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Being sure

Positive controls

27.02.2020 | Dr. M. Kahl

Inhalt
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36pt

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4. Verification

Fundamentals

What are postive controls?

What are positive controls?
Definitions

Definition USP 1207

Positive control: A positive control is a package with a known, intentional leak. Positive controls used for leak test method development and validation studies should duplicate those negative controls used for the same studies in terms of materials of construction, package assembly, and component processing. Positive controls are included for defect type and for larger-size defects (used for method development), as well as smallest-size defects (used for method development and validation studies). Microbial grow-through positive controls are used for microbiological challenge method development, validation, and routine testing of certain packaging systems uniquely at risk for microbial entry by grow-through processes.

What are negative controls?
Definitions

Definition USP 1207

Negative control: A negative control is a package with no known leak. Negative controls used for leak test method development and validation studies represent packages that were typically assembled using normally processed components.

What are functional test?

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Are all controls positive controls?

Differentiation from functional tests

Example: Multimeter for measuring voltages

Functional test for multimeter:

- e.g. attach to battery
- swich to AC 230V and attach to wall plug
- will display a voltage → multimeter works

Test with propose of **validation/qualification:**

- calibrated voltage source
- testing strategy
- measurement ranges
- well defined testing strategy
- definition of

Are all controls positive controls?

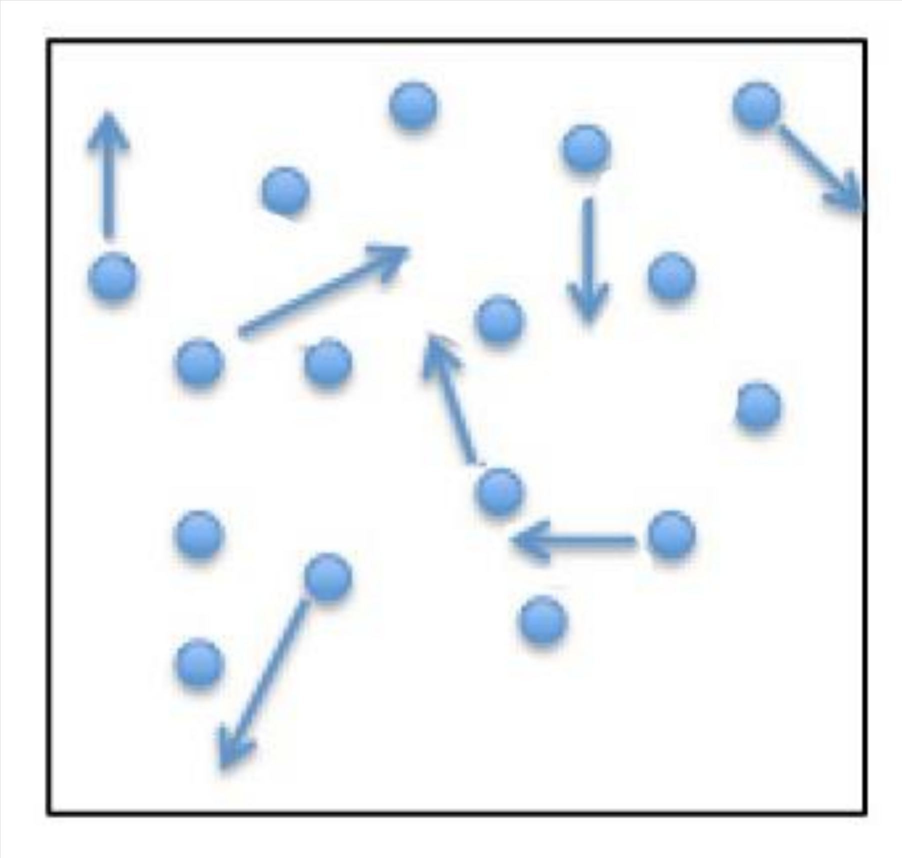
Differentiation from functional tests

What is a functional test?

A functional test is testing only the proper operation of the measuring / testing technology itself and not the entire testing equipment. For this test, the testing machine itself need not necessarily to be operated. Objects used for functional tests do not have to resemble the final testing samples.
Application: time to time tests, pre-batch tests, intra-batch tests

What are tests for validation / qualification?

In a **validation ore qualification** use a positive control that has all the properties of a negative sample (as they are produced) and in addition has a known artificial leak.
Application: qualification of equipment, validation of recipes, introduction of new formats



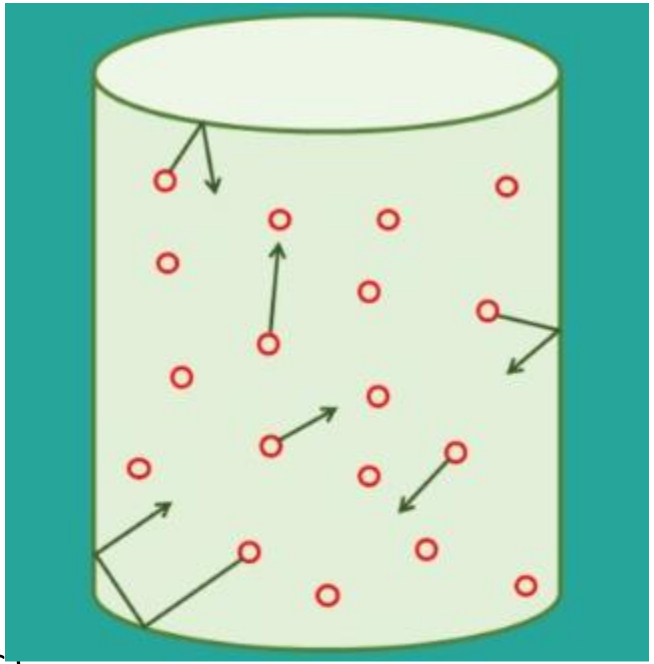
Ideal gas law

Positive controls – based on gas transfer

Gas dynamics – ideal gas law (equation of state)

The ideal gas law: $p * V = N * R * T$

▪ p: pressure	[bar]
▪ V: volume	[m3]
▪ N: amount of substance	[mol]
▪ R: ideal gas constant	8.314 J/K/mol
▪ $R = N_A * k_B$	
▪ N_A : Avogadro constant	$6.022 * 10^{23}$ 1/mol
▪ k_B : Boltzmann constant	$1.38 * 10^{-23}$ J/K
▪ T: temperature	[K]



- strictly speaking only valid for non interacting point like objects (Helium gas)
  - atomic gases

Application: use for calculating all gas transitions

Positive controls – based on gas transfer

Gas dynamics – ideal gas law (equation of state)

but

- very small errors for the temperature and pressure range used here
- deviations from the ideal gas only occur at dramatically different conditions
- can also be used for molecules

