



Risk of contamination through pinholes in gloves and how to prove the integrity

ISPE Nordic, Denmark

Yves Scholler

SKAN AG

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Agenda

- 1 Regulatory Requirements
- 2 Overview of Different Testing Methods
- 3 Microbial contamination risk of leaky gloves
- 4 Requirements for a Modern Glove Testing Unit
- 5 Conclusion

Regulatory Requirements



Regulatory Requirements

PIC/S (Aseptic processing isolators)

- “**A program** to minimize the risk of loss of integrity of gloves, sleeves and suits **should be present**.”
- This should include **operator practices, vigilance and the absence of sharp edges.**”

Regulatory Requirements

FDA (Sterile drug products produced by aseptic processing)

- "A breach in glove integrity can be of serious consequences."
- "A breach of isolator integrity should normally lead to a decontamination cycle."
- **"Integrity can be affected by.. holes in gloves.. or other leaks."**
- **"Breaches in integrity should be investigated."**
- "If it is determined that the environment may have been compromised, any product potentially impacted by the breach should be rejected."

Regulatory Requirements

FDA (Sterile drug products produced by aseptic processing)

- “A fault glove or sleeve (gauntlet) assembly represents a route of contamination and a critical breach of isolator integrity.
- With any use **gloves should be visually evaluated** for any macroscopic physical defect.
- **Physical integrity tests should be performed routinely.**
- The **monitoring and maintenance program** should identify and eliminate any lacking integrity.”

Regulatory Requirements

FDA (Sterile drug products produced by aseptic processing)

- “Due to the potential for microbial migration to microscopic holes in gloves and the lack of highly sensitive glove integrity tests, we recommend affording attention to the sanitary quality of the inner surface of the installed glove and to **integrate the use of a second pair of glove.**”

Regulatory Requirements

USP 30* <1208> validation of isolator systems

- “Gloves are another likely source of microbial contamination.
...
• **Very small leaks in gloves are difficult to detect until the glove is stretched during use.**
- There are several commercially available glove leak detectors; the operator ensures that the detectors test the glove under conditions as close as possible to actual use conditions.
- **Microbiological tests are used to supplement or substitute physical tests.”**



Different Testing Methods Overview

Background

- Cooperation of Novartis Pharma AG and SKAN AG
- Diploma Thesis of Angela Gessler
- Followed by additional evaluation and studies finalized in 08/2005

Technology/Application

How Risky Are Pinholes in Gloves? A Rational Appeal for the Integrity of Gloves for Isolators

ANGELA GESSLER^{1*}, ALEXANDRA STÄRK², VOLKER SIGWARTH¹, and CLAUDE MOIRANDAT³

¹*Skan AG, Allschwil/Switzerland, ²Novartis Pharma AG, Stein/Switzerland, and ³C. Moirandat Dienstleistungen, Basel/Switzerland* ©PDA, Inc. 2011

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Content of the Study

- Comparison of different physical methods for glove integrity testing
 - Realistic bioload of gloves used during routine work on isolator
 - Microbial contamination risk of leaky gloves used during routine work on isolators
 - A founded rational for justification of the impact of a leaky glove on product during routine production and testing
-
- Published 2011, PDA Vol. 65 no. 3

Materials and Methods

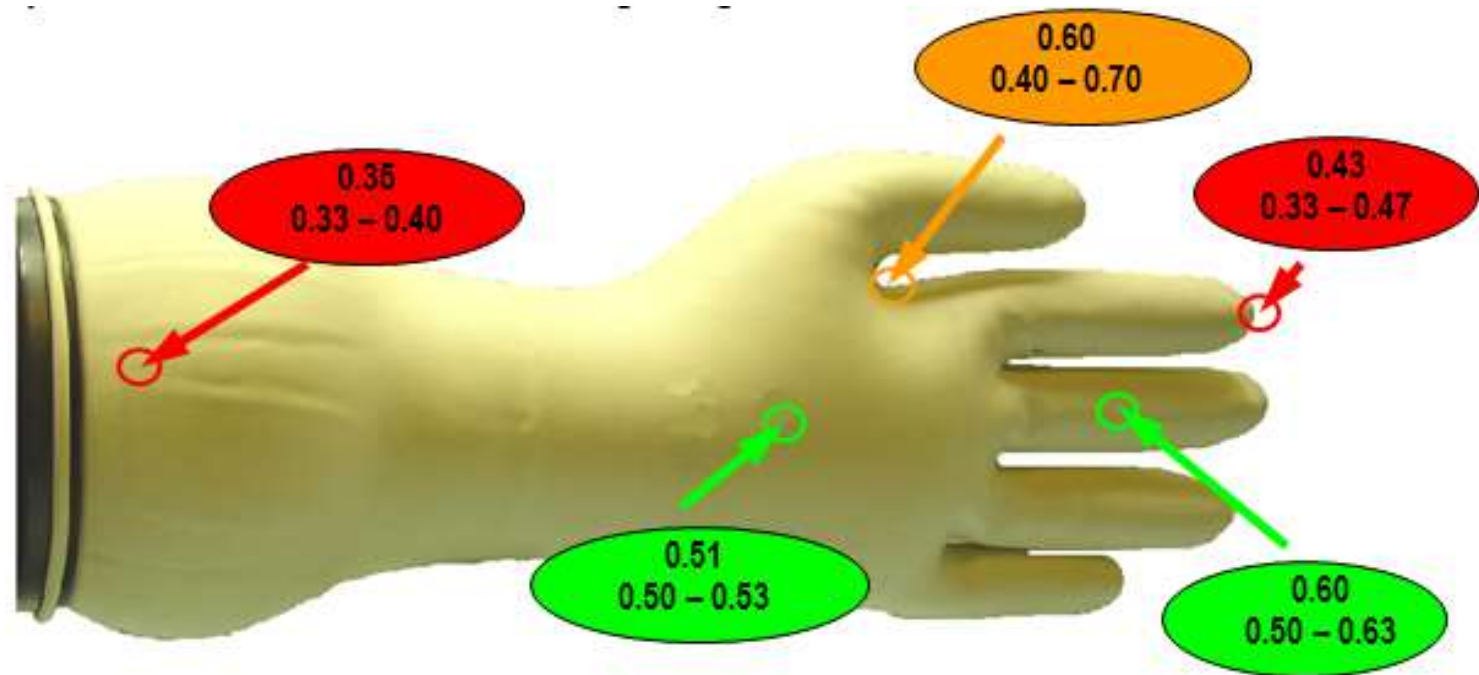
Gloves

- Specification: Material Hypalon Thickness 0.4 [mm]



Materials and Methods

- Measurement of Material Thickness [mm]
- Specified value: 0.4 [mm]
- Mean value and range out of 12 samples

