

# Glove Leak Testing in Isolators and RABS –

A foundation for reasonable testing and good practices for risk mitigation

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25-February-2014



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# Overview

- What the regulators say about gloves.
- Key technical points
  - What does a hole in a glove look like?
  - Methods used to test
  - Relating glove leaks to microbial contamination risk
  - Industry benchmarking – survey notes
- Key principles for good “Glove Management”



# What are the Regulations/Guidance

## ■ FDA Aseptic Processing Guidance:

### APPENDIX 1: ASEPTIC PROCESSING ISOLATORS

#### A. Maintenance

##### 1. General

Maintenance of isolator systems differs in some significant respects from the traditional, non-isolated aseptic processing operations. Although no isolator forms an absolute seal, very high integrity can be achieved in a well-designed unit. However, a leak in certain components of the system can constitute a significant breach of integrity. ***The integrity of gloves, half-suits, and seams should receive daily attention and be addressed by a comprehensive preventative maintenance program. Replacement frequencies should be established in written procedures*** that ensure parts will be changed before they breakdown or degrade. Transfer systems, gaskets, and seals are among the other parts that should be covered by the maintenance program.

- The integrity of gloves, half-suits and seams should receive daily attention (*note wording here*).
- Comprehensive PM program.
- Replacement frequencies should be established in written procedures.



# What are the Regulations/Guidance

## ■ FDA Aseptic Processing Guidance:

### APPENDIX 1: ASEPTIC PROCESSING ISOLATORS

#### A. Maintenance... 2. Glove Integrity

A faulty glove or sleeve (gauntlet) assembly represents a **route of contamination and a critical breach of isolator integrity**. A **preventative maintenance program** should be established. The choice of **durable glove materials**, coupled with a **well-justified replacement frequency**, are key aspects of good manufacturing practice to be addressed. With **every use**, gloves should be **visually evaluated for any macroscopic physical defect**. Physical integrity tests should also be performed routinely. A breach in glove integrity can be of serious consequence. The **monitoring and maintenance program** should identify and eliminate any glove lacking integrity and minimize the possibility of placing a **sterile product at risk**.

- Faulty glove or sleeve... route of contamination and a critical breach of isolator integrity.
- Preventative maintenance program should be established.
- Durable glove materials.
- Well-justified replacement frequency.
- Every use, visual inspection for macroscopic defects.
- Physical integrity tests performed **routinely**.
- Monitoring and maintenance program...minimize risk to product.



# What are the Regulations/Guidance

## ■ FDA Aseptic Processing Guidance:

### APPENDIX 1: ASEPTIC PROCESSING ISOLATORS

#### A. Maintenance

#### 2. Glove Integrity - continued

Due to the potential for ***microbial migration through microscopic holes in gloves*** and the ***lack of a highly sensitive glove integrity test***, we recommend affording attention to the ***sanitary quality of the inner surface of the installed glove*** and to integrating the use of a ***second pair of thin gloves***.

- Potential for microbial migration through microscopic holes in gloves.
- Lack of a highly sensitive glove integrity test (circa 2003).
- “Sanitary quality of the inner surface” of the installed glove.
- Second pair of thin gloves (under-glove)



# What are the Regulations/Guidance

## ■ EU Sterile Annex 1:

### Item 25:

**Monitoring** should be carried out *routinely* and should include *frequent leak testing* of the *isolator* and *glove/sleeve system*.

- Routine monitoring.
- Frequent leak testing of the isolator.
- Frequent leak testing of the glove/sleeve system.



# What are the Regulations/Guidance

- PICS (Pharma. Inspection Convention/Cooperation Scheme) - 2004:

## Section 9.5.3

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. There should also be an all encompassing preventative maintenance program that includes specification of examination and preemptive replacements.

- Program to minimize the risk of loss of integrity of gloves, sleeves and suits.
- Operator practices, vigilance and absence of sharp edges.
- Encompassing preventative maintenance program that includes:
  - Specification of examination
  - Preemptive replacements



# What are the Regulations/Guidance

## ■ PICS (Pharma. Inspection Convention/Cooperation Scheme) - 2004:

### **Details in Section 9.5.3.2 for analyzing risks (similar to FDA Guidance):**

The analysis of these risks should be documented and preventative actions such as the following should be considered:

- Selection of robust materials.
- Use of double skinned sleeves where puncture of one or both of the skins causes separation of the two layers and is easily detected by the operator.
- Operator training to avoid damage and vigilance to examine for damage.
- Frequent leak testing.
- Inner or outer sterile gloves.
- Sterile inner sleeves or garments.
- Preventative maintenance program that includes specification of examination and preemptive replacement.





## **Key Technical Points – Glove structure, holes and testing**

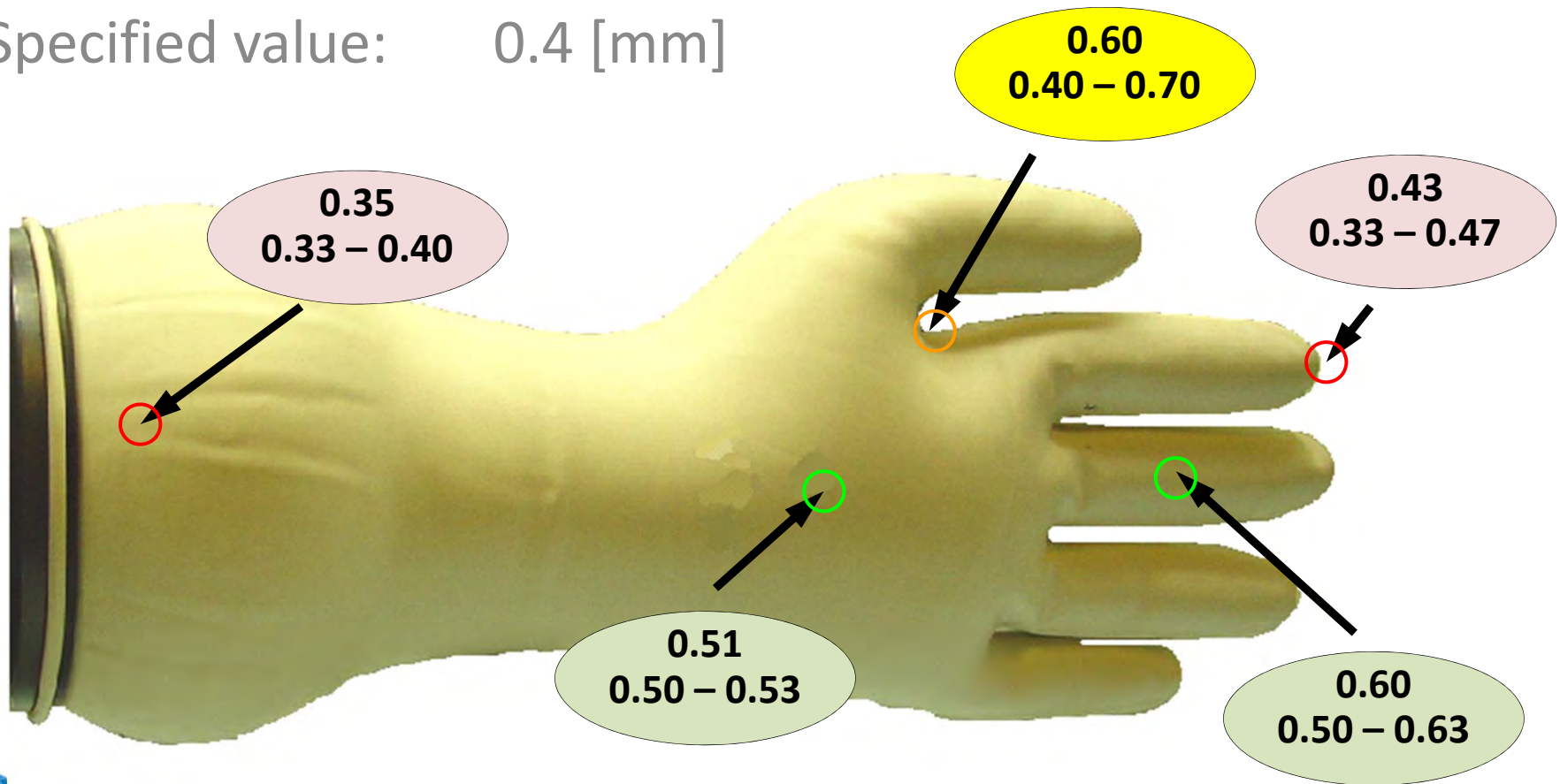
- What is the physical structure of a glove?
- Where do failures typically occur?
- What can be considered a “reasonable hole size” for testing?
- At what pressure should I test?
- How long does it take / should it take?



