



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



Jan van Burg

About Applikon Biotechnology



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



Thermo
SCIENTIFIC



applikon
BIOTECHNOLOGY

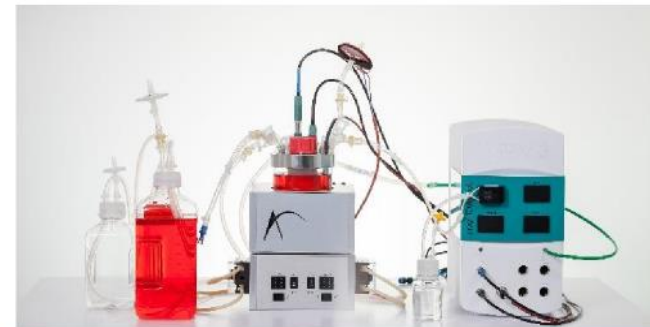
M
MERCK MILLIPORE

Powered by

applikon
BIOTECHNOLOGY



PALL Life Sciences

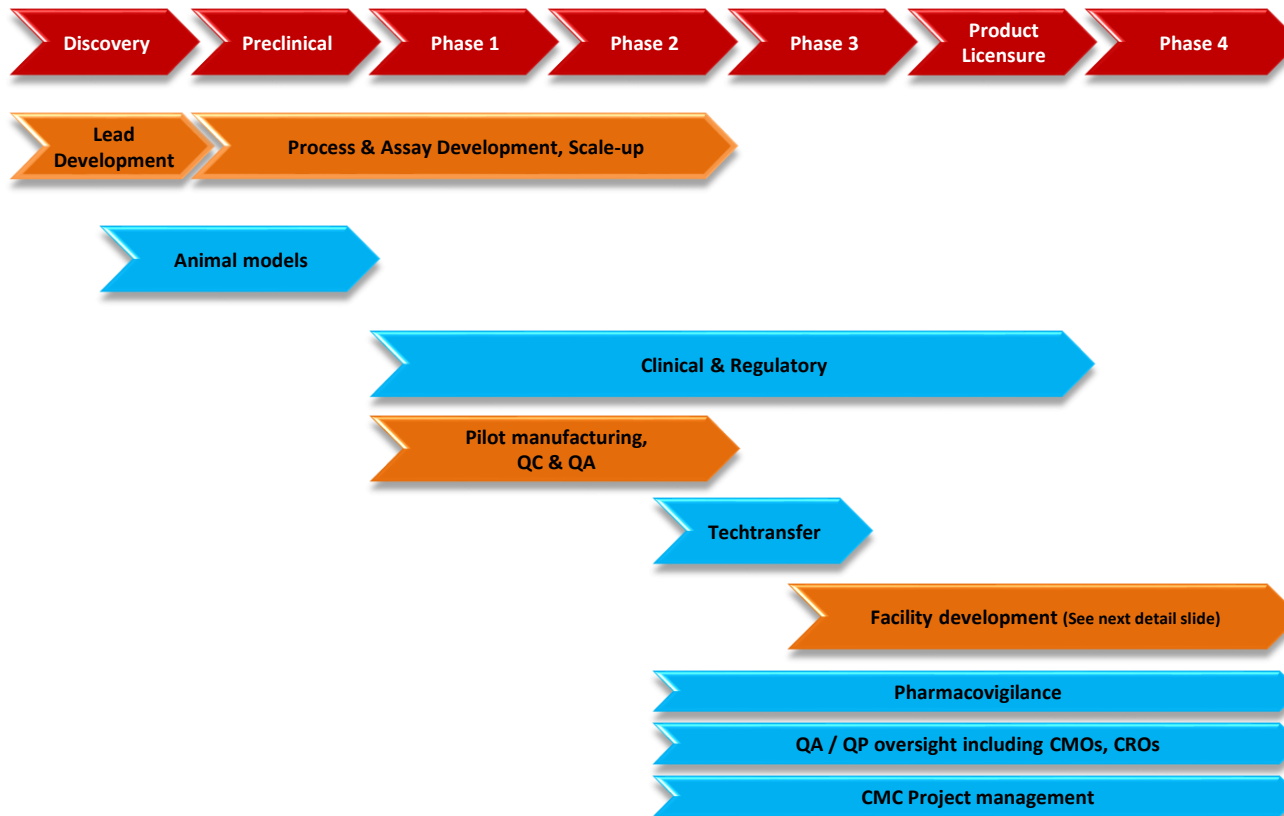


Applikon history in vaccine production

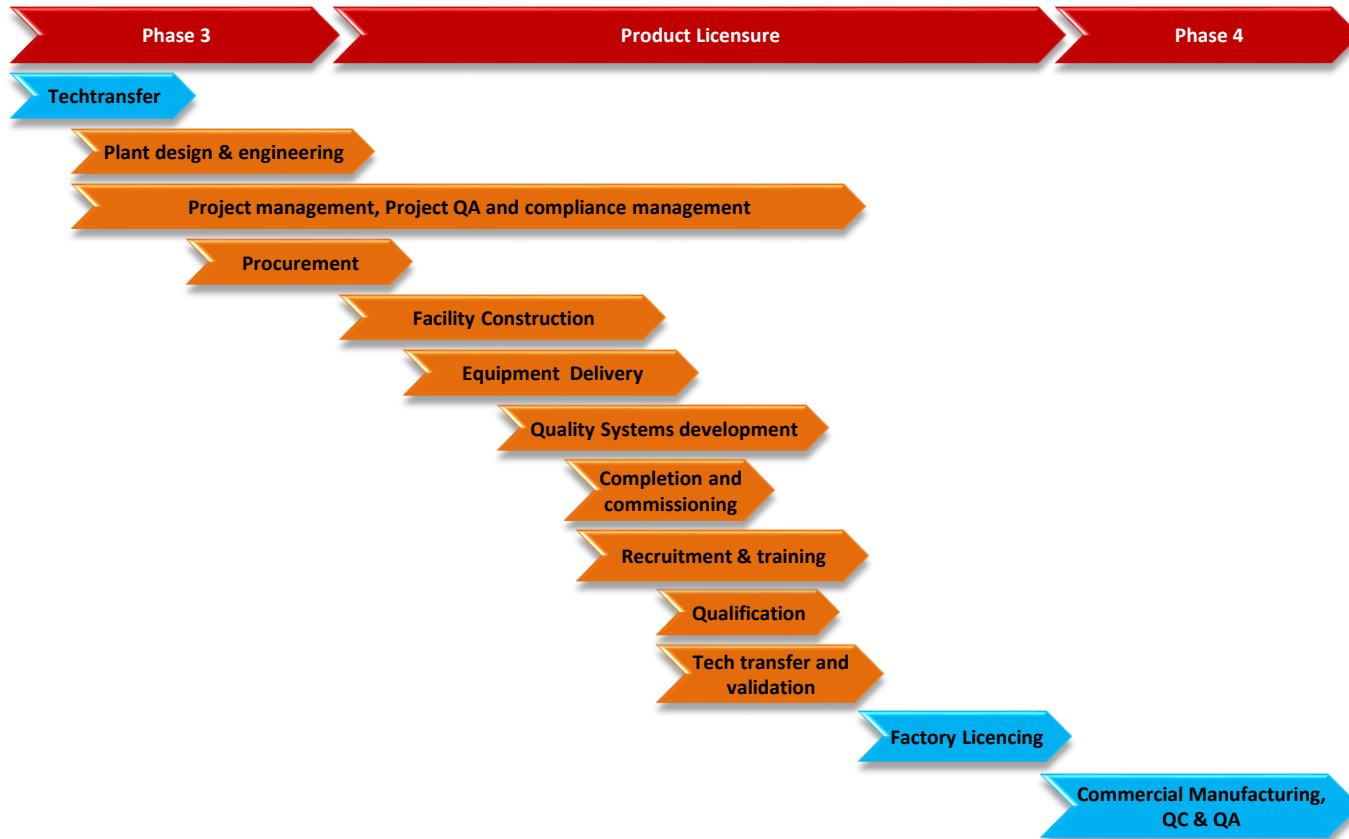
- 1970's: Bilthoven units
Dr. van Hemert & Ir. van Wezel
- 1989 Applikon and Contact Flow merger
- 1990's: China Vaccine Project RIVM, DHV,
Applikon (supply 3 complete vaccine plants)
- Last 30 years Multiple large scale vaccine projects



Vaccine Development



Facility Development



BioSafety & GMP

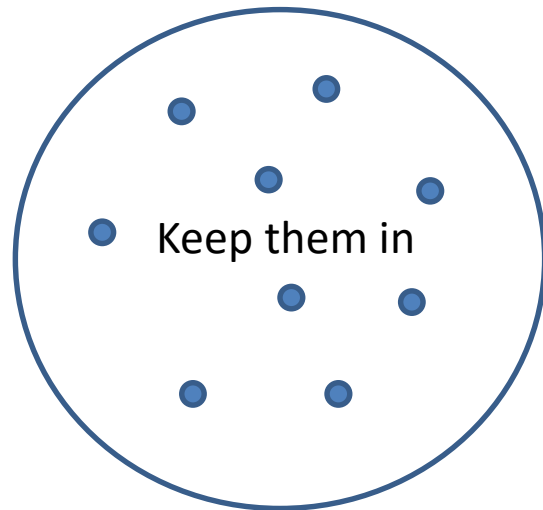
- Protect the operator
- Protect the consumer
- Reproducible results
- Controlled processes
- Documented processes



Biosafety vs GMP

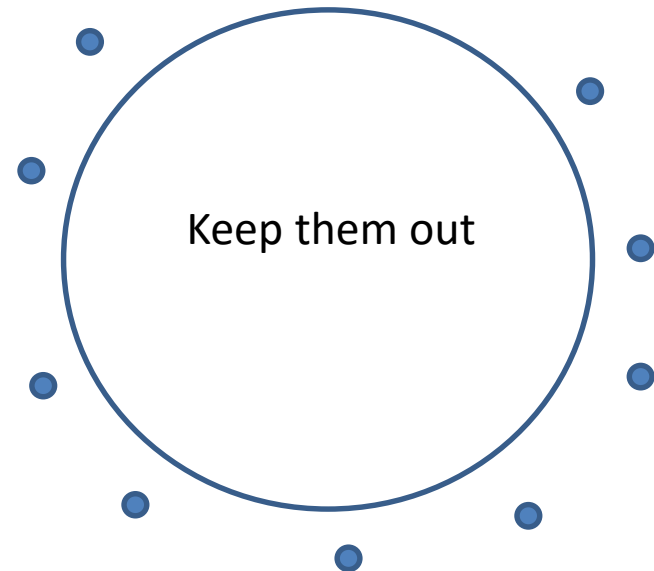
- Biosafety

- Protect the operator



- GMP

- Protect the consumer



GMP

- Enforced by law (CFR and Eudralex)
- Customer:
 - Provide safe products
 - GMP and Eudralex
- Supplier:
 - Eudralex - Volume 4 for equipment
 - Annex 2 and
 - Annex11 (computer systems)
 - 21CFR210 for equipment
 - Part11 electronic signatures
 - ASME BPE design guidelines



