

Experience with Artificial Leaks and Dye Ingress Container Closure Integrity Testing (CCIT)

AAPS NBC, Boston, May 17, 2016

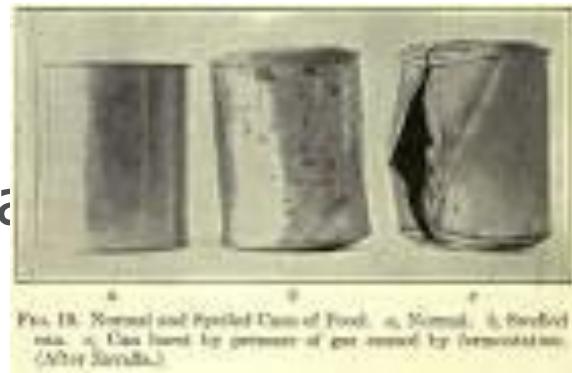
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DISCLAIMER

The perspectives and opinions in this talk are those of the presenter.



Food industry standard solutions – cans



sterile packaging available since 1880s



00 000 aseptic food products



Packaging should avoid microbial spoilage and infiltration of pathogens into products throughout the product shelf life

1970 – Septicemia problem

Nationwide Epidemic of Septicemia Caused by Contaminated Intravenous Products: Mechanisms of Intrinsic Contamination

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1975, p. 486-497

- Approx. 200 patients in hospitals showed septicemia (blood poisoning) with 20% mortality
- Hospitals used IV products from Abbott Labs
- Screw cap closure for infusion bottles with new elastomer liner



CCI addresses the maintenance of integrity to prevent microbiological ingress in sterile product packaging until the time of use (product opening)

Critical leak

- Void, gap, crack, hole, porosity or breach in a container closure system allowing the passage of microorganisms
- Main factor that affects critical leak size is whether or not the micro-leak is filled with liquid: microorganisms penetrate a liquid filled defect by motility or pressure differentials
- Still a lot of controversy discussions about the leak size at which the sterility may be jeopardized
 - 10 µm for fused silica capillary (Burrel, 2000)
 - 50 µm for channel leaks (Yam, K. 1995)
 - 0.4 µm Glass Micro-Pipettes (Kirsch, 1997)
 - 4 µm pinhole in steel plate (Morrical, 2007)
 - 20 µm OD Wire (Morrical, 2007)

Artificial leaks - Purpose of artificial leaks

- Used to assess sensitivity and performance of a technique, e.g., experimentally define limit between non-leaking and leaking CCS (CCIT method development)
- Correlation of pCCIT to microbial ingress (mCCCI)
- Assay validation purposes
- SST/assay controls

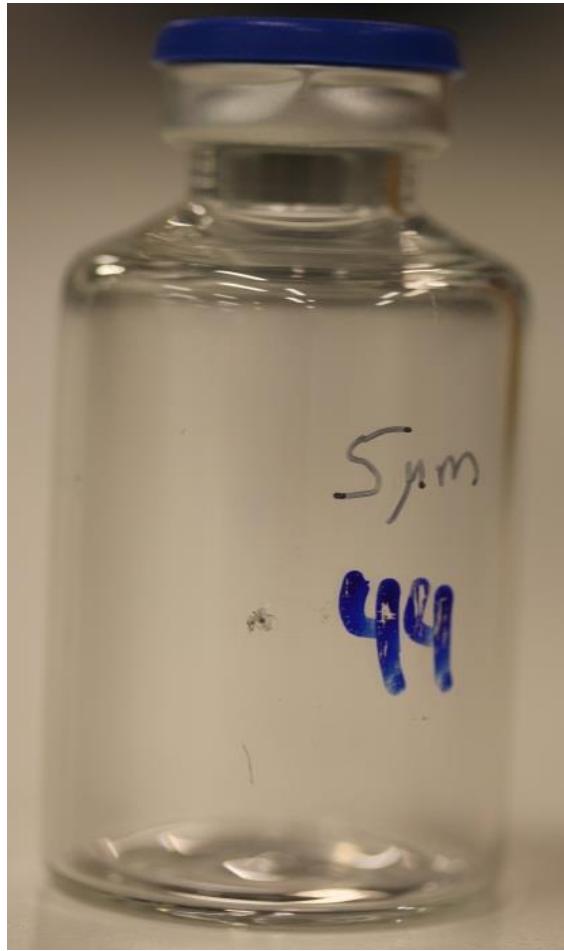
Types of artificial leaks

- Most commonly used methods of creating leaks:
 - Laser drilling into the body of the container
 - Laser drilling into a metal plate or tubing that is integrated to a CCS
 - Micron-wires inserted at the interface between the closure and container
 - Micropipettes (glass) inserted into the stopper or glued into an artificial hole of the container
 - Capillaries (fused silica, nickel, glass) or needles (steel) inserted into the stopper or glued into an artificial hole of the container