# Glove structure and variable thickness

*Measurement of Material Thickness [mm]*

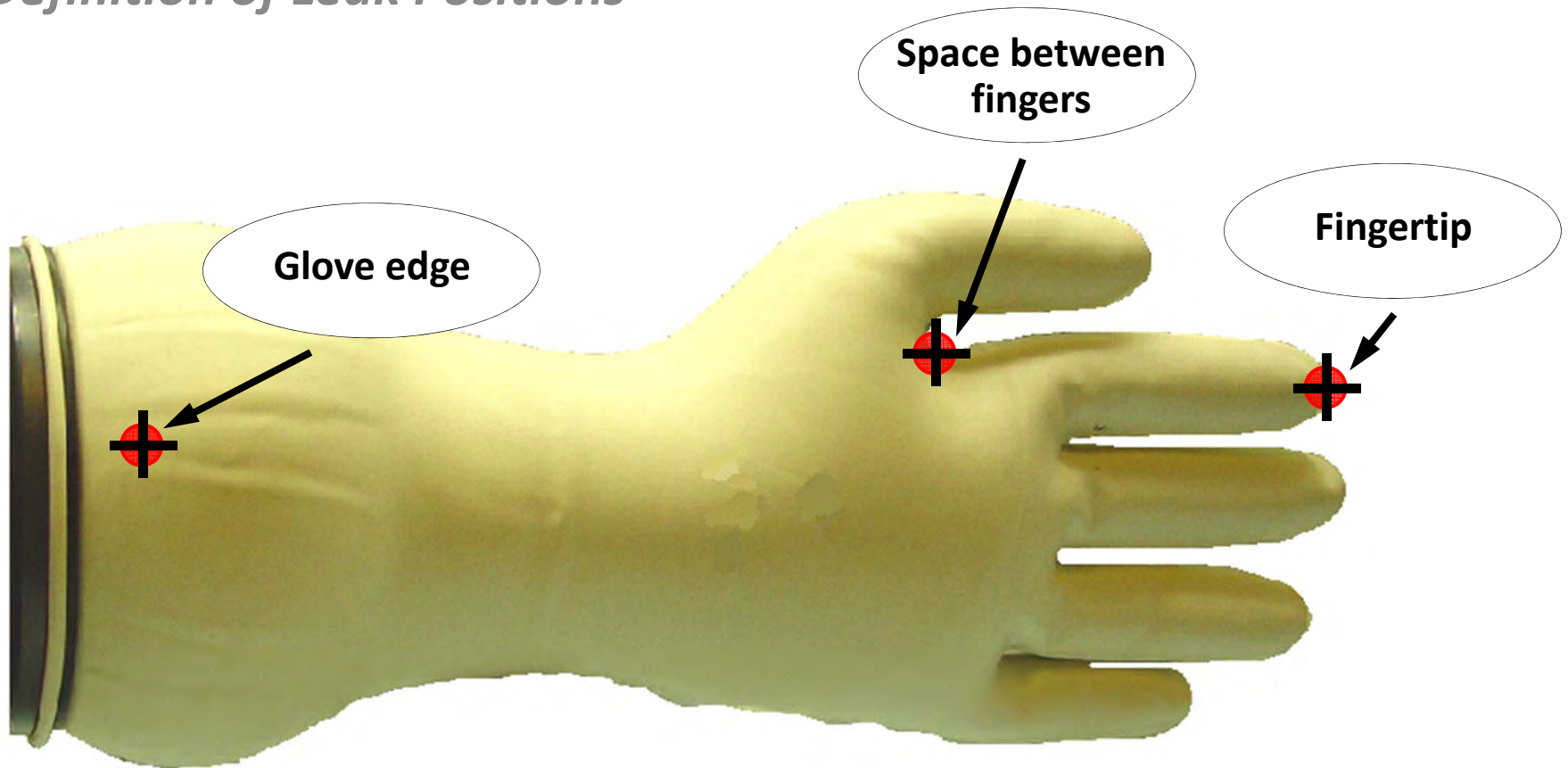
Specified value: 0.4 [mm]



Mean value and range out of 12 samples

# “Typical” glove hole locations

## *Definition of Leak Positions*



# “Typical” glove hole locations

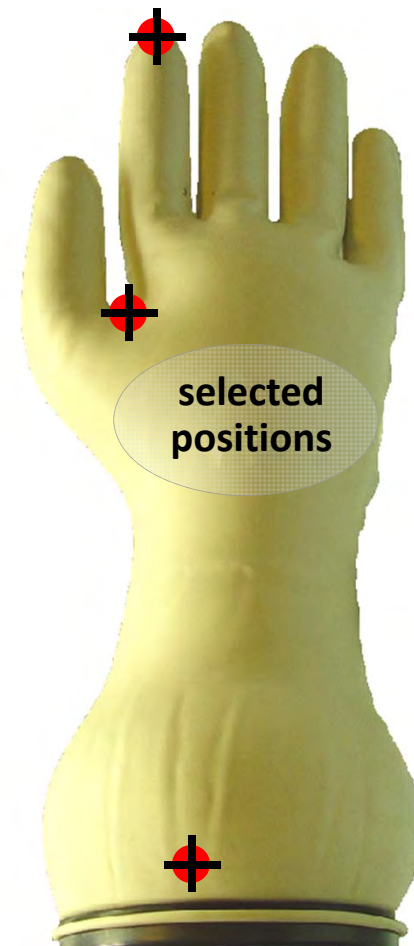
## *Definition of Leak Positions*

Selection based on:

Position with **frequent leaks** during  
production

Position of **thin glove material**

Position with a **high risk of contamination**



# “Typical” glove hole sizes and analysis

Leaks prepared using **syringe needles**

$\emptyset = 0.4$  [mm]

$\emptyset = 0.6$  [mm]

$\emptyset = 0.8$  [mm]

