Leakage protocol and measurements

Vania Silverio (INESC MN)

Workshop on Standardization of test methods in microfluidics
22 May 2024

The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

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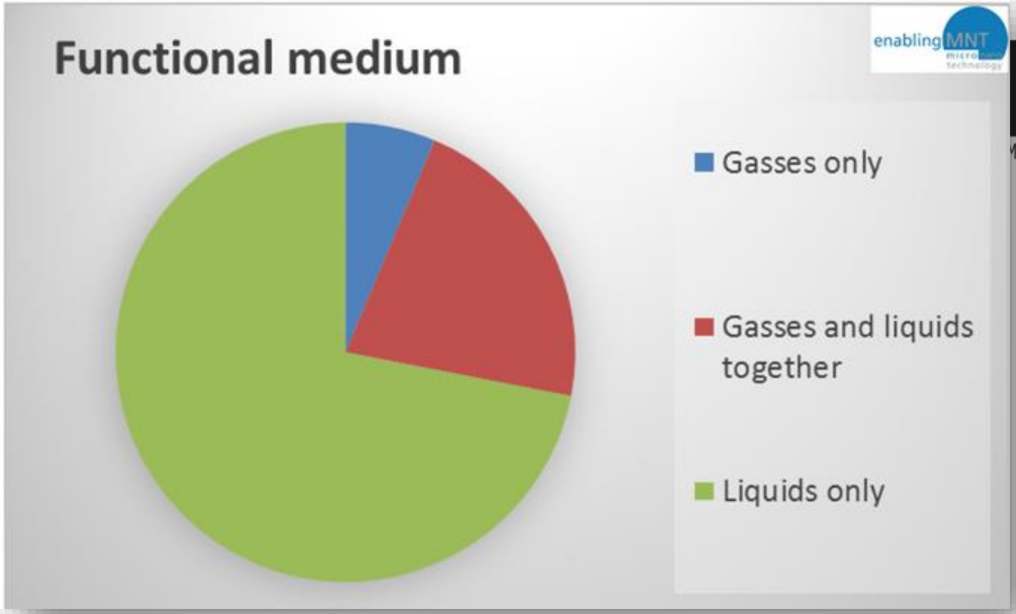
Establishing Metrology Standards in Microfluidic Devices

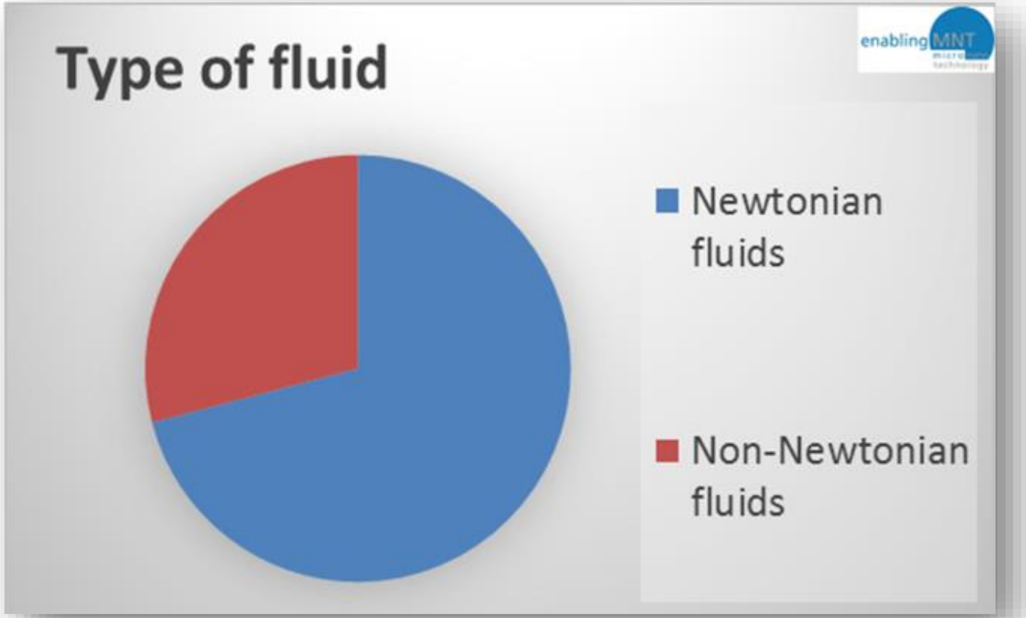
6.4. Leakage

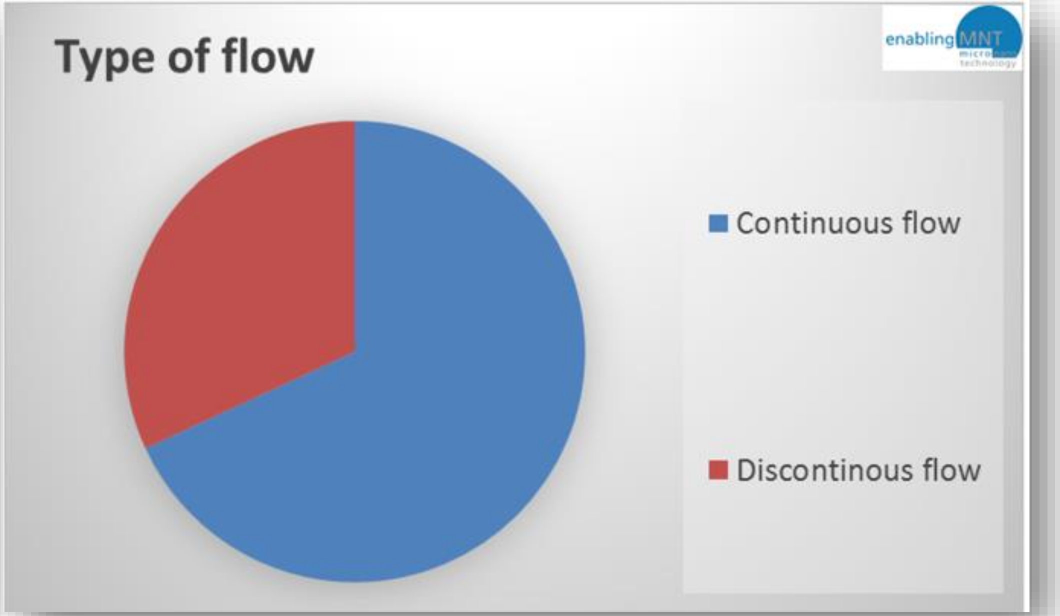
The flow rate can considerably be affected by leakage in the system, often this happens in the connecting points. Leakage can also occur in case of delamination of the chip, or when cracks appear due to overpressure or destructive modification of the chip material (due to over-heat for example).

Silverio V, Metaxiotou Z, Batista E, Kartmann S, Ogheard F, Lötters J (2022). MFMET A1.1.2 - Definitions Symbols and Vocabulary of Flow Control. https://doi.org/10.5281/zenodo.7092031