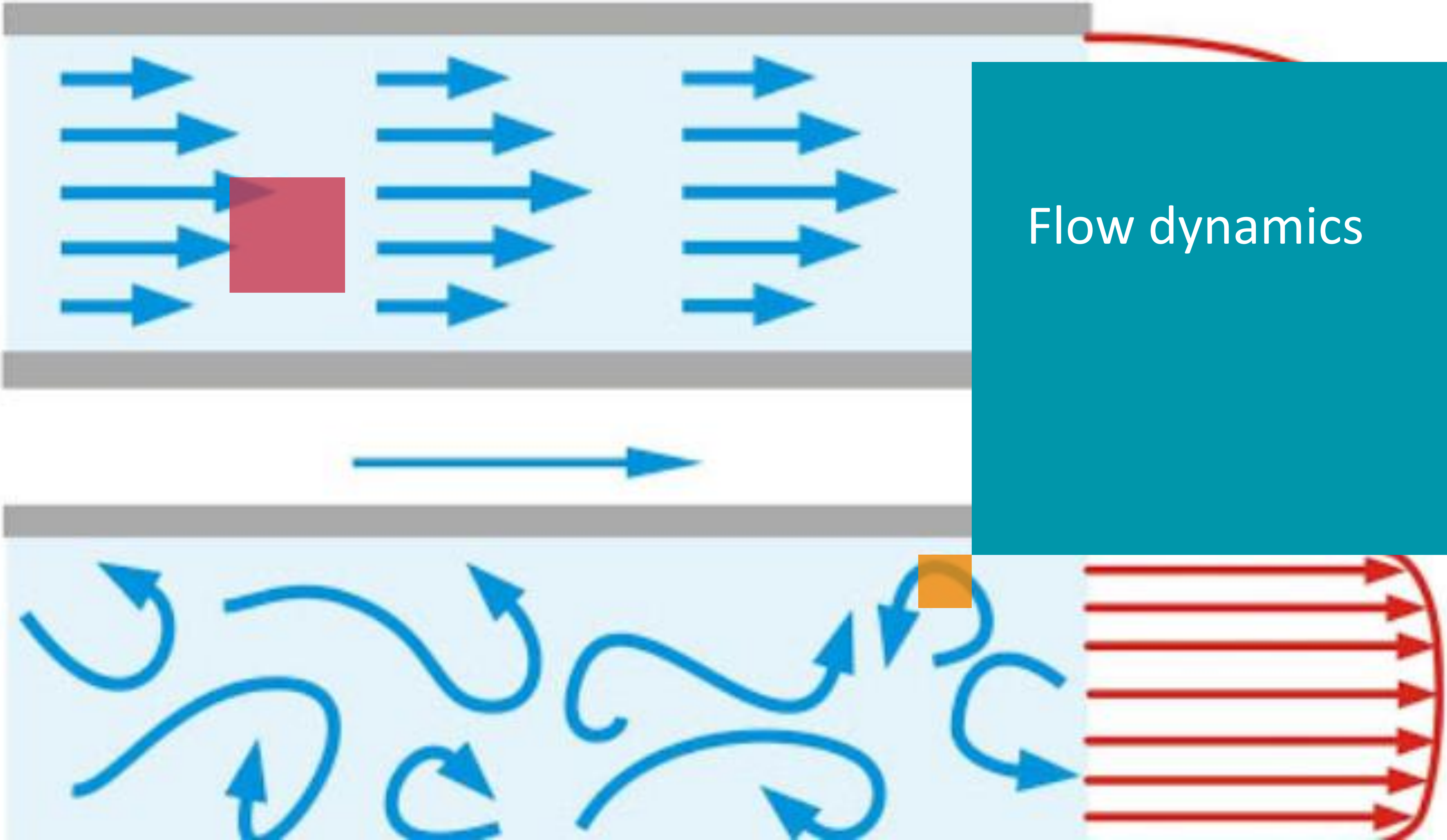
$$p * V = N * R * T$$

Extension on gas mixtures: $p_{tot} = p_1 + p_2 + \ldots; N_{tot} = N_1 + N_2 + \ldots;$

p_{tot}: total pressure

p_1, p_2, …: partial pressure of gas component 1, 2, …

Application: calculation of tracer gas concentrations for HSA.



Flow dynamics

Positive controls – based on mass transfer

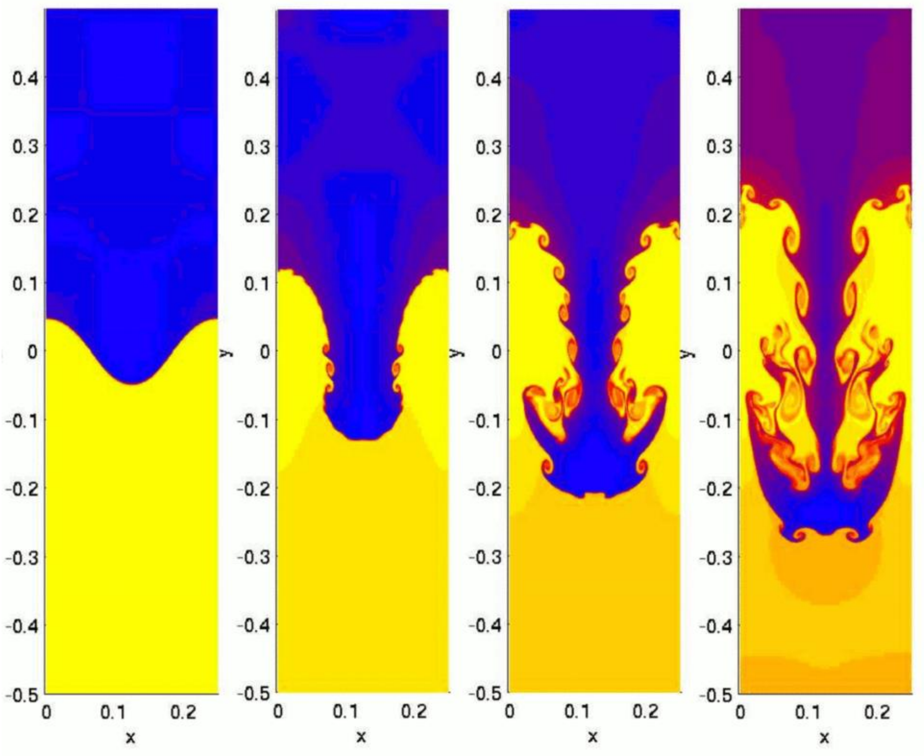
Some basics on flow dynamics

Equations are really complicated – the basics are:

- conservation of mass
- conservation of momentum
- conservation of energy

Some important properties for the application here are:

- **compressible** vs. incompressible flow (gas dynamics)
- **Newtonian** vs. Non-Newtonian fluids (related to viscosity)
- **Laminar** vs. turbulent flow



