



# 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



Jan van Burg

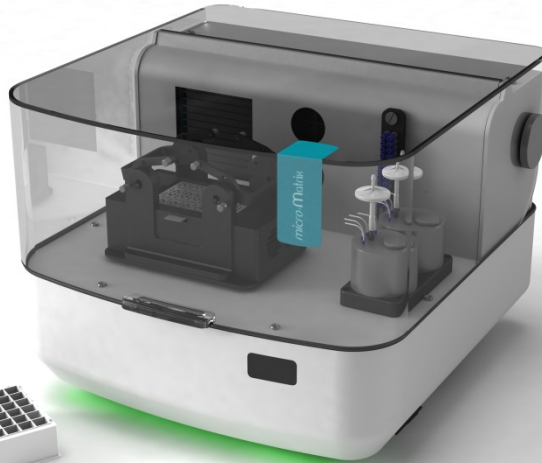
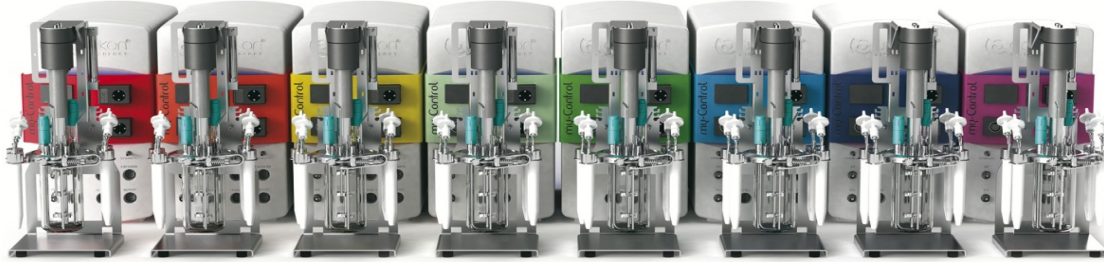
# Applikon history in vaccine production

- 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



**Thermo**  
SCIENTIFIC



**applikon**  
BIOTECHNOLOGY



**M**  
MERCK MILLIPORE

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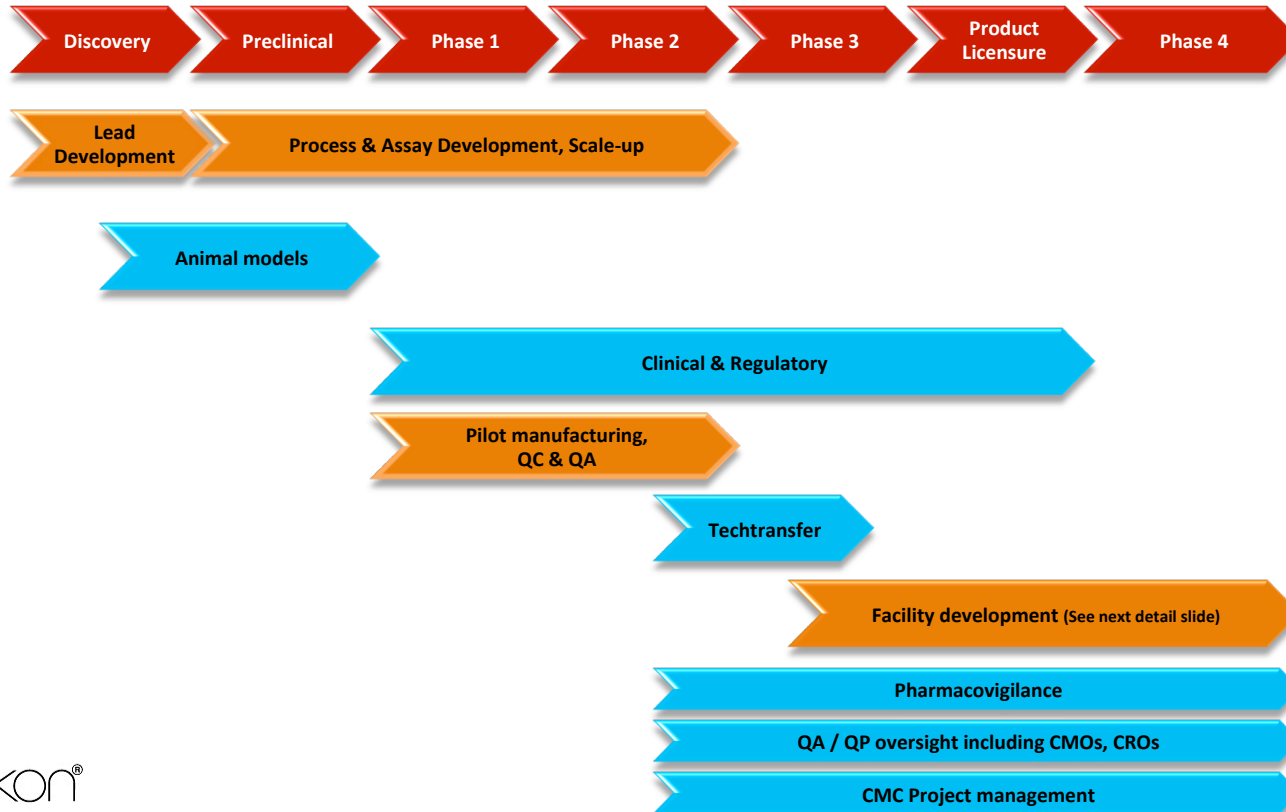
**applikon**  
BIOTECHNOLOGY