Laser drilled holes (orifices)

Advantages	Disadvantages
<ul style="list-style-type: none">• $\geq 1-2 \mu\text{m}$ in container (e.g., glass vial)• $\geq 0.5 \mu\text{m}$ in thin steel plates• Many materials can be used, e.g., glass container, steel plates/tubings• Leaks are closer to real world defects (no defined geometry)	<ul style="list-style-type: none">• Need to be prepared in specialized labs and are expensive• The size of laser-drilled void needs to be calibrated• Positive controls cannot be prepared directly on the product (product filled containers cannot be used for laser drilling)• Non-negligible risk of alteration of void post manufacture and/or calibration, prone to plugging during handling, shipment, storage: e.g., small holes can clog easily (silicone oil in siliconized syringes, due to micro-particles/fat from finger)

Laser drilled holes (orifice)



Laser-drilled
hole in glass vial wall

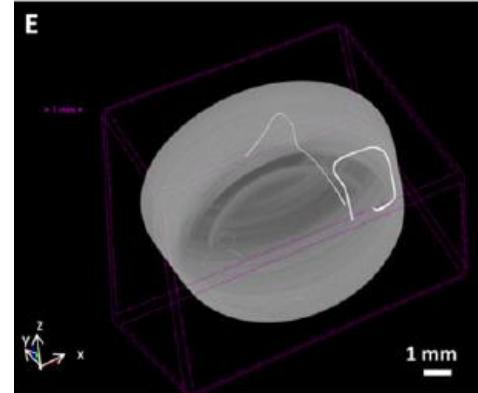
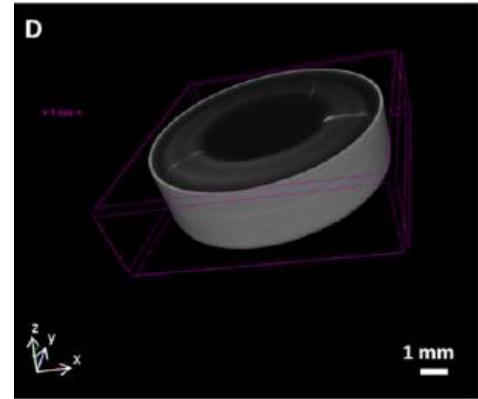
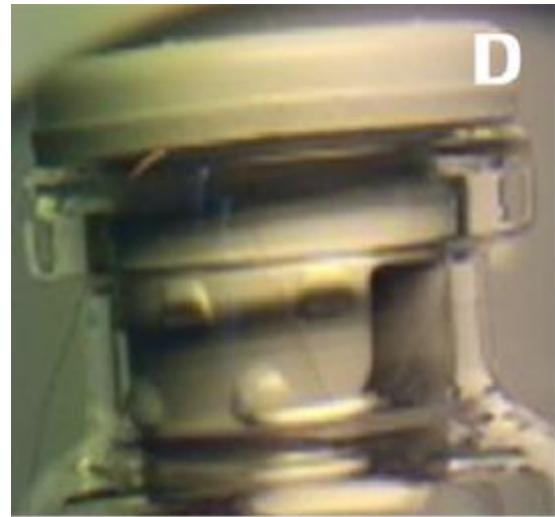
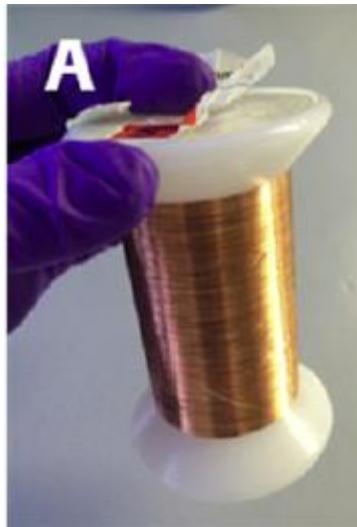