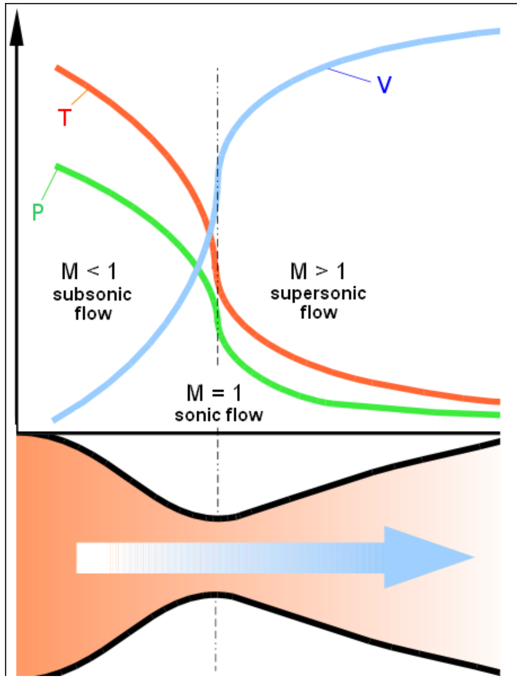
Positive controls – based on mass transfer

Compressive flow

Flow of compressible gases:

- needed for the calculation of flow using the model of an ideal orifice
- becomes important if duct length to with ratio is smaller than 5

- flow through the nozzle can become chocked
- speed in nozzle can get to supersonic
- increase in pressure difference will not further increase the flow
- ratio in pressures approx. 2



Application: calculation of flow via orifices

Positive controls – based on mass transfer

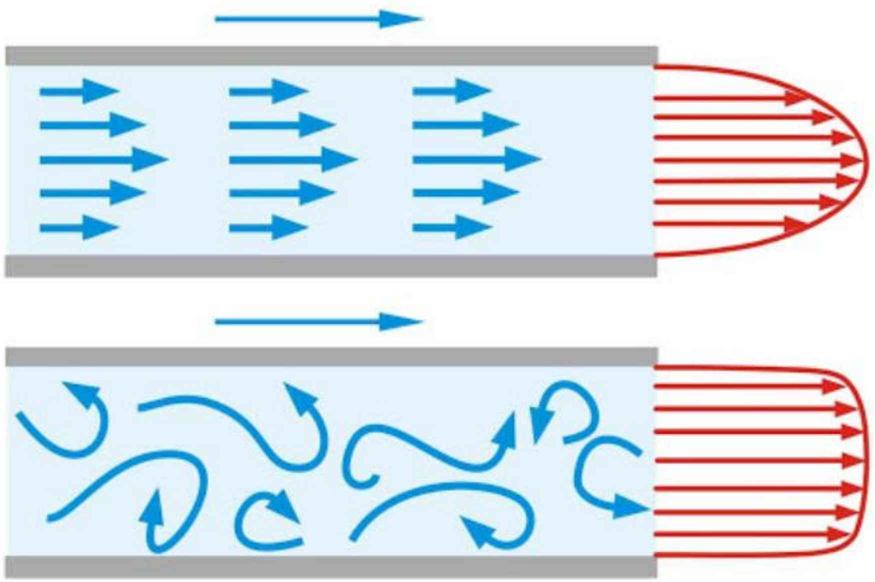
Laminar flow

Described by the Reynolds number

- relationship between inertial force and the shearing force
- for flow through a pipe
- Re = Q * D / ν / A
  - Q: mass flow
  - D: diameter
  - ν: kinematic viscosity
  - A: cross section

- transition range
  - critical Re = 1800 – 4000

Application: calculation of flow via tube structures



Background for all positive controls using some kind of tube structure as artificial leak

Positive controls – based on mass transfer

Balistic transport

Information in gas is transferred by inter-molecule collisions

- important parameter is the medium free path length
- medium distance a molecule travels in-between collisions

Pressure range	Pressure [mbar]	Medium free path length
ambient	1013	70 nm
Coarse vacuum	300 - 1	0.1 – 100 µm
Fine vacuum	1 – 0.001	0.1 – 100 mm

Application: Only for special cases with very low pressures

Flow via orifice

Positive controls – based on mass transfer

Flow model for orifice

Calculation of flow via ideal orifice:

$$Q = \frac{\mu * \pi * d^2 * \Psi * \sqrt{2 * p(low) * \rho(low)}}{4 * \rho(high)}$$

µ: expansion coefficient (µ approx. 0.82)
d: diameter
p(low): lower pressure
p(high): higher pressure
ρ: density
k: adiabatic constant

Flow function:

$$\Psi = \sqrt{\frac{k}{k-1}\left[\left(\frac{p(high)}{p(low)}\right)^{\frac{2}{k}} - \left(\frac{p(high)}{p(low)}\right)^{\frac{k+1}{k}}\right]}$$

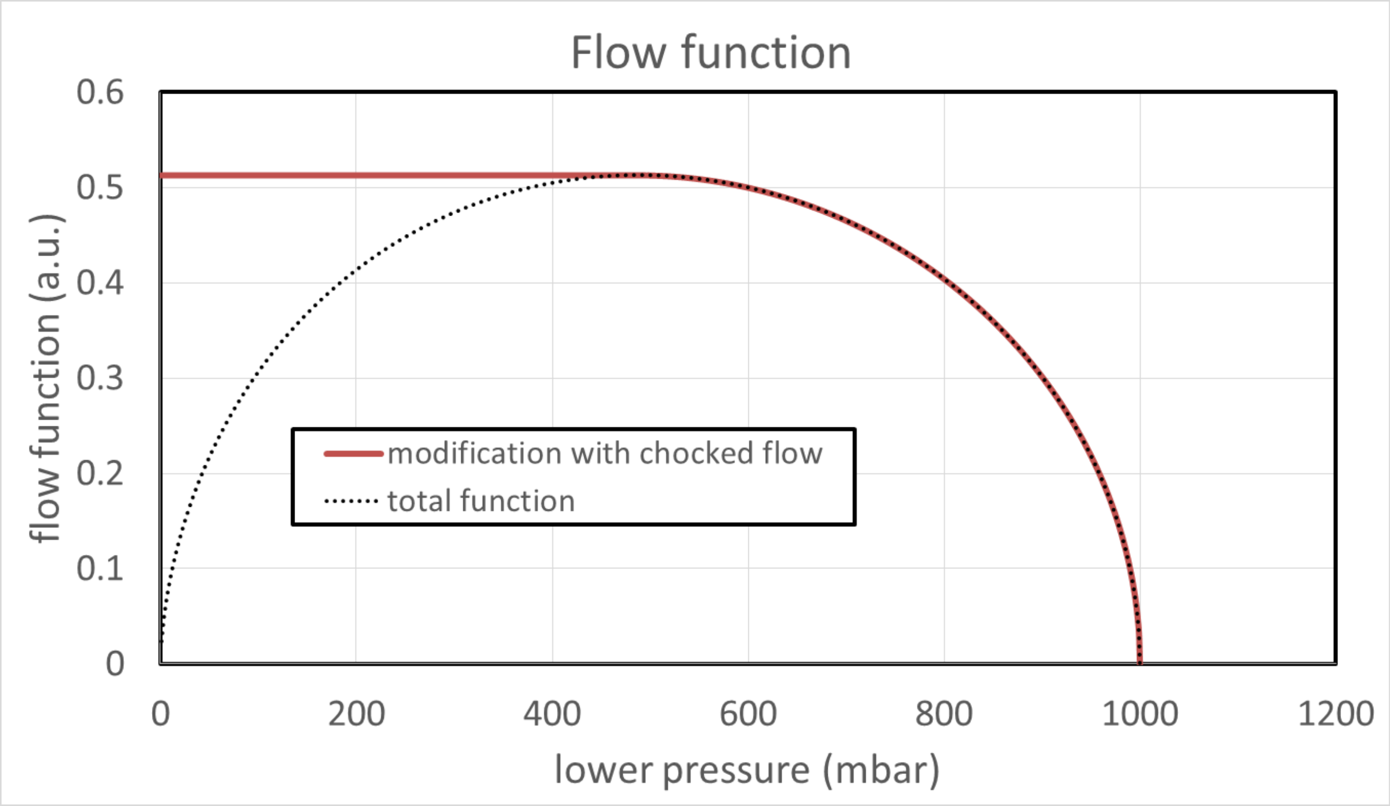
*Application: **Only one parameter** to tune the flow of positive control → diameter of hole*