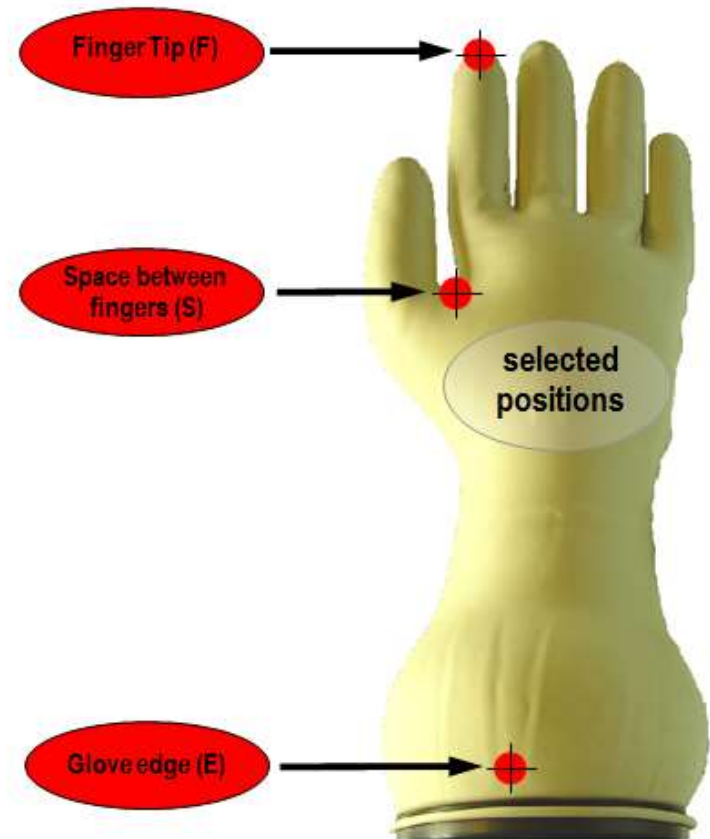


Materials and Methods

Definition of leak positions

Selection based on:

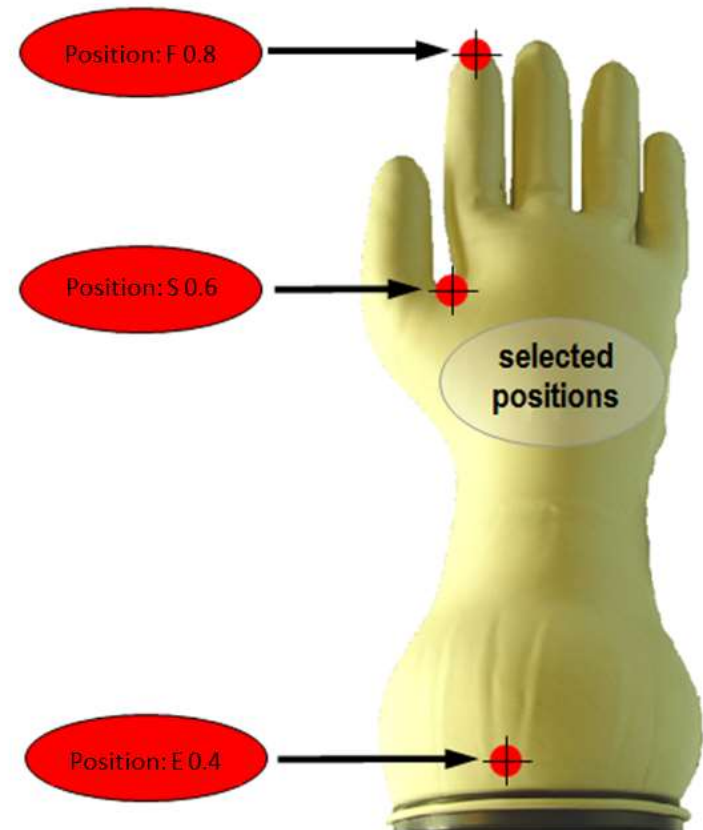
- Position with **frequent leaks** during production
- Position of **thin glove material**
- Position with a **high risk of contamination**



Materials and Methods

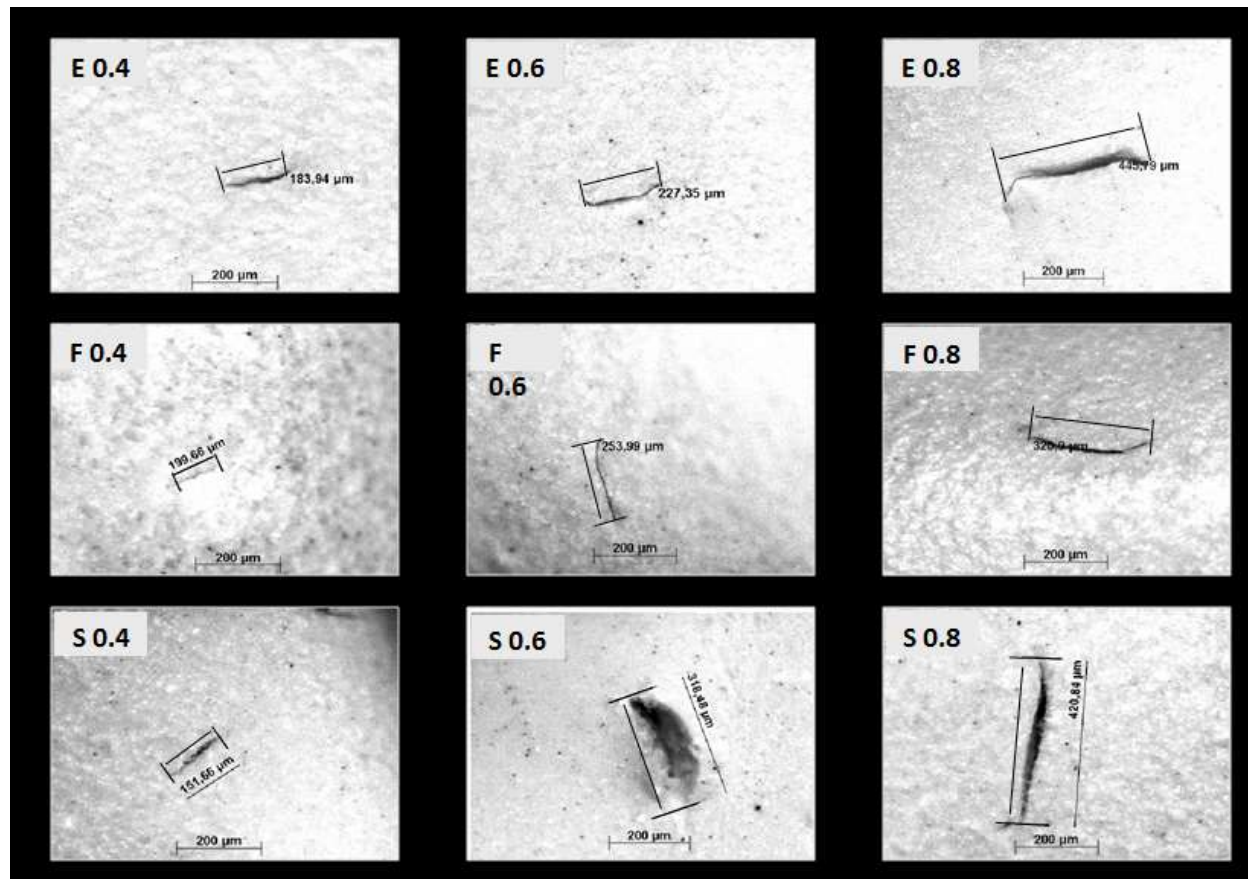
Preparation of Leaks

- Leaks prepared using syringe needles
 - $\varnothing = 0.4$ [mm]
 - $\varnothing = 0.6$ [mm]
 - $\varnothing = 0.8$ [mm]
- 3 gloves prepared per position and leak \varnothing
- 3 additional tight gloves as reference