**3 gloves** prepared **per position** and  $\emptyset$

**3 additional tight gloves** as reference

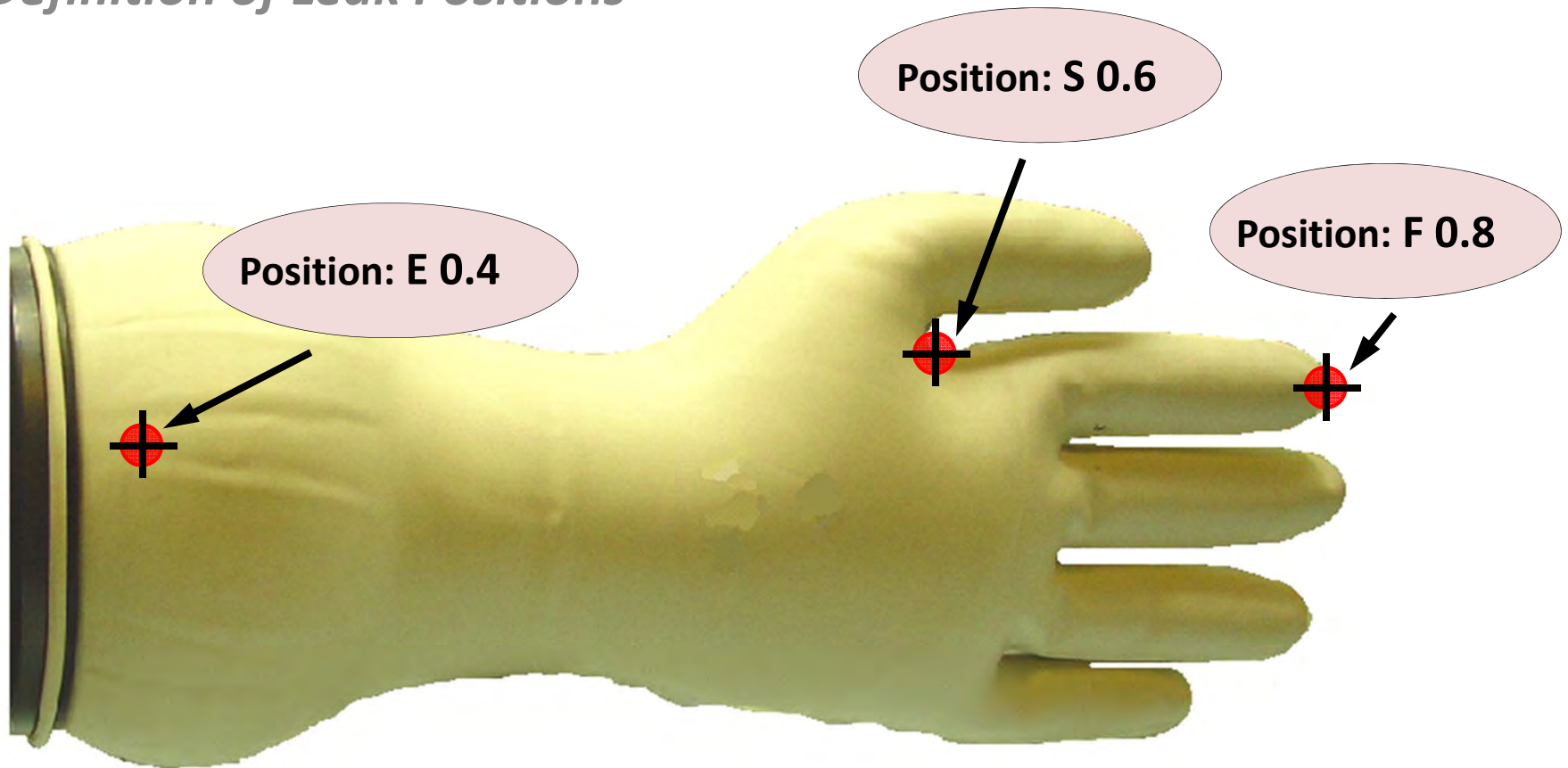
**30 gloves** prepared for the study

Gloves **defined stressed** before preparation



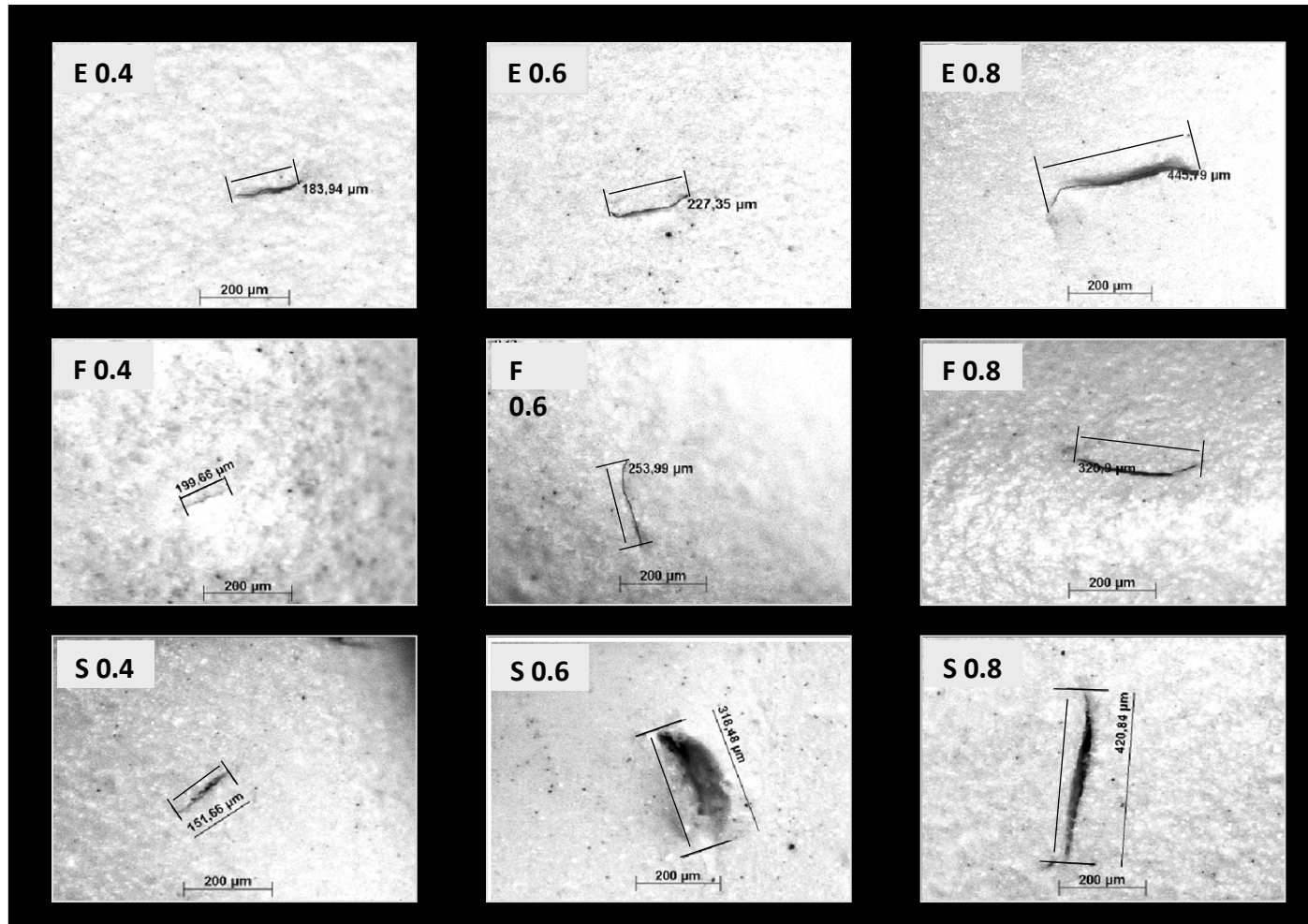
# “Typical” glove hole positions and analysis