Bioprocessing Equipment

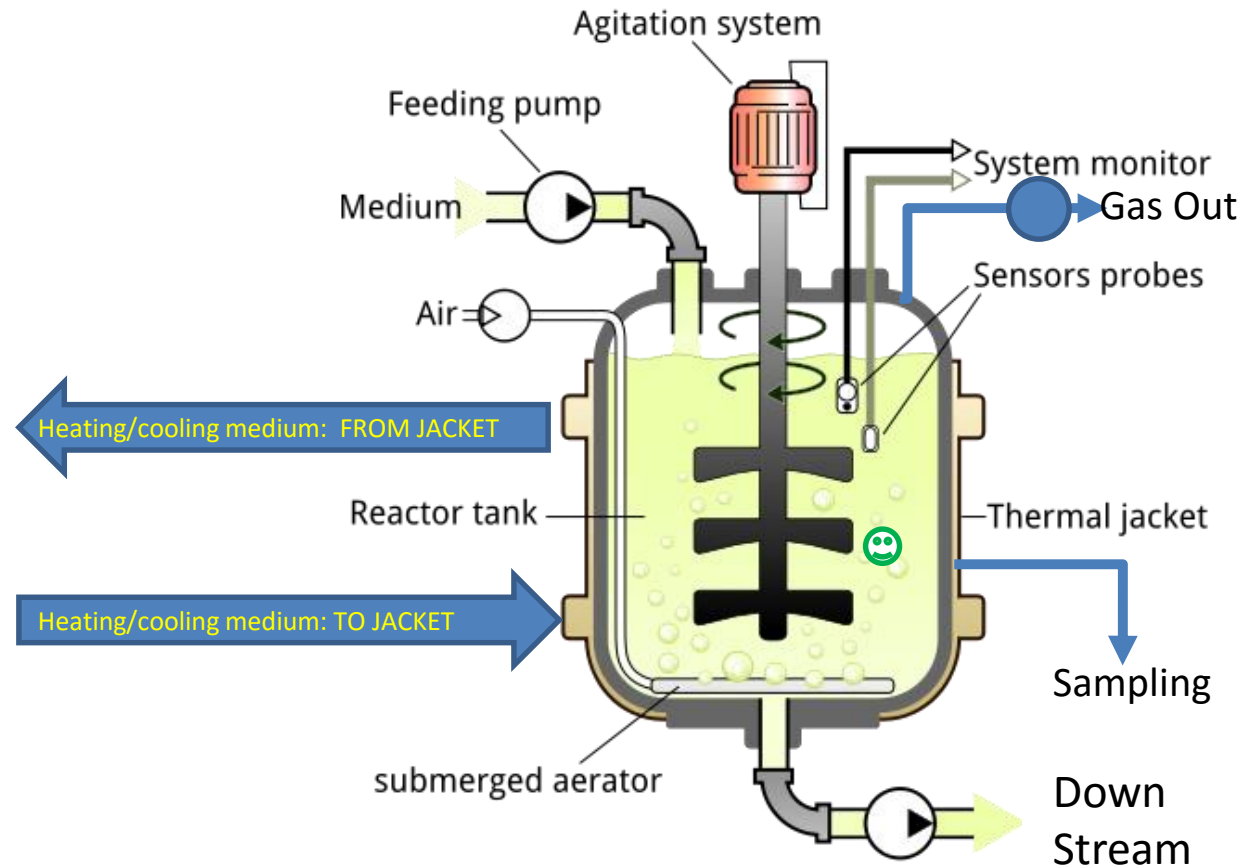


Biosafety

- 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



=Risk Area

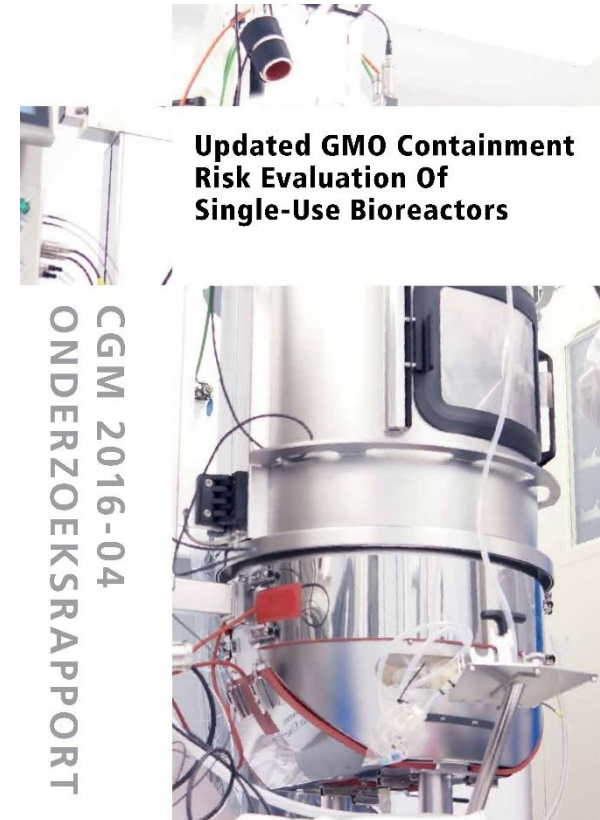
Single-Use or Re-Usable

- 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
- <http://www.cogem.net/index.cfm/en/publications/publication/researchreport-updated-gmo-containment-risk-evaluation-of-single-use-bioreactors?>