Materials and Methods

Leak Preparation



Materials and Methods

Resulting leak size microscopically measured and investigated

TABLE I

Summary Statistic of Pinhole Size after Perforation with Three Different Needle Sizes

	Count	Average (mm)	Standard deviation (mm)	Coefficient of variation	Minimum (mm)	Maximum (mm)
Leak_04	9	0.160	0.032	20.1%	0.101	0.20
Leak_06	9	0.258	0.076	29.5%	0.169	0.398
Leak_08	9	0.387	0.064	16.5%	0.286	0.465
Total	27	0.268	0.111	41.4%	0.101	0.465

Physical Tests

Comparison of different physical methods for glove integrity testing

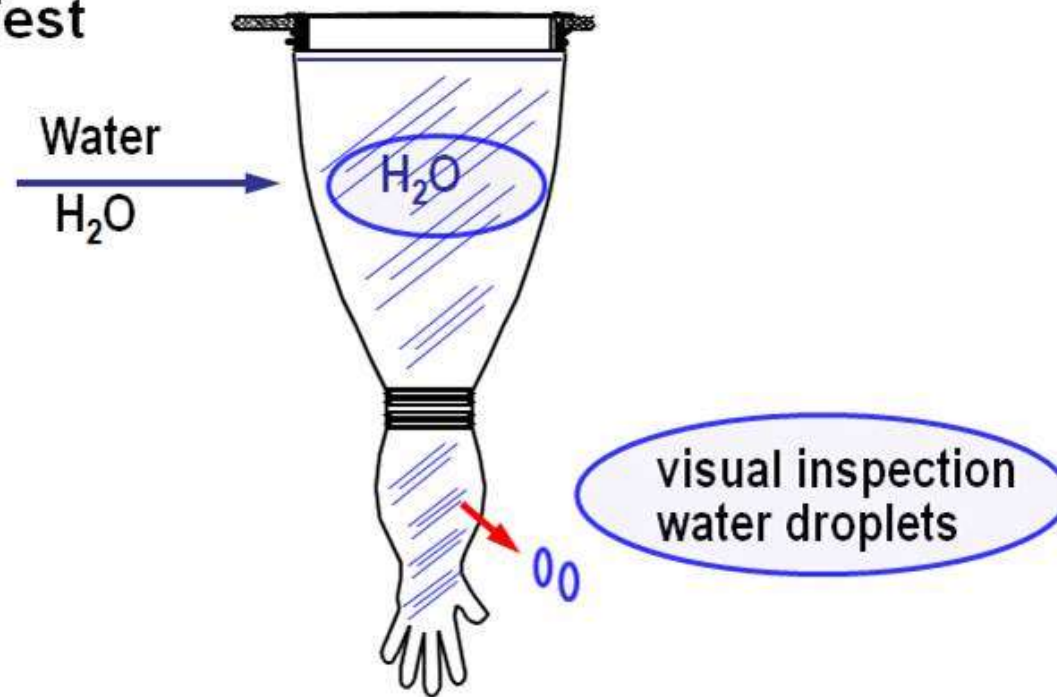
- **Capability** of **reliable** detection of glove leakage
- **Quantitative** or **qualitative** detection of leak size
- **Selective** or **cumulative** detection of leak position
- **Suitability** for **routine use** prior production / during production

Physical Tests

Comparison of different methods





Observed water droplets are used to justify the glove integrity

Water Test



Physical Tests

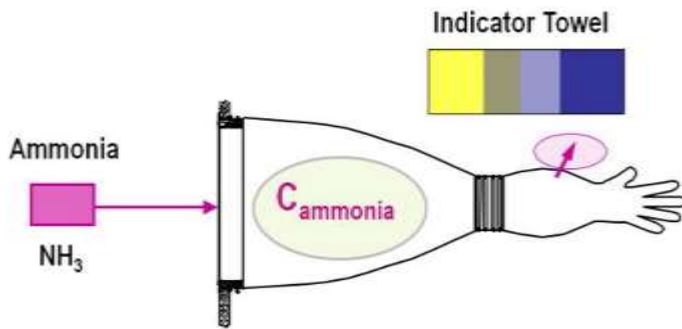
Water Test

Detected Leaks [%]	99		
Quantitative / qualitative	qualitative		
Selective / cumulative	100% selective	0% cumulative	
Suitability for routine use	 <ul style="list-style-type: none"> • weak point is detection of 0.4 finger tip leaks • only possible in vertical direction • water removal generates problems • wet glove surface • preparation in closed isolator not possible • contamination risk of closed isolator • not suitable during production, closed isolator 		