## *Definition of Leak Positions*



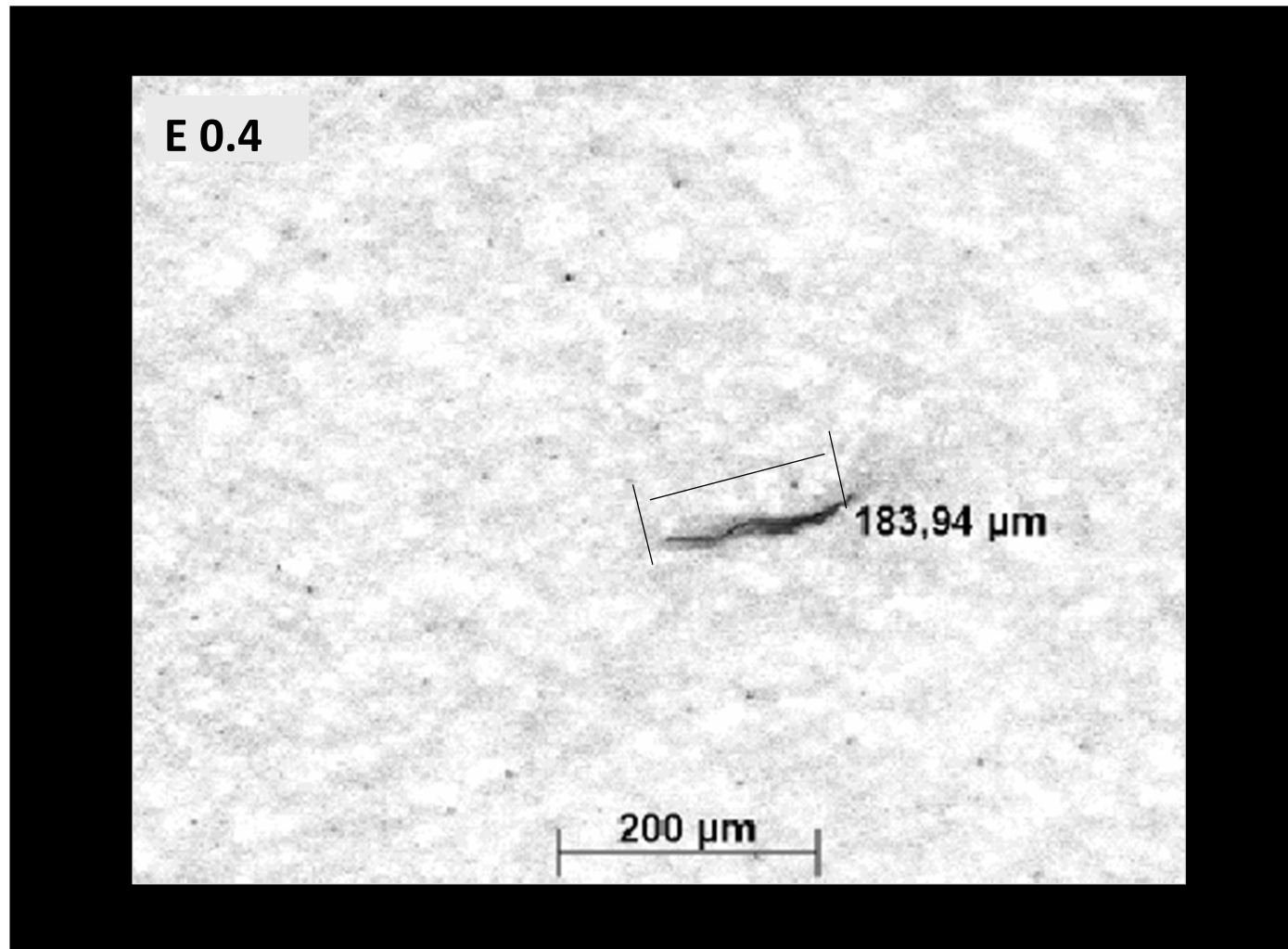


# “Typical” glove hole sizes and analysis



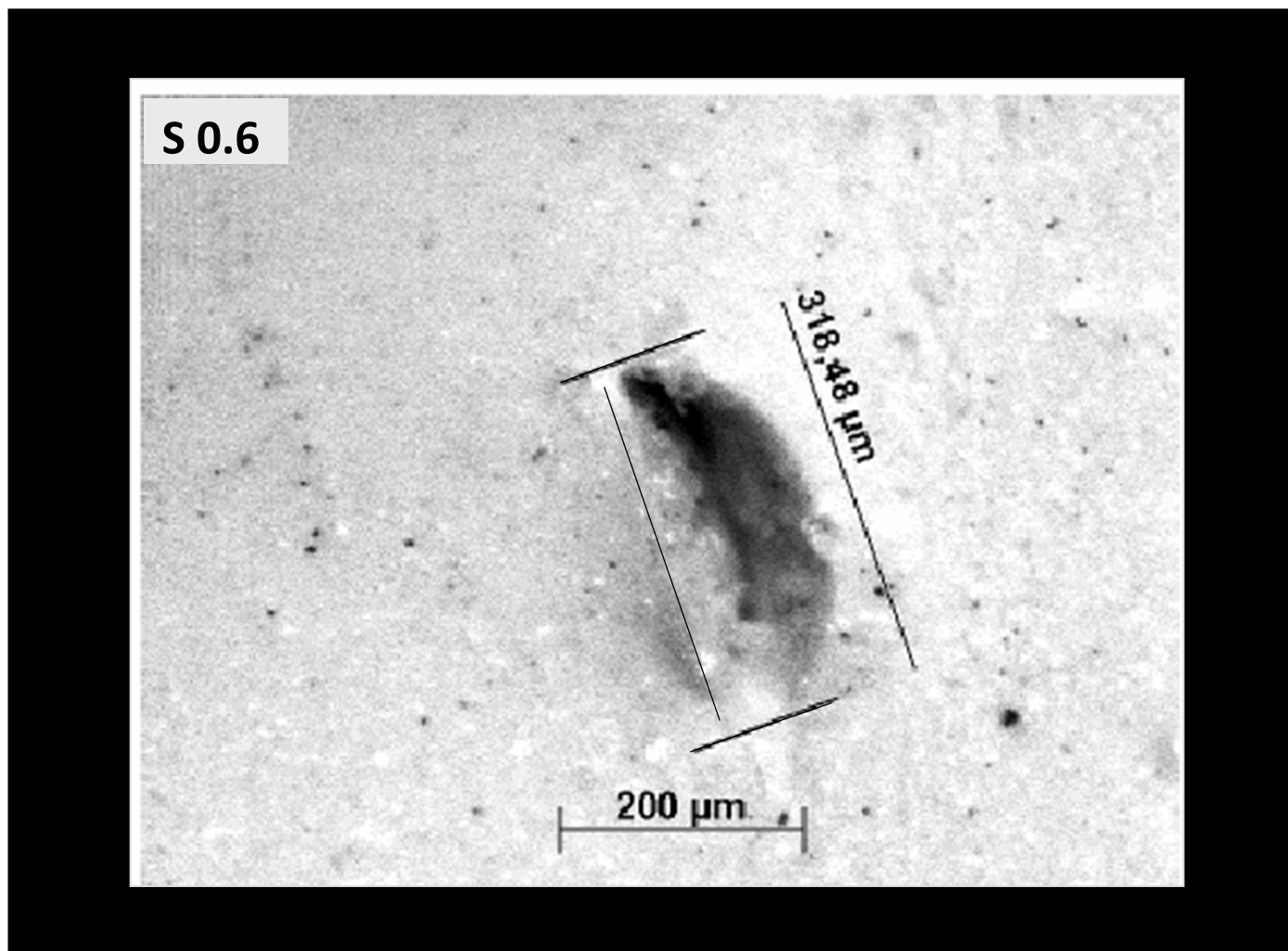
*Resulting leak size microscopically measured and investigated*