Single-Use or Re-Usable

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

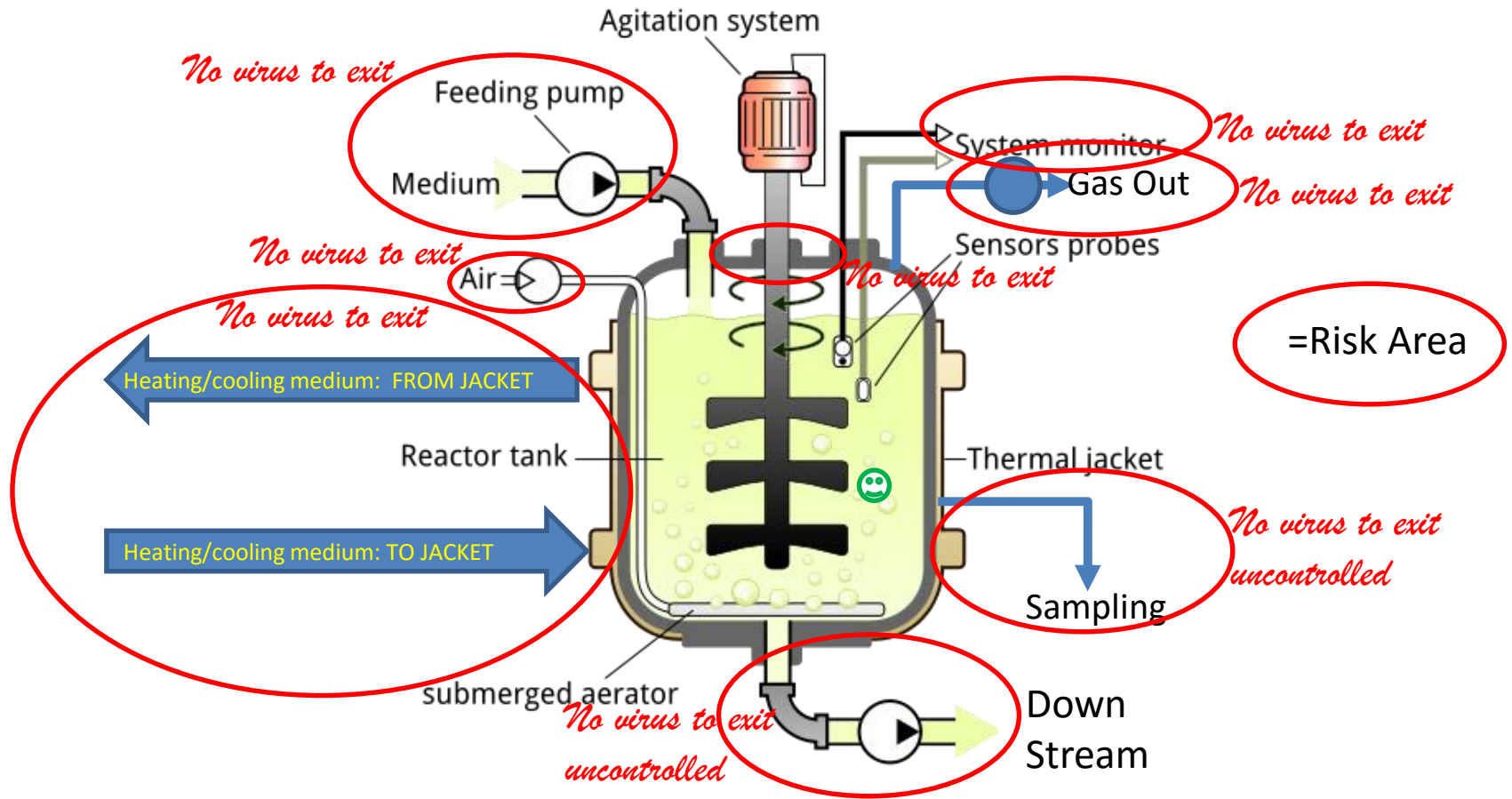


Re-Usable bioreactors

- 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

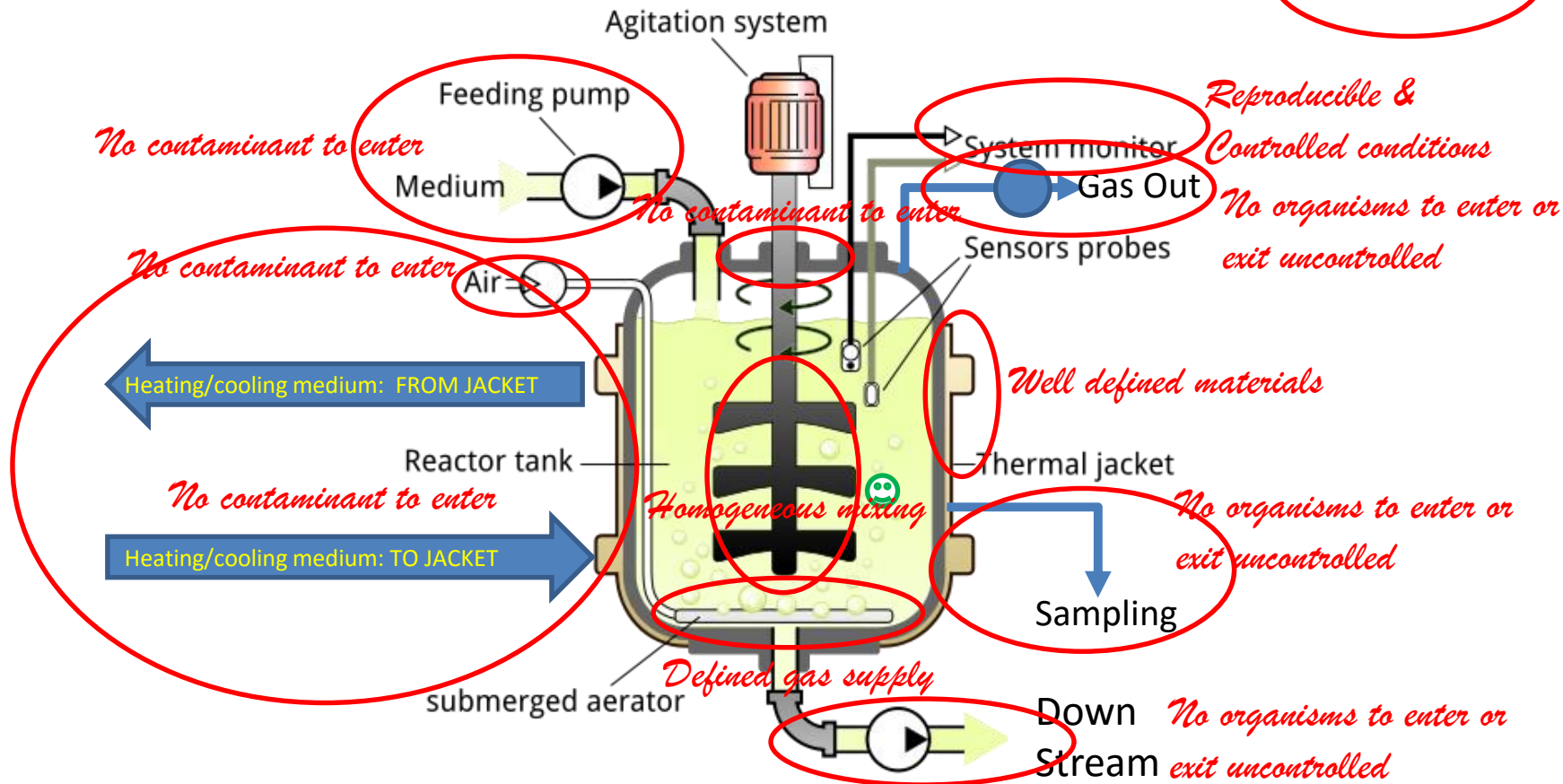


Recap: Bioreactor Biosafety



Recap: Bioreactor GMP

=Risk Area



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

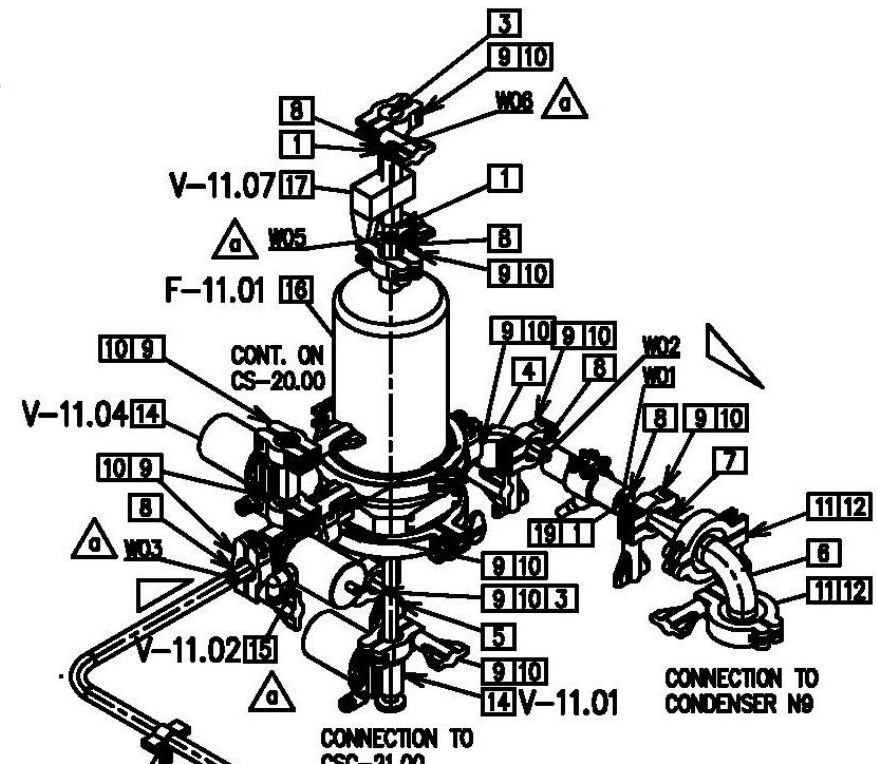


Off-gas Incinerator

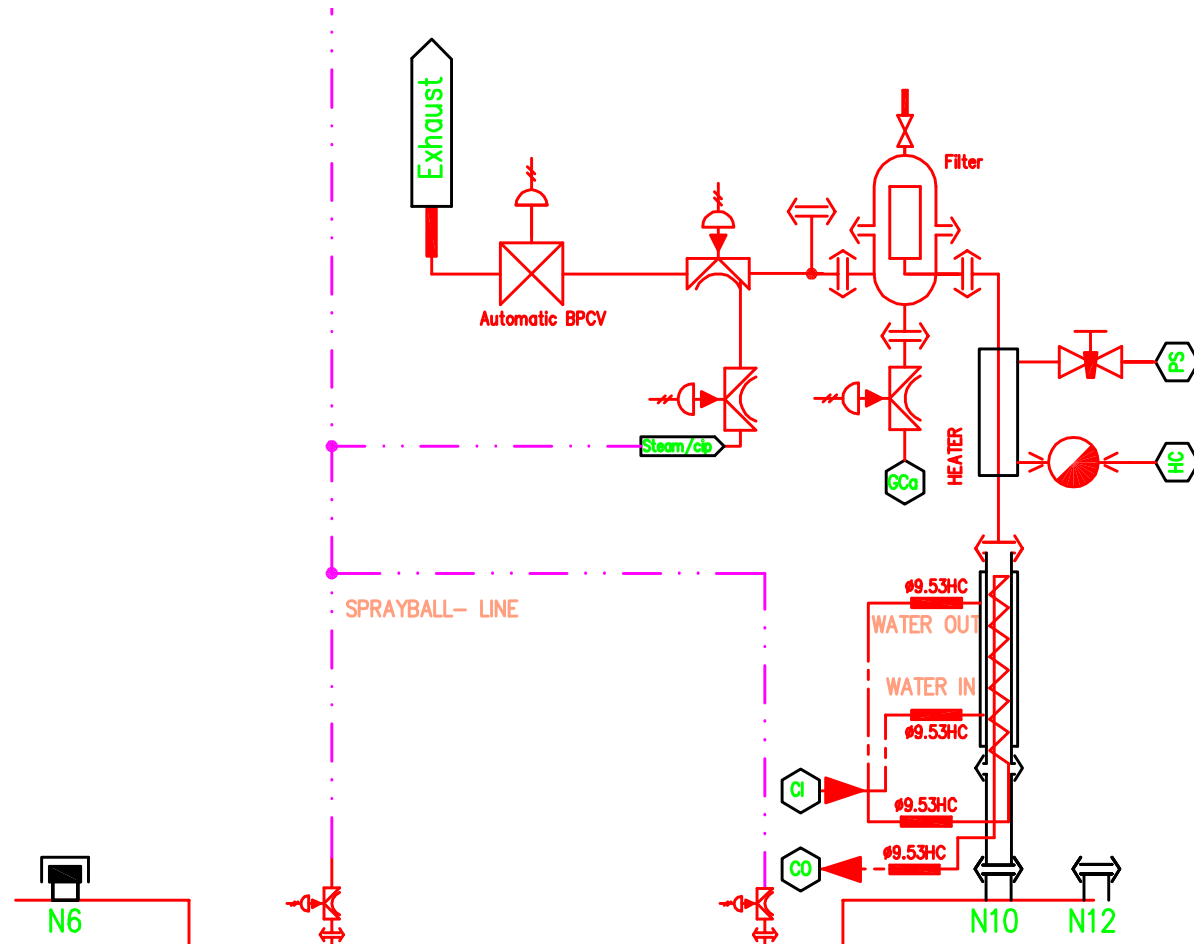
Gas Filters

Exhaust gas filter

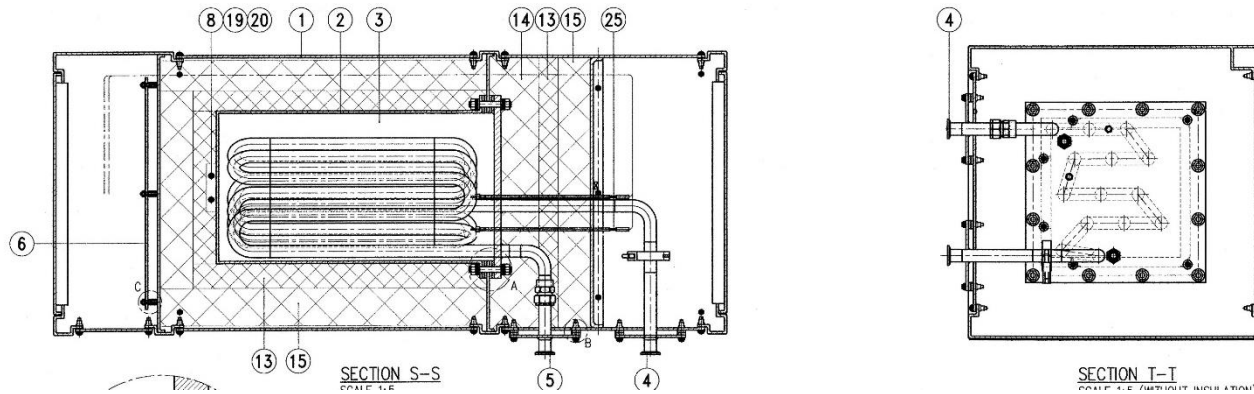
- 0.2 micrometer pore size
- Membrane filter
- Integrity test points
- Test integrity before and after process



Exhaust gas filter



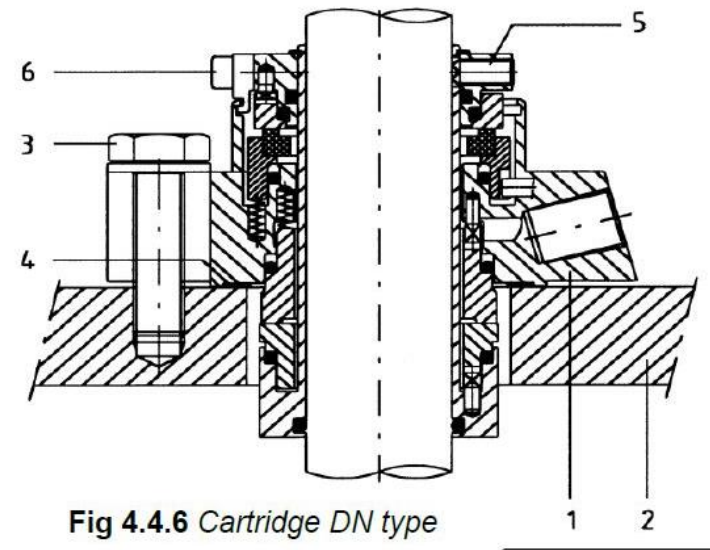
Exhaust gas incinerator



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

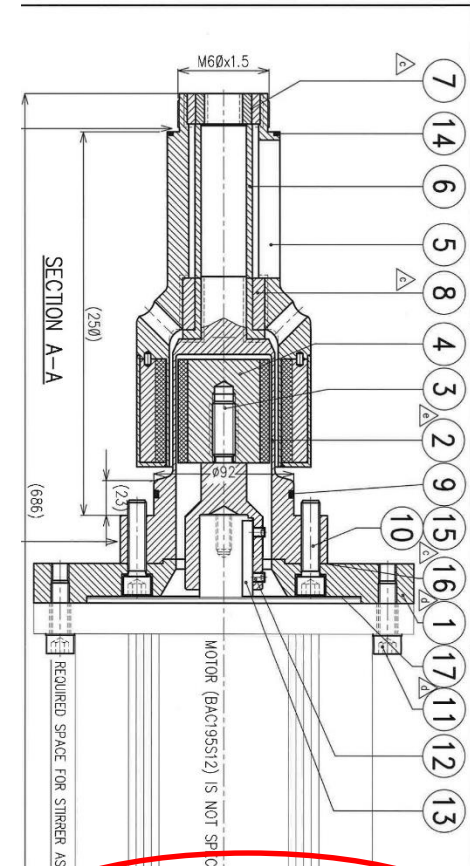
Agitator sealing

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



Agitator sealing

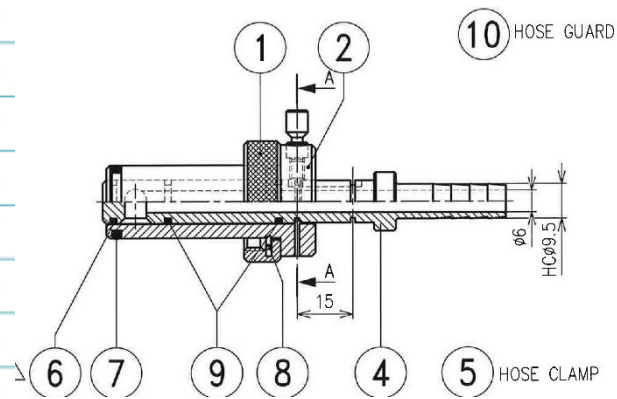
- Magnetic coupling: absolute sealing
- No direct contact between inside and outside of reactor
- Minimal maintenance
- Up to 40 Nm torque
- Cell culture up to 3000 liter volume
- Microbial up to 500 liter volume