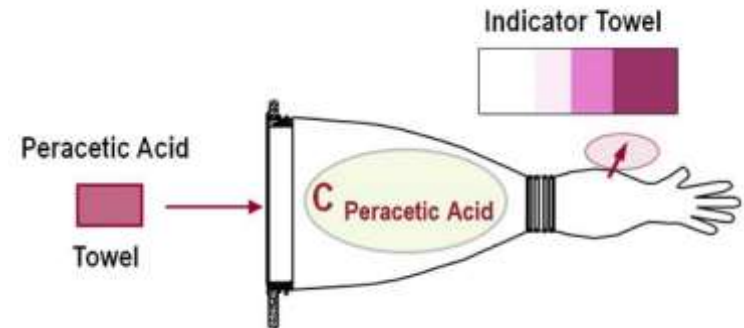
Physical Tests

Comparison of different physical methods

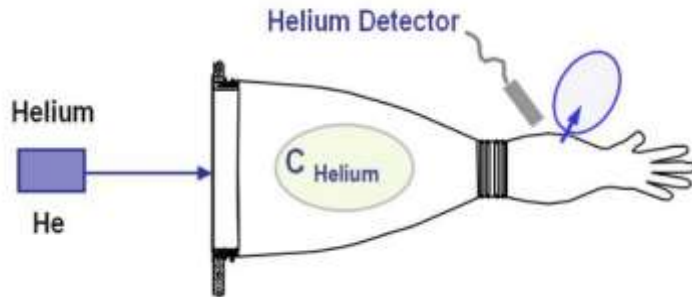
Ammonia Test



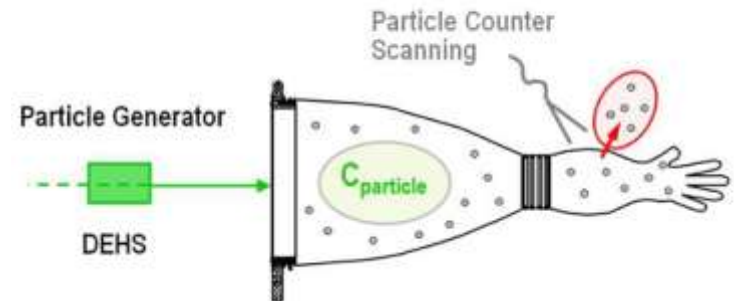
Peracetic Acid Test



Helium Test



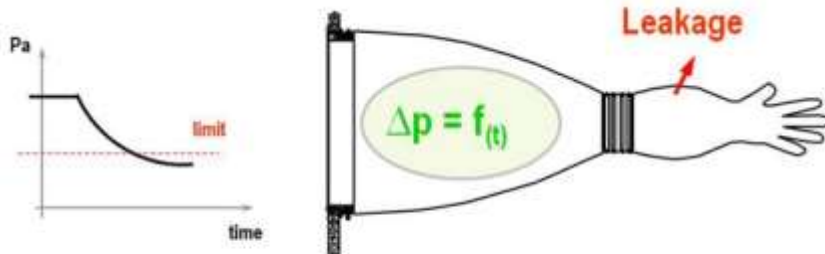
Particle Test



Physical Tests

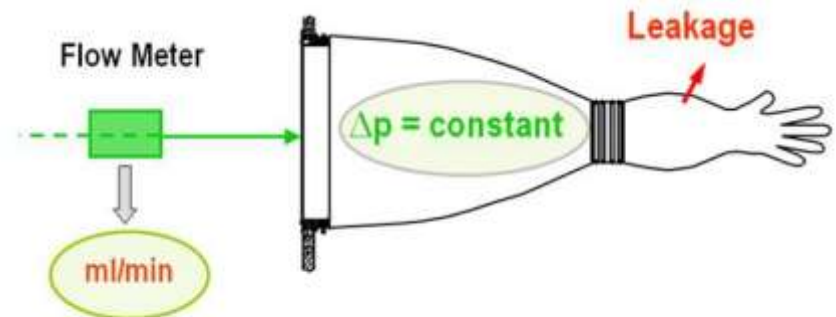
Pressure drop test

- Measured pressure drop is used to justify the glove integrity



Flow test

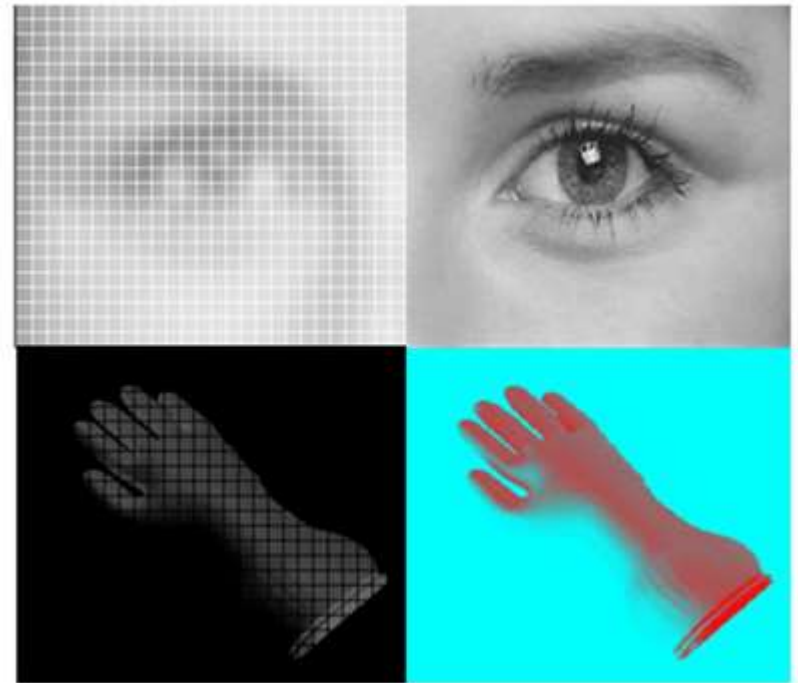
- Measured flow is used to justify glove integrity



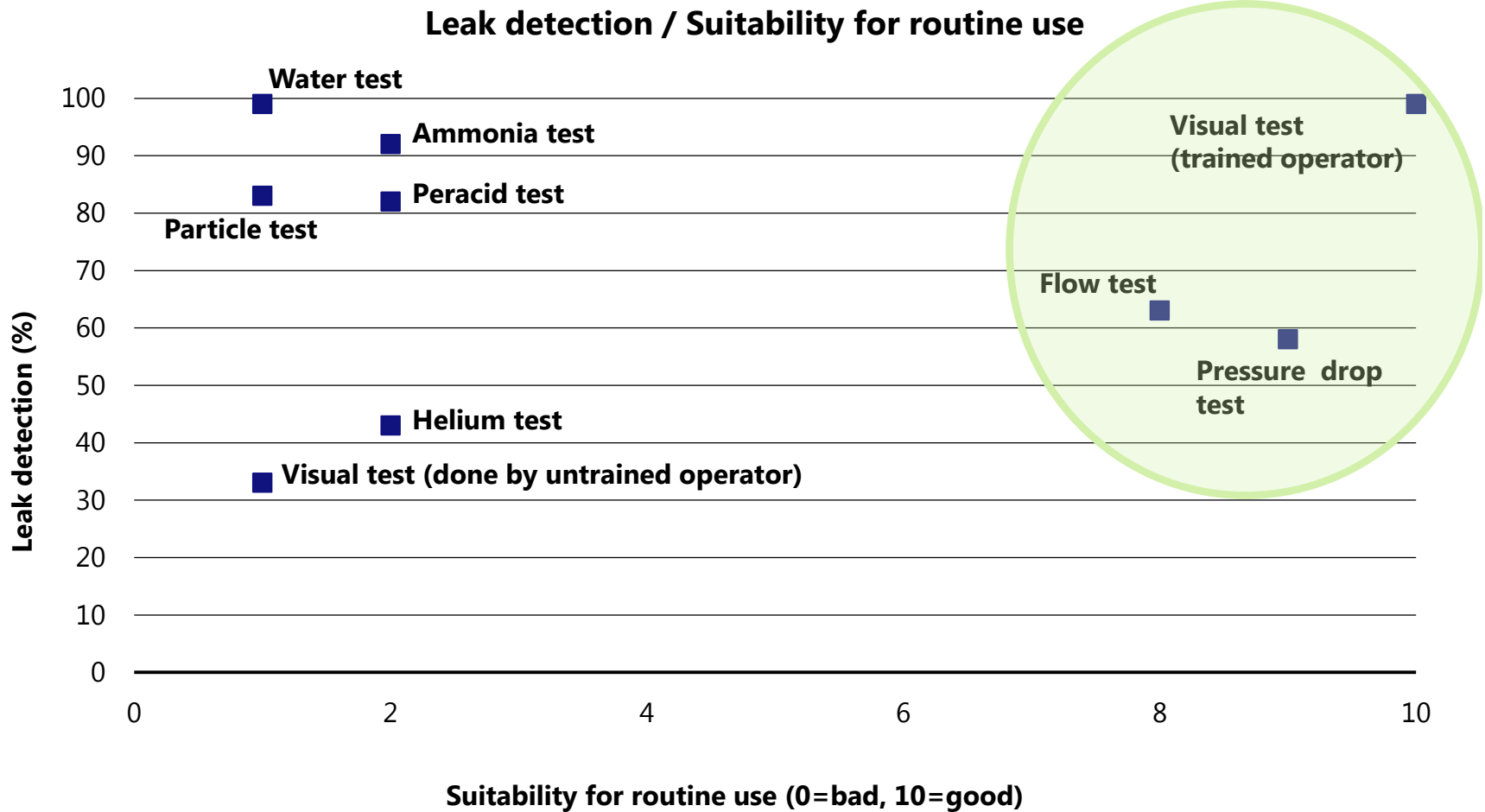
Visual Testing Methods

The detection of pinholes is performed visually by:

- Trained operator
- Not trained operator



Overview Testing Methods



Summary

Physical methods

- All suitable physical methods show limitations in leak detection
- Weak point of detection are in general leaks in finger tips
- Trained operators are able to detect almost every leak by visual inspection

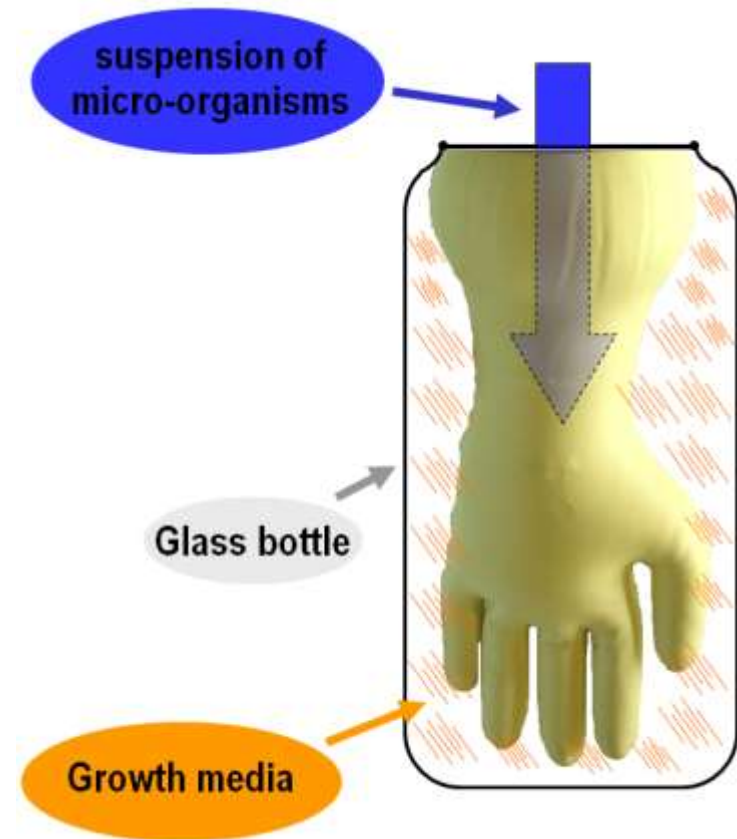
Microbiological Tests

- Microbiological growth penetration through leaky gloves
- Evaluation of realistic bioload on gloves used during routine production
- Determination of achieved Bioload
- Process simulation tests by handling in a isolator using leaky gloves

Growth penetration

Test preparation

- Glove, glass bottle and growth media steam sterilized
- Transferred into decontaminated isolator system
- Concentration: 1.6×10^8 [cfu/ml]
- Incubation time: 14 [days]
- Growth evaluation: 2, 7, 14 [day]



Growth penetration

Results

Growth Penetration 27 gloves with defined leaks and 3 tight gloves

- All tight gloves show no growth penetration
- 24 leaky gloves show penetration after 2 days
- 26 leaky gloves show penetration after 7 days
- All leaky gloves show penetration after 14 days

Tight gloves are a good barrier for microbial penetration
All defined test leaks represent a microbial contamination risk

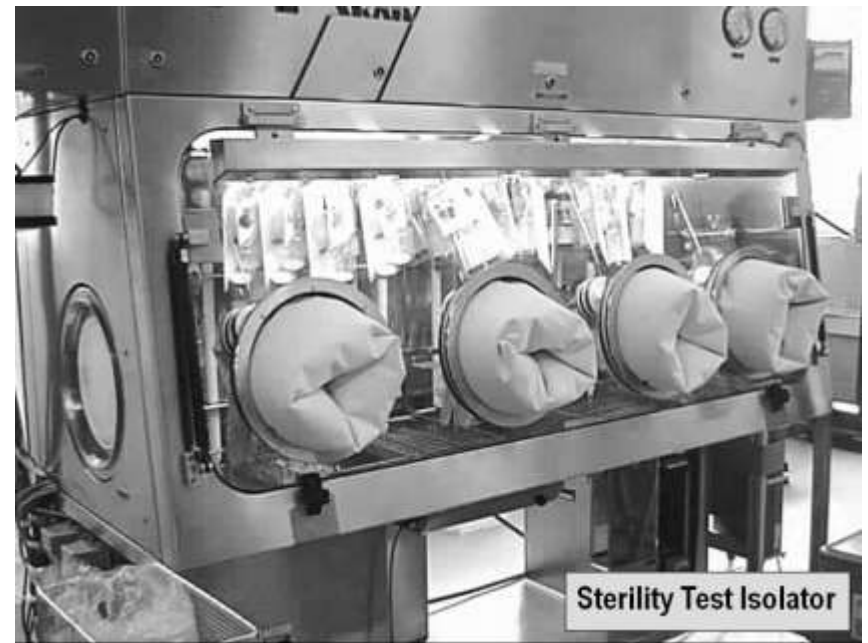
Realistic Bioload

Determination of realistic Bioload on existing Production Systems

- 5 x production isolators surrounding room, Class D
- 2 x sterility test isolators surrounding room, unspecified but controlled

- Determination of **current Bioload**
- Determination of **Bioload after disinfection** 70 [%]
Isopropanol, sprayed
- Determination of **Bioload after 11 production batches** ca. 45 [days]

Realistic Bioload



Realistic Bioload

Bioload on existing Production Systems

Total Number of Samples	Number of Samples with [cfu/sample]				max. Count
	0	1 - 5	6 - 10	> 10	
Current Bioload					
105	30	62	9	2	41
Bioload after Disinfection					
105	91	14	0	0	4
Bioload after 11 Production Batches					
55	10	32	5	7	69

Realistic Bioload

Bioload on existing Production Systems

Sample plate=25 cm² max. glove contamination per cm² =3

Total Number of Samples	Number of Samples with [cfu/sample]				max. Count
	0	1 - 5	6 - 10	> 10	
Current Bioload					
105	30	62	9	2	41
Bioload after Disinfection					
105	91	14	0	0	4
Bioload after 11 Production Batches					
55	10	32	5	7	69

Glove contamination

Determination of achieved bioload

- Different concentrations of test suspension 1.0×10^8 to 1.0×10^2
- Glove surface fully dipped into test suspension
- Determination of bioload on $1 \text{ [cm}^2\text{]}$ glove material
- After defined time intervals 0, 2, 4, 6 [h]

Glove contamination

Results: Achieved Bioload per cm²

Concentration of used Suspension [cfu/ml]	Time after contamination [h]			
	0	2	4	6
1×10^8	35'500	773	1'015	290
1×10^7	4'350	150	0	0
1×10^6	2'800	460	50	10
1×10^5	340	165	0	0
1×10^4	50	10	0	0
1×10^3	15	0	0	0
1×10^2	10	0	0	0

Realistic Bioload

Selected Contamination Levels / Bioload

- high bioload suspension 10^8 bioload 3×10^4
- lower bioload suspension 10^7 bioload 4×10^3
- realistic bioload suspension 10^4 bioload 5×10^1