# “Typical” glove hole sizes and analysis

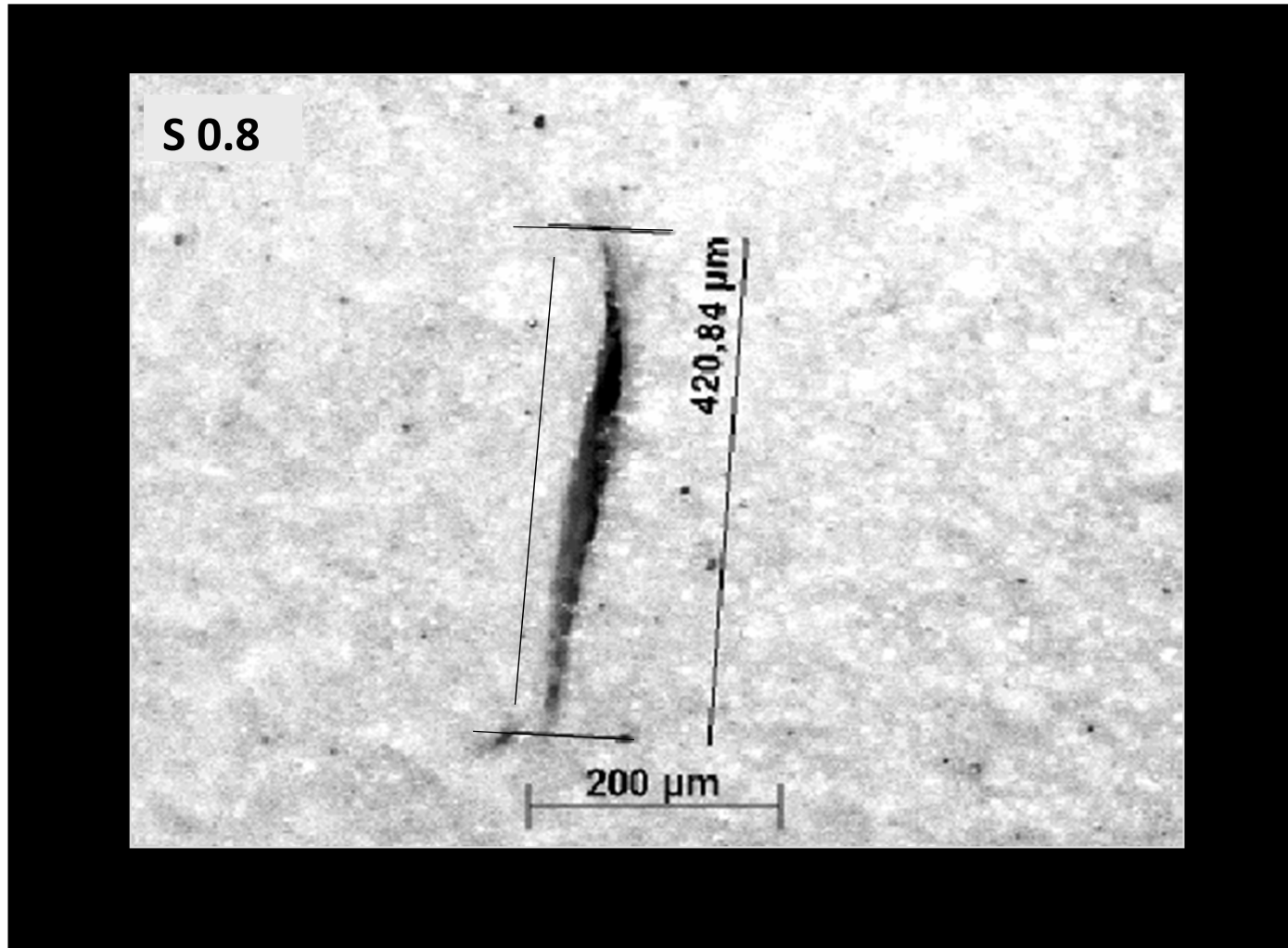




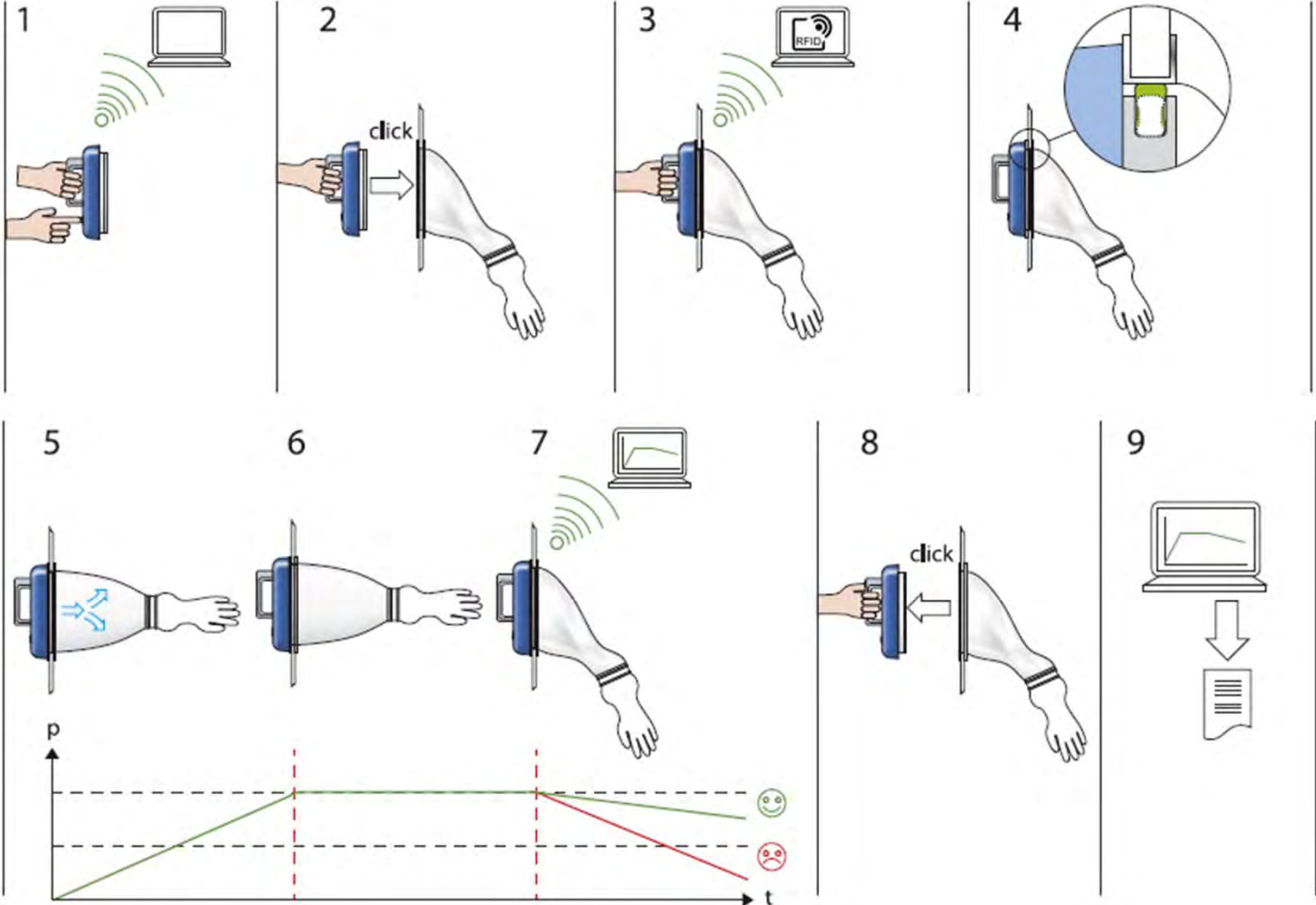
# “Typical” glove hole sizes and analysis