Laser drilled hole in
stainless steel capillary
which is glued into rubber
stopper of a vial

Wires

Advantages	Disadvantages
<ul style="list-style-type: none">• $\geq 10 \mu\text{m}$ (OD) wires in different materials available• Create micro-channels between elastomeric closure and container, e.g., similar to hair/foreign object between container and rubber closure (e.g., lip of vial) – mimic potential real world gap (undefined path)• Wide range of "leaks" can be prepared• Low cost	<ul style="list-style-type: none">• Actual size depends on many parameters (diameter of wire, elasticity of rubber, press fit forces)• Handling of the micron wires can be difficult and the size of the void needs to be calibrated• Wire need to be inspected to ensure that they were not broken during sealing process• The holes can close up over time depending on the relaxation of the materials (e.g., stopper)

Wires

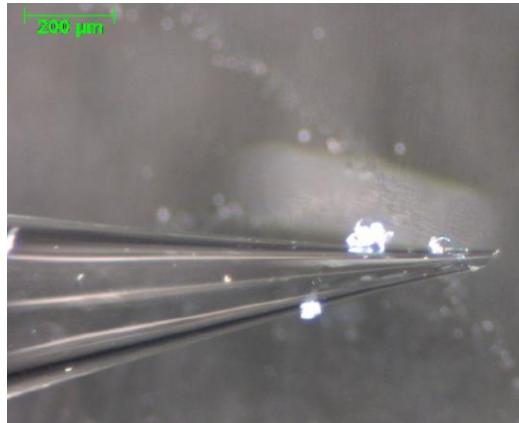


Nieto et al., PDA Journal of Pharmaceutical Science and Technology, 2016

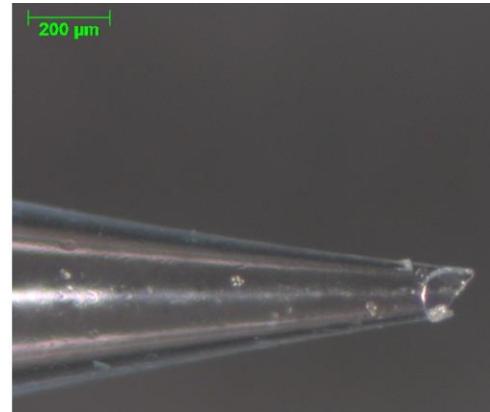
Glass pipettes

Advantages	Disadvantages
<ul style="list-style-type: none">• $\geq 0.1 \mu\text{m}$ (nominal internal diameter, tip)• “Consistent” pinhole type defects• Material matches primary packaging if glass pipettes are used	<ul style="list-style-type: none">• Break very easily, too fragile for routine use• Get larger, need to verify by microscope or flow rate measurements after sample preparation or handling (exposure to test conditions)• High risk of false sensitivity after preparation of a positive control (change of tip diameter)

Glass pipettes



10 μm new glass pipette
(approx. 50 μm opening
under microscope)



10 μm nominal glass pipette
(after handling (approx. 100 μm
opening under microscope))