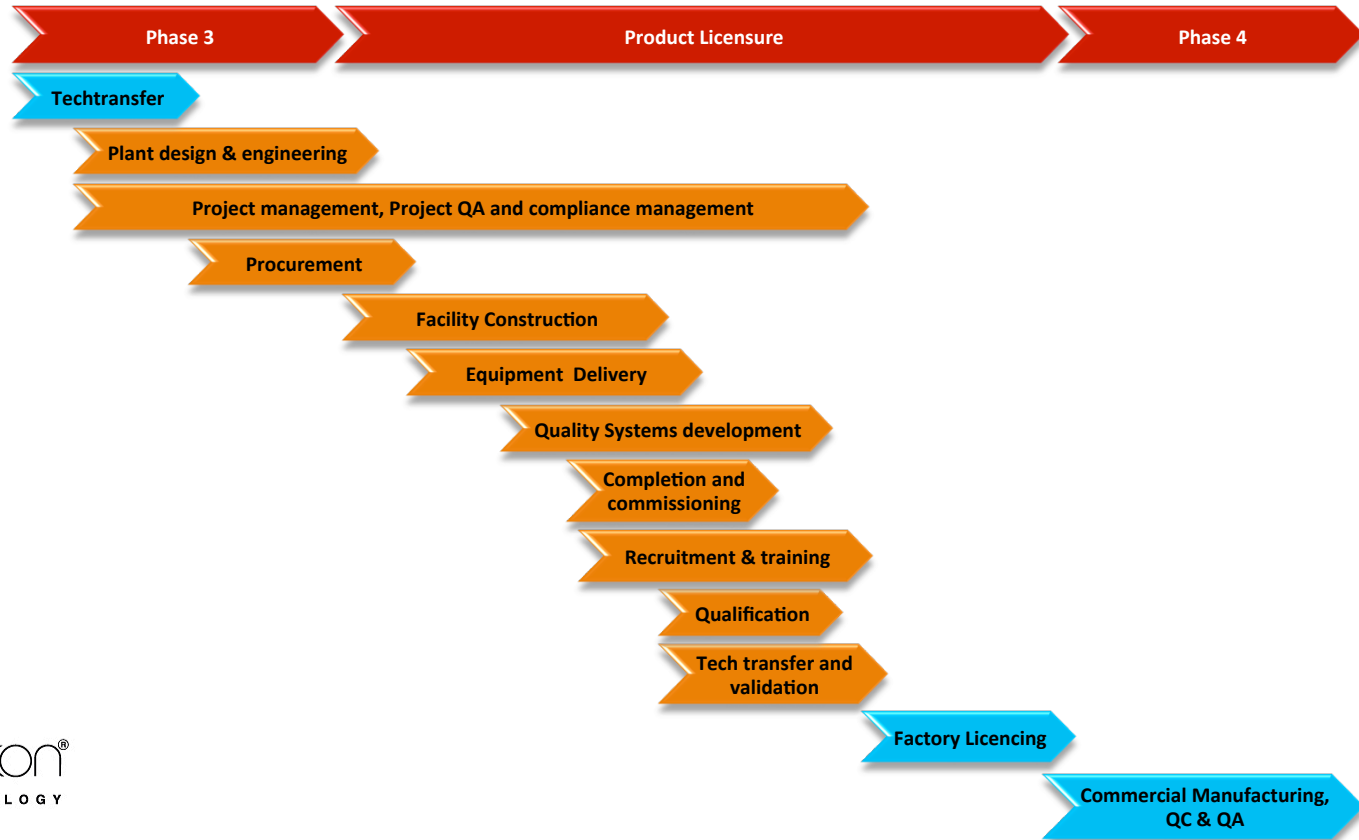
**PALL** Life Sciences



# Vaccine Development



# Facility Development

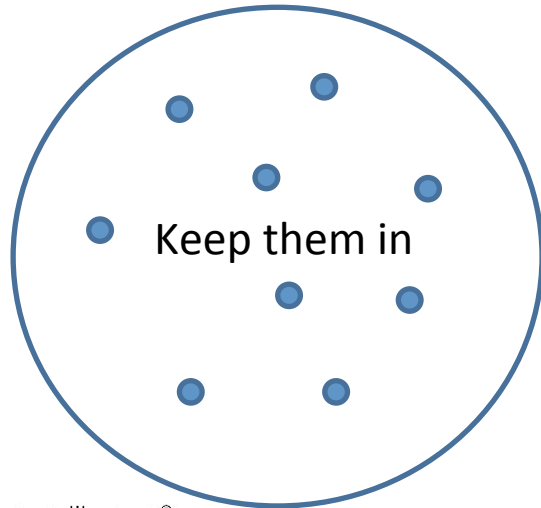




# Biosafety vs GMP

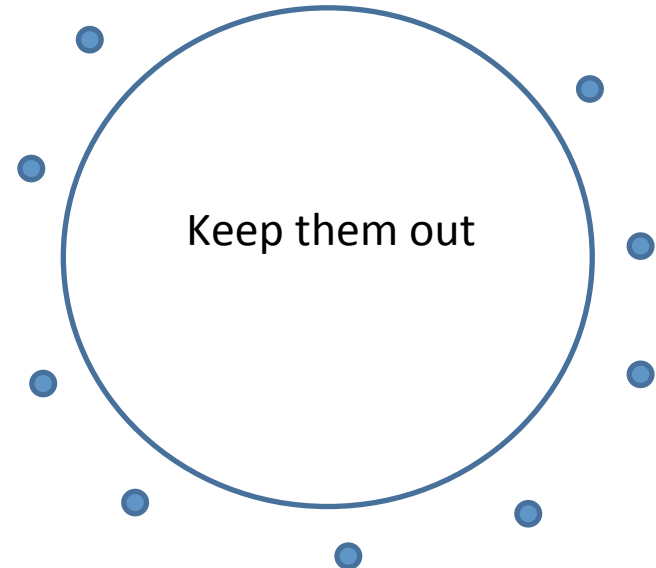
- Biosafety

- Protect the operator



- GMP

- Protect the consumer



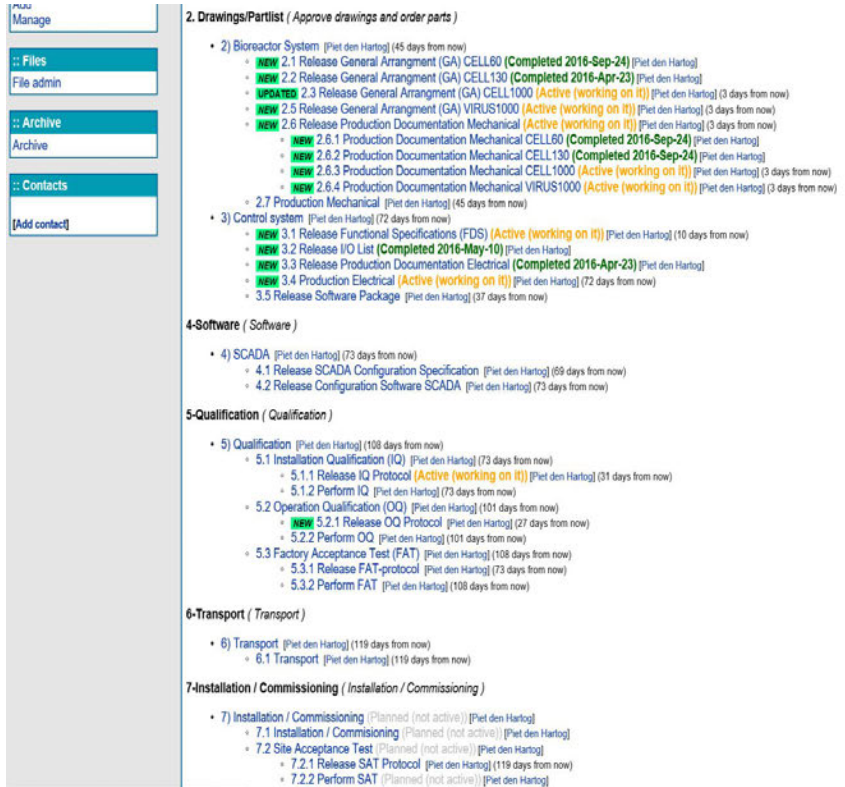
# Biosafety

- 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



The screenshot displays a web application interface for project communication. On the left, a sidebar contains navigation links: 'Manage', 'Files' (with a 'File admin' sub-link), 'Archive', and 'Contacts' (with an 'Add contact' button). The main content area is titled '2. Drawings/Partlist ( Approve drawings and order parts )' and lists various project items with their status and completion dates. For example, under '2) Bioreactor System', items include '2.1 Release General Arrangement (GA) CELL60' (Completed 2016-Sep-24), '2.2 Release General Arrangement (GA) CELL130' (Completed 2016-Apr-23), and '2.3 Release General Arrangement (GA) CELL1000' (Active