Positive controls – based on mass transfer

Flow model for orifice

Exemplary calculation of flow function

- external pressure 1000 mbar
- internal pressure is variated
- flow chocked below approx. 500 mbar
- flow will increase much slower for higher pressure differences



Flow via capillary

Positive controls – based on mass transfer

Flow model for capillary – laminar flow

Calculation of flow via capilary:

$$Q = \frac{\pi * d^4 * (p(high)^2 - p(low)^2)}{256 * \eta * l}$$

η : viscosity

d: diameter

p(low): lower pressure

p(high): higher pressure

l: length

Practical considerations:

Two parameters to tune the properties of flow dynamics

- pressure difference driven equalization
- concentration gradient driven processes (diffusion)

Application: use for designing positive controls when pressure driven flow and diffusion need modelling

Types of positive controls and preparation

Orifice type

Types of positive controls

Orifices – general remarks

Why use orifices?

- no resemblance to real defects
- perfectly shaped holes will never occur on real container

Reasons to do so none-the-less:

- can easily be modelled
- correlation between flow and diameter can be calculated
- USP gives a rough correlation between flow and leak diameter
  - technologies based on flow rate measurements can be correlated to a leak size
  - correlation to regulations is straight forward

Types of positive controls

Orifices – general remarks

Table 1. Package Integrity Test Method Leak Detection Index

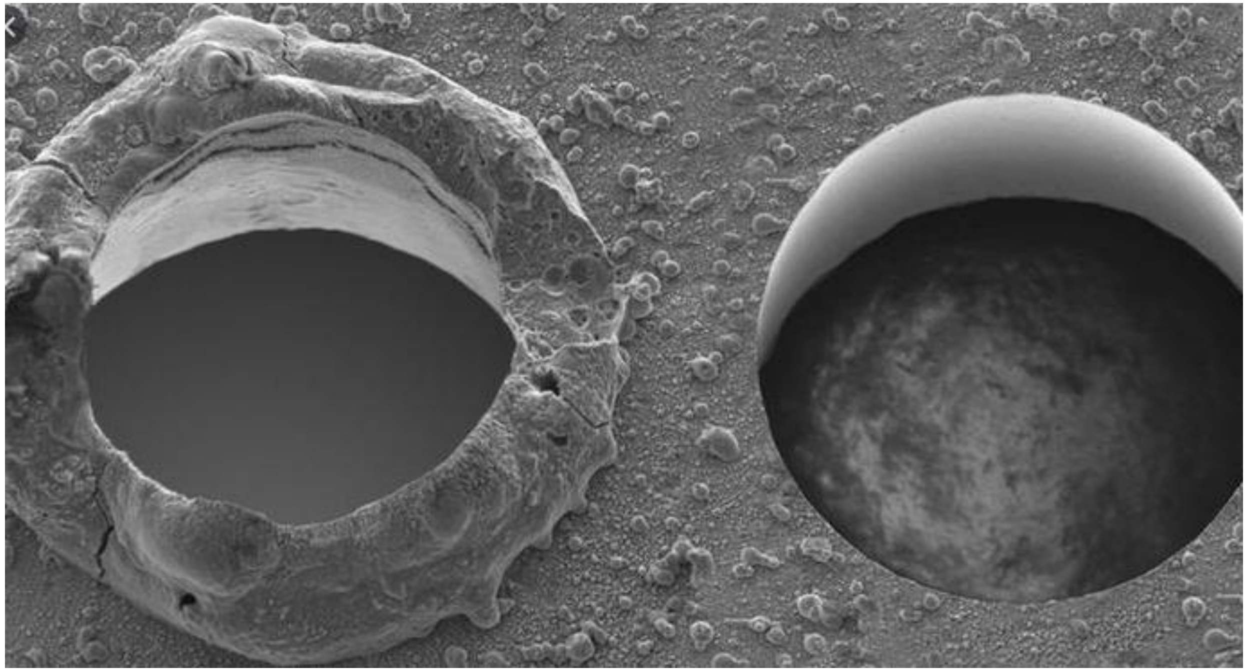
	Detectable Leaks Expressed in Two Different Units of Measure	
Limit of Detection Index Classification	Air Leakage Rate[a] (stdcm^3/s)	Orifice Leak Size[b] (µm)
1	$<10^{-6}$	<0.1
2	10^{-6} to 10^{-4}	0.1 to 1
3	6×10^{-4} to 4×10^{-3}	2 to 5
4	5.0×10^{-3} to 1.6×10^{-2}	6 to 10
5	0.017 to 0.360	11 to 50
6	>0.36	>50

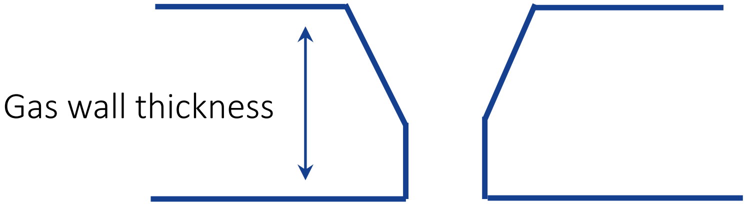
Types of positive controls
Laser drilled holes

Development in laser technology:
- ultrashort laser pulses available for machining
- thermal effect during machining neglectable
- machining of transparent media (glas)

Practical considerations:

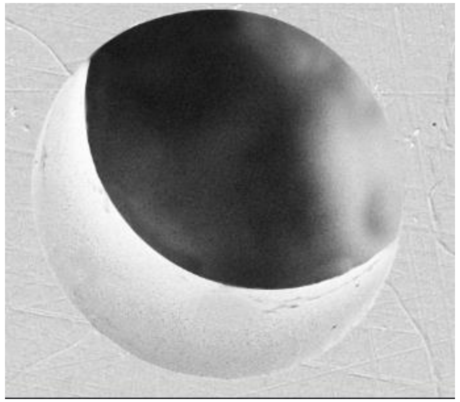
☹ final structure fill not have ideal orifice geometry
☹ quality of entrance and exit facet
☹ reliable manufacturing of very small flow rates
☹ leaks are limited to the container body (glas)
☹ / ☺ external manufacturing
☺ direct correlation to regulatory
☺ no protruding objects from container