# “Typical” glove hole sizes and analysis



# Glove Testing Method Example

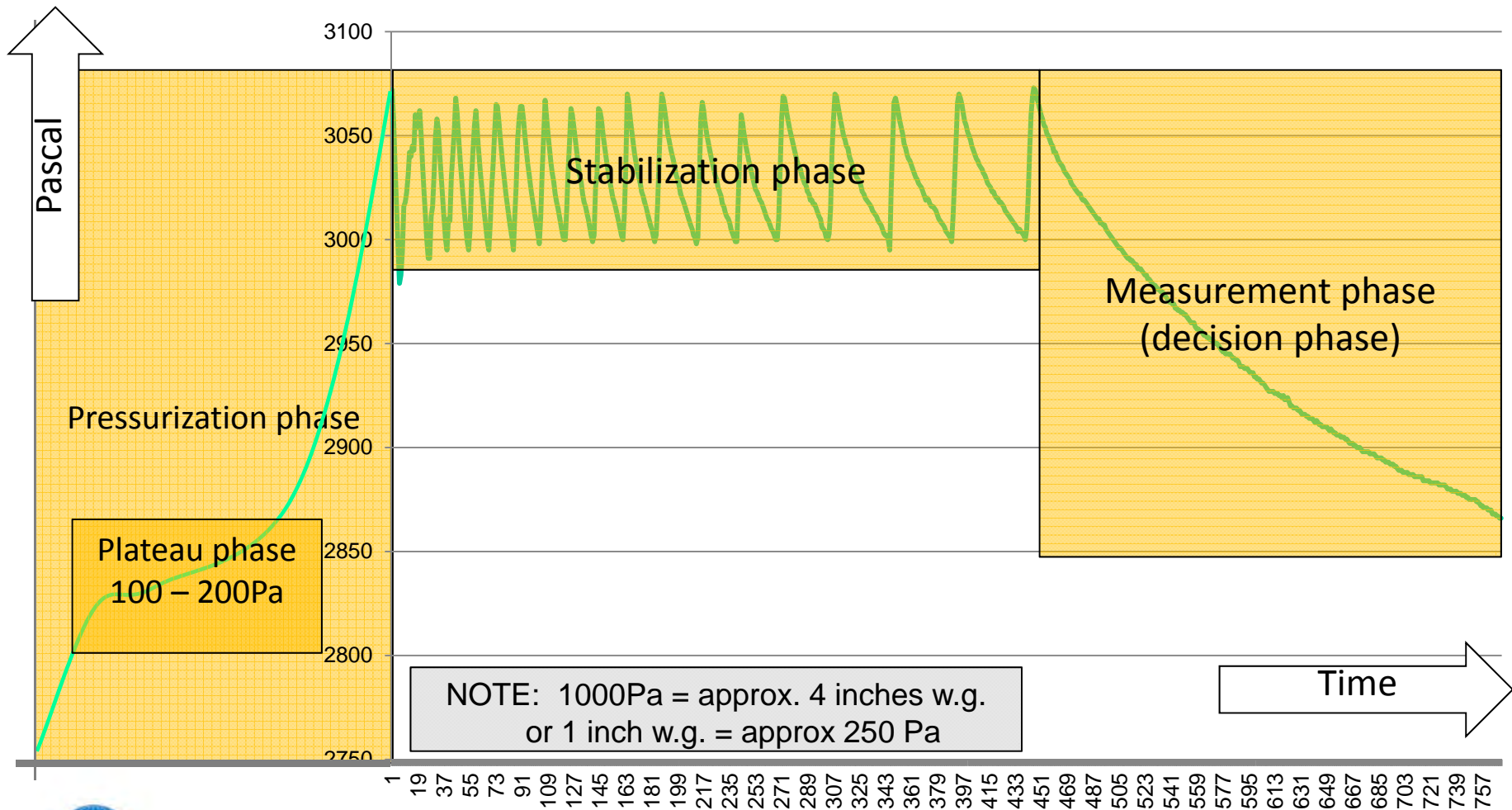


# Glove Testing Method Example





# Glove Testing Method and Timing Example



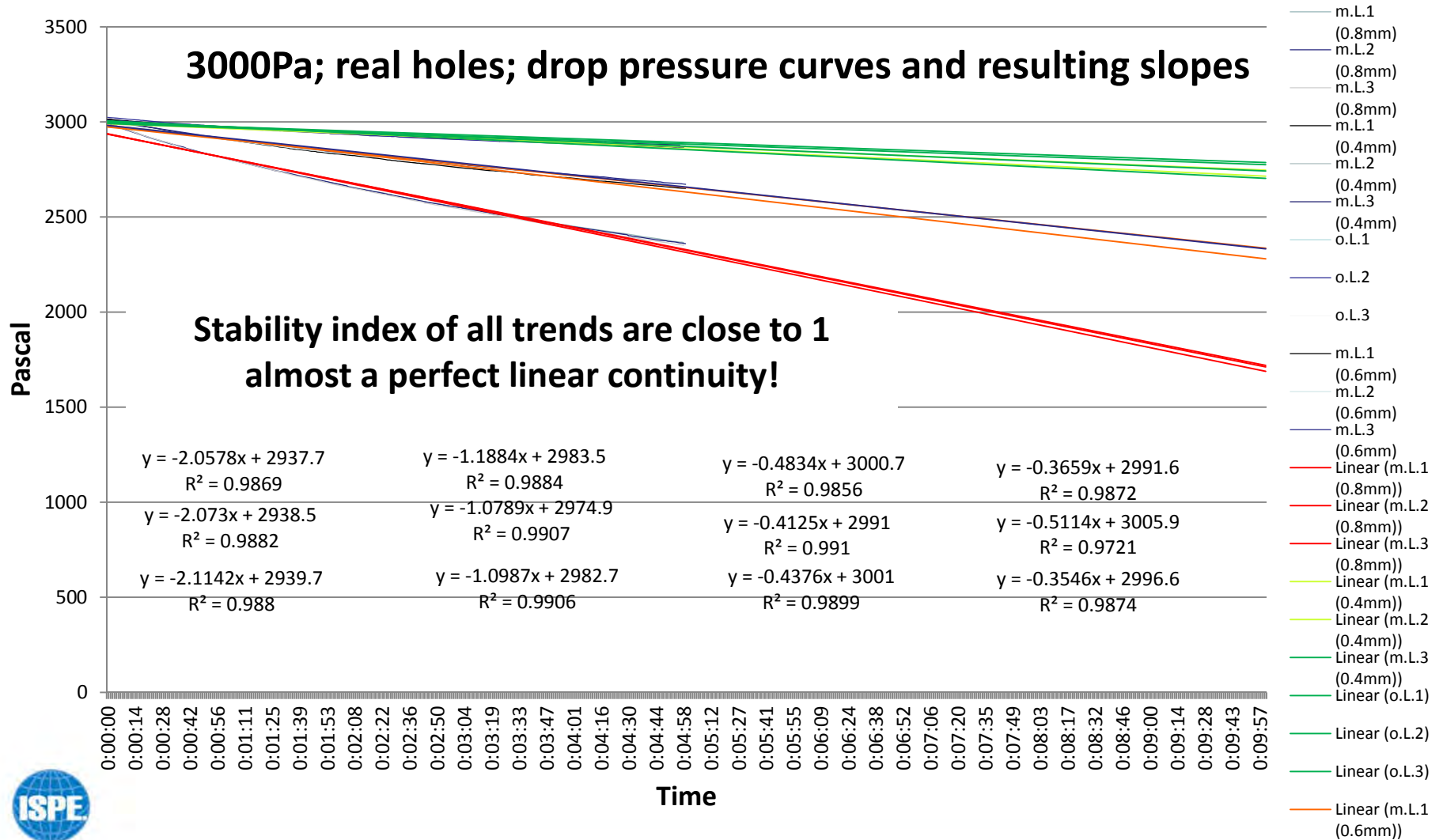
# Glove Integrity Test Example Results

*Extreme Example: Glove Material at 5000 [Pa] -- TOO HIGH!!,  
Hand distorted, but fingers not experiencing distortion*



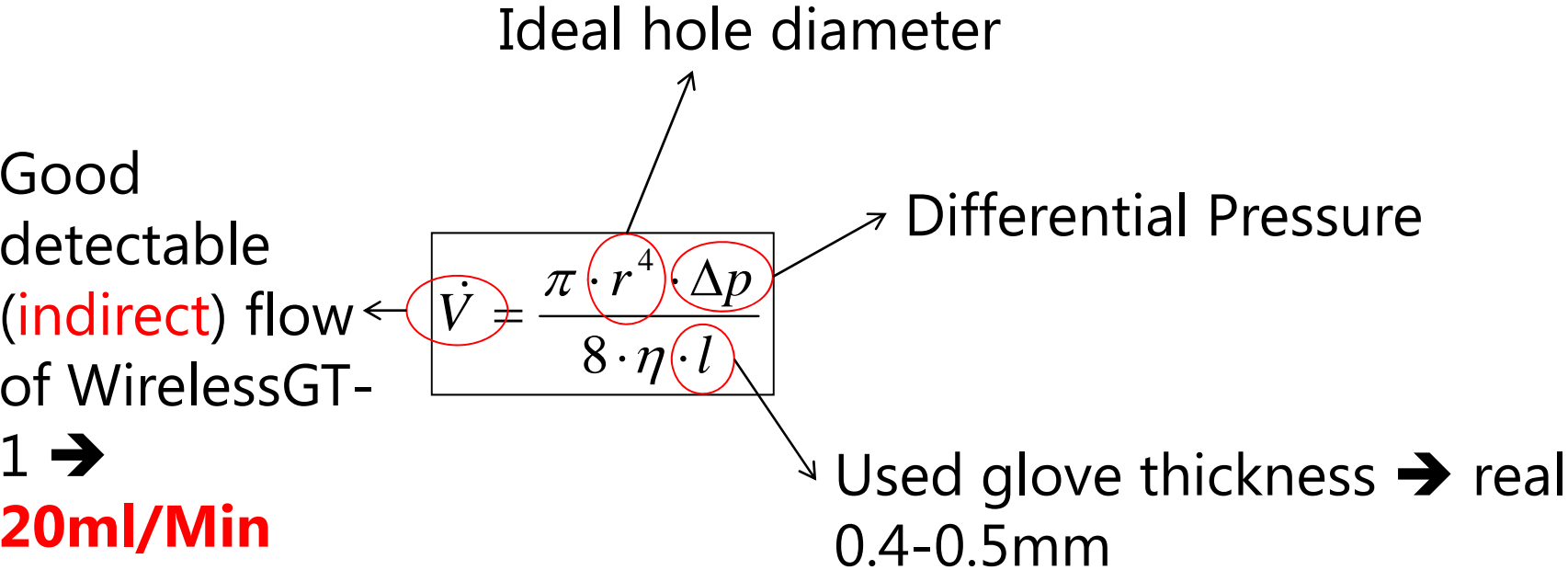
# Glove Testing Example

## Performance 'real holes'



# Glove Testing Theory

## Physics (Hagen-Poiseuille)



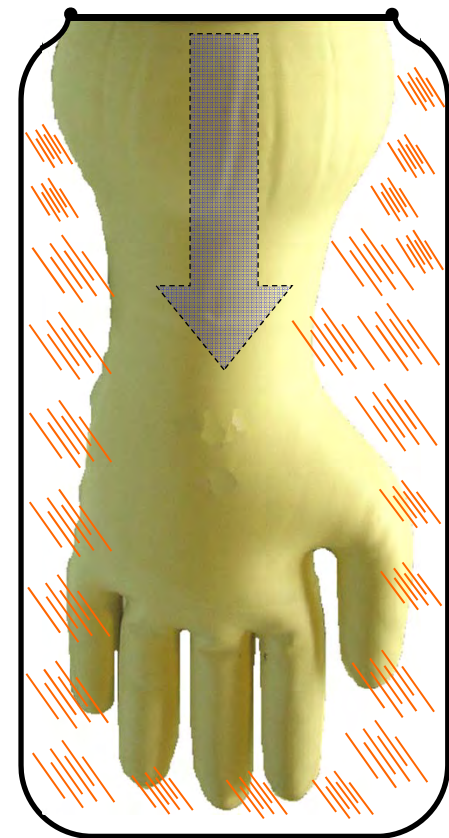


# Relating Glove Test to Risk of Microbial Contamination

## Glove Integrity defined as Growth Penetration

### *Test Preparation*

Test organism: **Brevundimonas diminuta**  
Concentration:  **$1.6 \times 10^8$  [cfu/ml]**  
Incubation temp.: **30 – 35 [°C]**  
Incubation time: **14 [days]**  
Growth evaluation: **2, 7, 14 [day]**  
Results: **growth even with small holes**  
**no growth with 'perfect' gloves**