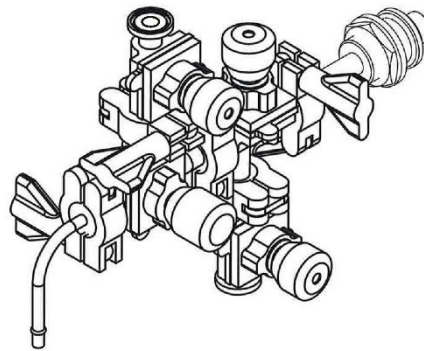
Tipspeed: $\pi * N * D_i$

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

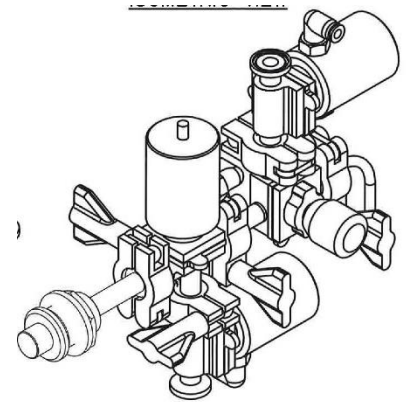
Liquid addition



Manual single shot addition



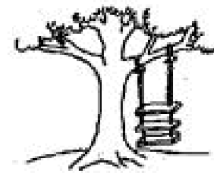
Manual resterilizable addition



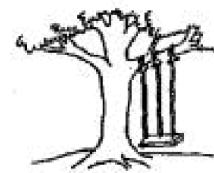
Automated resterilizable addition

Vaccine production scale systems

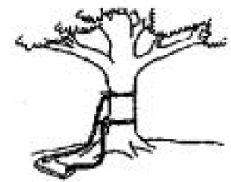
- Projects
 - One time process
 - Custom design
 - Fixed time
 - Fixed budget
- Communication is key!!



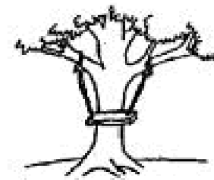
As proposed
by the project
sponsor.



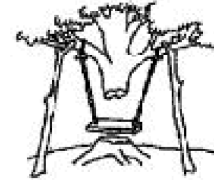
As specified
in the project
request.



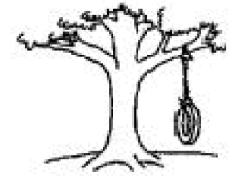
As designed
by the senior
architect.



As produced
by the
engineers.



As installed at
the user's
site.



What the
customer
really wanted.

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



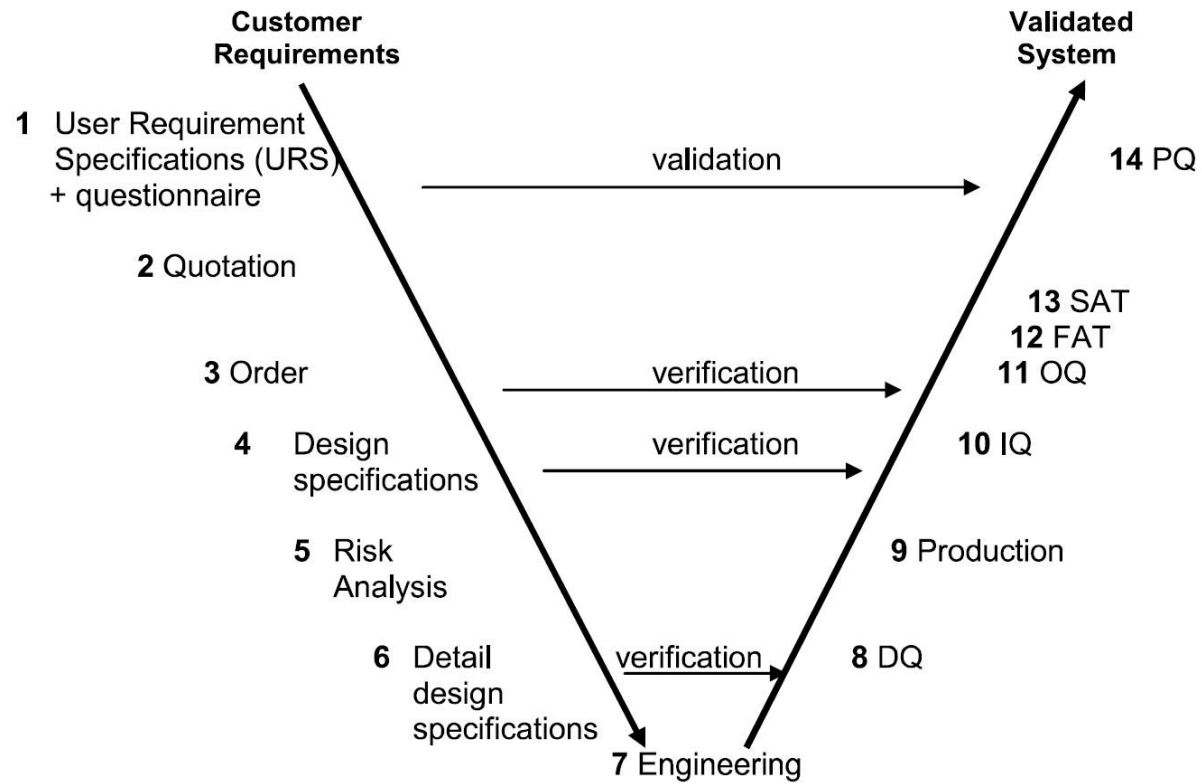
New vaccine production risk management

- 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

BF URS HIB FERM.pdf (BEVEILIGD) - Adobe Reader
Bestand Bewerken Beeld Venster Help
Openen Gereedschappen Invullen en ondertekenen Opmerking

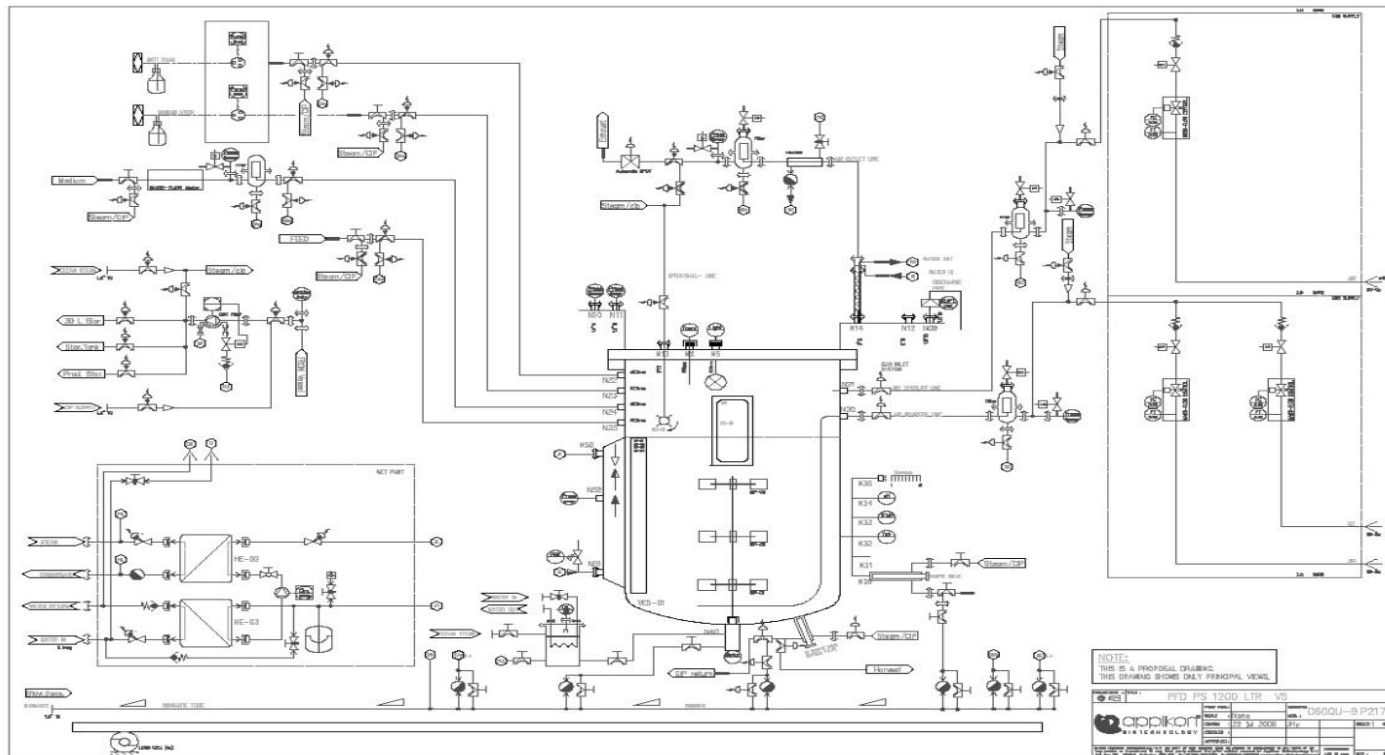
**URS FERMENTOR 1000 L
(GENERAL REQUIREMENTS)**

No.	Item	URS
1	Type	<ul style="list-style-type: none">- 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	<ul style="list-style-type: none">- 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 :<ul style="list-style-type: none">- Included housing filter for medium (housing filter compatible with filter cartridge Pall B1NF7PH4).