working on it). Other sections include '3) Control system', '4-Software ( Software )', '5-Qualification ( Qualification )', '6-Transport ( Transport )', and '7-Installation / Commissioning ( Installation / Commissioning )'. Each item is accompanied by a status indicator (e.g., 'NEW', 'UPDATE', 'Active working on it') and a timeline (e.g., '45 days from now', '10 days from now').

Manage

Files  
File admin

Archive  
Archive

Contacts  
Add contact

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]
  - UPDATE 2.3 Release General Arrangement (GA) CELL1000 (Active working on it) [Piet den Hartog] (3 days from now)
  - NEW 2.5 Release General Arrangement (GA) VIRUS1000 (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)
  - 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)

4-Software ( Software )

- 4) SCADA [Piet den Hartog] (73 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 )

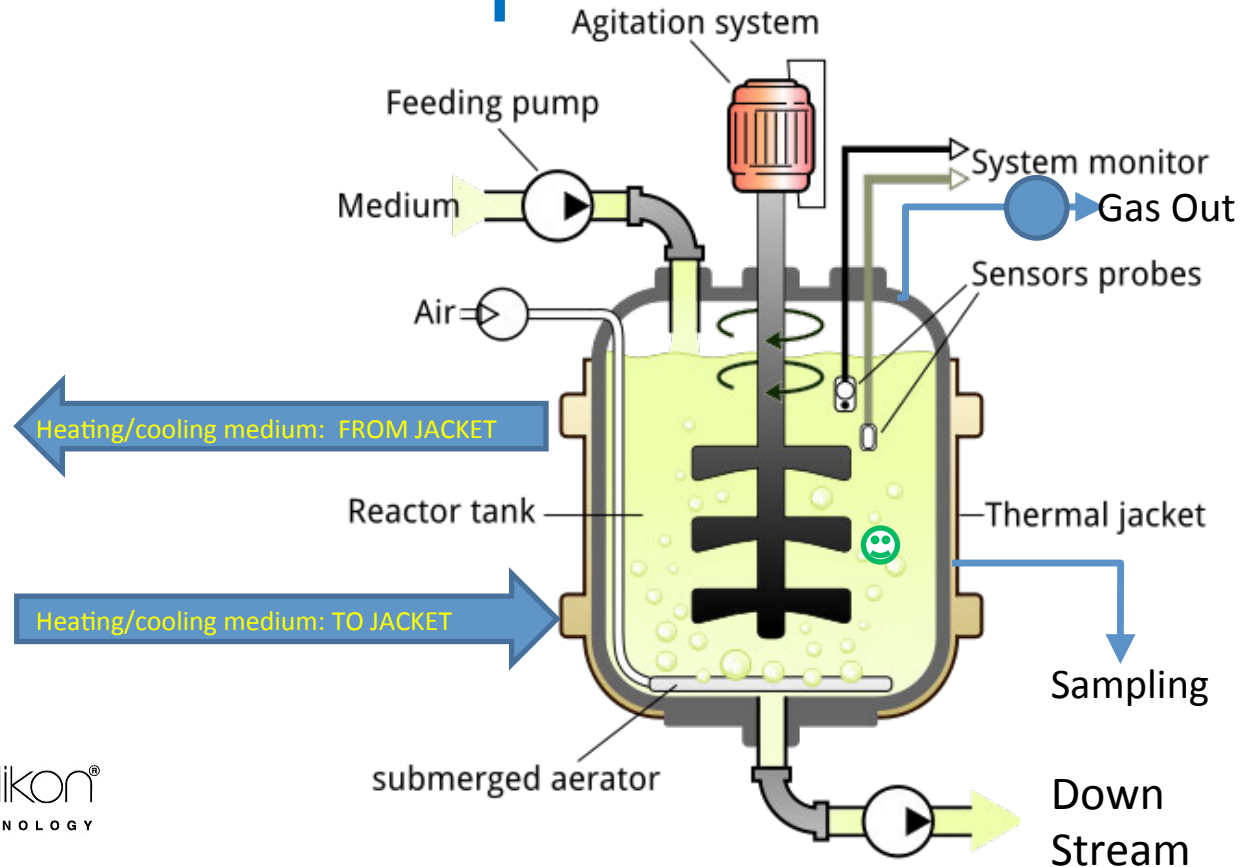
- 6) Transport [Piet den Hartog] (119 days from now)
  - 6.1 Transport [Piet den Hartog] (119 days from now)