Fluid and Flow in microfluidic devices

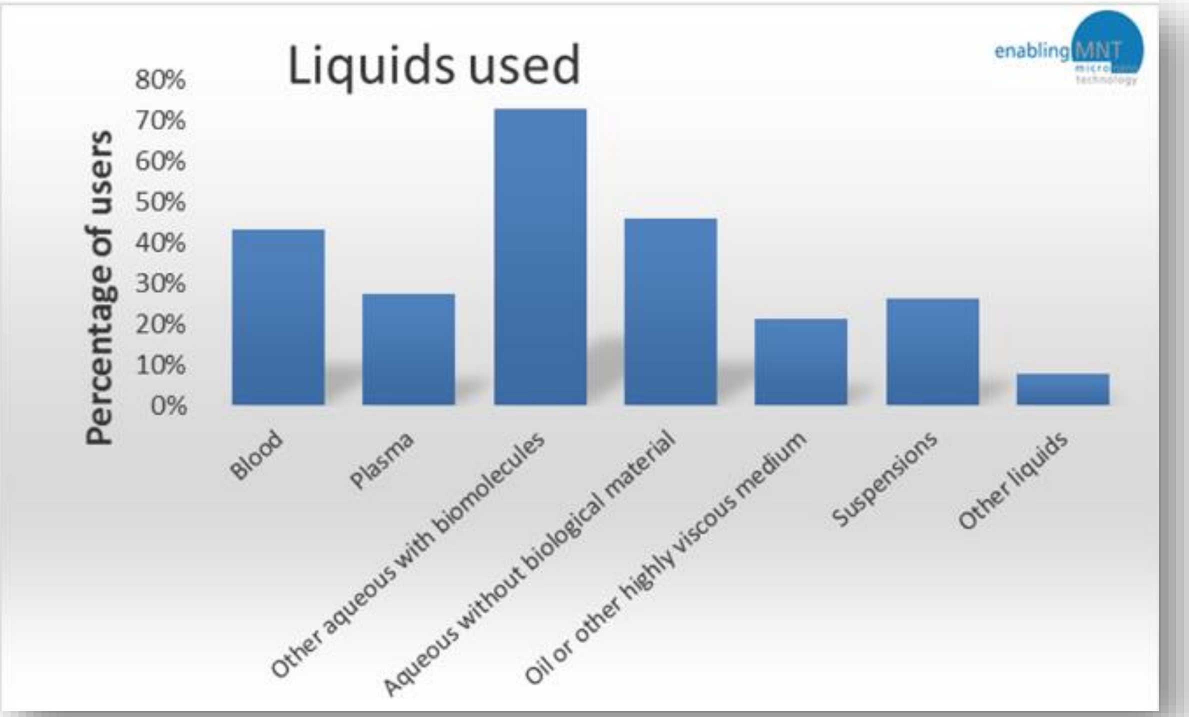


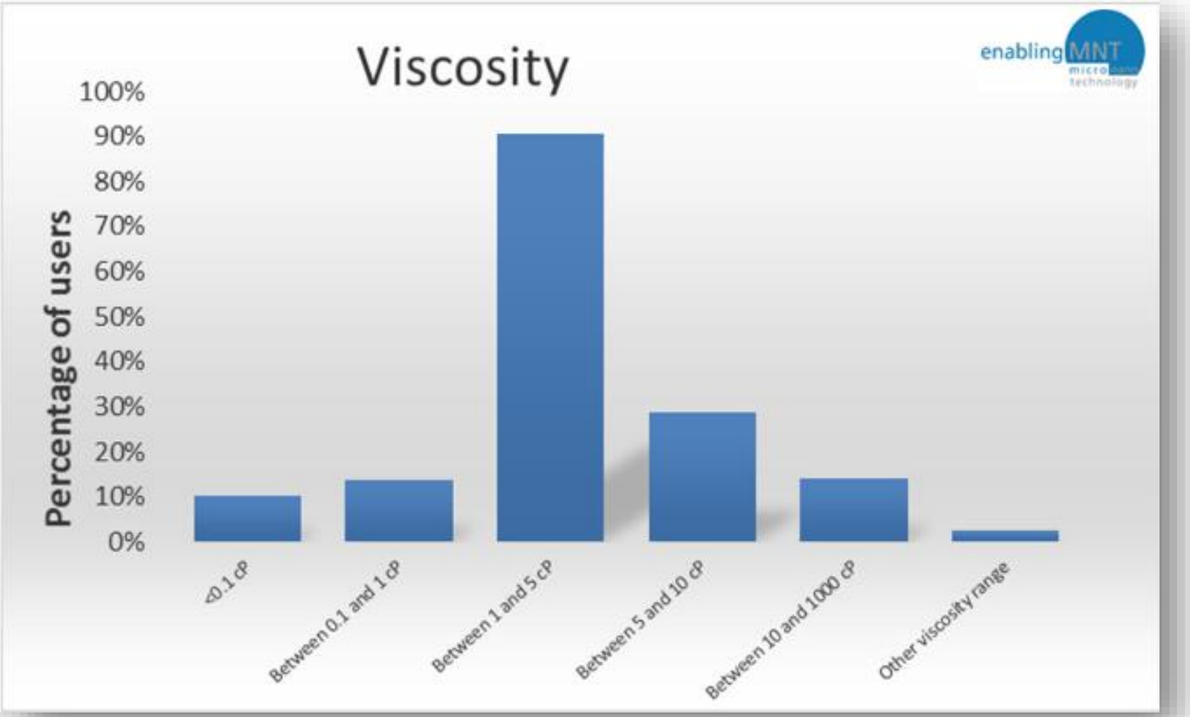




Henne van Heeren (2015) *Results survey on microfluidics flow control*, enablingMNT

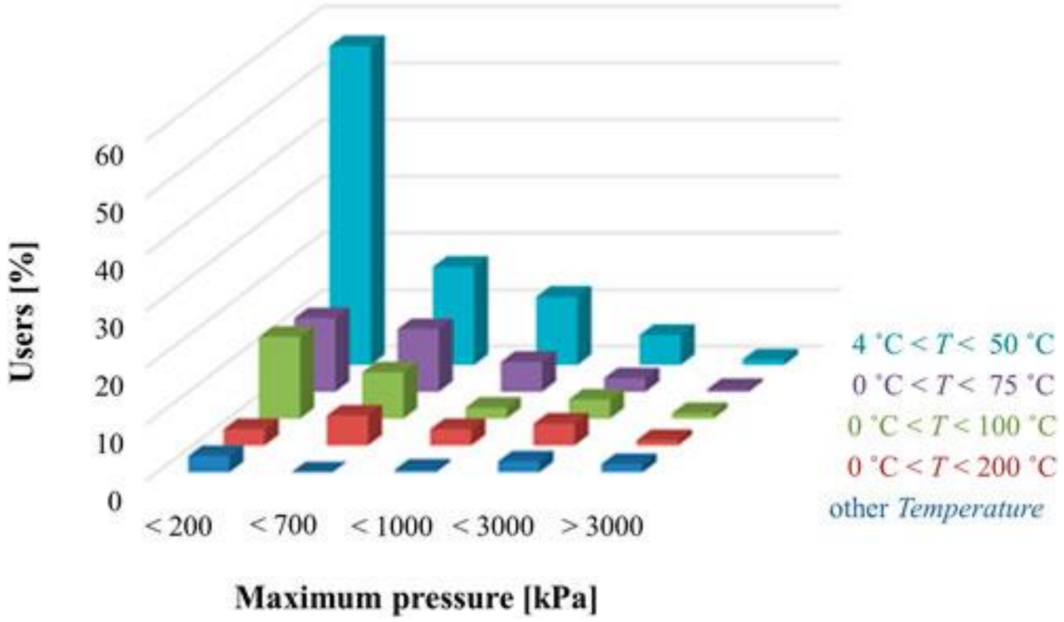
Fluid and Flow in microfluidic devices

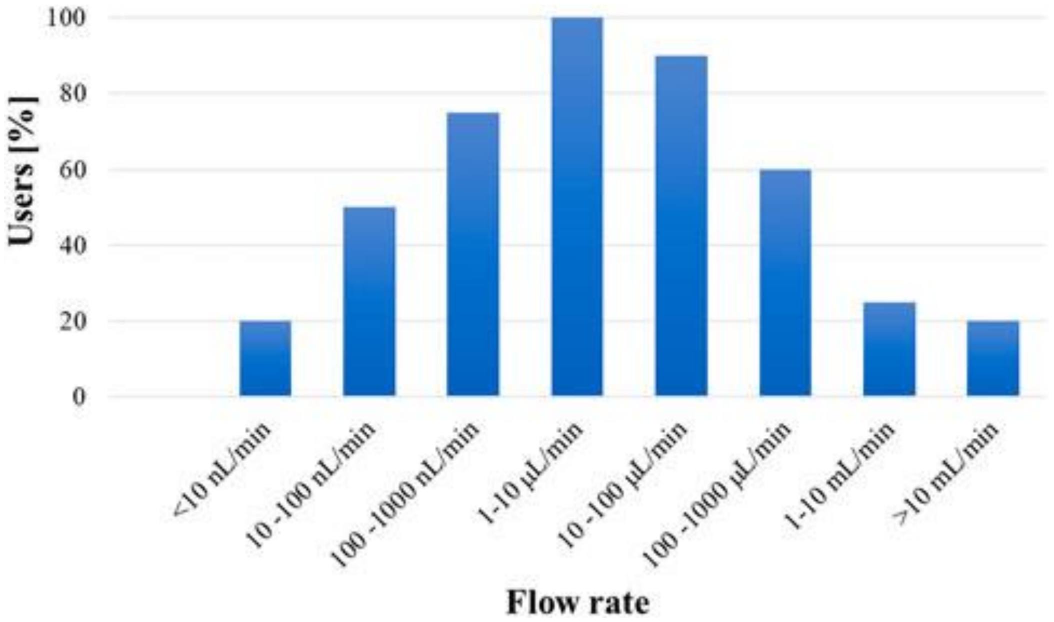




Henne van Heeren (2015) *Results survey on microfluidics flow control*, enablingMNT

Application classes



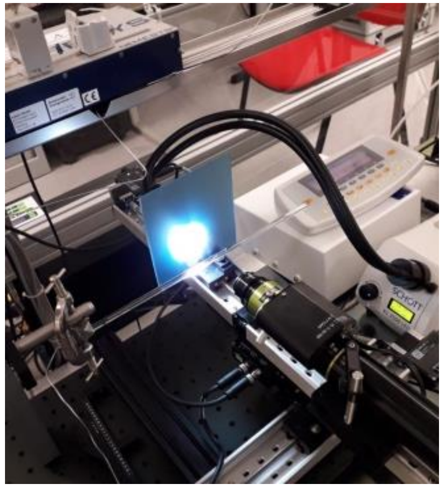


Class	Maximum pressure [kPa]	Maximum Temperature [°C]	Minimum Temperature [°C]
Capillary devices	-	50	4
PT 200/50	200	50	4
PT 200/75	200	75	4
PT 200/100	200	100	4
PT 700/50	700	50	4
PT 700/100	700	100	4
PT 3000/50	3000	50	4

Silverio *et al* (2022) Overcoming Technological Barriers in Microfluidics: Leakage Testing. Frontiers in Bioengineering and Biotechnology, 10: 958582 DOI: 10.3389/fbioe.2022.958582

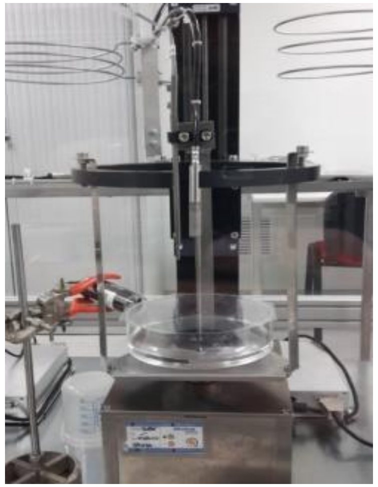
Flow classes (ongoing discussion)

Classes	Minimum flow	Maximum flow	Best accuracy
F1 'nano'	1 nl/min	1 µl/min	1 %
F2 'micro'	1 µl/min	100 µl/min	0.5 %
F3 'milli'	100 µl/min	10 ml/min	0.1 %