The road from specifications to operational product

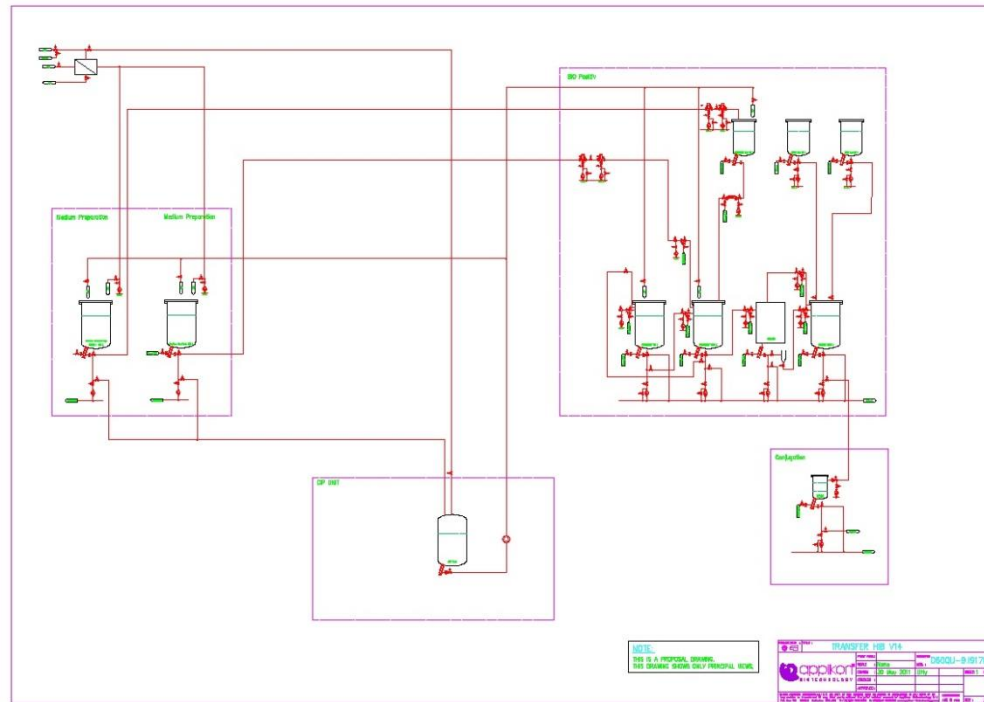
- Create Process Flow Diagram (PFD) and detailed quotation
- Order received
- Create project management website
- Piping and Instrumentation Diagram (P&ID)
- Functional Design Specifications (FDS)

PFD made according URS



Define the
interfaces

Schematic transfer lines



Define the
interfaces

Project management communication

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]
 - **UPDATED** 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]
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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)

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)

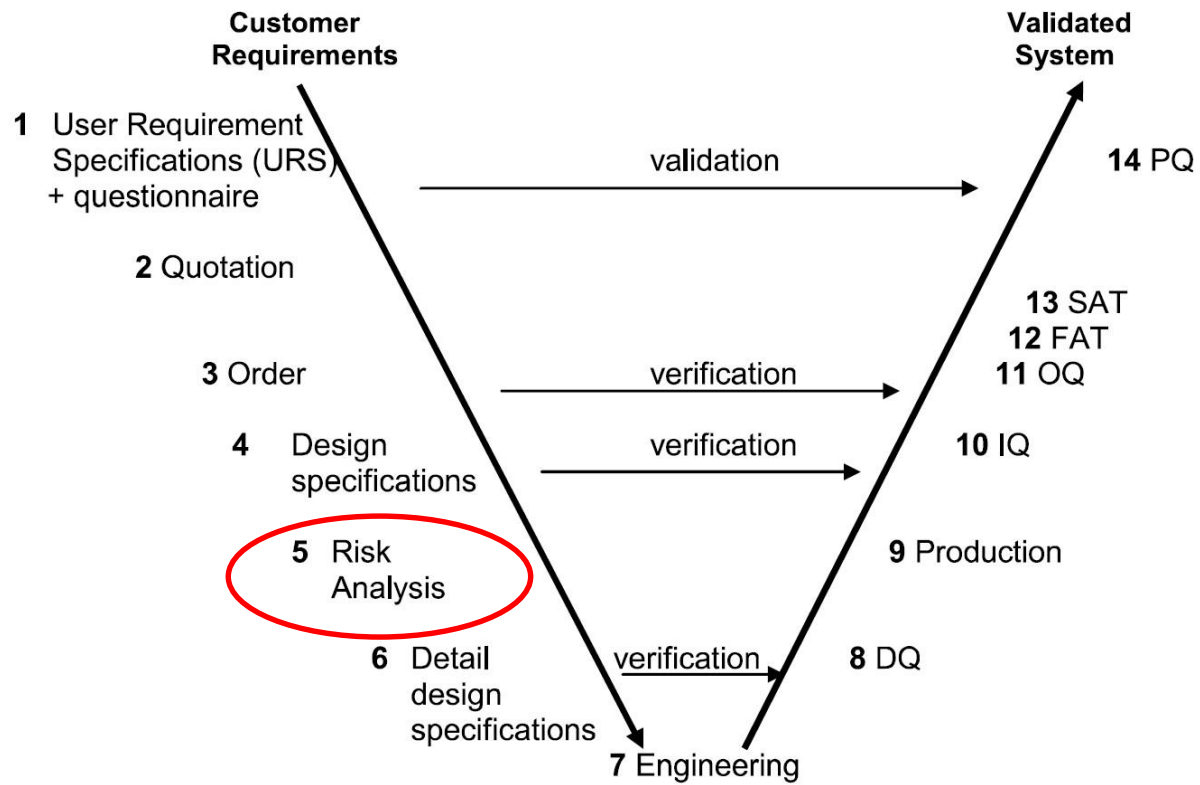
7-Installation / Commissioning (Installation / Commissioning)

- 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]

Project management communication

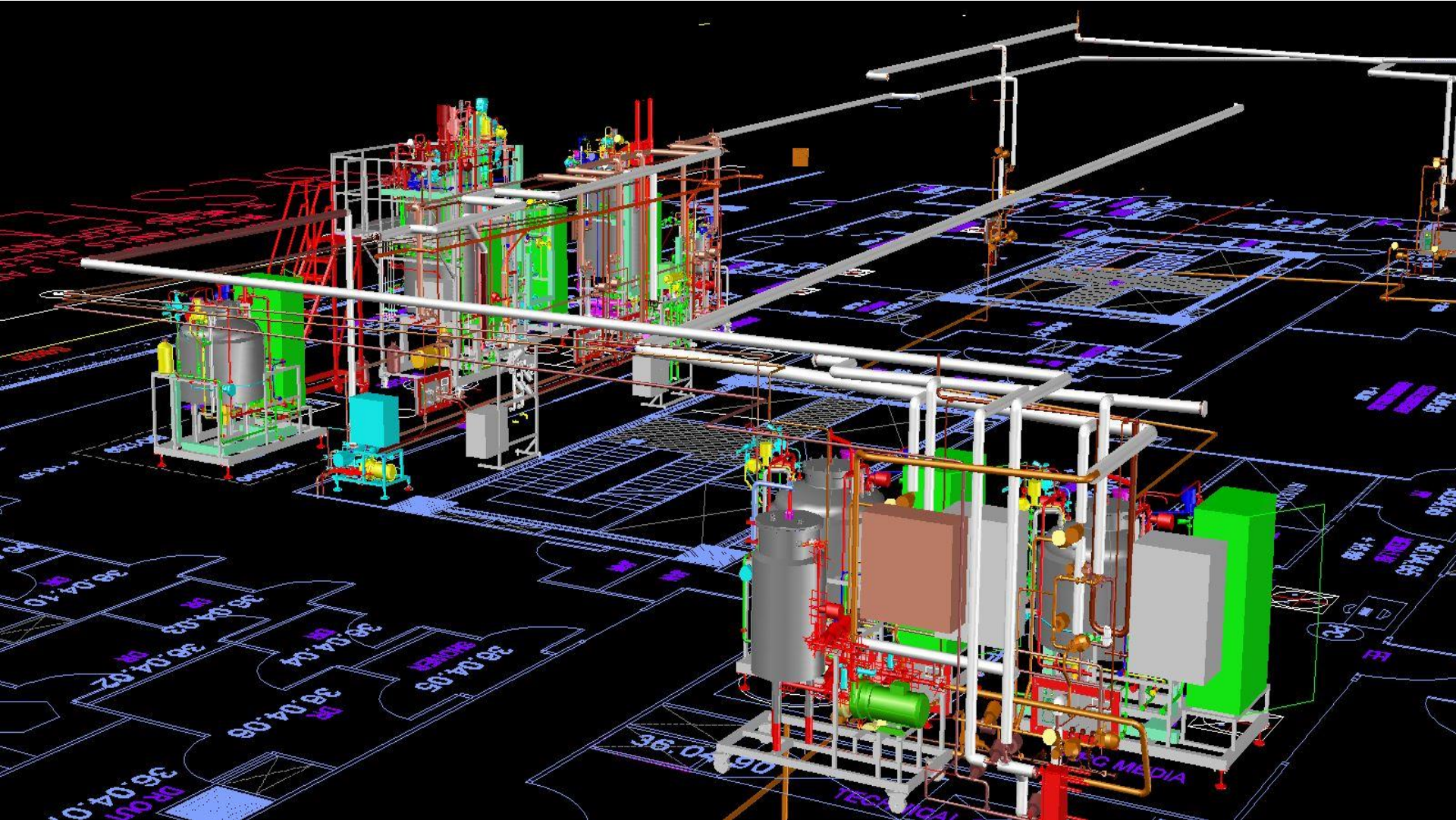
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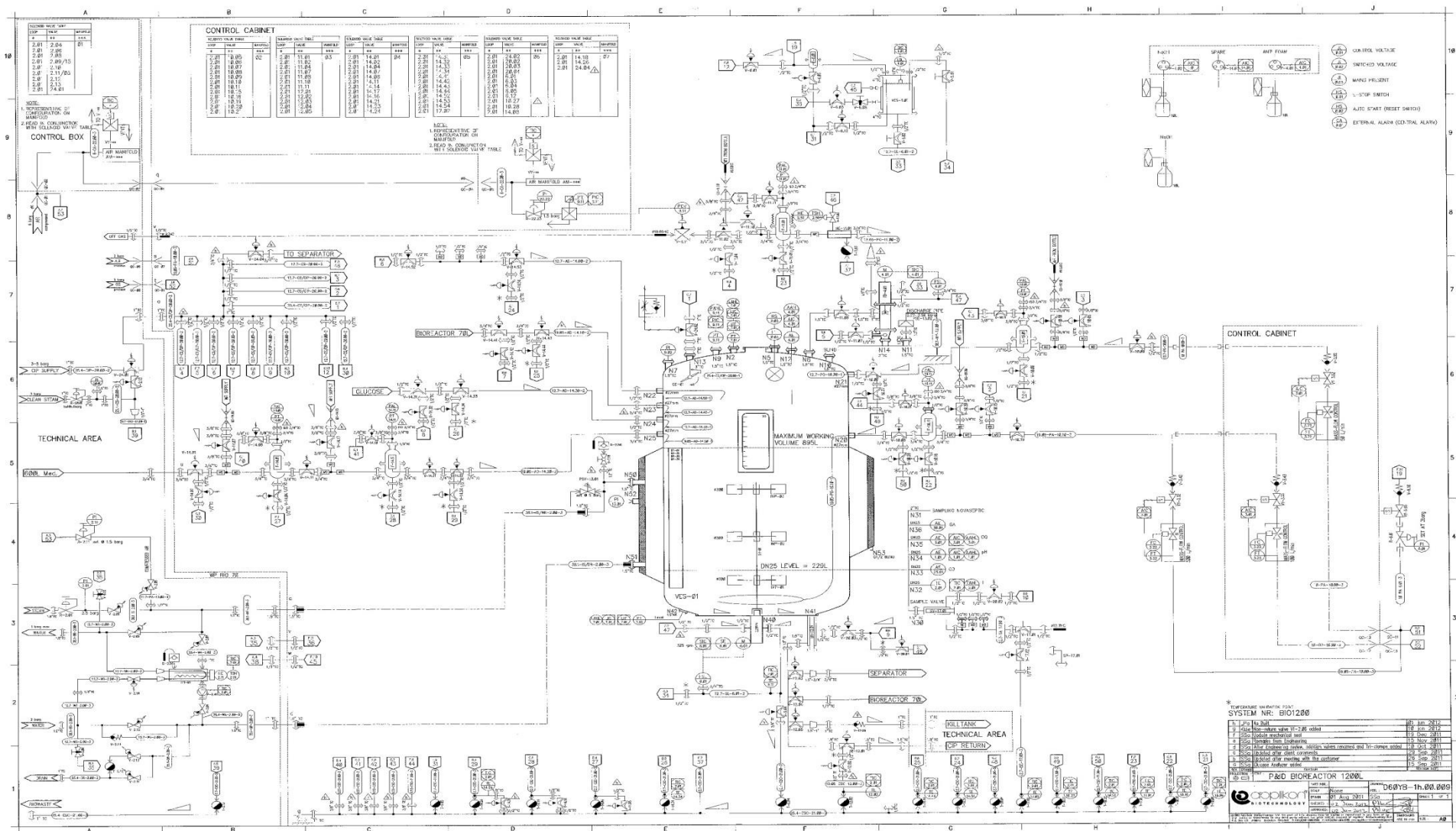
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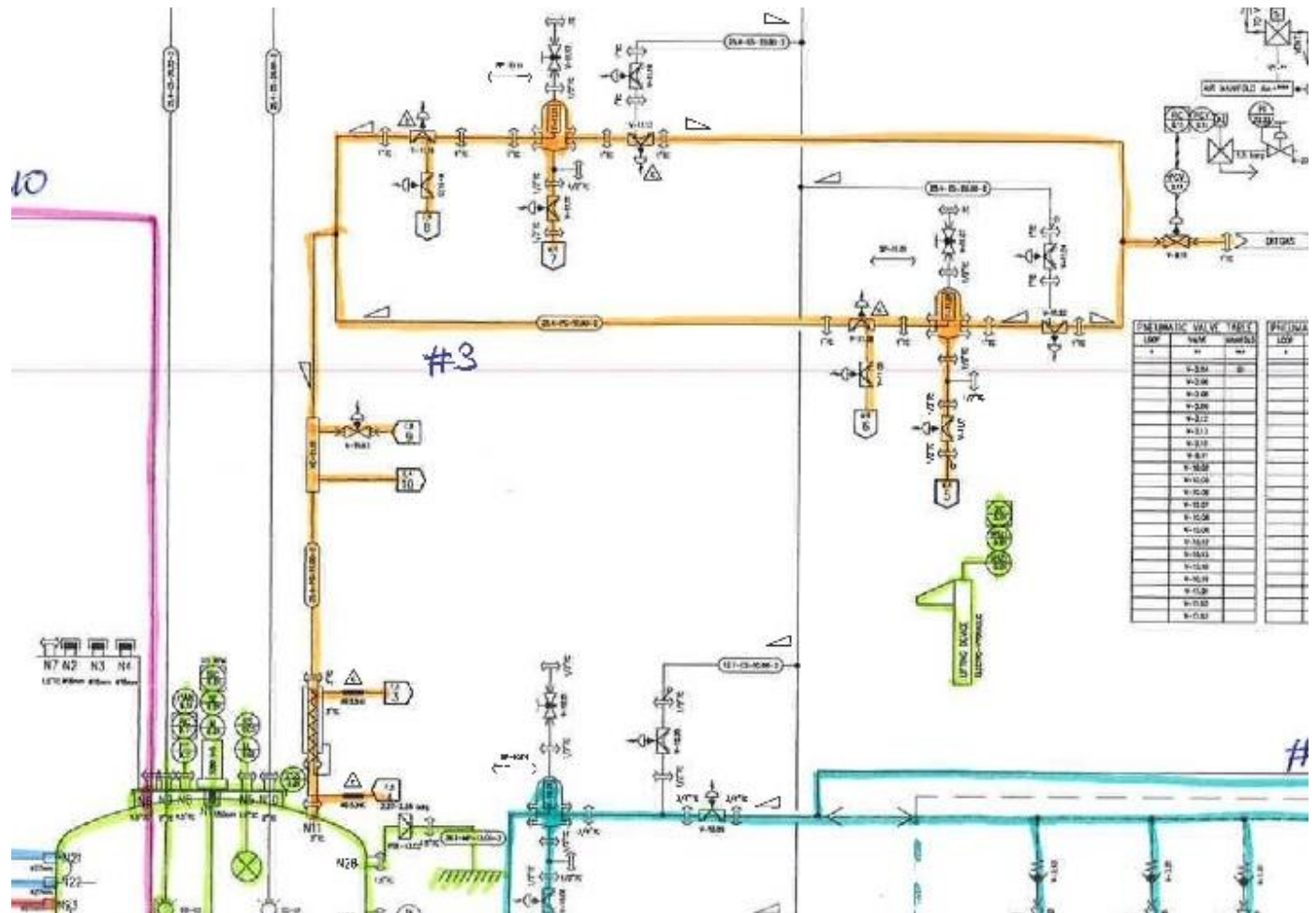