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]



# Recap: Bioreactor



=Risk Area



# 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

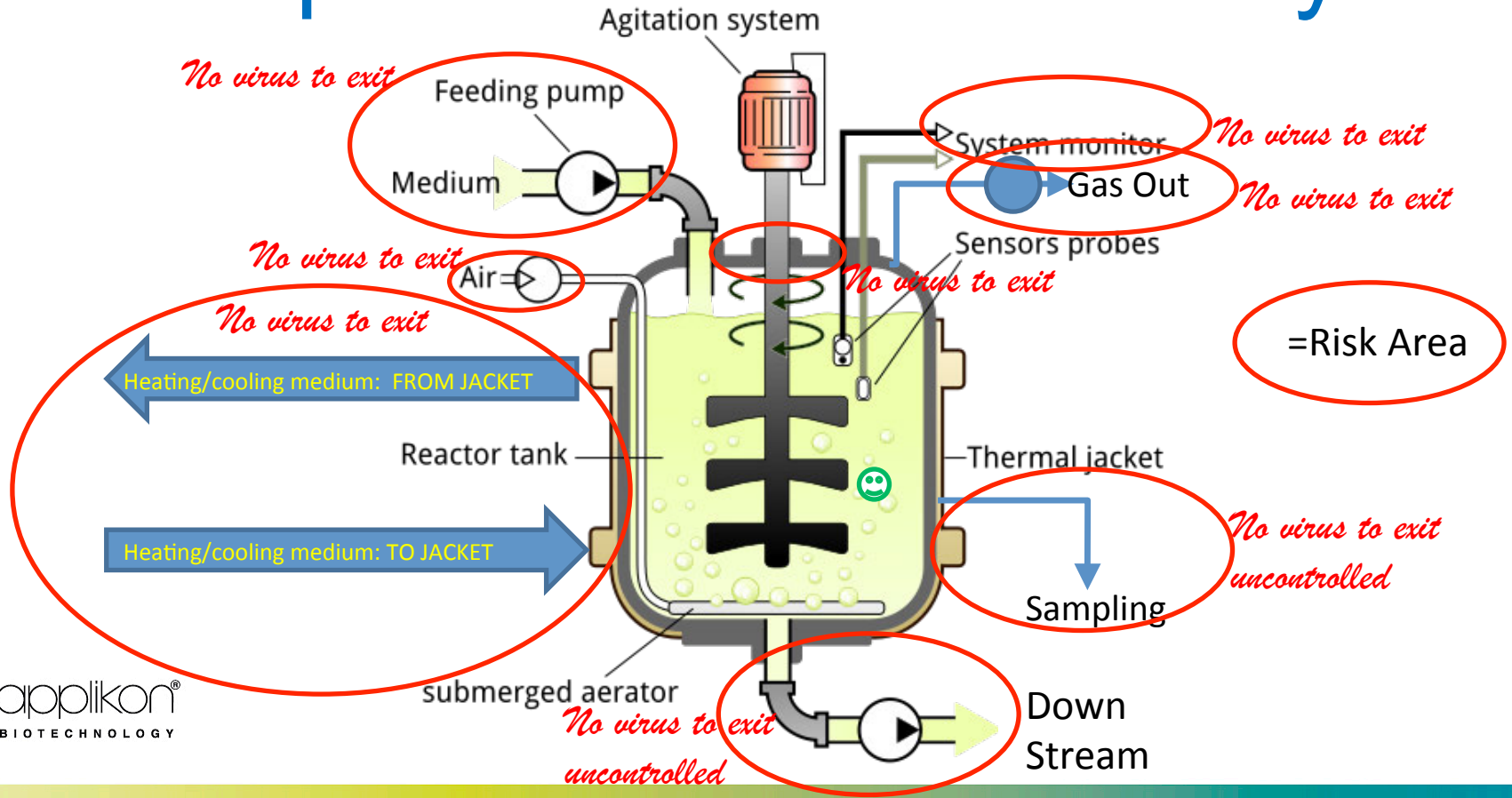


# Biosafety: Re-Usable bioreactors

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

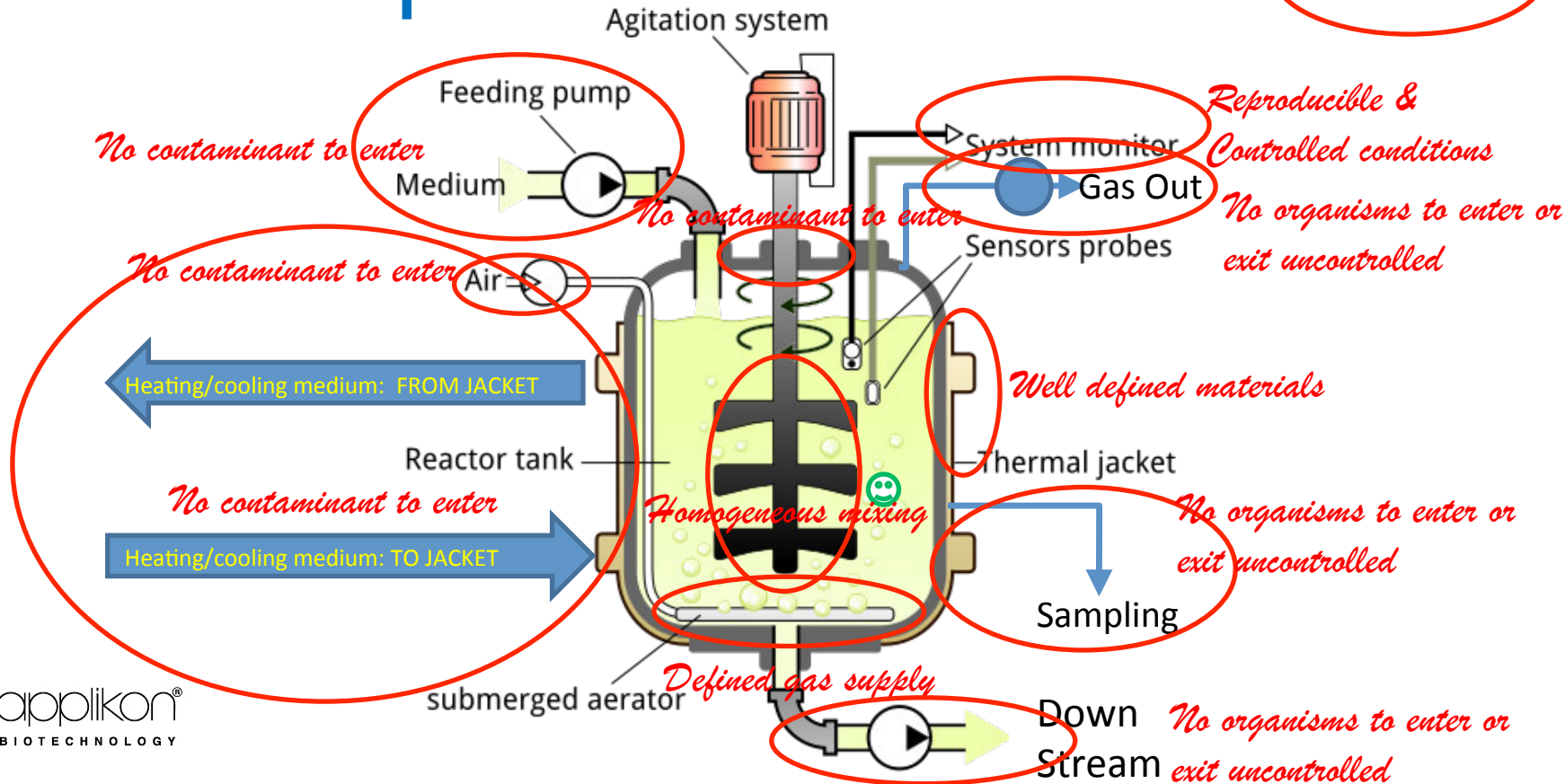