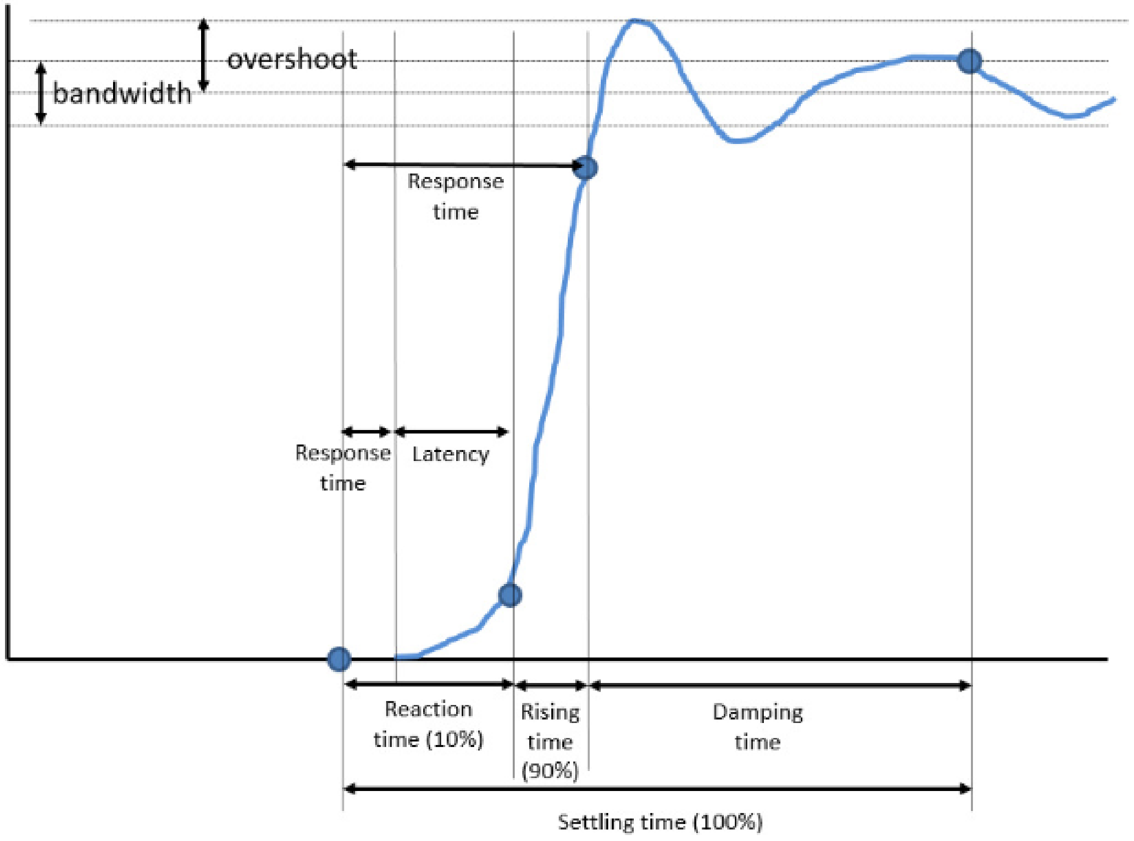
F1 'nano' flow traceability: optical methods

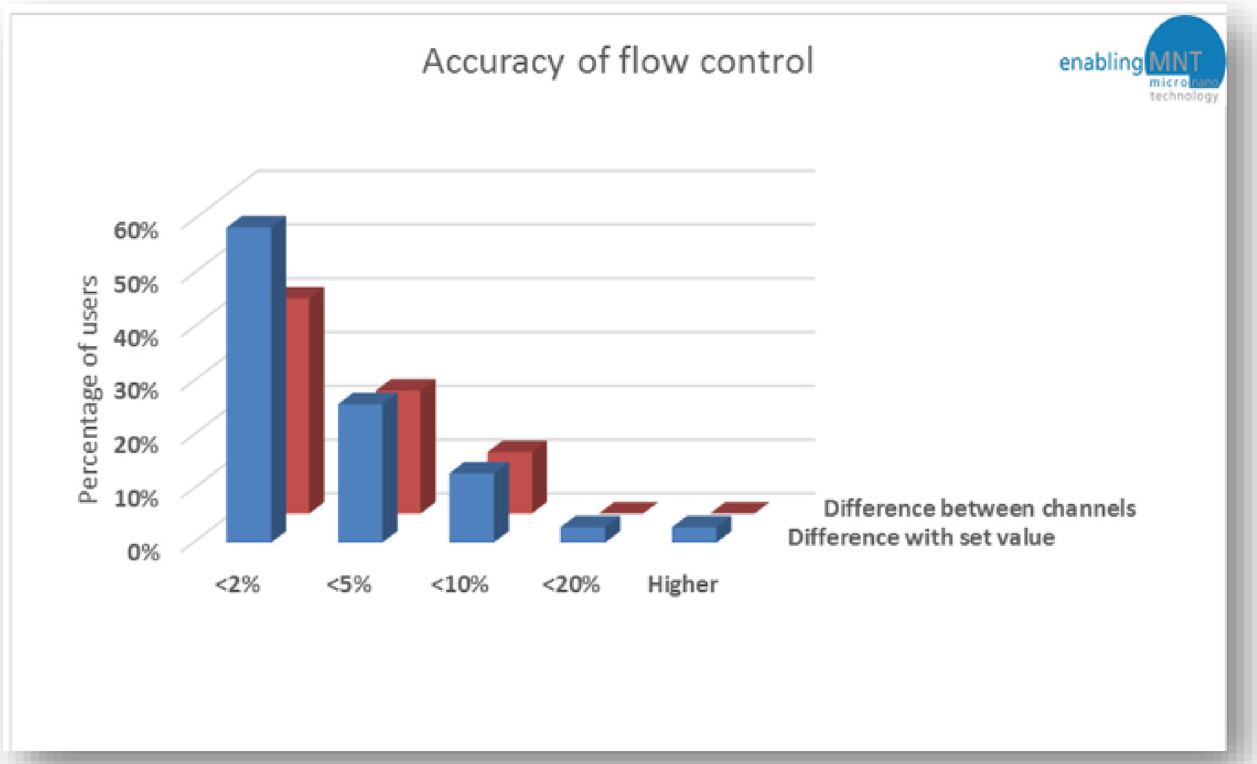
F2 'micro' flow traceability: gravimetric methods



F3 'milli' flow traceability: gravimetric methods

Fluid and Flow in microfluidic devices





Henne van Heeren (2015) *Results survey on microfluidics flow control*, enablingMNT

Malfunctioning risk analysis / FMEA for microfluidic devices

Establishing Metrology Standards in Microfluidic Devices

FMEA: Failure Mode and Effect Analysis

Table 1. FMEA for Microfluidic Devices

	Topic	Potential Failure Mode	Effect / severity (S: 1-10)	Potential Causes / Mechanisms / probability (P: 1-10)	Test / visual inspection /observable Effect of Failure Detection (D: 1-10)	Risk priority number S*P*D	Improvement / prevention actions	Actions taken for further Investigation
Materials	General	Delamination	Leakage / 6	Corrosion / 5	Visual inspection / 5	150	Selection of appropriate materials, limit exposure time	
	Polymer devices	Delamination	Leakage / 6	Contaminati on/ 4	Visual inspection / 1	24	Clean room procedures	
	Glass/silicon devices	Delamination	Etc.					
	Glass devices	Cracks	Etc.					
Building blocks	Valve	Misalignment	Leakage					
	Reaction chamber							
	Channel							
Components	Connector	Etc.						
	Blister							
	Tube							
Process step	Interfacing	Too low of a flow speed in flow cell	Incorrect measurem ent results / 7	Deformed ferrule used / 2	Leakage test / 7	98	Visual inspection	Stricter procedures ferule purchase and reusage
Operational conditions	Worst case use							
	Storage							
	Transport							
	End of Life							
	Environment al							

The identified actions may require **further analysis** or **corrective** and **preventative actions** especially if the risks are found to be unacceptable.

Severity, probability and detection → represented by number 1 to 10. The product of these numbers indicates the **risk priority**

van Heeren H, Davies M, Keiser A, Lagrauw R, Reyes D R, Silverio V, Verplanck N (2022) Protocols for leakage testing https://doi.org/10.5281/zenodo.6602162

Malfunctioning risk analysis / FMEA for microfluidic devices

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Table 1. FMEA for Microfluidic Devices

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Operational conditions	Worst case use							
	Storage							
	Transport							
	End of Life							
	Environment al							

Table 2. Rating description for effect and its severity (S)

Value	Description	Criteria
1	Minor	It is not likely to have much of an impact and will most likely be ignored by the stakeholder.
2	Low	It is likely to cause some amount of uneasiness to the stakeholder.
3	Low	Stakeholder will suffer some discomfort due to a minor problem with performance.
4	Moderate	User is uncomfortable due to performance not up to the desired level.
5	Moderate	User is dissatisfied or there is a reduction in performance affecting the overall process
6	Moderate	Quality cost due to warranty and repairs or there is a significant loss in performance
7	High	User is highly dissatisfied due to failure in some important parts of the process. There is also the likelihood of many defects affecting overall productivity.
8	High	User is highly dissatisfied due to failure in ALL parts of the process. There is also the likelihood of many defects affecting overall productivity.
9	Very High	Process becomes unstable which may create safety issues for the operator and exceed standard acceptance criteria.
10	Very High	Leakage will create safety issues for the operator or others.

Table 3. Rating description causes or mechanisms and probability (P)

Value	Description	Failure Rate	Percent Defective	Criteria
1	Remote	< 1 out of 1.5 x10⁶	<0.00007 %	Failure has never been seen in any relevant testing
2	Very Low	1 out of 1.5 x 10⁵	0.0007 %	
3	Low	1 out of 1.5 x 10⁴	0.007 %	Failure only seen once or twice in relevant scenarios
4	Unlikely	1 out of 2 x 10³	0.05 %	
5	Moderate	1 out of 400	0.25 %	Failure potential has been noted in several relevant scenarios or tests. If procedures are followed, the failure potential is minimal.
6	Moderate	1 out of 100	1 %	
7	High	1 out of 20	5 %	Failure potential has been noted in many relevant tests/scenarios. In-process control may be required to avoid failure.
8	Very High	1 out of 8	12.5 %	
9	Very High	1 out of 3	33 %	Failure potential has been noted in many scenarios/tests. An active non-standard feedback control loop may be required.
10	Extremely High	>1 out of 2	50 %	Failure potential has been noted in most scenarios/tests. The process should be re-evaluated, and/or a redesign should be considered.