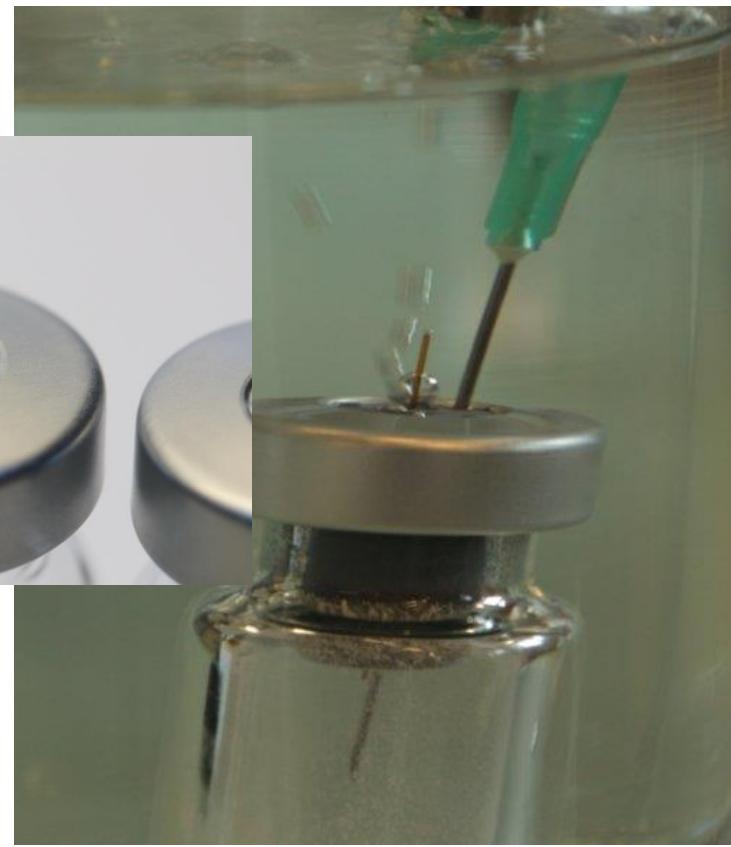
Capillaries

Advantages	Disadvantages
<ul style="list-style-type: none">• $\geq 0.2 \mu\text{m}$ internal diameter (fused silica)• Robust, wide size range and different materials available (fused silica, glass, nickel)• Easy preparation directly at the testing location• Leakage rate can be fine-tuned through length of capillaries• Consistent dimensions/leaks• Due to defined dimensions, no need to calibrate each individual leak	<ul style="list-style-type: none">• Geometry different from real world leaks• Uncertainty on actual diameter for very small tubings e.g., $0.2 \mu\text{m} \pm 0.1 \mu\text{m}$

Capillaries - Practical Aspects



10µm Capillary glued
Only defined leakage



10µm Capillary not glued
Undefined leakage

Capillaries – Practical Aspects

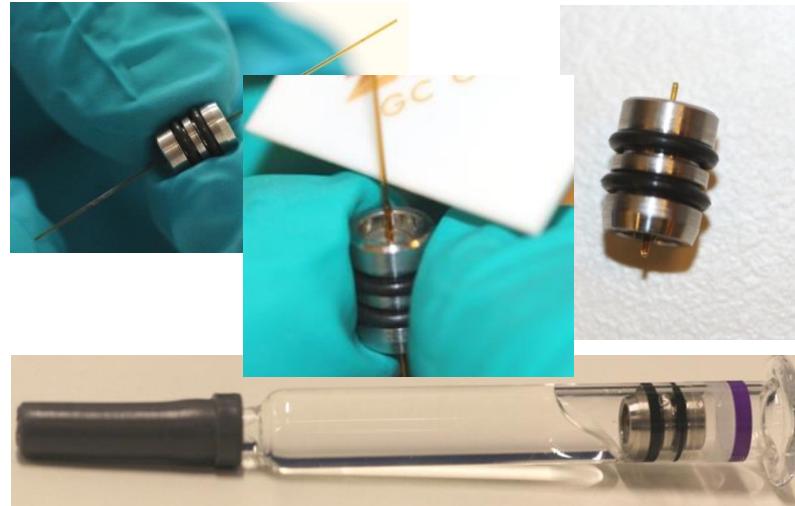
Through syringe rubber stopper



- Length of capillary varies as a function of fill volume (stopper position)
- Difficult to glue, only at outer part of stopper

Placement of capillary may be difficult and high risk for damage or inadvertent closure by glue or handling; dimensions of capillary need to be controlled irrespective of product fill volume

Optimized Stopper Replacement Part



- Consistent Length of capillary for each stopper position
- Improved preparation of positive leakage controls

Tubings – Gross leaks

Other types of gross leaks

- Injection needles of various internal diameters
- Cutting part of or piercing of rubber stopper
- Absence of a closure



Cut steel needle of 1.6 mm ID glued into rubber stopper

Methods need to be evaluated for response to large leaks (limitation of specific method, e.g. laser based headspace analysis can not directly detect larger leaks because of fast equilibration of head space with environment)