Concentration of used Suspension [cfu/ml]	Time after contamination [h]			
	0	2	4	6
1×10^8	35'500	773	1'015	290
1×10^7	4'350	150	0	0
1×10^6	2'800	460	50	10
1×10^5	340	165	0	0
1×10^4	50	10	0	0
1×10^3	15	0	0	0
1×10^2	10	0	0	0

Process simulation

Test Preparation

- Defined gloves installed on isolator system
- Needed test material transferred into isolator system
- Isolator H₂O₂ decontaminated

Start contamination of gloves



Process simulation

Glove Contamination

- Each day prior testing
- Outer glove surface fully dipped into defined test suspension
- 5 [min] drying phase

Start test handling



Process simulation

Test Handling

- During each test period, daily
- Handling of 20 sterile glass balls using the contaminated gloves
- From left to right and from right to left
- 2 hours per day
- Over a 5 days period

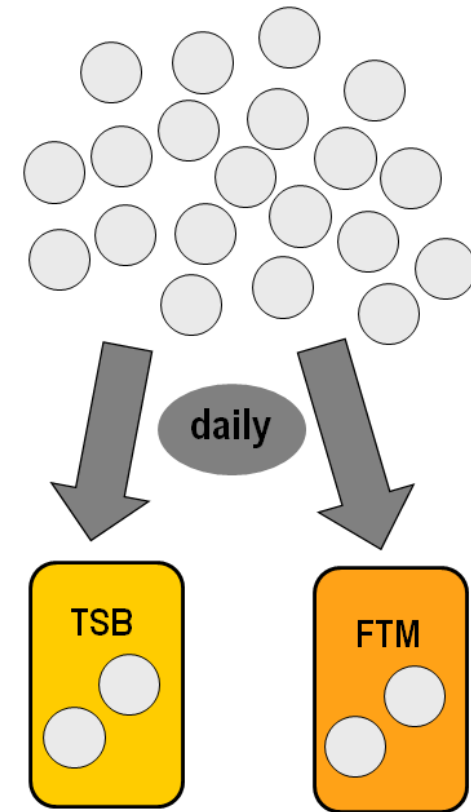


Process simulation

Contamination Control of Glass Balls

- Daily after each test period
- 4 of the glass balls transferred into growth media
- 2 into TSB for aerobic bacteria and molds
- 2 into FTM for anaerobic + aerobic bacteria
- Incubated period 7 [day]

Growth / No Growth Evaluation



Process simulation

Environmental Controls

Daily after each test period using contact plates

- Finger tip of each glove
- Sleeve of each glove
- Bottom of isolator chamber
- Side wall of isolator chamber

Daily during each test period using two settling plates

- Air born contamination

Daily after each test period using swabs

- The defined leak position

Process simulation

Results: Glove: F 0.4; Suspension: 10^8 ; Bioload: 3×10^4

Test 1	day 1	day 2	day 3	day 4	day 5
Glass ball TSB	neg	neg	neg	pos	pos
Glass ball FTM	neg	neg	neg	neg	pos
Fingers glove 1	1	0	0	0	0
Fingers glove 2	0	0	0	0	8
Fingers glove 3	0	0	0	0	6
Fingers glove 4	0	0	0	0	1
Sleeve glove 1	0	0	0	0	0
Sleeve glove 2	0	0	0	0	2
Sleeve glove 3	0	0	0	0	0
Sleeve glove 4	0	0	0	0	0
Bottom isolator	0	0	0	0	0
Sidewall isolator	0	0	0	0	0
Settling plate 1	0	0	0	0	0
Settling plate 2	0	0	0	0	0

Special Controls	day 1	day 2	day 3	day 4	day 5
Glove leak: Fingertip F 0.4					
Leak 1	0	0	0	0	2
Leak 2	3	0	0	0	0
Leak 3	0	0	0	0	0

Overview Bio Contamination

TABLE VII
Summary of the Practical Process Simulation Tests

Bioload (t = 0) On Glove	Pinhole Position and Size	Contamination Detected (On Product Or By Plate)
High (3.6×10^4 CFU/cm ²)	F, S, and E for 0.4 mm	Few positive
Medium (4.3×10^3 CFU/cm ²)	F, S, and E for 0.6 and 0.8 mm	All negative
Realistic (5.0×10^1 CFU/cm ²)	F, S, and E for 0.6 and 0.8 mm	All negative



Summary

Microbiological tests

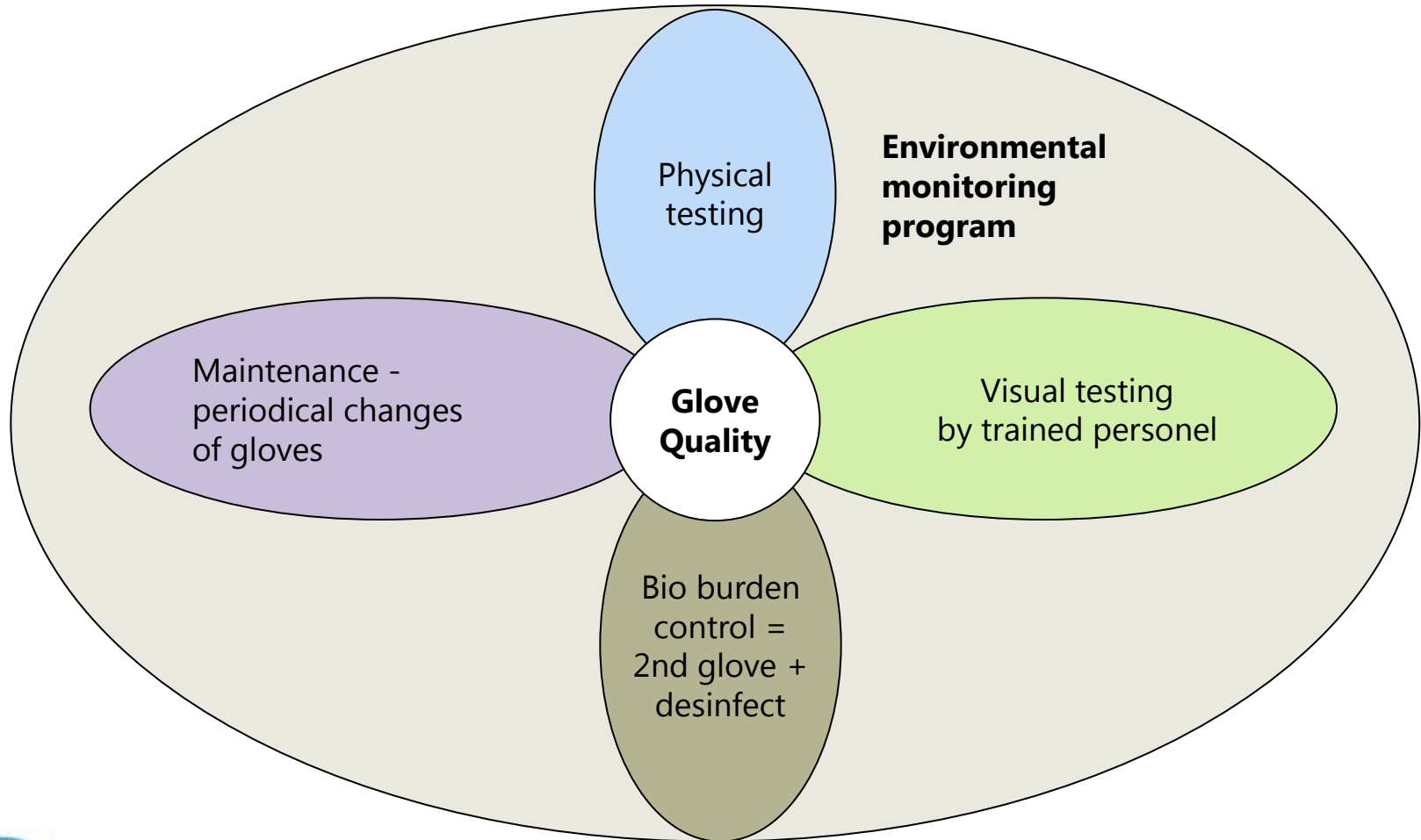
- Micro-organisms are in general able to penetrate glove leaks
- High bioload leads to contamination of the isolator through glove leaks
- Lower bioload and bioload in range of realistic value shows no contamination risk