Thermal vs. non-thermal laser machining



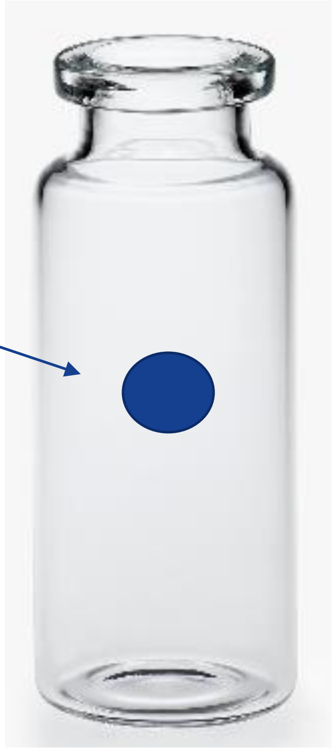
Cross section of laser drilled leak path

Types of positive controls
Metallic orifices (laser drilled)



- orifices drilled in thin metallic foil
- metal film is then fixed to the container (glued)
- technology available from high vacuum components (vacuum stages)

Metal foil with laser drilled hole



Practical considerations:

☹ mounting at limited areas
☹ handling and mounting extremely complicated
☹ / ☺ external manufacturing
☺ best available quality for the hole geometry
☺ best match for the model of an ideal orifice

Micro tube type

Positive controls – based on mass transfer

Capillaries

- calibrated capillaries available
  - ceramic capillaries
  - glas capillaries
- metal rods (with laser micro drilled hole at one end)
- micro pipette
- combination of models might be needed

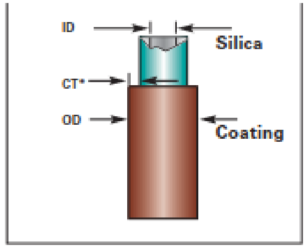
Practical considerations:

☹ mechanical handling

☺ mounting on cite, easy to mount

☺ verification on site

ID µm		OD µm		CT* µm
002	± 01	150	± 06	12
005	± 02	150	± 06	12
005	± 02	363	± 10	20
010	± 02	150	± 06	12
010	± 02	363	± 10	20
015	± 02	150	± 06	12
015	± 02	363	± 10	20
020	± 02	090	± 06	12
020	± 02	150	± 06	12
020	± 02	363	± 10	20
025	± 02	150	± 06	12
025	± 02	363	± 10	20
030	± 02	150	± 06	12
030	± 02	363	± 10	20
040	± 03	105	± 06	12



TSP Characteristics

- Standard polyimide coating
- Synthetic fused silica
- 100% proof tested at 100kpsi**

Special types

Types of positive controls

Wire under the stopper

- introduce wire in-between stopper and neck
- different wire materials possible

Practical considerations:

☹ no direct correlation between wire size and leak size
☹ leak path is not easily modulated
☹ final leak rate is not determined before hand
☺ easy in-house manufacturing
☺ fast and cost effective
☺ best match for the model of an ideal orifice

Types of positive controls

Cracks

- generate crack in container body
- crack can be introduced
  - mechanically
  - thermally
  - combinations

Practical considerations:

☹ no possibility to yield the flow rate to be produced

☹ yield of final output is “uncertain” at best

☹ every sample needs requalification to determine final leak rate

☹ leaks (cracks) tend to reseal

☺ “easy” in-house manufacturing

☺ fast and cost effective

☺ matches best real occurring defects

Positive controls vs. test technologies

Study with LONZA

Positive controls vs. test technologies
Study with Lonza

Systematic Assessment of pCCIT Method performance and Commonly Used artificial Leaks

published in PDA journal

- **4 different CCIT methods**
  - Dye ingress
  - He Leak test
  - vacuum decay
  - head space analysis

- **4 different types of artificial leaks**
  - Laser drilled micro holes
  - Capillary leaks
  - Copper wire leaks

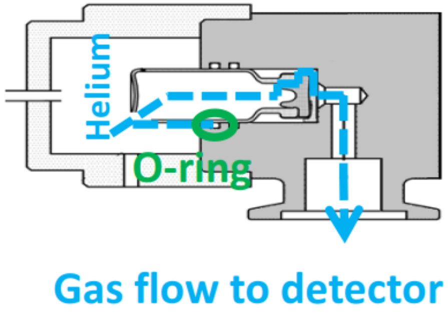
Positive controls vs. test technologies

Study with Lonza

Helium Leak test

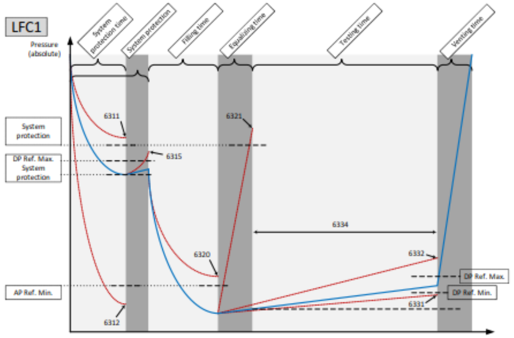
Vacuum mode

Method acc. to Morical et. al



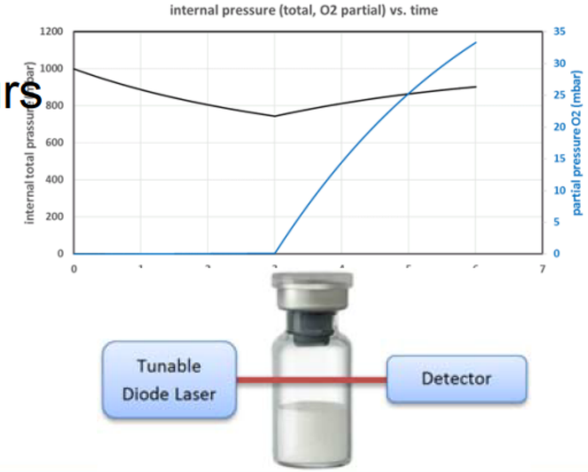
Vacuum decay

5mbar abs. pressure



Head Space Analysis

O_2 concentration
500 mbar for 3 hours
Atm for 3 hours



Blue dye ingress

500mbar for 1 hour

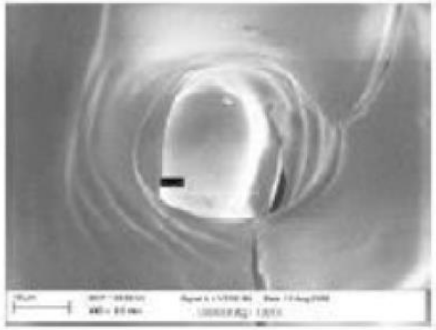
Atmospheric pressure for 1 hour

Positive controls vs. test technologies

Study with Lonza

Micro holes

Laser-drilled micro holes with different inner diameters

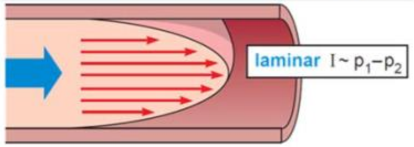


Copper wire

Wire with certain diameter between stopper and glas

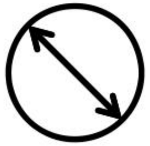
Capillaries (orifice)