Comparison of artificial leaks

	Replicates	He-Leak [mbar*L/s]
Background Level	4	3.0×10^{-10} to 3.7×10^{-9}
Negative Leakage Control (tight empty vial)	4	1.5 to 4.7×10^{-9}
Laser Drilled Holes		
5 µm steel capillary (nominal flow effective diameter; glued into rubber stopper)	2	4.4×10^{-4} to 1.5×10^{-3}
2 µm in vial glass body (nominal flow effective diameter; empty vial)	2	9.0 to 9.1×10^{-5}
3 µm in vial glass body (nominal flow effective diameter; empty vial)	2	1.1 to 2.3×10^{-4}
5 µm in vial glass body (nominal flow effective diameter; empty vial)	2	2.5 to 6.1×10^{-4}
Copper Wires		
10 µm OD (placed between rubber stopper and vial, capped at different compression)	12	7.3×10^{-9} to 7.0×10^{-7}
30 µm OD (placed between rubber stopper and vial, capped at different compression)	12	1.1×10^{-8} to 1.4×10^{-4}
Micro-glass Capillaries (glued into rubber stopper of vial)		
2, 5, 10 µm tip (nominal ID)	11	2.5×10^{-4} to 4.4×10^{-2}
Procedural Negative Leakage Control (closed, glued fused silica capillary in rubber stopper of vial)	4	1.8 to 4.2×10^{-9}
Fused Silica Capillary (glued into rubber stopper of vial)		
2 µm (ID) x 11.8-12.9 mm (L)	3	7.7×10^{-9} to 4.0×10^{-8}
5 µm (ID) x 10.2-10.9 mm (L)	3	2.2 to 4.4×10^{-7}
10 µm (ID) x 10.1-13.0 mm (L)	7	1.0×10^{-6} to 5.4×10^{-5}
15 µm (ID) x 10.0-10.2 mm (L)	4	2.9 to 5.6×10^{-5}
25 µm (ID) x 10.5-13.5 mm (L)	6	1.0×10^{-4} to 1.0×10^{-3}

Comparison of artificial leaks

- Non leaking levels were approx. $\leq 5 \times 10^{-9}$ mbar*L/s (background, tight vials, procedural blanks with glued capillaries)
- Same very low flow rates (approx. $\leq 4.0 \times 10^{-8}$ mbar*L/s) observed for 2 μm fused silica capillaries (L:~12 mm), 92% of 10 μm and 42% of 30 μm wires
- Comparable flow rates ($1-9 \times 10^{-4}$ mbar*L/s) for most of "5 μm " laser drilled holes, 17% of 30 μm wires and 25 μm fused silica capillaries (L:~11 mm)
- 10 μm fused silica capillaries (L:~10-13 mm) show flow rates (1.0×10^{-6} to 5.4×10^{-5} mbar*L/s) lower than 2 μm laser drilled holes (9.0×10^{-5} mbar*L/s)
- It is difficult to achieve consistent leakages using wires and even more glass micro-pipettes

Dye Ingress – USP <1207>

Dye ingress (tracer liquid submersion test) is stated as one of the CCIT useful in any of the product life cycle phases

Tracer liquid test method is a destructive approach ***providing an indication*** of leak presence and may provide a measure of relative leak size.

Successful liquid tracer detection relies on a combination of tracer solution wicking, tracer solution effusion, and tracer element diffusion through a liquid-filled leak path and are events that are difficult to predict or control, especially for detection of smaller leaks. These events are influenced by numerous factors, including the package materials of construction, leak path tortuosity and topography, tracer liquid surface tension, and leak path blockage by product, extraneous debris, and air locks.

Tracer liquid tests ***may fail to reliably detect small leaks*** due to any one of a number of factors including air locks, product, or debris in the leak path; liquid surface tension; leak path geometry; or insufficient differential pressure test conditions. The same is true for microbial ingress tests that are further subject to the inherent variability of living microorganisms.

Dye Ingress – Industry Experience

Dye ingress (tracer liquid submersion test) is one of the most used test method to demonstrate CCI within industry:

- Versatile and can be used on primary and secondary packaging in support of development, manufacturing and stability testing
- Detects directly relevant leaks of concern
- Different dyes can be used to tailor the method
- Improved sensitivity when optimized vacuum/pressure cycles are used. LOD varies depending on the leak size, materials, dye concentration and challenge conditions
- The tracer liquid must be miscible and not chemically reactive with the product
- Correlation to microbial ingress can be established using the same challenge conditions
- Has been seen to work well for liquids but depending on the dye it may not be suitable for lyophilized products

Dye Ingress Test – Method Execution

Prepare positive leakage controls using drug product (with e.g. fused silica capillary of defined internal diameter and controlled length)



Place positive leakage controls and samples into pressure vessel and add dye solution that all units are completely immersed