Hazop study

- 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

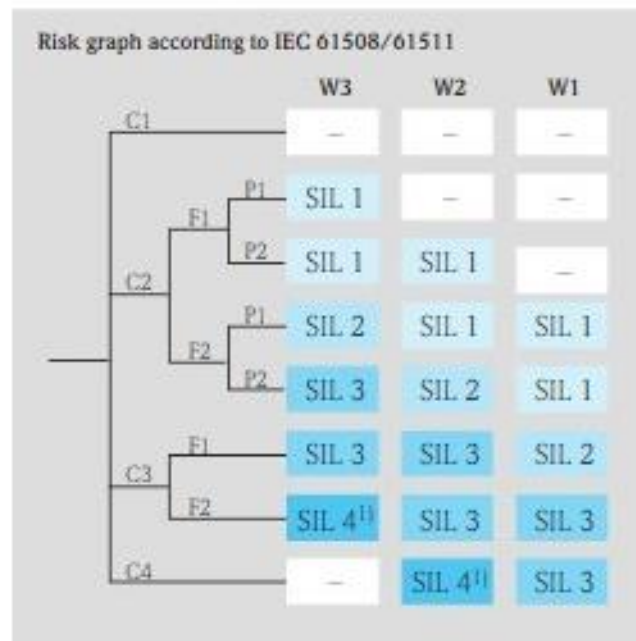
-1 to 3.1 barg
0-150°C
OP: 0.2-0.7 bargSIP: 1.1-1.3 bargCIP: 0-0.2 barg
OP: 25-37°C SIP: 121-125°C CIP: 70-90°C
WV: 706LTV: 1000L
product contact: SS 316L (1.4404); Silicone/ EPDM/ PVDF
CS: 1.5 bargCIP: 4-6 barg 0.4-5 m3/h water with 2% detergent (alkali/acid)Medium out 2: 12L/min 0.5 bargNutrient / 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; steam valve failure; continued fermentation; liquid overflow (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 1 Blocked filter: F-11.X1	Elevated P due to gas supply without venting, leading to pressurize up to 2barg 1 (P setting of R-3.52)	Safety	C1	F2	P1	W2	-	PSE-13.02 @ 2.43barg PSH-9.11 @ 1.5barg PAH-9.11						
1	1	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	PSE-13.02 @ 2.43barg PAH-9.11 BSL2 (max), no permanent injury 1 expected	C1	F2	P1	W2	-	
1	2 PT-9.11 fails low closing V-9.11	Increase of P beyond 3.1barg by continued cultivation Possible operator exposure due to rupture of silicone tubing of additions. Increased pressure to max 1barg. No P	Safety	C2	F2	P1	W2	SIL1	PSE-13.02 @ 2.43barg BSL2 (max), no permanent injury expected	C1	F2	P1	W2	-	

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.



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

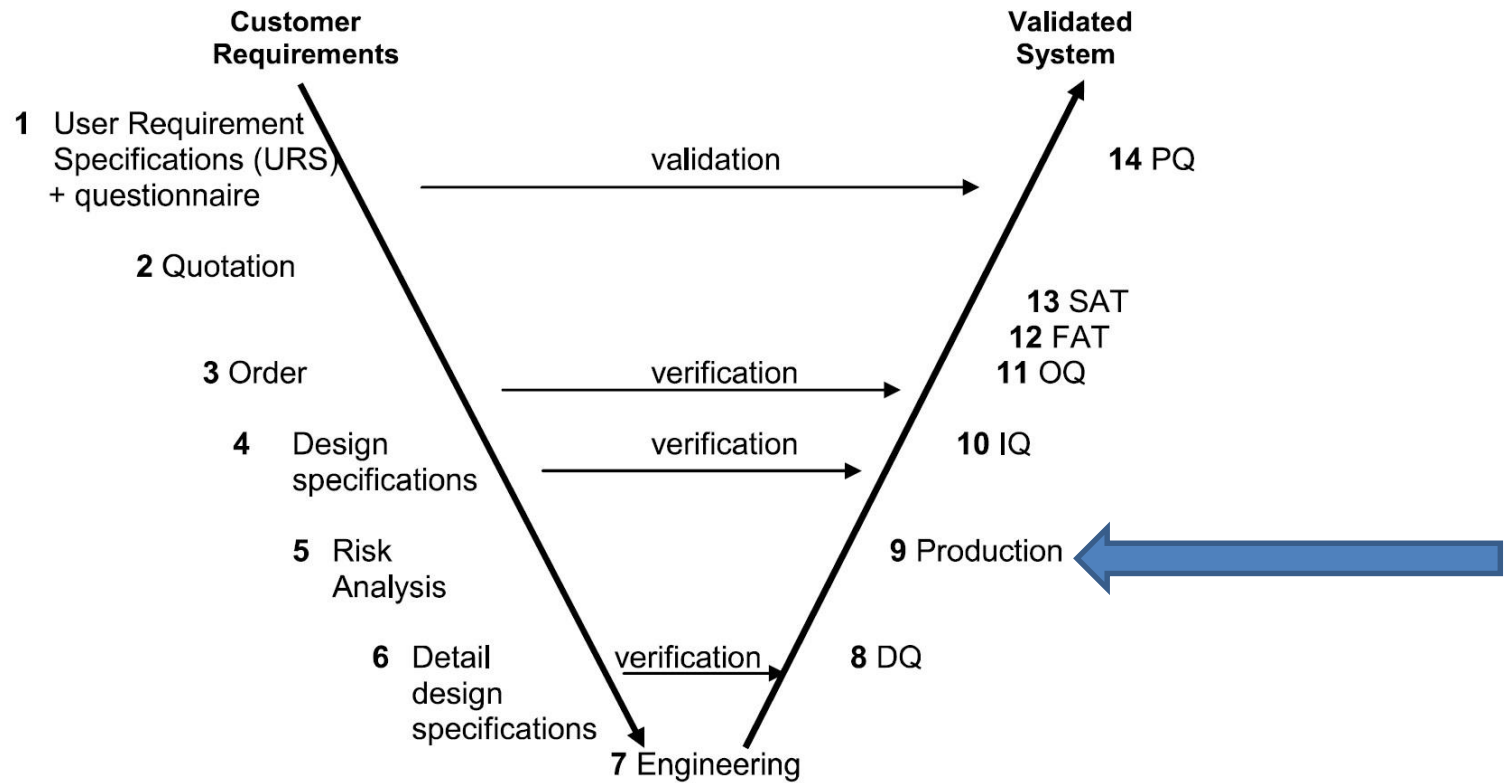
- P1 possible under certain circumstances
- P2 almost impossible