Capillaries with different inner diameters cut to length of the corresponding flow rates acc. to Hagen-Poiseuilles law



Capillaries (nominal diameter)

Capillaries with different inner diameters cut to the length of 10 mm



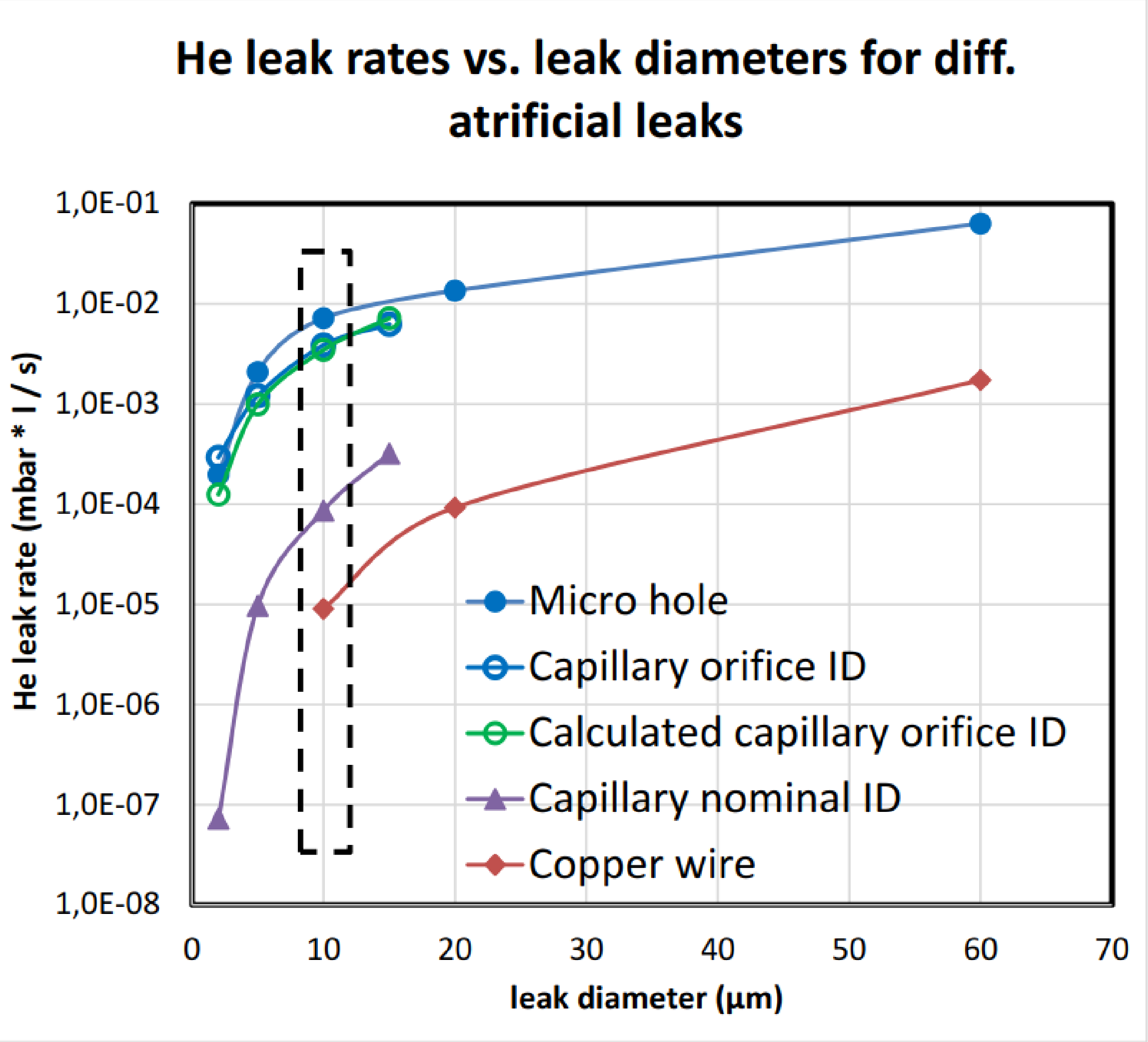
Source: Image micro holes: Dana Guazzo, presentation “Sterile product integrity testing”, May 2010

Positive controls vs. test technologies

Study with Lonza

Robustness of artificial leaks:

- Significant variation in flow rate of different artificial leak types
- 10 µm leaks indicate a variation of up to three magnitudes (factor=1000)
- Significant deviations from nominal to target leak size (calculated vs. measured HE leak)
- Capillaries (orifice diameter) show high degree of correlation to theoretical flow rate

