| 2   | Capacity       | Working volume : 1000 Liter  
|      |                | Total volume : 1500 Liter                                             |
| 3   | Design/Feature | - cGMP  
|      |                | - Agitation : Stirrer  
|      |                | - Air inlet 1 : Overlay, air  
|      |                | - Air inlet 2 : Sparger, air + O2  
|      |                | - Air outlet : Housing filter with heating element  
|      |                | - Mechanical foam breaker : 200 L/min, From MBR  
|      |                | - Addition line 1 :  
|      |                |   - Included housing filter for medium (housing filter compatible with filter cartridge Pall B1NF7PH4). |
The road from specifications to operational product

- Create Process Flow Diagram (PFD) and detailed quotation
- Order received
- Create project management website
- Piping and Instrumentation Diagram (P&ID)
- Functional Design Specifications (FDS)
PFD made according URS

Define the interfaces
Schematic transfer lines

Define the interfaces
Project management communication

2. Drawings/Partlist (Approve drawings and order parts)
   - 2) Bioreactor System [Piet den Hartog] (45 days from now)
     - NEW 2.1 Release General Arrangement (GA) CELL60 [Completed 2016-Sep-24] [Piet den Hartog]
     - NEW 2.2 Release General Arrangement (GA) CELL130 [Completed 2016-Apr-23] [Piet den Hartog]
     - UPDATED 2.3 Release General Arrangement (GA) CELL1000 [Active (working on it)] [Piet den Hartog] (3 days from now)
     - NEW 2.6 Release Production Documentation Mechanical [Active (working on it)] [Piet den Hartog] (3 days from now)
       - NEW 2.6.1 Production Documentation Mechanical CELL60 [Completed 2016-Sep-24] [Piet den Hartog]
       - NEW 2.6.2 Production Documentation Mechanical CELL130 [Completed 2016-Sep-24] [Piet den Hartog]
       - NEW 2.6.3 Production Documentation Mechanical CELL1000 [Active (working on it)] [Piet den Hartog] (3 days from now)
       - NEW 2.6.4 Production Documentation Mechanical VIRUS1000 [Active (working on it)] [Piet den Hartog] (3 days from now)
   - 3) Control system [Piet den Hartog] (72 days from now)
     - NEW 3.1 Release Functional Specifications (FDS) [Active (working on it)] [Piet den Hartog] (10 days from now)
     - NEW 3.2 Release IQ List [Completed 2016-May-10] [Piet den Hartog]
     - NEW 3.3 Release Production Documentation Electrical [Completed 2016-Apr-23] [Piet den Hartog]
     - NEW 3.4 Production Electrical [Active (working on it)] [Piet den Hartog] (72 days from now)
     - 3.5 Release Software Package [Piet den Hartog] (37 days from now)

4-Software (Software)
   - 4) SCADA [Piet den Hartog] (71 days from now)
     - 4.1 Release SCADA Configuration Specification [Piet den Hartog] (69 days from now)
     - 4.2 Release Configuration Software SCADA [Piet den Hartog] (73 days from now)

5-Qualification (Qualification)
   - 5) Qualification [Piet den Hartog] (108 days from now)
     - 5.1 Installation Qualification (IQ) [Piet den Hartog] (73 days from now)
       - 5.1.1 Release IQ Protocol [Active (working on it)] [Piet den Hartog] (31 days from now)
       - 5.1.2 Perform IQ [Piet den Hartog] (73 days from now)
     - 5.2 Operation Qualification (OQ) [Piet den Hartog] (101 days from now)
       - NEW 5.2.1 Release OQ Protocol [Piet den Hartog] (27 days from now)
       - 5.2.2 Perform OQ [Piet den Hartog] (101 days from now)
     - 5.3 Factory Acceptance Test (FAT) [Piet den Hartog] (108 days from now)
       - 5.3.1 Release FAT-protocol [Piet den Hartog] (73 days from now)
       - 5.3.2 Perform FAT [Piet den Hartog] (108 days from now)

6-Transport (Transport)
   - 6) Transport [Piet den Hartog] (119 days from now)
     - 6.1 Transport [Piet den Hartog] (119 days from now)

7-Installation / Commissioning (Installation / Commissioning)
   - 7) Installation / Commissioning [Planned (not active)] [Piet den Hartog]
     - 7.1 Installation / Commissioning [Planned (not active)] [Piet den Hartog]
     - 7.2 Site Acceptance Test [Planned (not active)] [Piet den Hartog]
       - 7.2.1 Release SAT Protocol [Piet den Hartog] (119 days from now)
       - 7.2.2 Perform SAT [Planned (not active)] [Piet den Hartog]
2. Drawings/Partlist (Approve drawings and order parts)