Table 4. Rating description for detection (D)

Value	Description	Failure Rates	Percent Defective	Criteria
1	Remote	< 1 out of 1.5 x10⁶	<0.00007 %	Failure has never been seen in any relevant testing
2	Very Low	1 out of 1.5 x 10⁵	0.0007 %	
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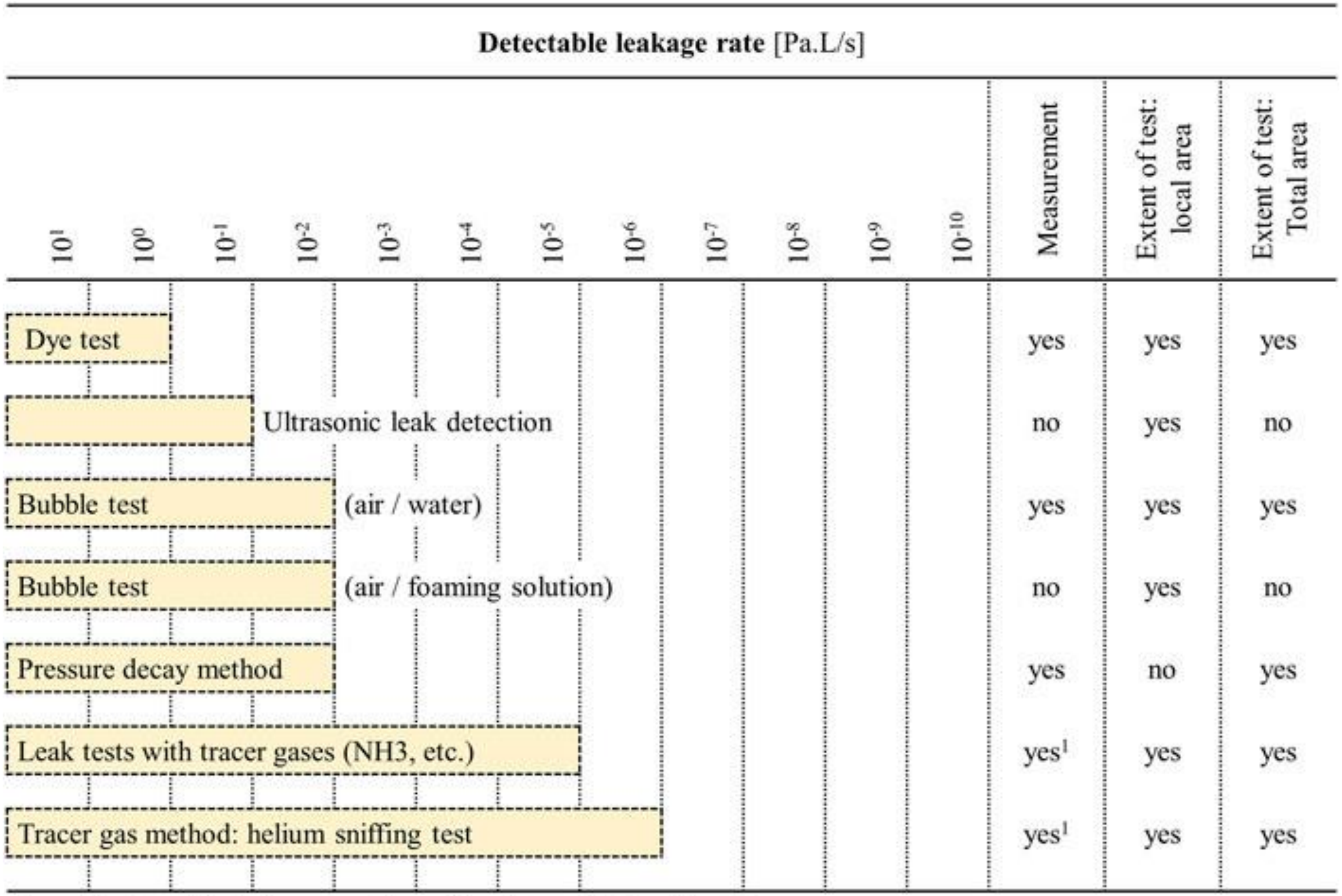
van Heeren H, Davies M, Keiser A, Lagrauw R, Reyes D R, Silverio V, Verplanck N (2022) Protocols for leakage testing https://doi.org/10.5281/zenodo.6602162

How to detect leakage ?

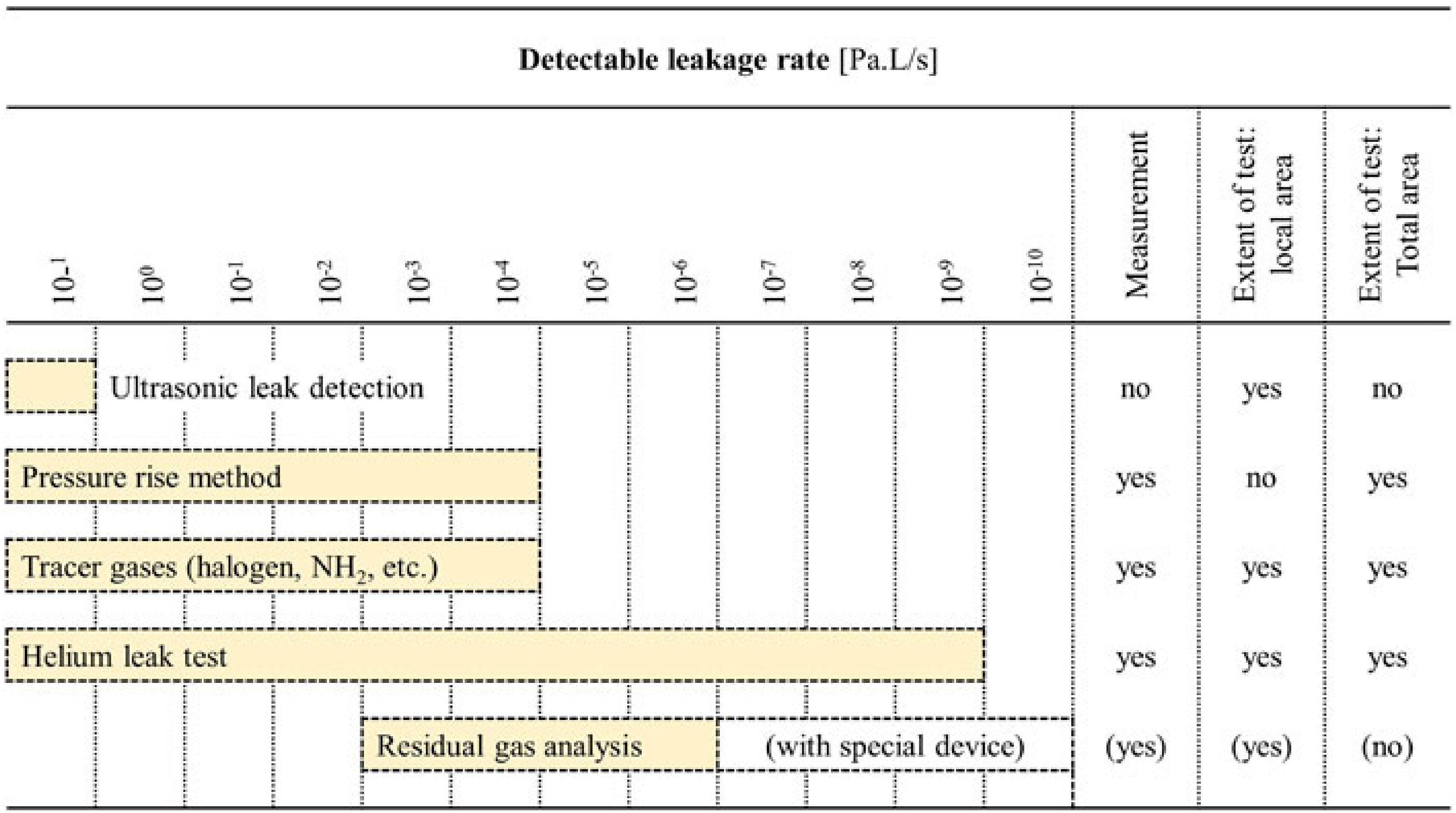
Leakage testing – detection limits



[1] quantification of the leakage rate only conditionally possible in sniffing tests

Silverio V, Guha S, Keiser A, Natu R, Reyes D R, van Heeren H, Verplanck N, Herbertson L H (2022) Overcoming Technological Barriers in Microfluidics: Leakage Testing. Frontiers in Bioengineering and Biotechnology, 10: 958582 **DOI: 10.3389/fbioe.2022.958582**

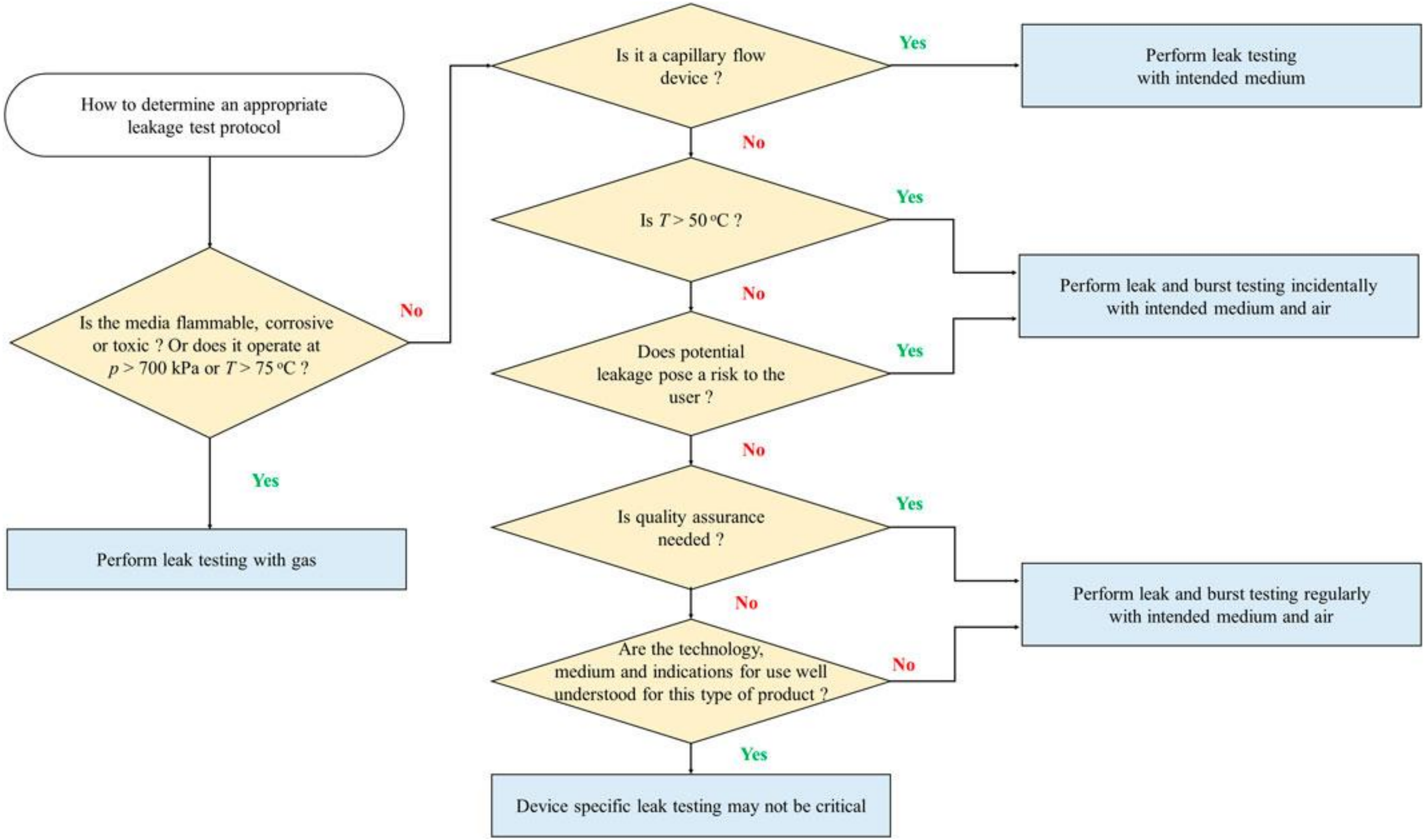
Leakage testing – detection limits



leak detection and leak tightness testing methods under vacuum

Silverio V, Guha S, Keiser A, Natu R, Reyes D R, van Heeren H, Verplanck N, Herbertson L H (2022) Overcoming Technological Barriers in Microfluidics: Leakage Testing. Frontiers in Bioengineering and Biotechnology, 10: 958582 **DOI: 10.3389/fbioe.2022.958582**