IS THIS A REALISTIC TEST? **TOO RIGOROUS?**

# Relating Glove Test to Risk of Microbial Contamination

## Glove Integrity defined in 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

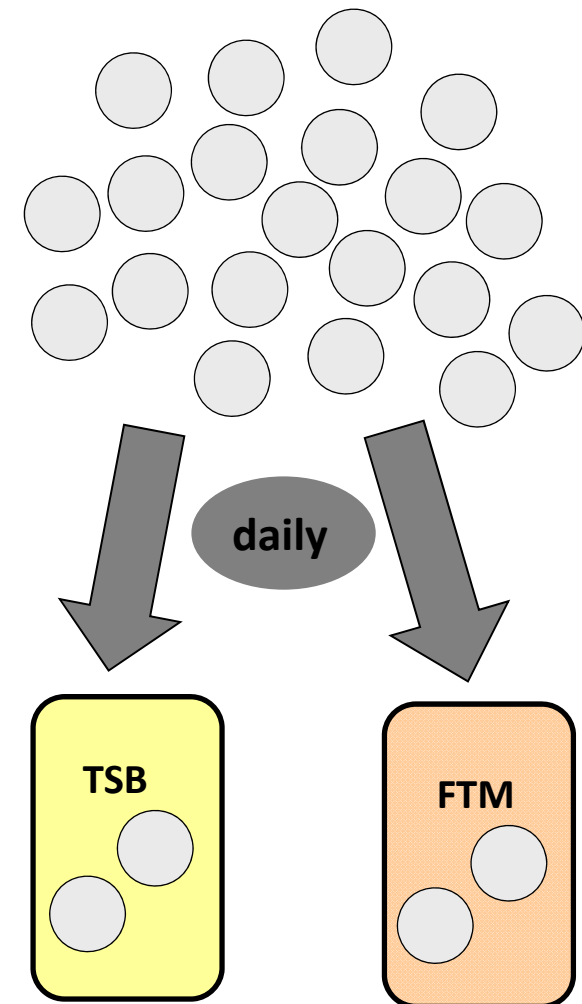
Incubated period 7 [day]

### **Growth / No Growth Evaluation**



TSB: Trypticase Soy Broth – typically for aerobic bacterial growth

FTM: Fluid Thioglycollate Media – typically for anaerobic bacteria



# Relating Glove Test to Risk of Microbial Contamination

## Glove Integrity defined in Process Simulation

*Further Process Simulation Tests*

*Realistic Bioload at 0.8mm hole*

Suspension: **1 x 10<sup>4</sup>** [cfu/ml]

Bioload: **5 x 10<sup>1</sup>** [cfu/cm<sup>2</sup>]

Glove Leak:	Finger Tip	<b>F 0.8</b>	<b>all negative</b>
Glove Leak:	Space between fingers	<b>S 0.8</b>	<b>all negative</b>
Glove Leak	Edge of Glove	<b>E 0.8</b>	<b>all negative</b>



# Relating Glove Test to Risk of Microbial Contamination

## Glove Integrity defined in Process Simulation

*Interpretation: Process Simulation Tests Lower / Realistic Bioload*

With lower bioload  $4 \times 10^3$  and bigger leak **F 0.8, S 0.8, E 0.8**

➔ no contamination observable

Contamination levels in the range of realistic bioload  $5 \times 10^1$

➔ lead to no observable contamination  
with any defined leak position and biggest leak size



# Benchmarking – What are others doing?

*Question 1 - Do you perform visual inspection of the glove for the detection of a hole?*

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
No							
Yes, before attaching the glove to the port (for new gloves)			x				
Yes, Just before the doors are closed		x	x	x	x		
Yes, after sterilisation			x				
Yes, during batch					x		
Yes, in between batch (if in campaign)		x					
Yes, after the batch (before declassification)			x			x	
Yes, after door opening	x	x	x				

Site 1 - Hole size unknown

Site 4 - not defined, we take representative pinholes from production

Site 5 - Once per day (24h), inspection done by trained people (gmp training)



# Benchmarking –

Question 2 - Do you perform automatic glove testing on the glove for the detection of a hole?

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
No							
Yes, before attaching the glove to the port (for new gloves)							
Yes, Just before the doors are closed		x					
Yes, after sterilisation							
Yes, during batch							
Yes, in between batch (if in campaign)							
Yes, after the batch (before declassification)			x				
Yes, after door opening	x	x		x	x	x	



Site 4 - equal or more than 100µm

# Benchmarking –

Question 3 - Do you ever re-test if the automatic glove tester fails?

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
No	x				x		
Yes,		X – 3x max	X – 1x max	x		x	

Site 4 - there is a procedure in the SOP what to check if the test fails (e.g. connection of glove tester etc.).



# Benchmarking –

## Question 4 - What is the trigger to change the glove?

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Time	X - 12weeks	x	X- 1 month		x	X twice a year	
Number of cycles							
Hole / leak	x	x		x	x	x	

Site 5 - 2 years after installation (based on qualification) or at expiry date 4 years after manufacturing as per vendor recommendation





# Benchmarking –

Question 5 - What is the material of the glove?

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
CSM-Hypalon	x	x	x	x	X (prod)	x
PVC					X (sterility)	
Neoprene			x			



# Benchmarking –

Question 6 - What is the material of the sleeve (if used)

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
CSM-Hypalon				x	x	x	
PVC	x	x	x				
Neoprene							

Site 5 - PU/CSM for "exposed" or at risk positions, CSM for Prod and PVS/Divetex for Sterility test



# Benchmarking –

Question 7 - How are the gloves treated prior to assembly on the isolator?

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Washed	x			x	X		
Visual inspection				x	X		
Tested for integrity	x				X		
Irradiated			x				
Steam sterilised							
Ethylene oxide							
Disinfected with alcohol		x					



# Benchmarking –

Question 8 - If a hole (pinhole) is detected during production, what action is taken ?

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Nothing							
Contact monitoring of the hole					X	x	
Stop using the glove (continue production if possible)	x		x	x	X	x	
Repair the glove	x				X		
Replace the glove		x	x				
Stop the batch							

Site 4 - Holes are hard to detect during production, that never occurred. Detection is always during glove test after production

Site 5 - Action depends of the criticality of the position as per risk analysis



# Benchmarking –

Question 9 - Do you monitor the Grade C part of the isolator glove?

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
No	x	x		x	x	x	
Yes,			x				

Question 10 - Do ALL of the answers above apply:

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Globally to my organisation							
To my site only	x	x	x	x	x	x	



# Glove Management – Top Ten List

- Glove Management as a term goes beyond just glove leak testing. It is a comprehensive program that includes topics such as:
  1. Glove Cleaning
  2. Glove Disinfection
  3. Use of “under-gloves”
  4. Procedure for Glove Entry and Exit
  5. Glove Visual Inspection
  6. Glove Mechanical Testing (Glove Leak Tester)
  7. Environmental Monitoring of Glove Surfaces and Personnel
  8. Glove/Sleeve Change-Frequency
  9. Documentation of Glove Maintenance / Log-Books
  10. Product Disposition and Investigation Procedure for Glove Deviations



# Glove Management

- **Glove Cleaning:**

- Cleaning is a separate issue from disinfection, although related.
- Standards should be adopted to define how 'clean' a glove must be and look before it should be changed.
- For example, if a few marks are left from a Sharpie Marker for a glove that is often used for handling product samples or environmental monitoring, then a certain level of cosmetic defects are fully acceptable and even expected.
- If these cosmetic defects get to the point that they make the visual inspection detection of mechanical glove defects difficult, then perhaps a glove change is warranted.



# Glove Management

## ■ Glove Disinfection:

- Glove disinfection is a hot topic because isolator and RABS gloves are still considered the 'weakest link' due to the operator intervention into the aseptic process.
- It is important to address periodic disinfection of BOTH SIDES of the glove (the 'sterile' side used in the Grade A space and the 'operator side' which can be in anything from Grade A to D space, depending upon the application).
- A high level disinfectant should be used on both side periodically and a sanitization agent (such as 70% IPA) is also often used on a more frequent basis in order to control bioburden.
- In case a pin-hole leak or breach event occurs, you are reducing RISK of contamination.





# Glove Management

## ■ Use of Under-Gloves:

- Many users of isolators and RABS elect to use an under-glove.
- Here, an operator dons a glove (sometimes sterile, sometimes non-sterile), typically sprays their gloved hand with sterile IPA (isopropyl alcohol), then inserts their gloved hand into the isolator or RABS glove.
- This provides a lower bioburden and reduced product risk if a glove leak or breach occurs.
- Also reduces / eliminates hand perspiration transfer to pin-hole leaks in gloves.
- CAUTION: Some users 'over-spray' the 70% IPA and leave a lot of residual moisture in the gloves – this is "too much of a good thing" and should be avoided.



# Glove Management

- Procedure for Glove Entry and Exit:
  - As discussed above, an 'under-glove' is often used and sprayed with IPA prior to glove entry.
  - This eases entry into the glove and facilitates exit as well.
  - Additionally, some prefer to keep the position of the glove controlled (off the floor of the isolator/RABS and/or keeping the 'hand' of the glove under the Grade A unidirectional air flow to better maintain bioburden control.
  - Rapid or sloppy glove exiting can lead to inverting of the gloves and glove fingers, making it difficult for the next operator to enter.
  - This increased handling also increases risk of glove damage or contamination



# Glove Management

## ■ Glove Visual Inspection:

- It is good practice to perform a gross visual inspection of the glove EVERY TIME a glove is entered.
- Here you can detect gross defects in a glove easily.
- A 'close visual inspection' should be performed periodically (typically before and/or after each batch) to see if there are any glove defects evident.
- Studies have shown that a well-trained operator can detect very small defects in a glove better than expensive glove leak testers upon close visual inspection.
- It should be noted, though, that the FDA and others, have stated that a periodic mechanical glove leak test should be performed on a routine basis in order to have an objective determination of glove leak detection.