# Recap: Bioreactor Biosafety



# Recap: Bioreactor GMP

=Risk Area







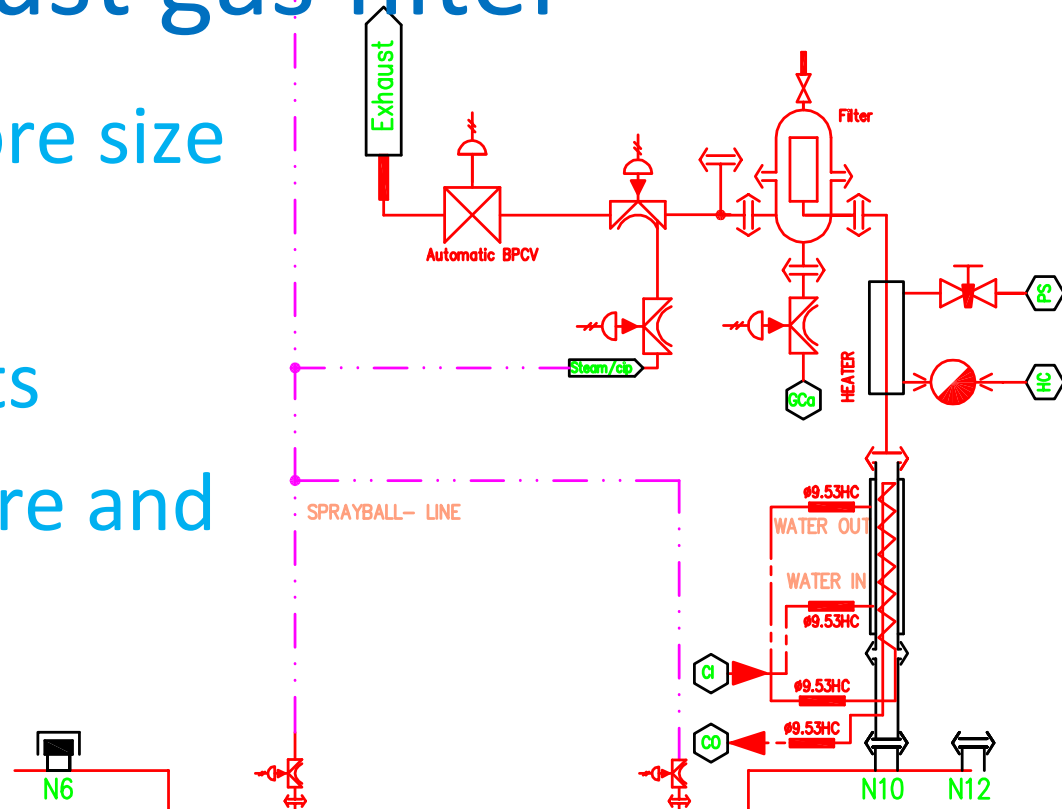
*Off-gas Incinerator*

*Gas Filters*

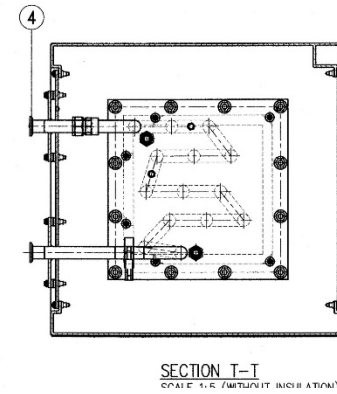
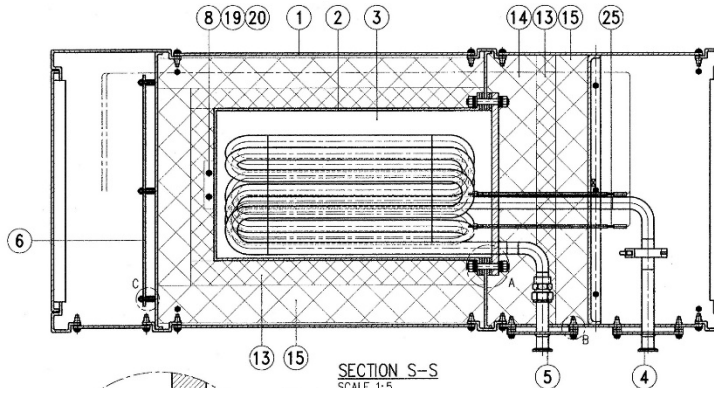


# Exhaust gas filter

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



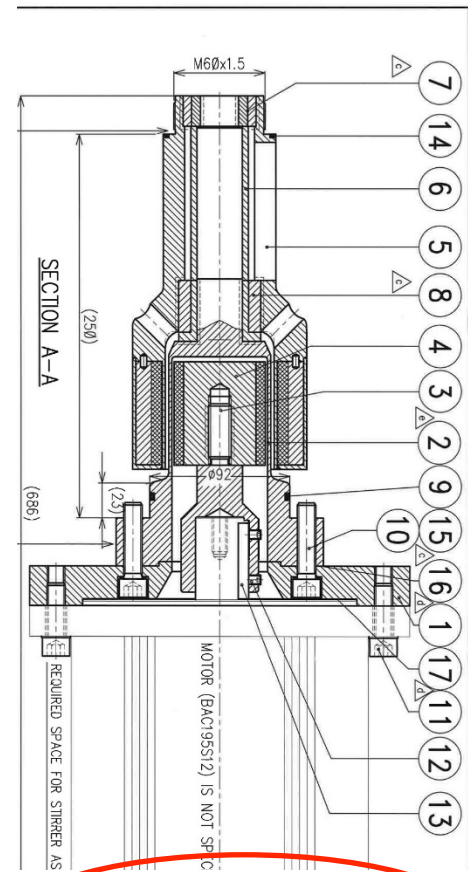