Challenge with successive pressure and vacuum cycles



Wash (and dry if needed) exterior surface of challenged units



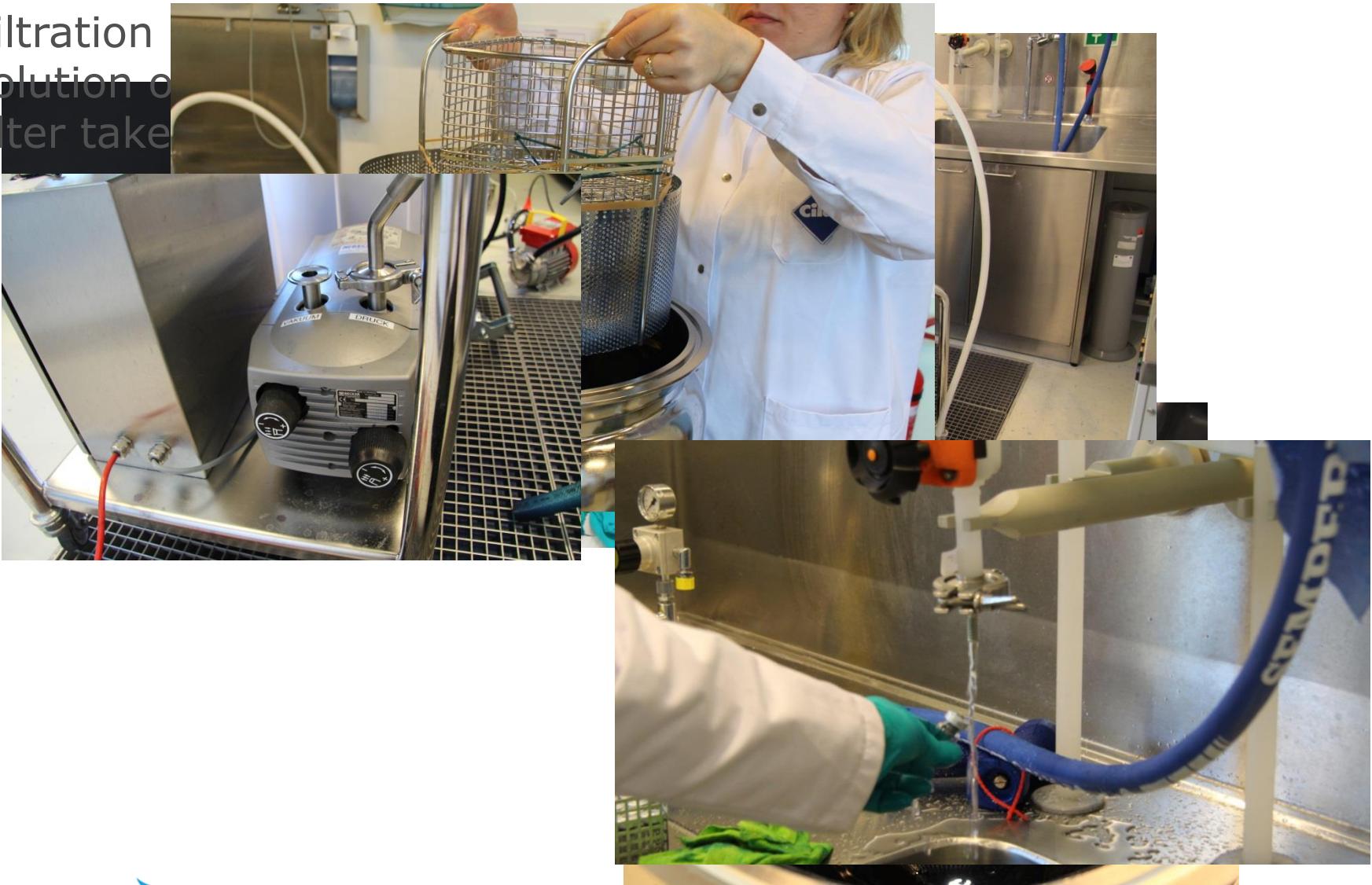
Visually inspect challenged units by comparing to positive and negative dye units to determine if discoloration (dye penetration)



In case of doubt, measure UV absorbance (e.g. for methylene blue at 670nm)