- **2) Bioreactor System** [Piet den Hartog] (45 days from now)
  - **NEW** 2.1 Release General Arrangement (GA) CELL60 *(Completed 2016-Sep-24)* [Piet den Hartog]
  - **NEW** 2.2 Release General Arrangement (GA) CELL130 *(Completed 2016-Apr-23)* [Piet den Hartog]
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    - **NEW** 2.6.4 Production Documentation Mechanical VIRUS1000 *(Active (working on it))* [Piet den Hartog] (3 days from now)
  - **2.7 Production Mechanical** [Piet den Hartog] (45 days from now)

- **3) Control system** [Piet den Hartog] (72 days from now)
  - **NEW** 3.1 Release Functional Specifications (FDS) *(Active (working on it))* [Piet den Hartog] (10 days from now)
  - **NEW** 3.2 Release I/O List *(Completed 2016-May-10)* [Piet den Hartog]
  - **NEW** 3.3 Release Production Documentation Electrical *(Completed 2016-Apr-23)* [Piet den Hartog]
  - **NEW** 3.4 Production Electrical *(Active (working on it))* [Piet den Hartog] (72 days from now)
  - 3.5 Release Software Package [Piet den Hartog] (37 days from now)
1 User Requirement Specifications (URS) + questionnaire
2 Quotation
3 Order
4 Design specifications
5 Risk Analysis
6 Detail design specifications
7 Engineering

Customer Requirements

validation

Verified System

14 PQ
13 SAT
12 FAT
11 OQ
10 IQ
9 Production
8 DQ
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
# Hazop study report

- **Temperature:** 1 to 3.1 barg, 0°C to 150°C
- **OP:** 0.2-0.7 barg, SIP: 1.1-1.3 barg, CIP: 0.0-0.2 barg
- **OP:** 25-37°C, CSIP: 121-125°C, CCIIP: 70-90°C
- **Washing:** 70L, TV: 100L
- **Product Content:** SS 316L (5/4/4/4), Silicone EPDM, PVDF
- **CS:** 1.5 barg CIP, 4-6 barg 0-4.5 m³/h waste water with 2% detergent (alkaline)/Medium out: 2-12L/min 0.5 barg, Nutrient feed: 2.3-12L/min 0.5 barg, Alkaline (20 rpm pump)/Medium in: 4L/min (0.5 barg)/Feed: 30L/min Medium out: 1-30L/min 0.5 barg, Blow-out: atm back
- **Test for Product:** Production of IPV vaccine, relief valve, air over-pressured, steam valve failure, continued fermentation, liquid overflow (CIP)

<table>
<thead>
<tr>
<th>Deviation</th>
<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>1 Pressure high</td>
<td>Blocked off: V-11.X2/V-11.X5 or V-9.11 fail closed</td>
<td>Elevated P due to gas supply without venting, leading to pressure up to 2 barg (setting of R 3.52)</td>
<td>Safety</td>
<td>C (sev.)</td>
<td>F (exp.)</td>
<td>P (evol)</td>
<td>W (prob)</td>
</tr>
<tr>
<td>1 Pressure high</td>
<td>Blocked filter: F-11.X1</td>
<td>Continued cultivation at high P, extra CO2 production, possibility to pressure beyond design P=8.3 barg, Possible operator exposure due to rupture of silicone tubing of additions</td>
<td>Safety</td>
<td>C2</td>
<td>F2</td>
<td>P1</td>
<td>W2</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Increase of P beyond 3.1 barg by continued cultivation Possible operator exposure due to rupture of silicone tubing of additions Increased pressure to max 1 barg, No P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 lifetime.
- 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**

- **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>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</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>
Factory Acceptance Test

- Mutually agreed tests
- Test of performance & safety (interlocks etc.)
- Supplier utilities
- Supplier location
- End of manufacturing
- Packing and shipping (risk analysis)
Transport
Arrival and unpacking
Installation
Site Acceptance Test

- Mutually agreed tests
- Test performance & safety
- Customer utilities
- Customer location
- Next is PQ by customer
Operation and performance testing
Conclusions

• Biosafety is responsibility of supplier & customer
• GMP and Biosafety should be combined in design
• Automation can help reducing risks
• Standard solutions reduce project and safety risks
• Risks are in every step of the process and need to be identified and minimized
• Experienced partners reduce risks
• Open communication during project is key to success
Thank you!