# Exhaust gas incinerator



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

# Agitator sealing

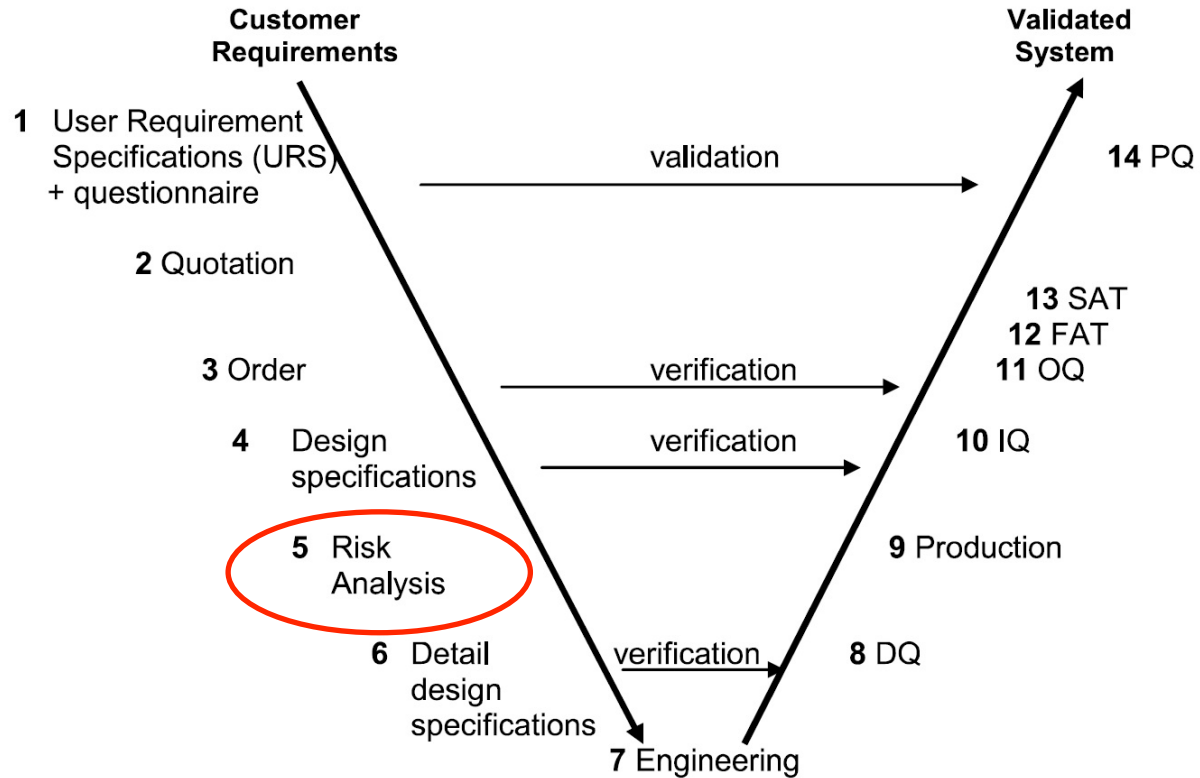
- Magnetic coupling
- No direct contact between inside and outside of reactor
- Minimal maintenance
- Up to 40 Nm torque
- Cell culture up to 3000 liter volume
- Microbial up to 500 liter volume