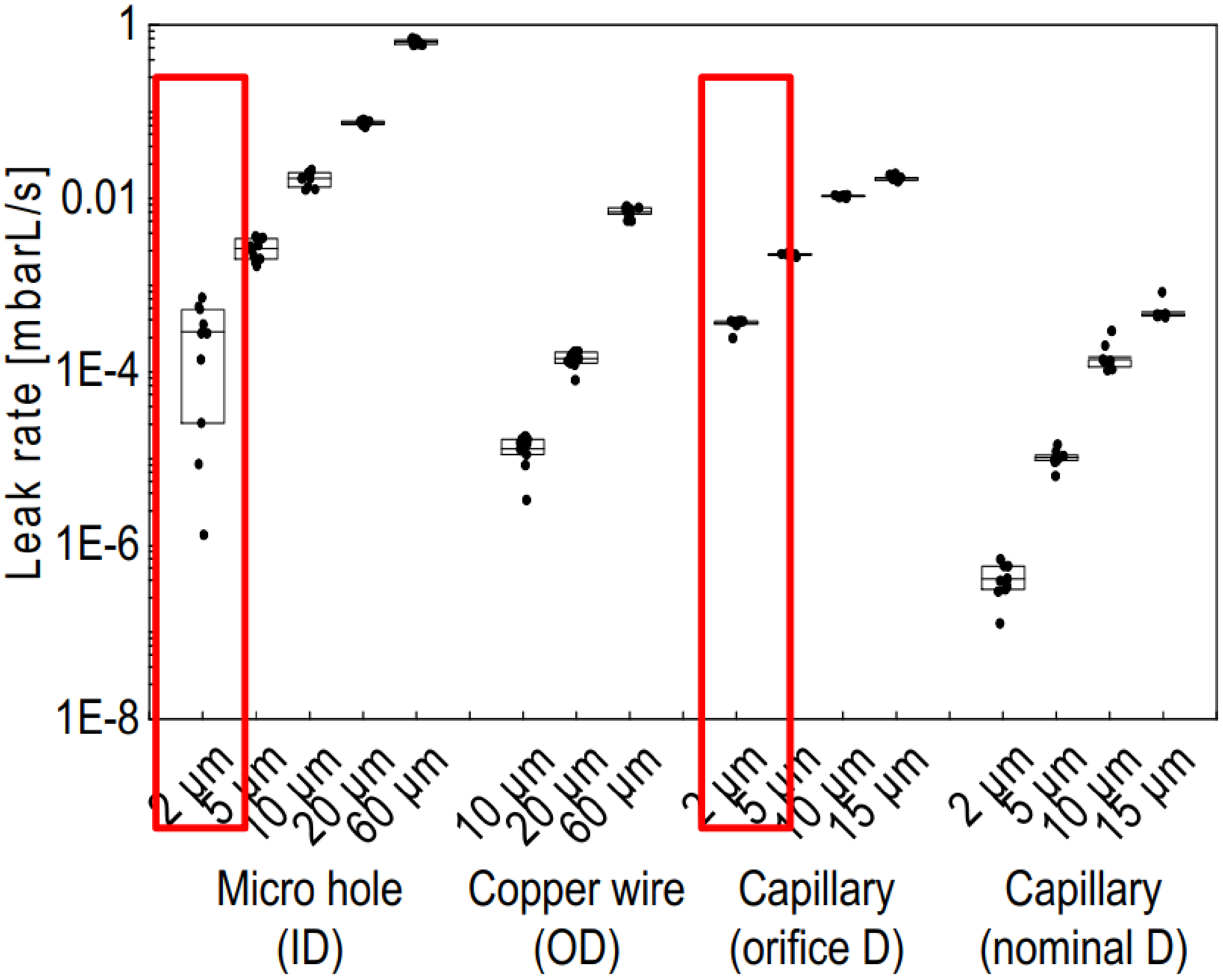


Positive controls vs. test technologies

Study with Lonza

Robustness of artificial leaks:

- Laser drilled micro-holes can result in irregular channels and show increased variability for smaller leaks
- Artificial leaks need to be verified by a sensitive method, such HE-Leak prior to the use as positive controls in order to confirm the target leak rate / size



Positive controls vs. test technologies

Study with Lonza

Detection rate of applied methods

Comparison of methods with applied parameters on the basis of artificial leaks with capillaries (orifice diameter).

Capillary orifice diameter (µm)	Target orifice HE-flow rate (mbar*l/s)	Helium Leak (vacuum mode)	Vacuum decay	Head Space Analysis (O_2)	Dye Ingress
2	1.4E-4	100%	0%	100%	0%
5	8.9E-4	100%	100%	100%	50%
10	3.6E-3	100%	100%	100%	100%
15	8.0E-3	100%	100%	100%	90%

Note: Method parameters have significant impact on sensitivity
Only positive controls with the targeted flow rate have been considered for the results

Positive controls vs. technologies

Positive controls selection

Depending on technologies

Technology / Positive controls	Orifice Laser drilled	Orifice Metal plate	Capillary Glas / ceramic	Capillary Metallic (pipette)	Wire under stopper	Crack
Differential pressure	OK	OK	OK	OK	OK	OK
Headspace analysis	OK	OK	OK	OK	OK	OK
Force sensor	OK	OK	OK	OK	OK	OK
High Voltage	OK	NOK	NOK	NOK	NOK	OK
Mass Spectroscopy	OK	OK	OK	OK	OK	OK

Positive controls selection

Depending on packaging components

Technology / Positive controls	Orifice Laser drilled	Orifice Metal plate	Capillary Glas / ceramic	Capillary Metallic (pipette)	Wire under stopper	Crack
Glas body	OK	NOK	OK	OK	NOK	OK
Neck	OK	OK	NOK	NOK	NOK	NOK
Sealing area	NOK	NOK	NOK	NOK	OK	NOK
Stopper	NOK	NOK	OK	OK	NOK	NOK
Plunger	NOK	NOK	OK	OK	NOK	NOK
Flexible container	OK	NOK	OK	OK	NOK	NOK
RNS	NOK	NOK	NOK	NOK	NOK	NOK

Verification

Helium leak testing

Types of positive controls

Verification of flow – Helium leak testing

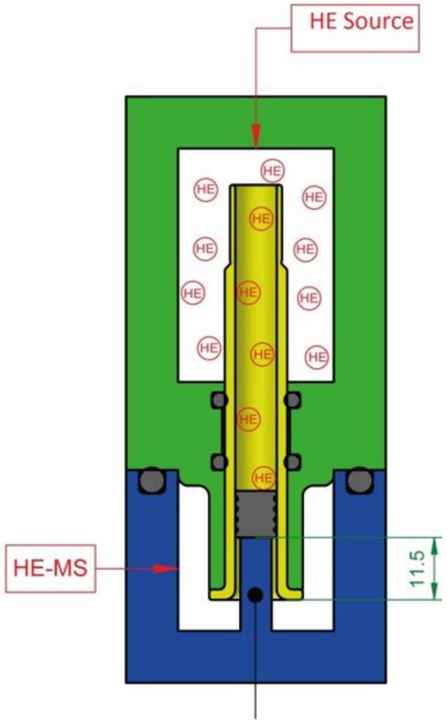
- Use Helium mass spectroscopy
- Calculation of flow similar to model description -

Practical considerations:

☹ not suitable for all containers – permeation can occur
☹ practical application issues – Helium tightness of fixture / setup
☹ final flow of positive container needs to be converted

☺ most precise detection strategy available – suitable for inherent CCIT
☺ "destructive" method – usually needs a second access to the container
☺ internationally standardized conditions for testing
  - Pressures: 1000 mbar / < 10^{-2} mbar
  - Temperatures: 293 K
  - Concentrations: 100 % Helium / 0 % Helium