# Glove Management

- **Glove Mechanical Testing:**
  - As noted above, glove mechanical testing is encouraged by the regulators, but no specific testing interval is prescribed.
  - The more other monitoring and visual inspections that one does, the less often mechanical testing should be needed.
  - Mechanical testing systems are available from SKAN and others from simple one-glove, non-automated testers to multi-glove, fully automated glove testing systems with formal batch reports.
  - The selection of a system should be performed in concert with a risk analysis and an overall glove management plan taking into consideration all of these items.



# Glove Management

- Environmental Monitoring of Glove Surfaces and Personnel:
  - Periodic environmental monitoring of gloves and sleeves (where applicable) should be performed, typically at the end of every batch operation or when it makes sense for your process.
  - It is important to immediately remove any media/agar residuals from the glove fingers to prevent the spread of media and promotion of the growth of organisms – this can easily be achieved via an IPA wipe or other disinfectant cleaning post-monitoring.



# Glove Management

- **Glove and Sleeve Change Frequencies:**
  - There is no set change interval for gloves, although typically, gloves and sleeves are changed MINIMALLY on an annual basis on many isolator systems during an annual PM.
  - Depending upon use, gloves and sleeves may need to be changed anywhere from daily to weekly to monthly. Not all gloves in a system need to be changed at the same time.
  - Perhaps a certain section of an isolator or RABS is often used and gloves are changed there weekly while the remainder of the system is changed only every three months.
- An **example** of the guiding principles from one of SKAN's clients is as follows: *"If gloves are used daily or on multiple shifts, the gloves should be inspected after each production cycle and leak tested at least on a weekly basis. With moderate to heavy usage, the gloves should not need to be replaced more than once per quarter. Lighter usage may require less frequent, semi-annual replacement."*



# Glove Management

- **Glove Maintenance Documentation / Log Books:**
  - It is important to have some method to identify your glove position (glove number, door number, etc...) to accurately track which gloves have been monitored, tested or changed.
  - A simple logbook can be employed to document glove change-out and noting the reason for the glove change is important too (routine versus defect/damage).
  - Periodically, these records should be reviewed and appropriate PM or change-out intervals should be adjusted to the actual production conditions observed.



# Glove Management

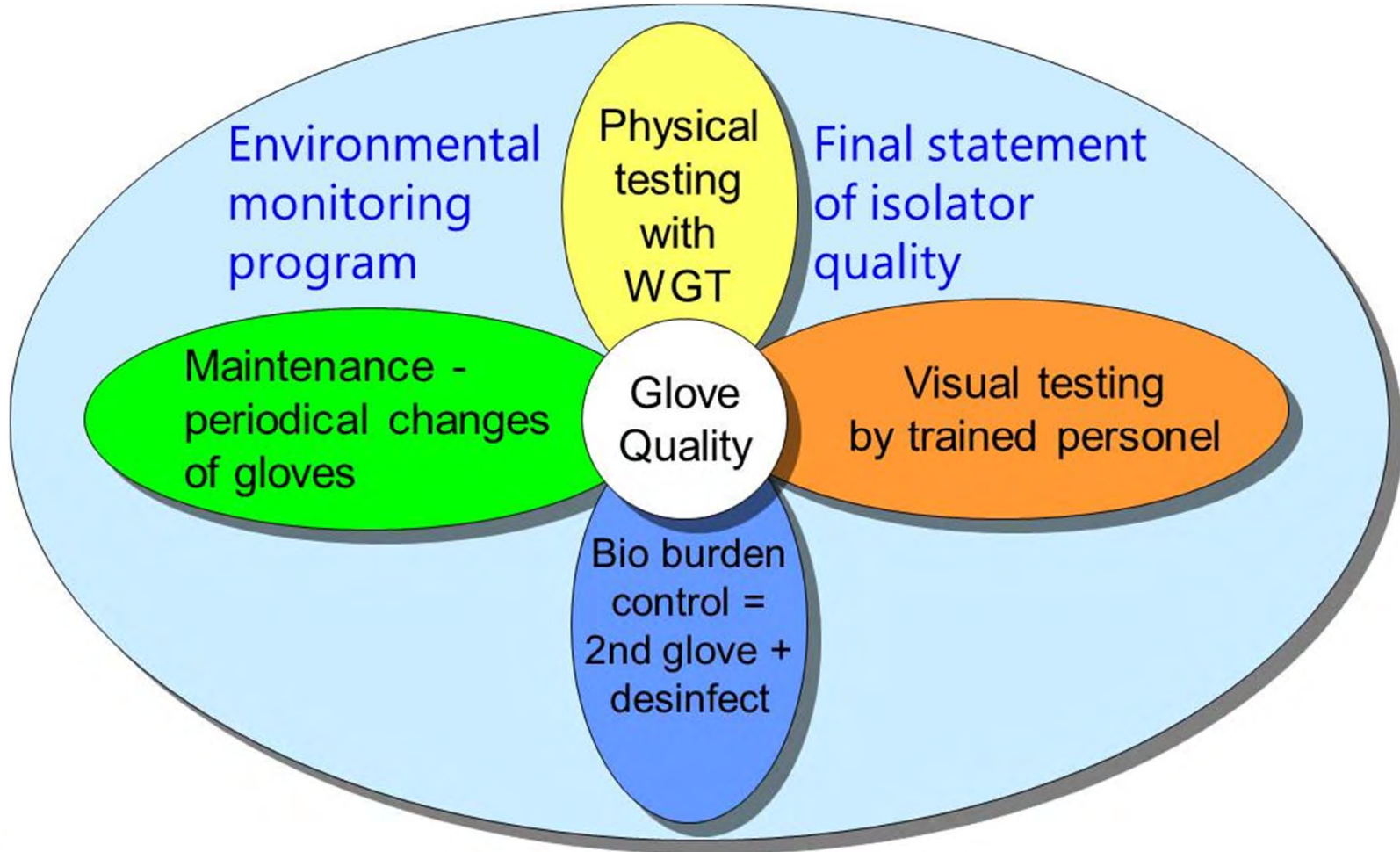
- Product Disposition and Investigation Procedure for Glove Deviations:
  - It is good to have a plan ready in case of a glove problem.
  - How will you respond?
  - Is additional monitoring required for an investigation?
  - If a glove fails in a critical area, how will the product risk be determined?
  - Setting up these procedures and investigation methods will help to determine the root cause for any glove problem and allow you to prevent or reduce the risk of future problems.





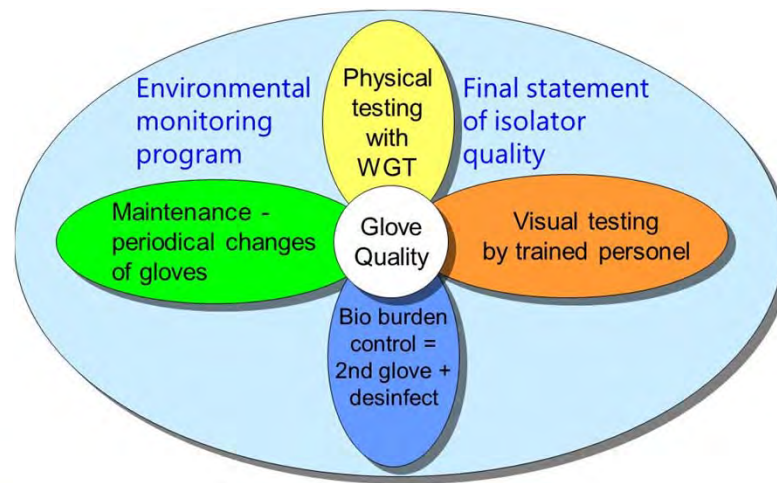
# Glove Management

- Conclusions:



# Glove Management

- **Conclusions:**
  - Look at glove management in a more global sense, taking into account all of the items discussed in this presentation.
  - Develop a 'risk-based' glove management program that fits your situation and application.
  - Define a written SOP and rationale for your glove management strategy – this is the best defense.
  - Yes, a physical glove test is an expectation of regulators – make sure that your method makes sense and is efficient as possible.



# References / Acknowledgements

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- PIC/S – Pharmaceutical Inspection Convention / Pharmaceutical Inspection Cooperation Scheme, PI 014-1, Recommendations for Isolators Used for Aseptic Processing and Sterility Testing, June 2002.
- Patrick Vanhecke, GSK – thank you☺

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