$$P_s = N_p * \rho_f * N^3 * D_i^5$$

# Equipment risk management

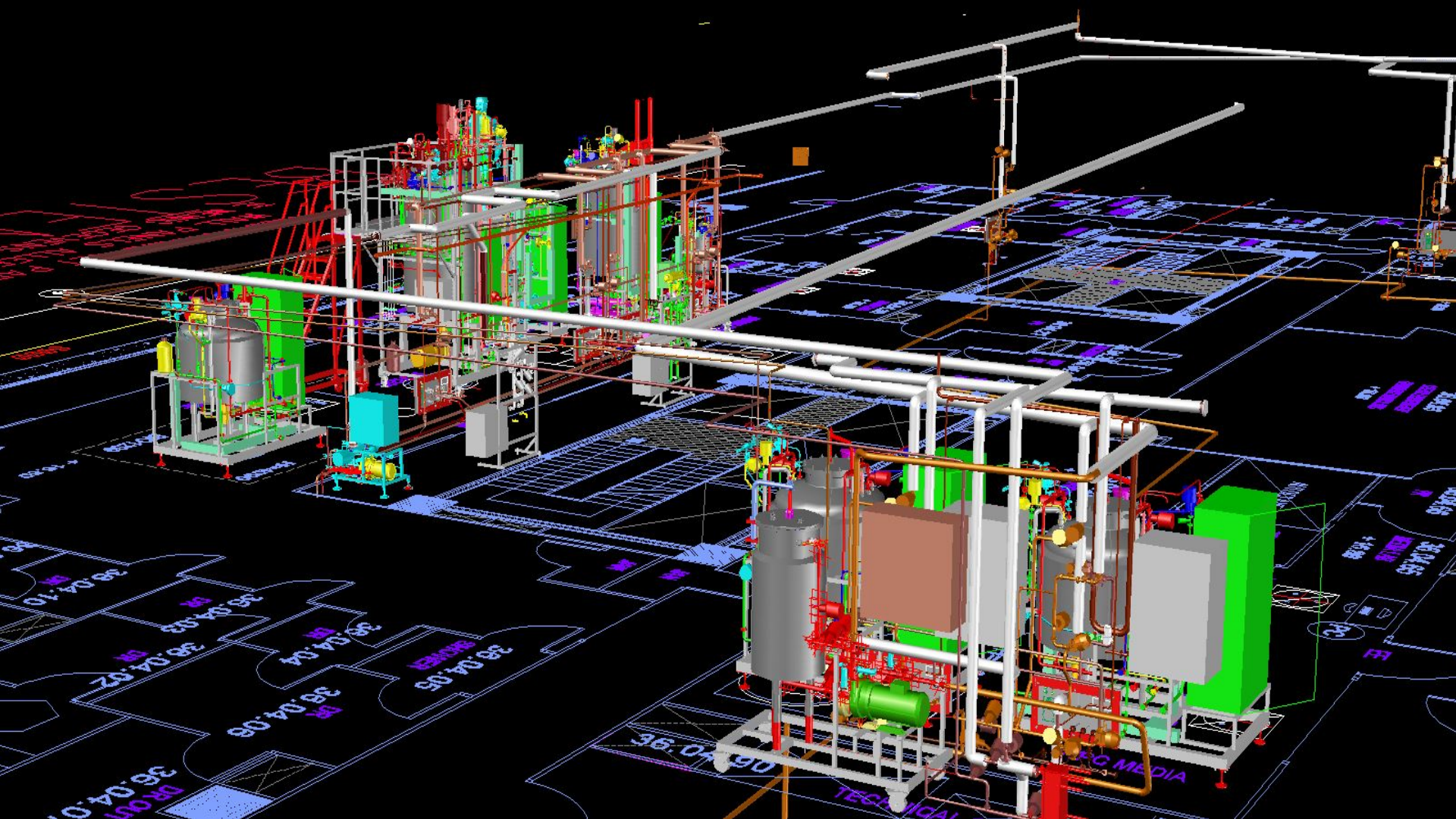
- Focus on the interfaces!!!
  - Different suppliers
    - Building, upstream and downstream equipment
  - Different equipment
    - Liquid flow path, connection types, temperatures, flows
  - Different software solutions
    - Handshakes between devices, communication and data integration, validation,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











# Hazop study report

## Introductions

-1 to 3.1 barg

0-150°C

OP: 0.2-0.7 bargSIP: 1.1-1.3 bargCIP: 0-0.2 barg

OP: 25-37°C SIP: 121-125°C CIP: 70-90°C

WV: 706LTV: 1000L

product contact: SS 316L (1.4404); Silicone/ EPDM/ PVDF

CS: 1.5 bargCIP: 4-6 barg 0.4-5 m<sup>3</sup>/h water with 2% detergent (alkali/acid)Medium out 2: 12L/min 0.5 bargNutrient / feed: 2.3-12L/min 0.5 bargAlkali (20rpm pump)Inoculum: 4L/min (0.5 barg?)Feed: 30L/minMedium out 1: 30L/min 0.5 bargBiowaste: atm back

tent Production of IPV vaccine.relief cases: air overpressure; steamvalve failure; continued fermentation; liquid overfill (CIP)