☺ Results are comparable – intra / inter organization, packaging systems



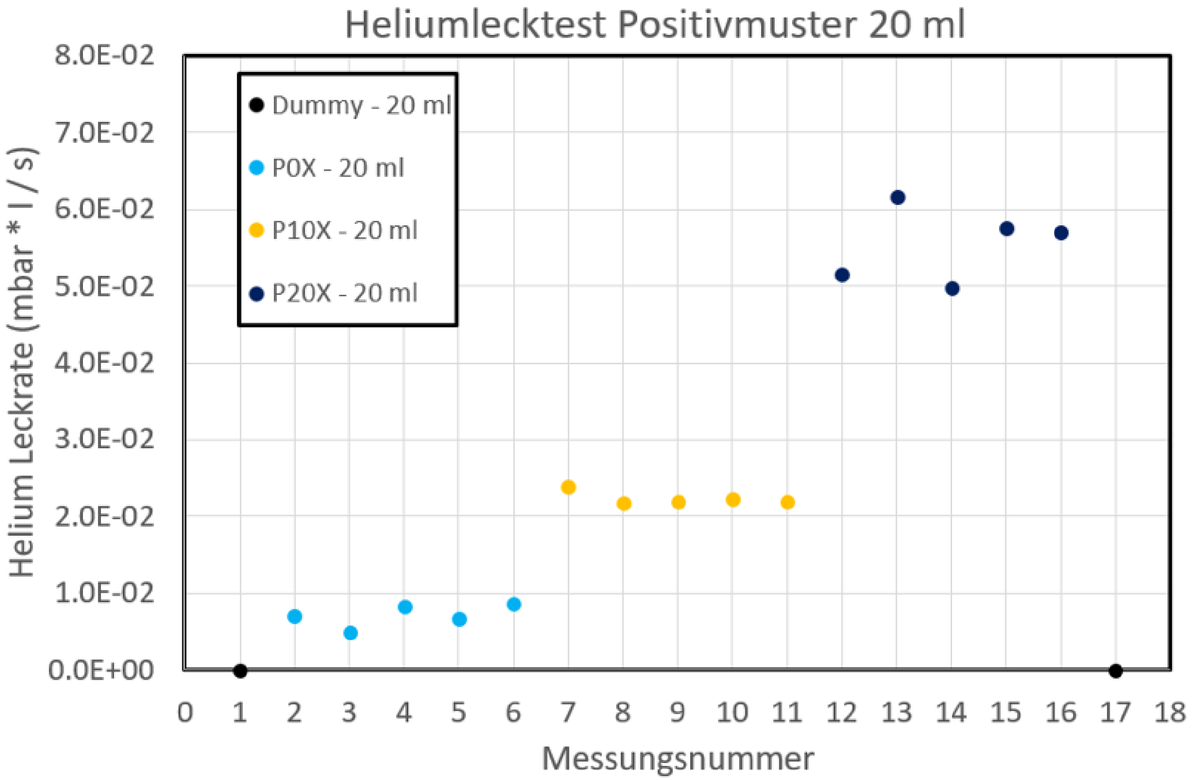
Stopper fixture

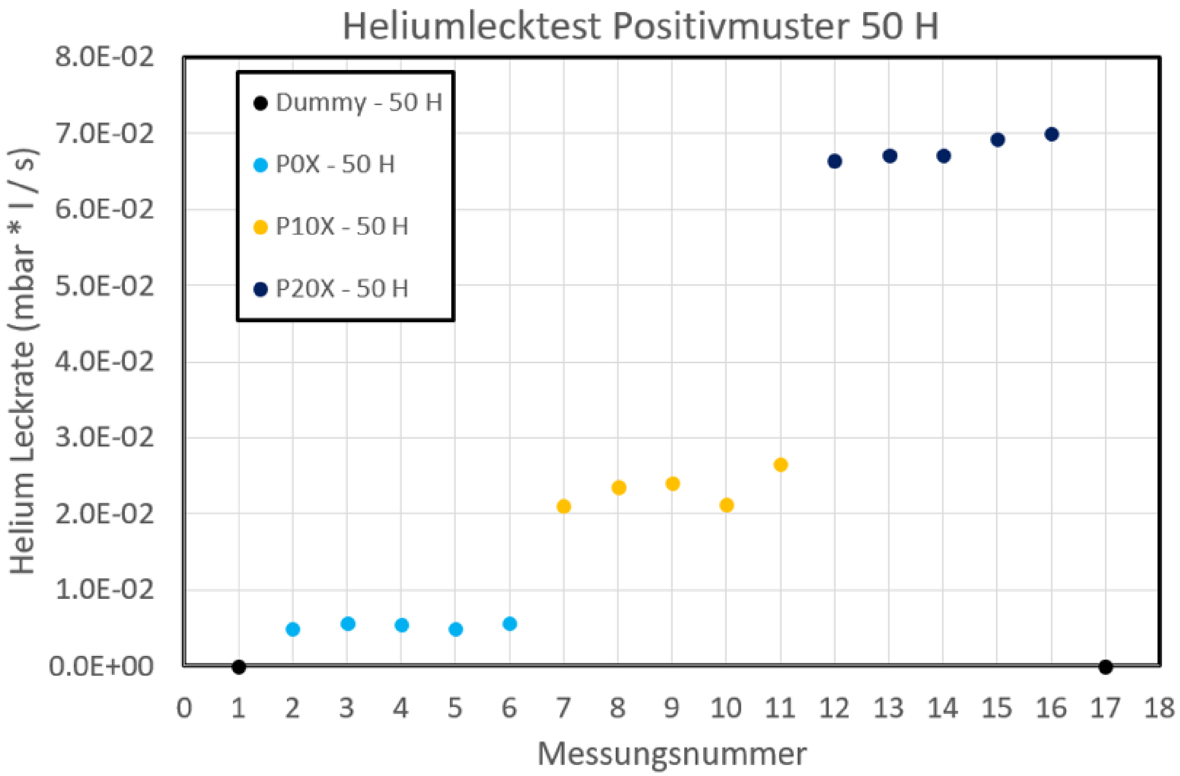
Example for Helium test fixture

Types of positive controls

Verification of flow – Helium leak testing – Example data

- Helium leak testing for positive controls



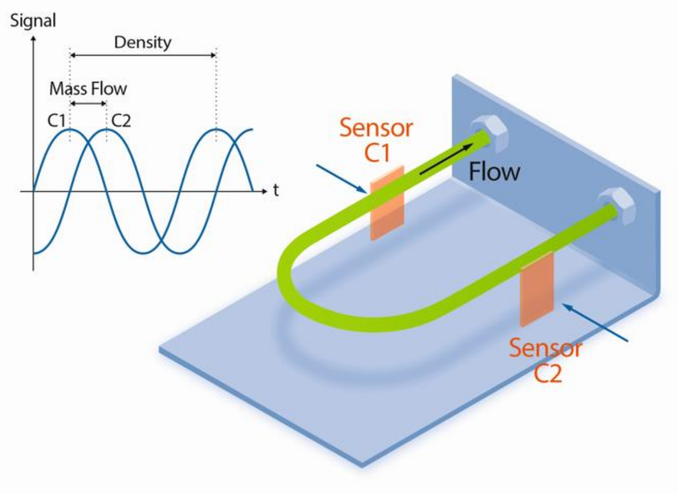


Flow rate measurment

Types of positive controls

Verification of flow – Flow rate measurement

- Measure air flow using mass flow tester
- Sample must be accessible from two sides



Practical considerations:

☹ conditions of test must be controlled thoroughly

☹ pressure and environmental conditions must be controlled

☺ certification conditions of positive samples can be adapted to test conditions

☺ can be implemented quickly – suited for functional test for positive sample

☺ check quickly function of positive controls before use

Bubble test

Types of positive controls

Verification of flow – Bubble test

- Measure air flow with bubble test
- connect positive container to pressure source, pressurize
- put sample under water
- collect air over defined interval of time

Practical considerations:

☹ prone to errors (human error, process errors, …)
☹ sample is put under water – influence of humidity to control
☹ pressure conditions can not be adapted to some later test conditions
☺ quick and fast method
☺ easy to realize on site
☺ no complicated equipment needed

Being sure.

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