Dye Ingress Test – Test Equipment

Filtration
solution o
filter take



Dye ingress test – Enhanced Equipment and Improved Method Performance Measures

Improvements of dye ingress procedure to improve reliability and robustness and make it QC friendly (FMEA performed):

- Filtration of dye solution prior each run over 1 µm filter in short time
- Positive leakage control preparation (capillaries to be glued to reduce false positives)
- Challenge vessel with programmed pressure / vacuum cycles to automate method execution
- Optimized fixtures for samples (to avoid damage of positive controls)

Improvements of Dye Ingress Method



Visual Inspection



From left to right:
Negative dye control, negative leakage control,
4x positive leak controls (10 μm fused silica),
4x positive leakage controls (25 μm fused
silica),
positive dye control (blue equivalent to
penetration of 0.10 μL dye)



Vials in Visual Inspection Station:
left vial: negative dye control vial,
mid vial: challenged Sample vial,
right vial: positive dye control vial (blue
equivalent to penetration of 0.25 μL dye)

Dye Ingress Test – Performance Characteristics

- Penetration of 0.10 μL of dye into e.g., 1 mL filled drug product syringe can be consistently detected by visual inspection
- >80% of positive leakage controls with 10 μm fused silica capillary of a length 11 ± 1 mm demonstrate robustness of dye ingress at 1.0×10^{-6} to 5.4×10^{-5} mbar*L/s (He, orifice leak 0.1 to 1 μm per USP)
- Very flexible use of optimized system, with sample sizes between 20 to a couple of hundreds units, enabling testing of vials of different size and pre-filled syringes during the same run

Conclusions

- The leakage flow rate does not necessarily correlate to a specific hole size
- Referring to leak size in micrometer (μm) may suggest an absolute unit which can be compared to the size of bacteria, but this assumption is misleading!
- Dye ingress is robust and versatile when optimized (dye concentration, surfactants, pressure/vacuum cycles, equipment)
- Is the dye ingress method a probabilistic method (associated with random outcomes)?



Thanks

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- European Industry colleagues
- BioPhorum colleagues

Back-up slides

Definition of Container Closure Integrity

Container closure integrity (CCI) can be defined as the ability and quality of a container closure system (primary and secondary packaging) to protect a product from loss and contamination (synonymous with *Package Integrity*, USP)

CCI addresses the maintenance of integrity to prevent microbiological ingress in sterile product packaging until the time of use (product opening)

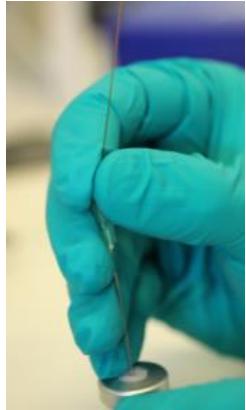
Dye Ingress Test - Requirements

- Extremes of pressure and handling, e.g., shipment and storage of product should be considered
- Positive controls should demonstrate dye ingress and should be of a defect size that approaches a critical leak
- Concentration of challenge solution must be sufficient for detection (via chosen detection method)
- Dye ingress using visual determination of ingress must establish each inspector's dye detection limit.
- Dye ingress method is a probabilistic method (associated with random outcomes, reliability?)

CCIT during life cycle of a parenteral product

- Package development and validation
 - Critical dimensional tolerances for each component material, challenges with worst-case critical dimensions and maximum stress conditions
 - Package processing (package material cleaning, sterilization) and assembly (sealing e.g. vials-capping forces or assembly)
 - Package integrity evaluation as part of process validation
- Product Manufacturing
 - Packaging or capping processes provide consistent products (process controls, on-line controls)
- Commercial Product Stability
 - Physical test methods are preferred and are to be implemented during commercial product stability

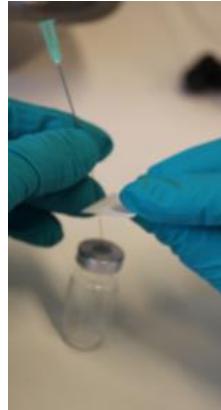
Preparation of Positive Controls



Pierce stopper of vial with 21G needle with capillary inserted (but not protruding through beveled needle tip)



Withdraw 21G needle with capillary remaining in vial



Cut capillary leaving approx. 2 cm beyond stopper surface



Apply glue around capillary on stopper surface



UV cure glue



Cut capillary to final total length of <1.5 cm