Conclusion

Routine program for glove integrity testing

- Physical integrity test using pressure drop or flow test after production and change of gloves
- Additional visual glove inspection after physical testing
- Establish operator training and qualification for visual inspection
- Defined disinfection program to control bioload on outer glove surface
- Use of second pair of glove to control bioload on outer surface

Glove Integrity Insurance



Requirements for a Testing Unit

- Short testing cycle
- Not sensitive against surrounding influences
- Fast preparation-, stabilisation- and testing phase
- Flexible system (e.g. recipes)
- Wide and adjustable pressure range parameter (pneumatic gasket / gloves)
- Wireless
- RFID technology (e.g. automatic port detection)
- User friendly
- Clean room conform

WirelessGT



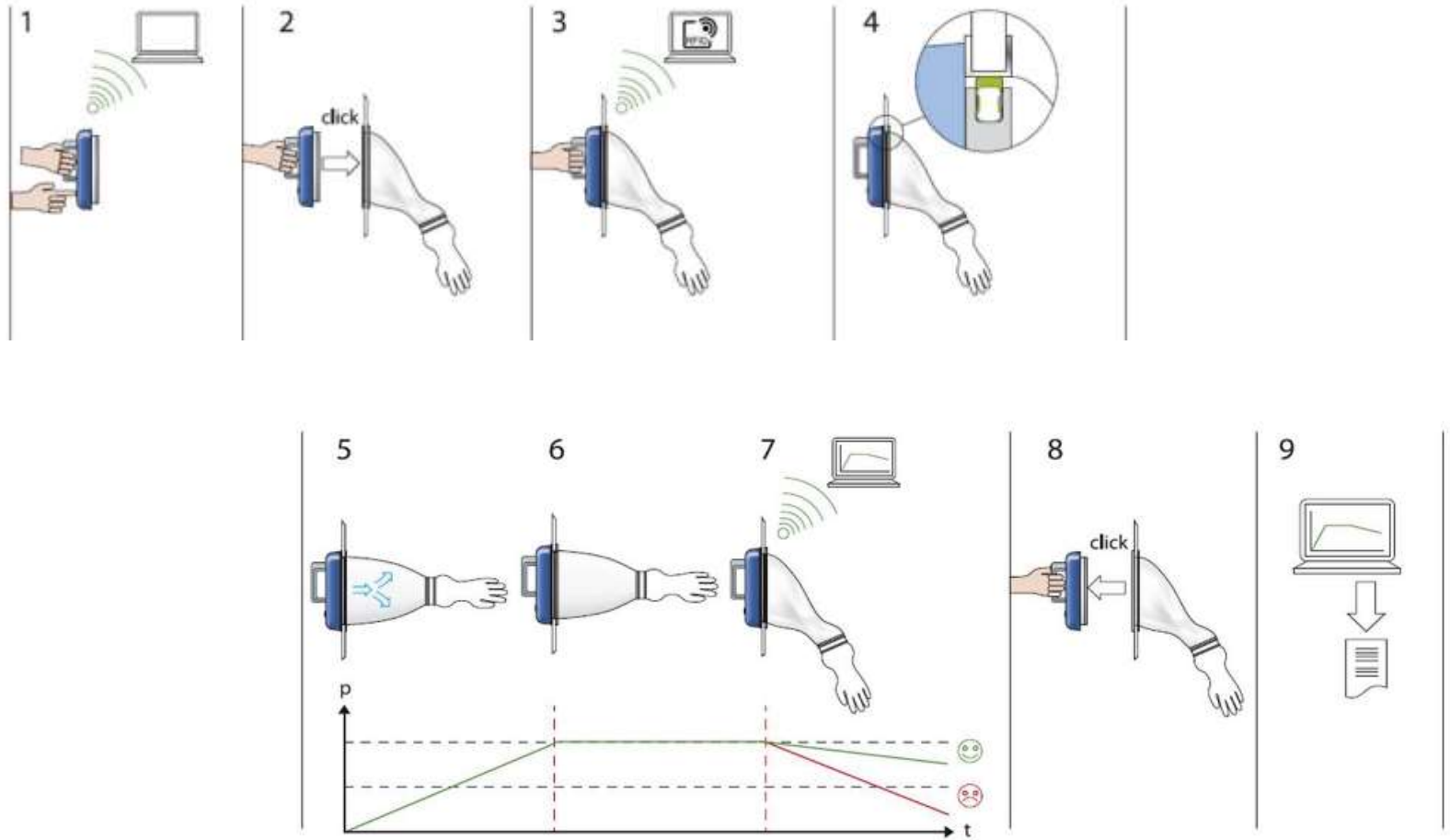
Features of the WirelessGT

- Completely autonomous, no cables, no wires
- Wireless transmission to PC
- Safe recognition of the tested glove port by RFID
- Testing time from 15 minutes, depending on glove material and accuracy required
- Specific test recipes for different types of gloves