Leakage testing



Ogheard F, Daugbjerg T S, Romieu K, Silverio V (2023)

MFMET A1.2.3 - Documented example of the test protocol for leakage and burst pressure

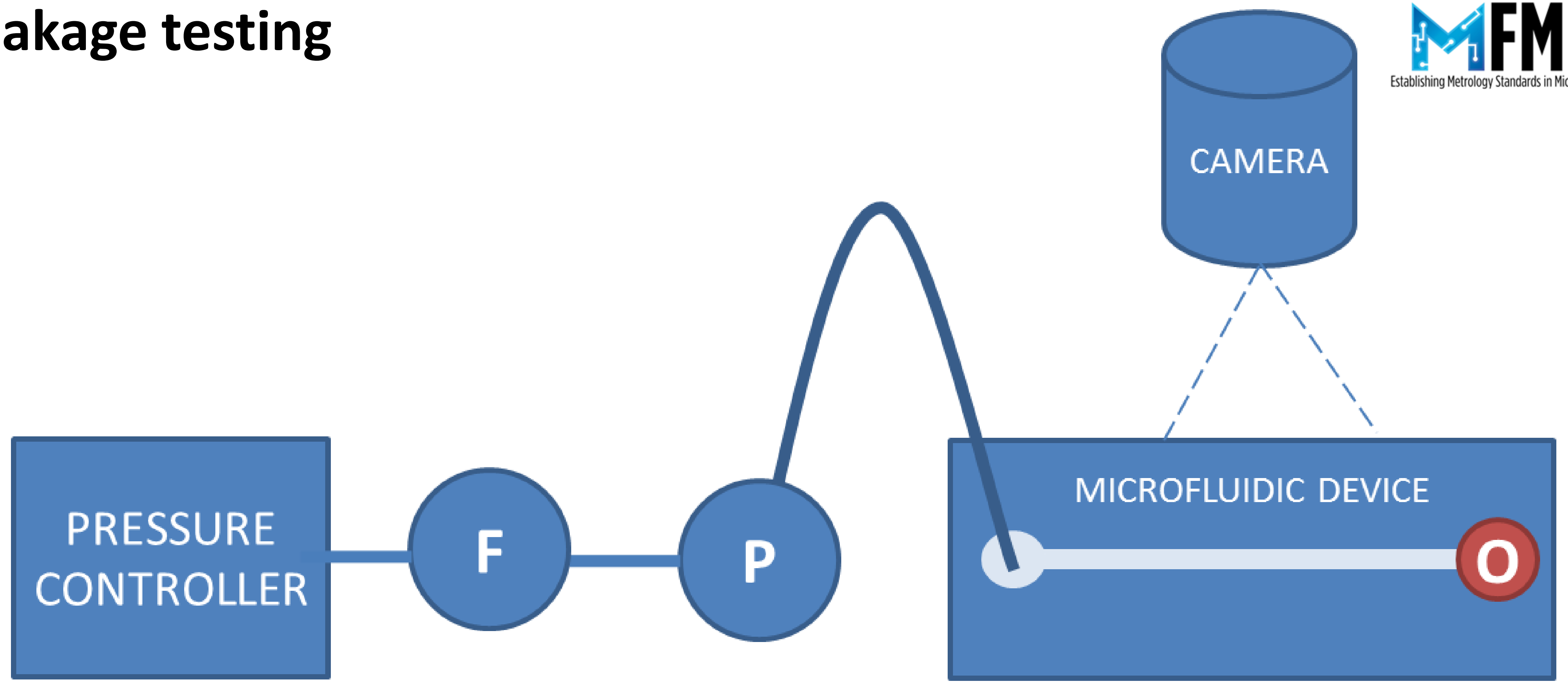
https://doi.org/10.5281/zenodo.10114802

Daugbjerg T S, Ogheard F, Batista E, van Heeren H, Silverio V (2023)

MFMET Deliverable 1 - Guidelines and a test protocol for flow control evaluating leakage and burst pressure in microfluidic devices

https://doi.org/10.5281/zenodo.7901265

Leakage testing



https://mfmet.eu/publications

- Deliverable 1 – Guidelines and a test protocol for flow control evaluating leakage and burst pressure in microfluidic devices
- A1.2.3 Documented example of the test protocol for leakage and burst pressure
- The MFA & MFMET – Protocols for leakage testing
- Overcoming Technological Barriers in Microfluidics: Leakage Testing. Front. Bioeng. Biotechnol. 10: 958582 DOI: 10.3389/fbioe.2022.958582

Leakage testing

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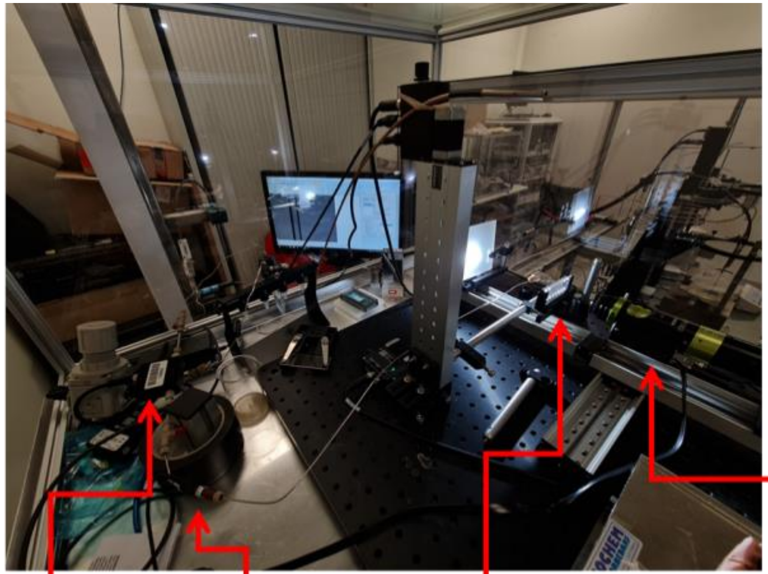
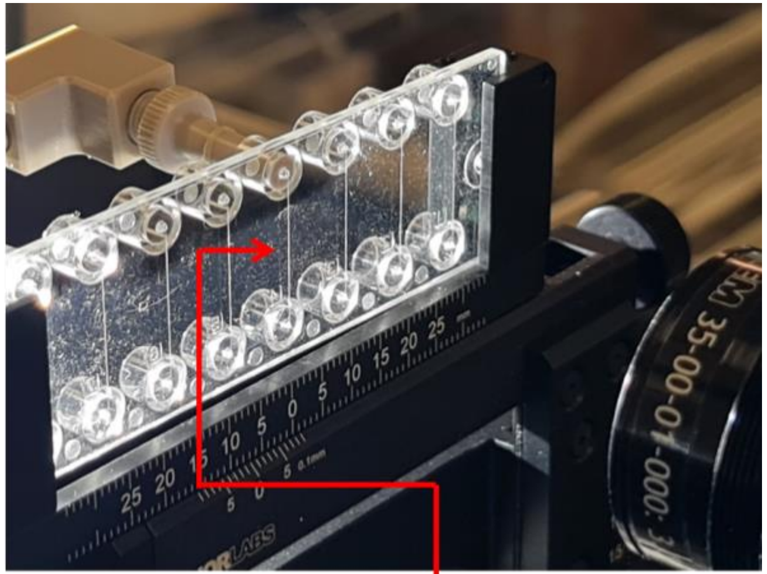
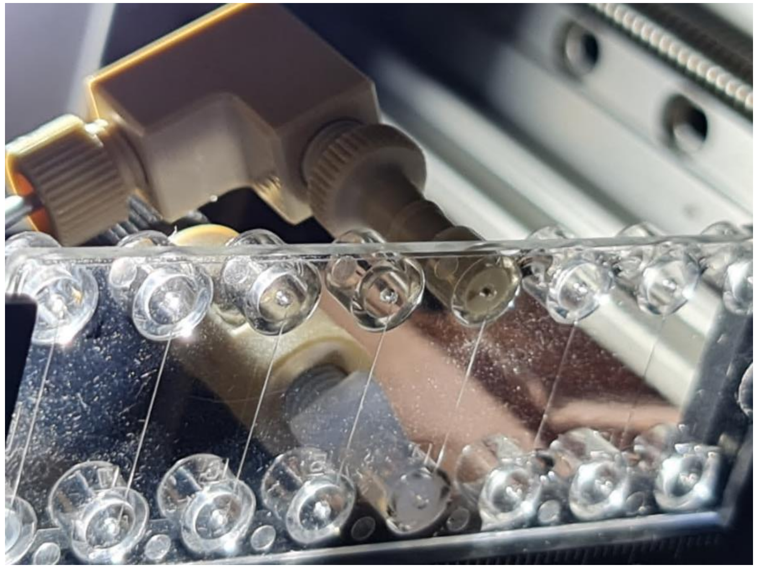
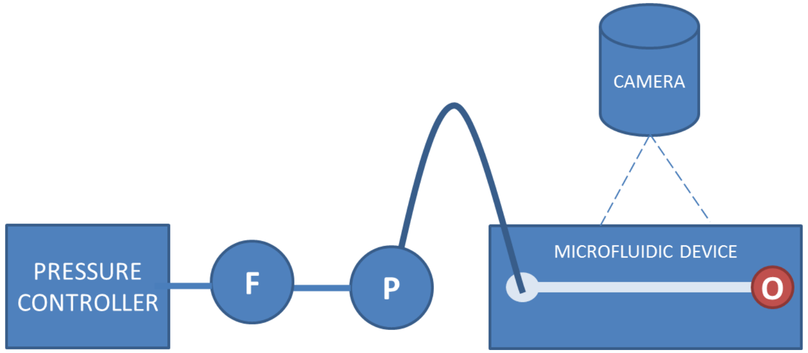
Establishing Metrology Standards in Microfluidic Devices