Probability of unwanted occurrence

- W1 very slight
- W2 slight
- W3 relatively high

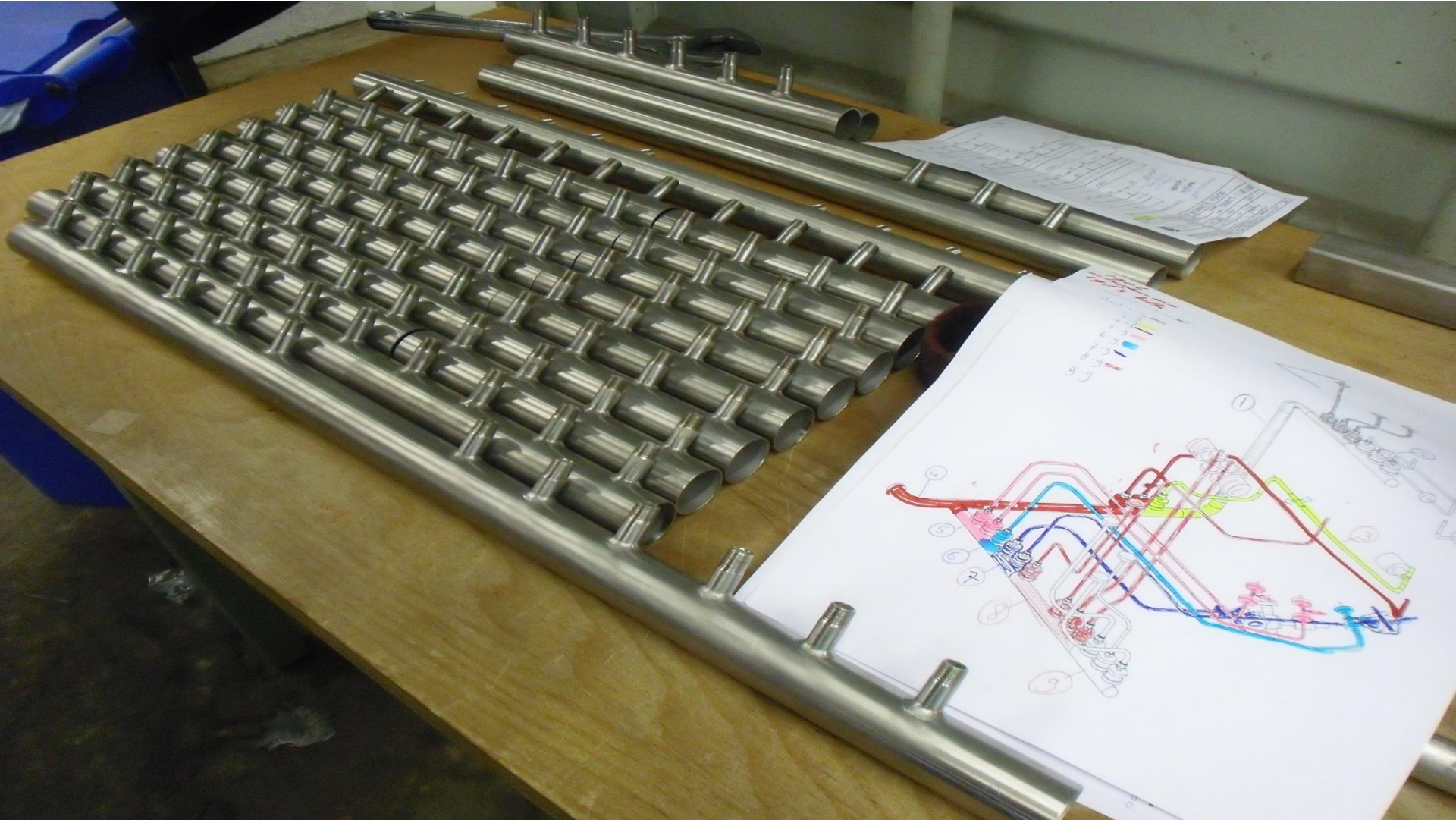
Hazop actionlist

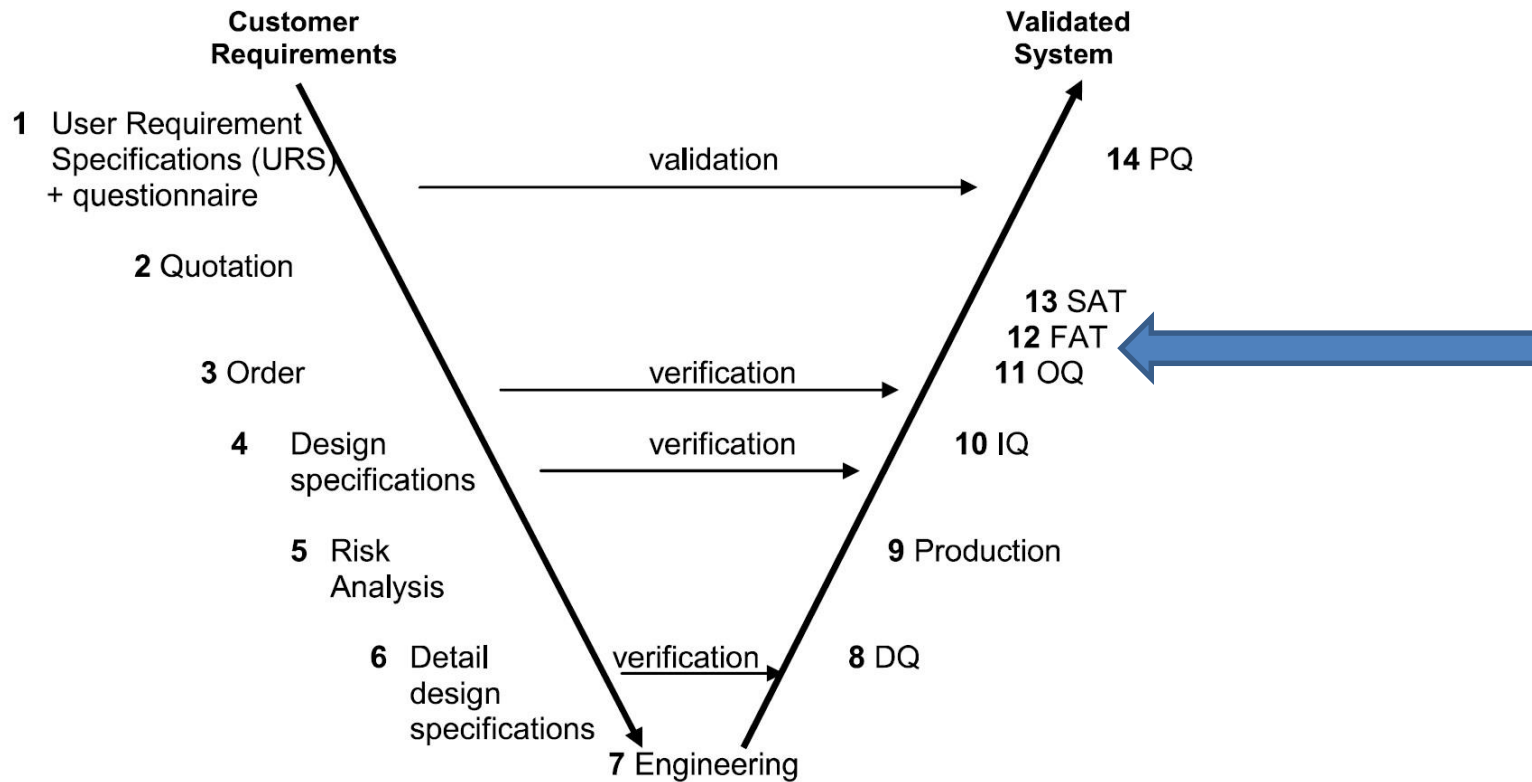
		prior to use the equipment.	
3	1.11.1.1.	Do not use chlorides in the vessel if $T > 50^{\circ}\text{C}$. In case chlorides are required, do not perform full sterilization with chlorides present, sterilize this feed in a separate vessel. Describe appropriate use in SOP.	Customer
4	1.12.1.1.	Unable to 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









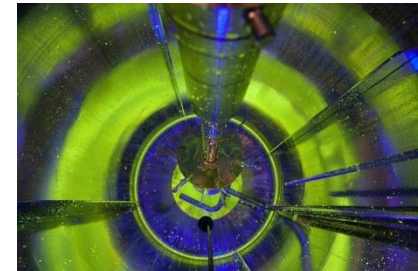




Factory Acceptance Test

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

applikon [®] BIOTECHNOLOGY		FAT PROTOCOL CONTROL LOOPS		NO: FAT/HARVEST 1000, Control loops	
		Version: 1. Approved		1 (1/3)	
		Page: 1		Order # 310151	
				Syst.: HARVEST 1000	
<hr/>					
Site : HARVEST 1000 with i Control (Siemens)					
System ref.: 310161					
Applikon ref.: FAT.HARVEST 1000.Control loops A1.doc					
Document ref.:					
<hr/>					
SIGNING SECTION FOR PROTOCOL REVIEW					
Project management					
Name : 					
Function : PROJECT MANAGER					
Signature : Approved by: P. den Hartog					
Date : 23 Dec 2012					
<hr/>					
SIGNING SECTION FOR PROTOCOL RELEASED					
Quality control department					
Name : 					
Function : QUALITY CONTROL DEPARTMENT					
Signature : Manager Quality Control/Quality Assurance					
Date : 23 Dec 2012					
<hr/>					
SIGNING SECTION FOR PROTOCOL APPROVED					
After this protocol is filled in completely and all defect list items are recorded, the approval section can be signed.					
The protocol is :					
<input type="checkbox"/> accepted without remarks					
<input type="checkbox"/> accepted with remarks (see defect list)					
<input type="checkbox"/> not accepted. Modifications and re-test are required					
<hr/>					
Quality control department					
Name :					
Function :					
Signature :					
Date :					