Deviation		Cause		Consequence		Risk category	before risk reduction					Effective Safe guards					after risk reduction					Re
							C (sev.)	F (exp.)	P (avoi)	W (prob.)	RR			C (sev.)	F (exp.)	P (avoi)	W (prob.)	RR				
1	Pressure high	1	Blocked offgas: V-11.X2/V-11.X6 or V-9.11 fail closed Blocked filter: F-11.X1	1	Elevated P due to gas supply without venting, leading to pressurize up to 2barg (P setting of R-3.52)	Safety	C1	F2	P1	W2	-	1	PSE-13.02 @ 2.43barg PSH-9.11 @ 1.5barg PAH-9.11									
1		1		2	Continued cultivation at high P: extra CO2 production; possibility to pressurize beyond design P=3.1barg Possible operator exposure due to rupture of silicone tubing of additions.	Safety	C2	F2	P1	W2	SIL1	1	PSE-13.02 @ 2.43barg PAH-9.11 BSL2 (max), no permanent injury expected	C1	F2	P1	W2	-				
1		2	PT-9.11 fails low closing V-9.11	1	Increase of P beyond 3.1barg by continued cultivation Possible operator exposure due to rupture of silicone tubing of additions.	Safety	C2	F2	P1	W2	SIL1	1	PSE-13.02 @ 2.43barg BSL2 (max), no permanent injury expected	C1	F2	P1	W2	-				
					Increased pressure to max 1barg. No P																	

# 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

			W3	W2	W1
C1			—	—	—
C2	F1	P1	SIL 1	—	—
		P2	SIL 1	SIL 1	—
	F2	P1	SIL 2	SIL 1	SIL 1
		P2	SIL 3	SIL 2	SIL 1
C3	F1		SIL 3	SIL 3	SIL 2
	F2		SIL 4 <sup>1)</sup>	SIL 3	SIL 3
C4			—	SIL 4 <sup>1)</sup>	SIL 3

## 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

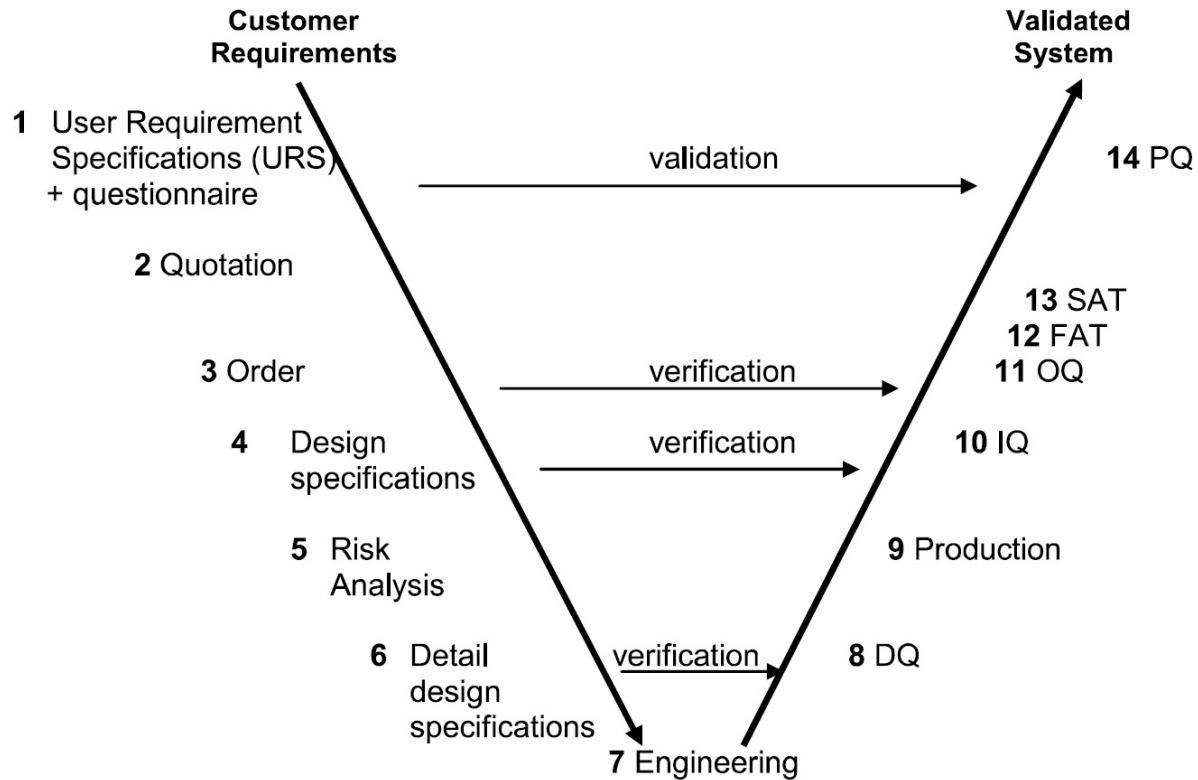
- P1 possible under certain circumstances
- P2 almost impossible

## Probability of unwanted occurrence

- W1 very slight
- W2 slight
- W3 relatively high

# Hazop actionlist

		prior to use the equipment.	
3	1.11.1.1.	Do not use chlorides in the vessel if $T > 50^{\circ}\text{C}$ . 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.	Customer
4	1.12.1.1.	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.	Customer
5	1.35.1.1.1	SOP must be defined for operation of sterilization routine.	Customer
6	1.39.1.1.1	Confirm CIP pump specs and maximum CIP supply P	Customer
7	1.39.1.1.1	Consider opening other route to drain during clean offgas to bioreactor flowpath (i.e. V-14.43&44)	Applikon







# 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!