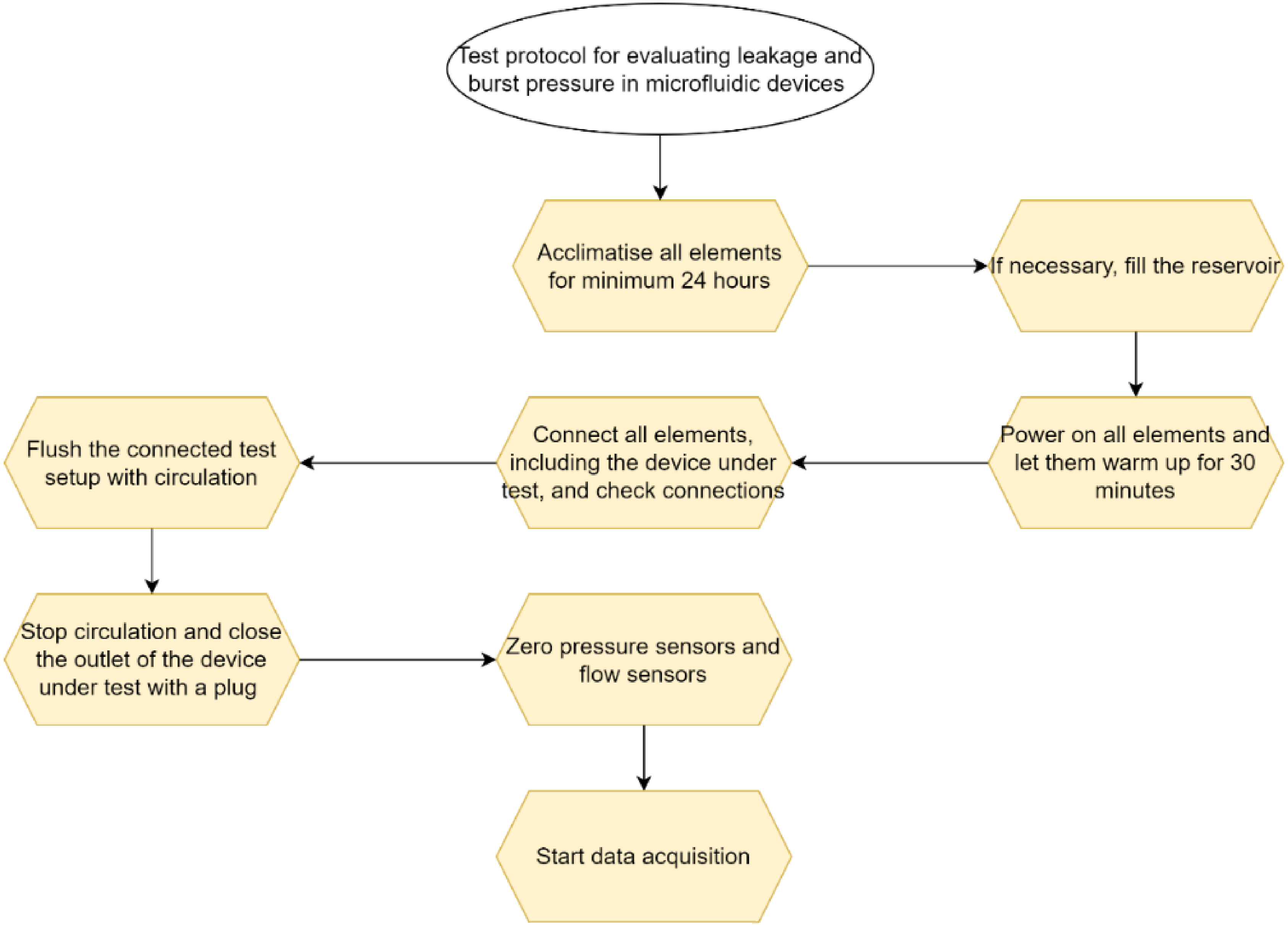




Fluidic 157 microfluidic ChipShop
Luer male to threaded Upchurch adapter
Threaded female-female Upchurch elbow



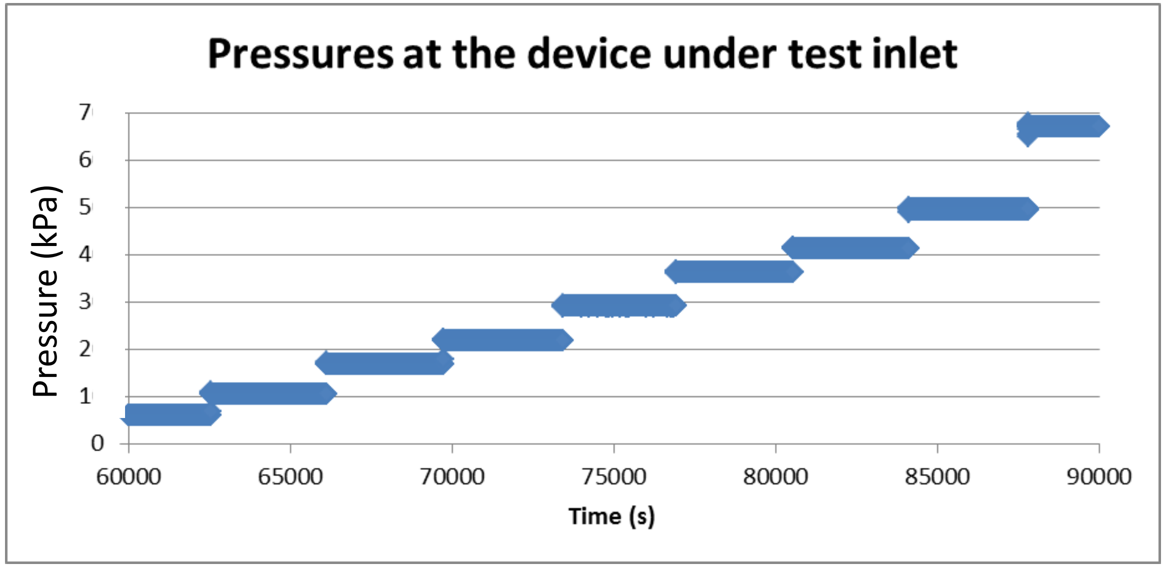
- Material: Topas (Topas is a COC – cyclic olefin copolymer – resin)
- 8 parallel channels of 100 µm width, 100 µm depth, 18 mm length
- 75.5 mm by 25.5 mm microscope slide format
- Luer fluidic interface

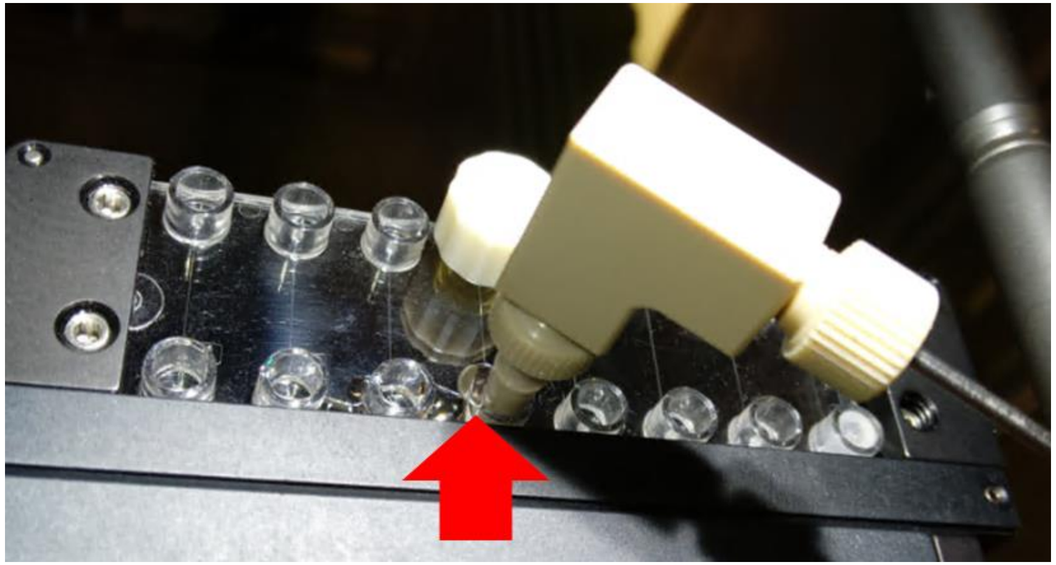

Test protocol for evaluating leakage and burst pressure in microfluidic devices
Acclimatise all elements for minimum 24 hours
If necessary, fill the reservoir
Power on all elements and let them warm up for 30 minutes
Connect all elements, including the device under test, and check connections
Flush the connected test setup with circulation
Stop circulation and close the outlet of the device under test with a plug
Zero pressure sensors and flow sensors
Start data acquisition

Leakage testing – test protocol

I. Visual inspection test

1. Power ON
2. 30 min stabilization time
3. Set pressure to increase by steps from 0 to 20 kPa
4. Record images, p_{in}, T_{amb}, p_{amb}, humidity





Leak appearing at the device under test
Inlet during the visual leakage test
Set pressure: 7 kPa