Transport



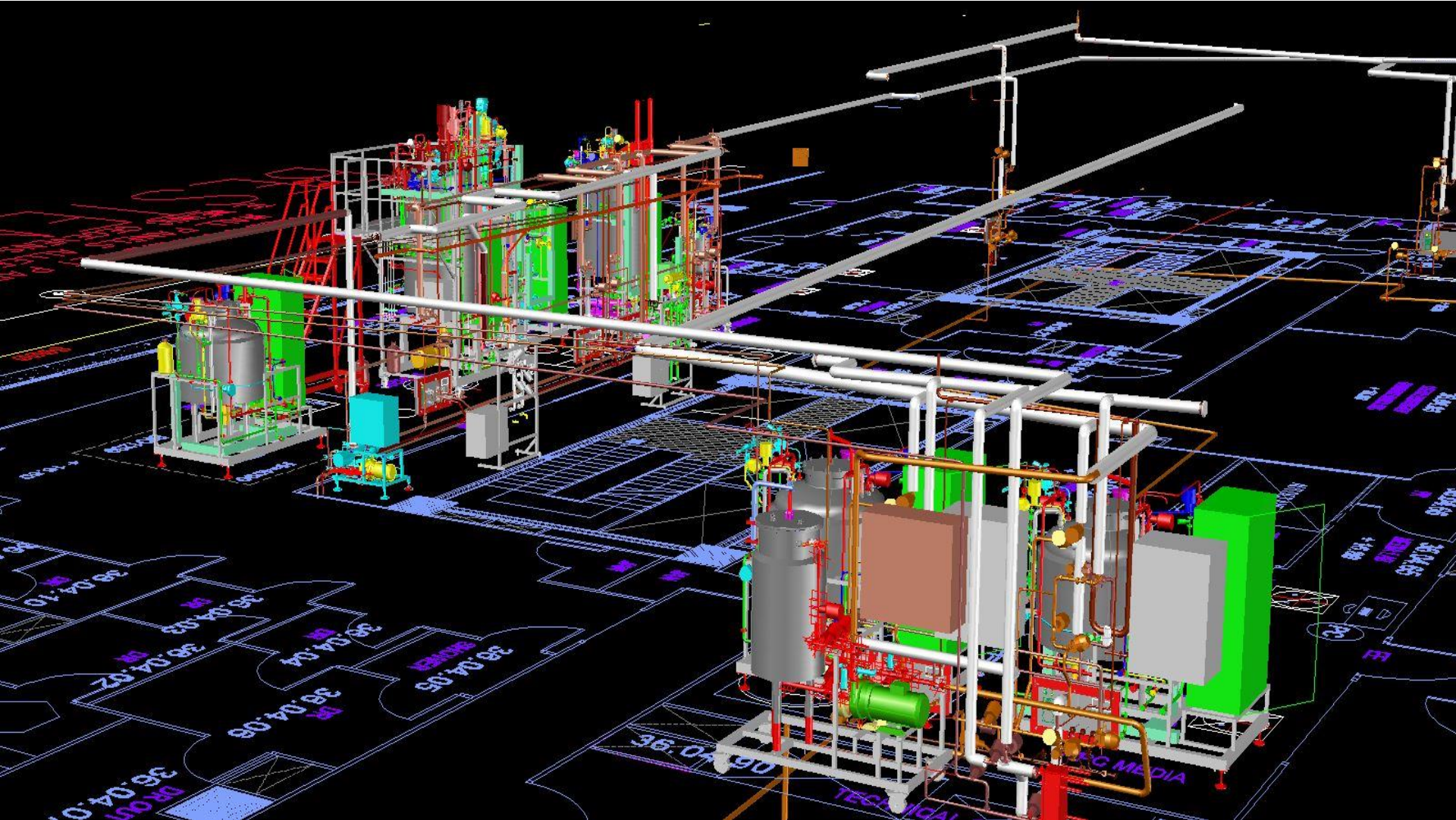


Arrival and unpacking

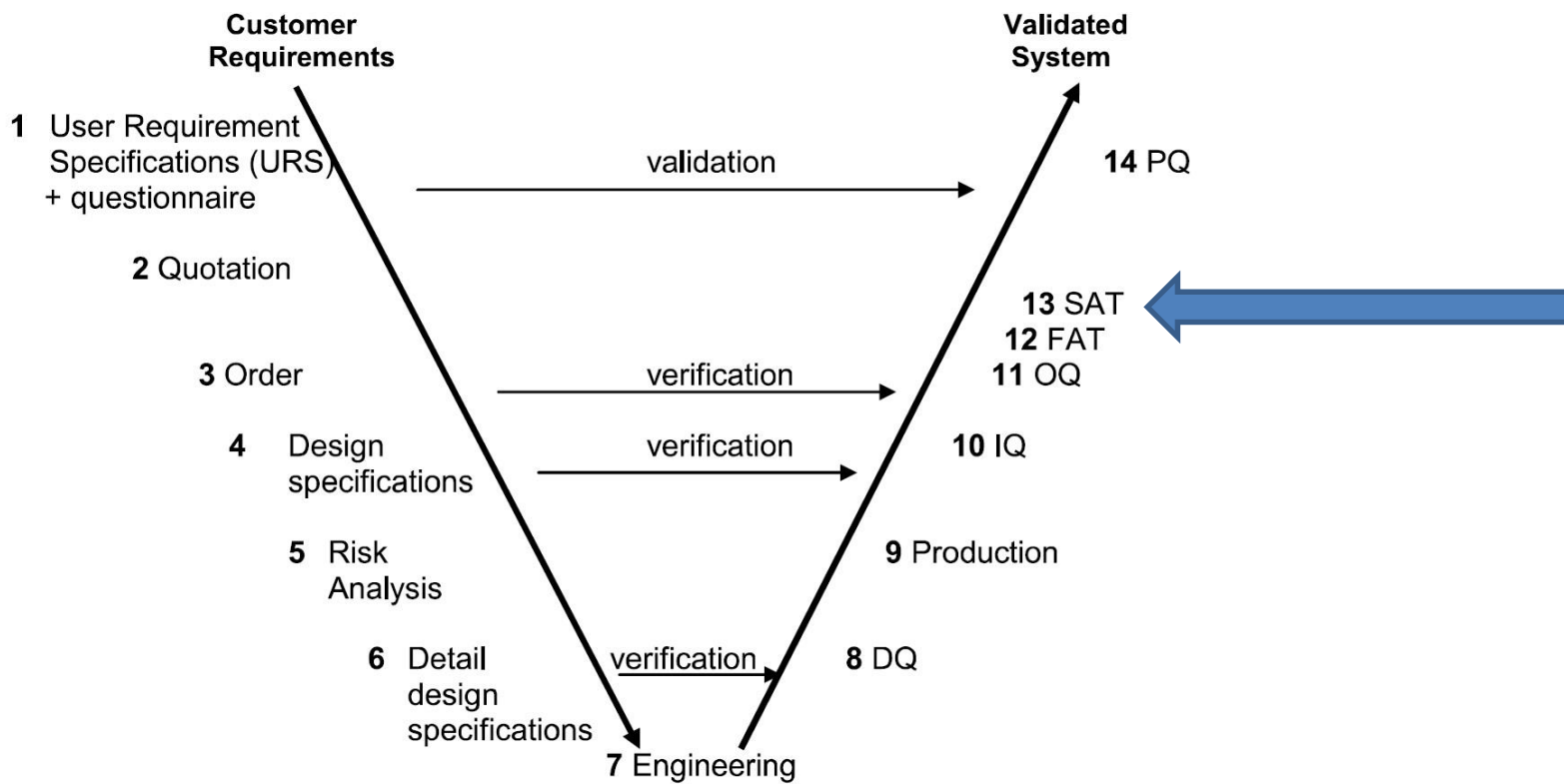


Installation








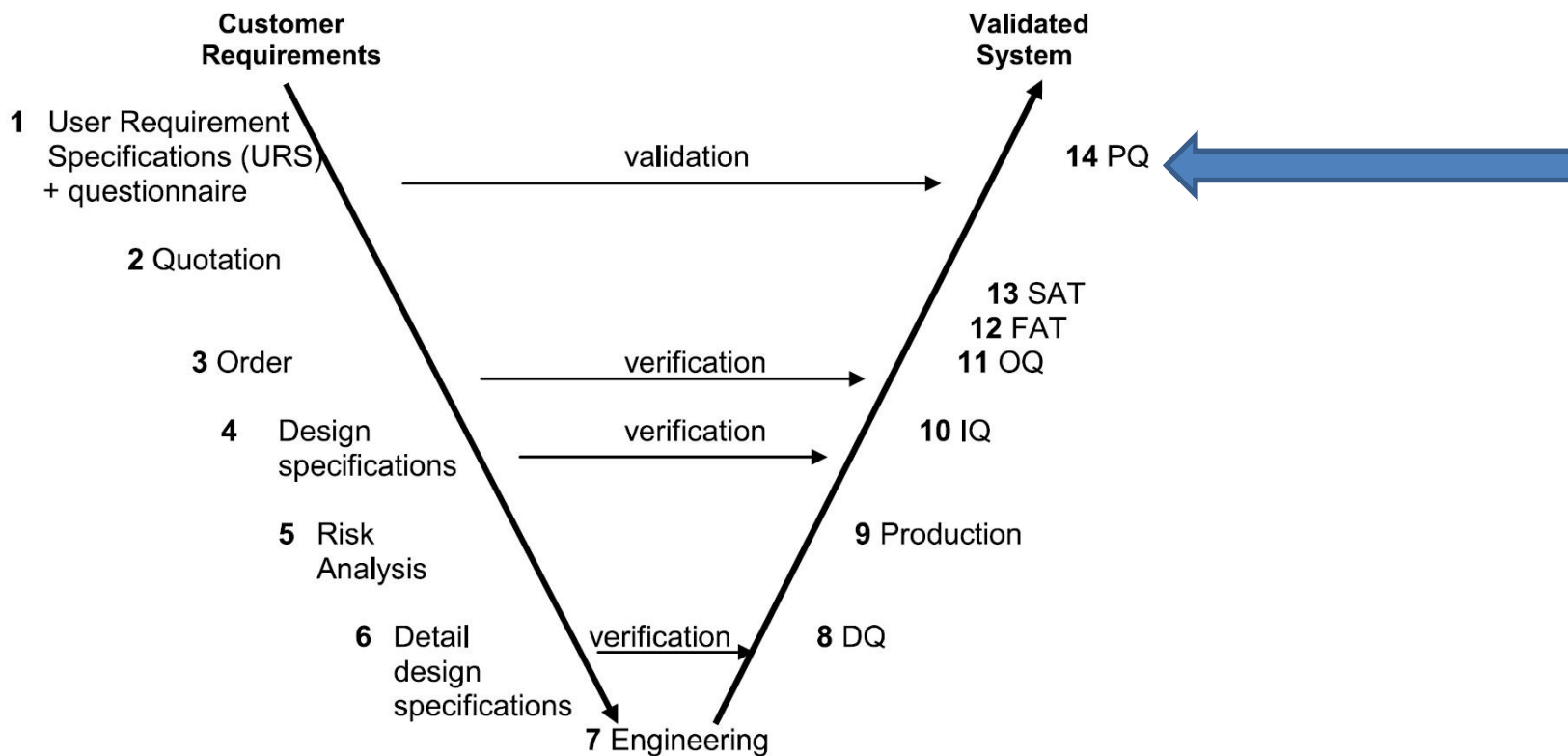




Site Acceptance Test

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

		SITE ACCEPTANCE PROTOCOL CONTROL LOOPS		NO: SAT-PQ-001/PM/0550 Control: eggs	
				Version: A1	
				Page: 1 (14)	
				Order #: 313262 Sys.: BIO550	
Customer information: Customer : BIO550 with i Control (Siemens) System ref. : 310262 Applikon ref. : SAT.P5550.12903805B Control loops A1 Document ref. :					
SIGNING SECTION FOR PROTOCOL REVIEW					
Project management Name :  Function : PROJECT-MANAGER Signature : Approved by: P. den Hartog Date : 01 May 2012					
SIGNING SECTION FOR PROTOCOL RELEASED					
Quality control department Name :  Function : QUALITY CONTROL DEPARTMENT Signature : Approved by: L. Van Bruggen Date : 14 May 2012 Quality Control Engineer					
SIGNING SECTION FOR PROTOCOL APPROVED					
After this protocol is filled in completely and all defect list items are recorded, the approval section can be signed.					
The protocol is : <input type="checkbox"/> accepted without remarks <input type="checkbox"/> accepted with remarks (see defect list) <input type="checkbox"/> not accepted. Modifications and re-test are required					
Quality control department Name : Function : Signature : Date :					



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!



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BIOTECHNOLOGY

Type: Pilot System

EQUIPMENT No: U6LU51000