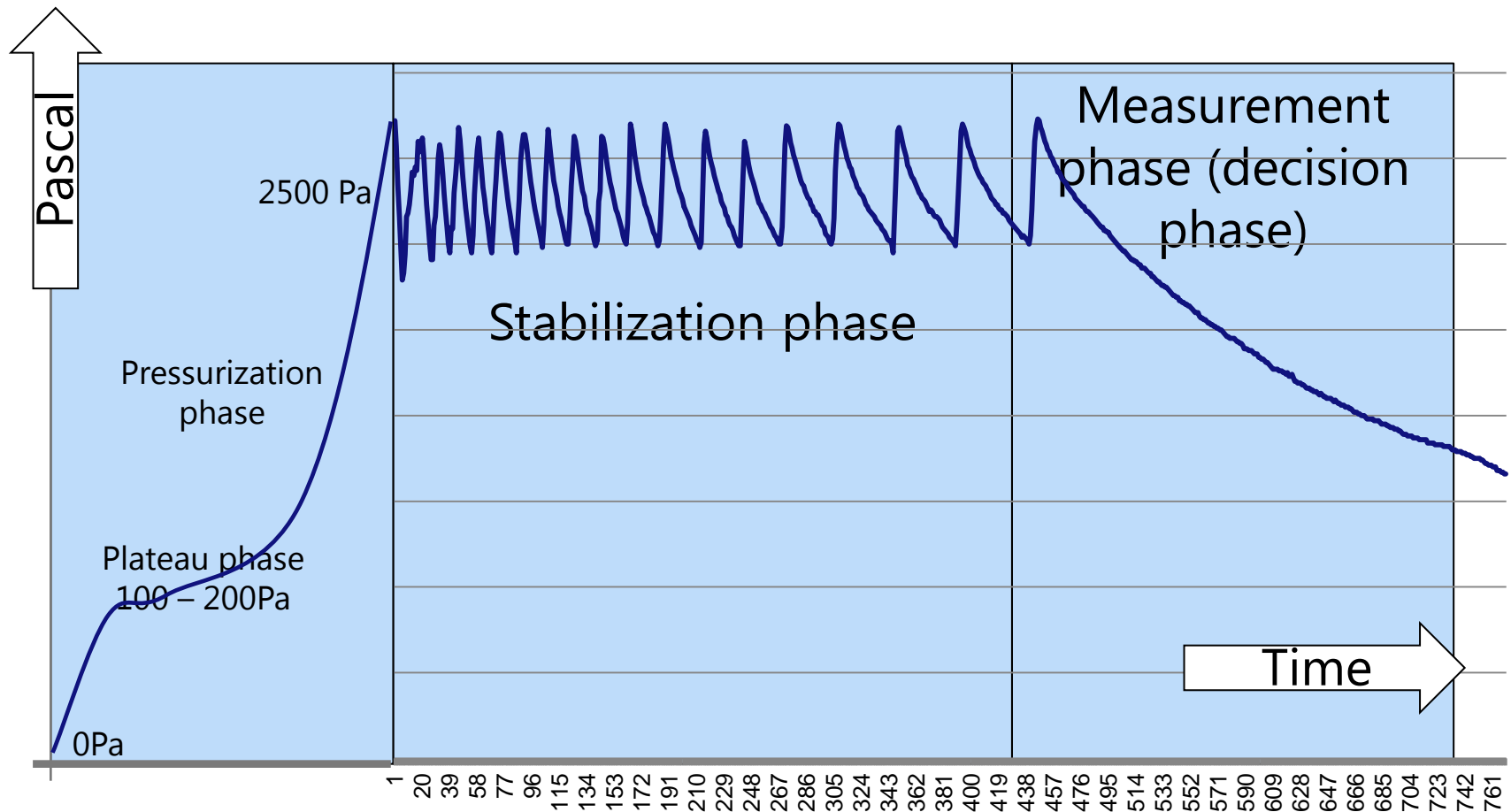
Features of the WirelessGT

- User friendly operation, cGMP compliant testing
- Detects holes larger than 150 μm
- Test pressure up to 3500 Pa
- All gloves of an isolator can be tested simultaneously
- In situ testing without removal of the gloves
- Generates a Batch and Configuration Report

Wireless Glove Tester



Features of the WirelessGT



Picture: SKAN WirelessGT

Features of the WirelessGT

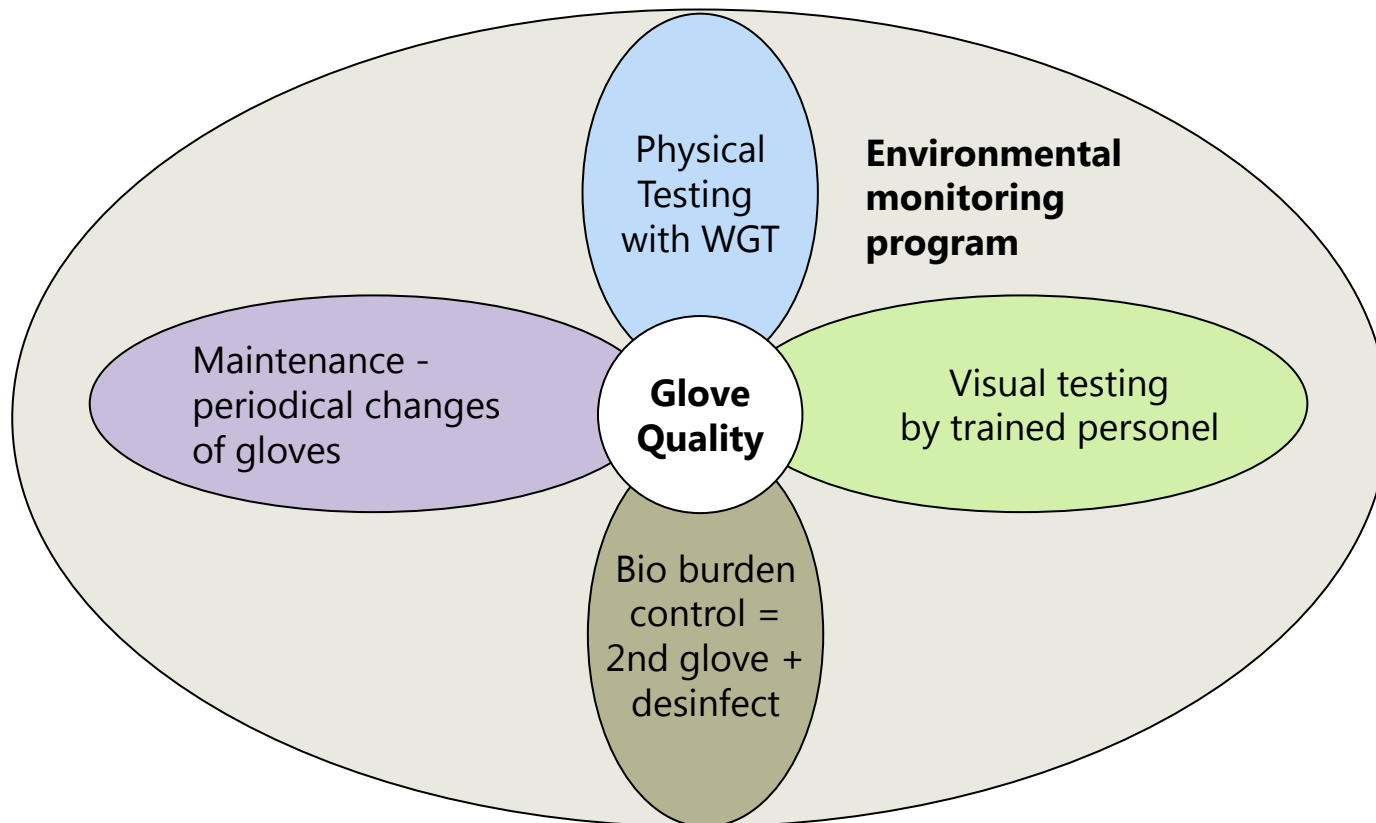


Conclusion

- A program to minimize the risk of loss of integrity of gloves should be present.
- Visual inspection is still the most reliable glove testing method.
- Appropriate complementary physical test methods (regulatory) are pressure drop or flow test.
- Bio-contamination is - with minor leakage of a glove – not to be expected at GMP compliant application

WirelessGT

An important contribution





Thank you!

Questions?

Yves Scholler, Sales Manager
SKAN AG, Binningerstr. 116
CH-4123 Allschwil

yves.scholler@skan.ch
Tel +41 61 485 44 44

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