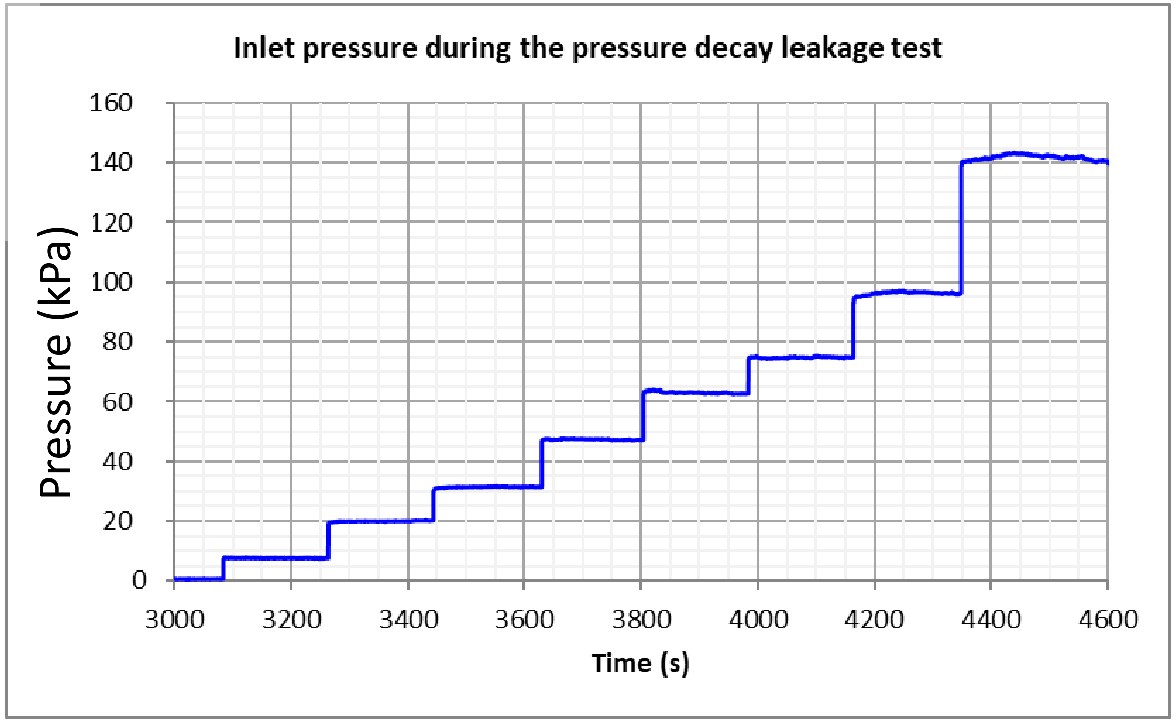
Leakage testing – test protocol

I. Visual inspection test

1. Power ON
2. 30 min stabilization time
3. Set pressure to increase by steps from 0 to 20 kPa
4. Record images, p_{in}, T_{amb}, p_{amb}, humidity

II. Pressure decay test

1. Power ON
2. 30 min stabilization time
3. Set pressure to increase by steps from 0 to 140 kPa
4. Fix the pressure at a given setpoint by the pressure controller for 3 min
5. Record p_{in}, T_{amb}, p_{amb}, humidity



Leakage testing – test protocol

Establishing Metrology Standards in Microfluidic Devices

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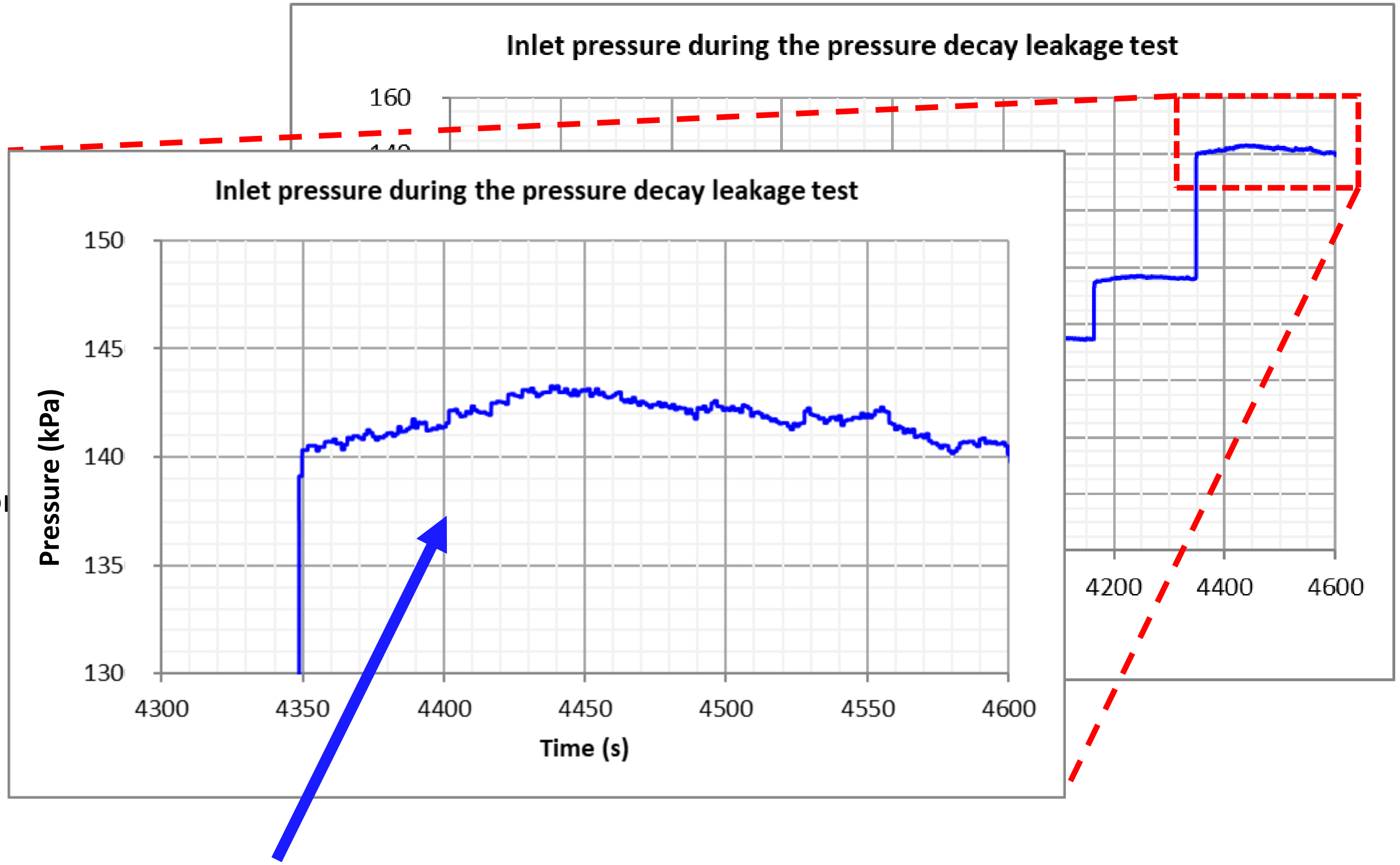

Variability and subsequent decay in pressure indicate leakage

Leakage testing – test protocol

I. Visual inspection test

1. Power ON
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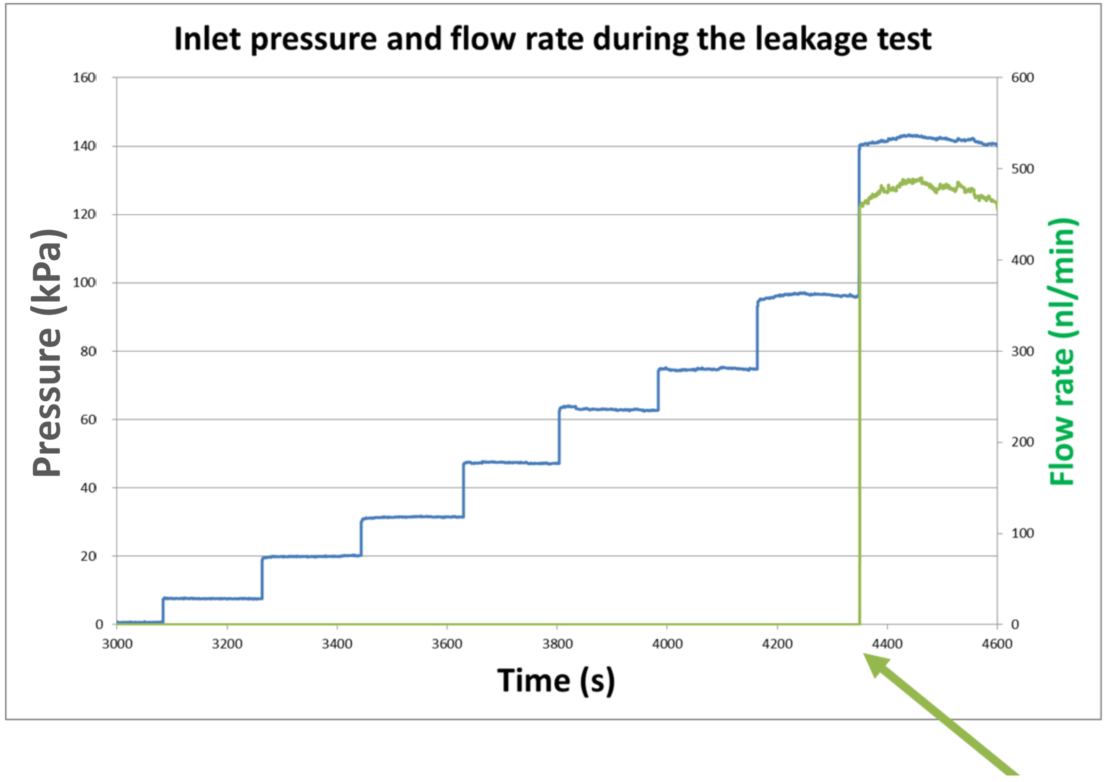
3. Set pressure to increase by steps from 0 to 20 kPa
4. Record images, p_{in}, T_{amb}, p_{amb}, humidity

II. Pressure decay test

1. Power ON
2. 30 min stabilization time
3. Set pressure to increase by steps from 0 to 140 kPa
4. Fix the pressure at a given setpoint by the pressure controller for 3 min
5. Record p_{in}, T_{amb}, p_{amb}, humidity

III. Flow rate measurement test

1. Power ON
2. 30 min stabilization time
3. Set pressure to increase by steps from 0 to 140 kPa
4. Record $\dot{m}$, p_{in}, T_{amb}, p_{amb}, humidity



Step in flow rate indicates leakage

Establishing Metrology Standards in Microfluidic Devices

Visual inspection test	Pressure decay test	Flow rate measurement test
Localizes the leakage	Cannot localize the leakage	Cannot localize the leakage
Easy to interpret	Hard to interpret	Easy to interpret
Slow	Fast	Fast

- Liquid-based testing → destructive testing
- Inappropriate –> contamination, sterilisation, or single-use devices

All **instruments calibrated** on their full range, and by **traceability** to national standard at CETIAT laboratory or by ISO 17025 accredited laboratories

Transfer standards for leakage

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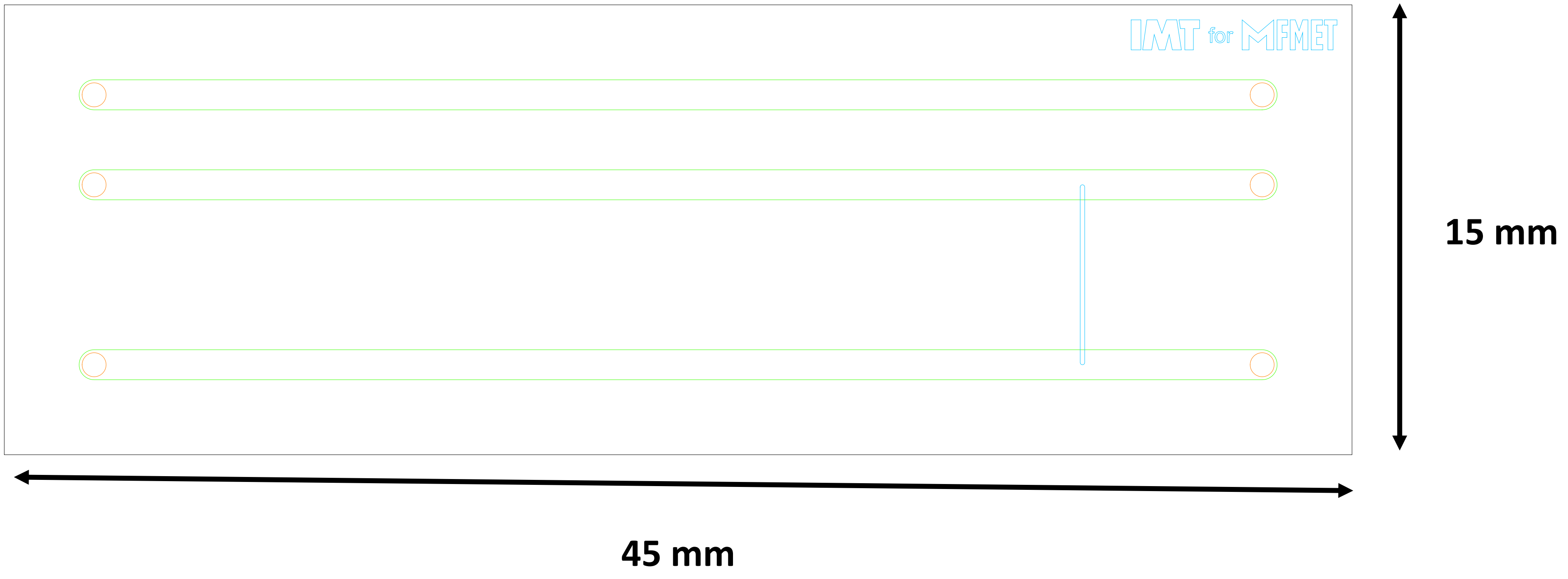
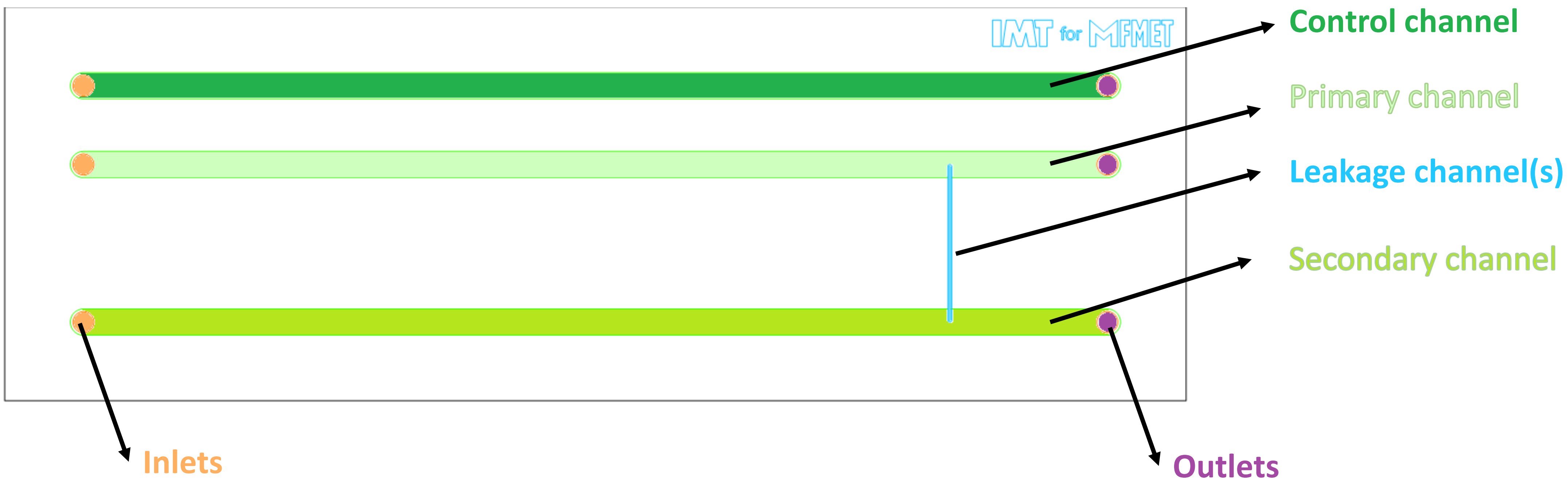
GLASS



Transfer standards for leakage

GLASS



Transfer standards for leakage

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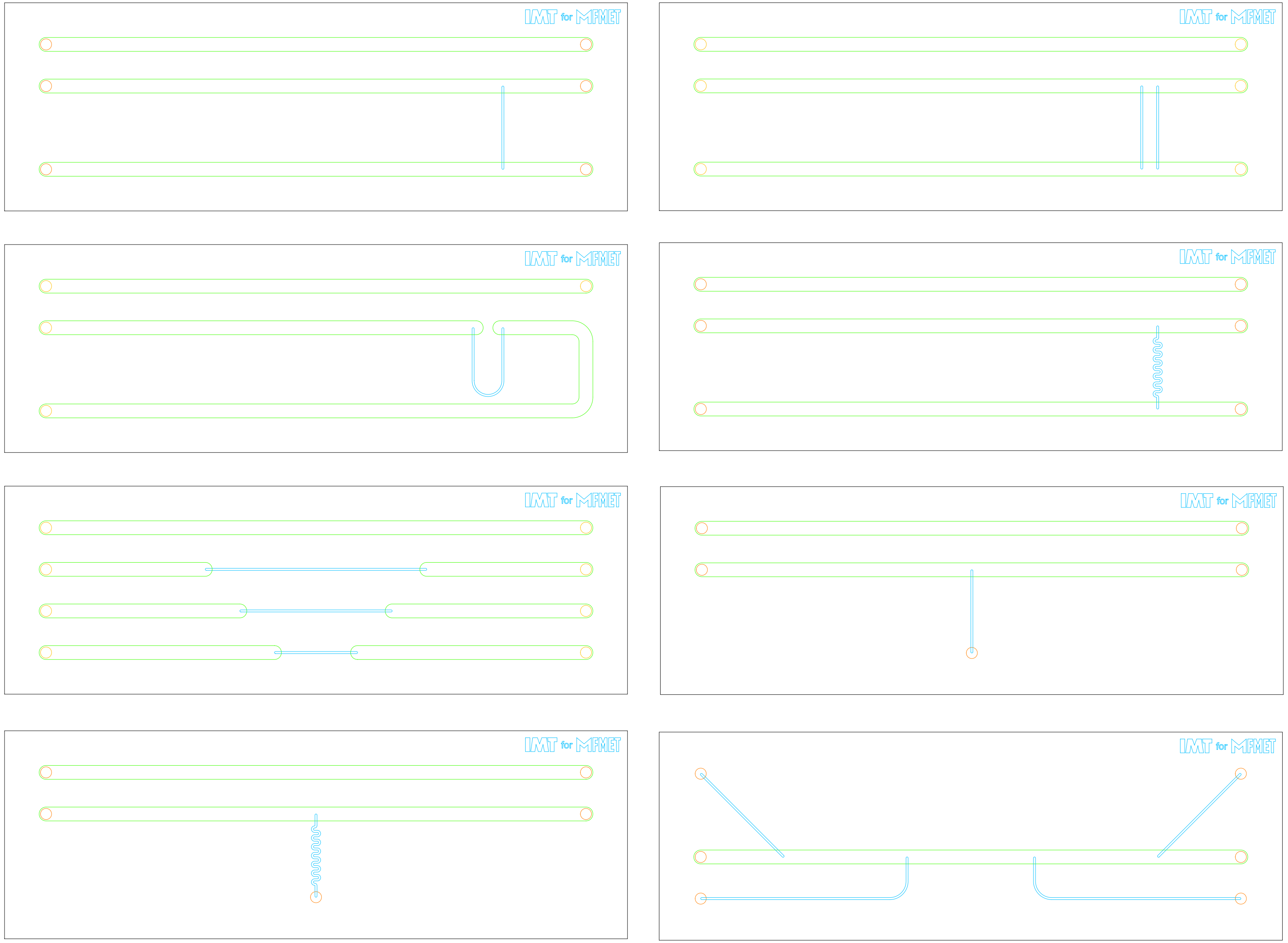
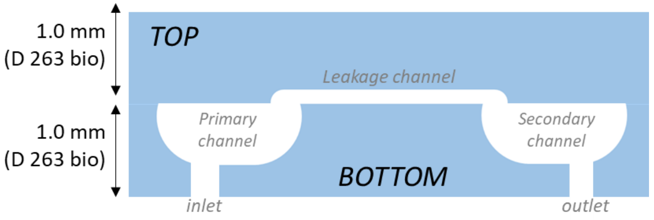
IMT



GLASS

	Inlet/outlet	Control/Primary/ Secondary channels	Leakage channel
Height (µm)		100	2
Width (µm)		1000	150
Length (mm)		33	5; 10; 15
Diameter (µm)	800		

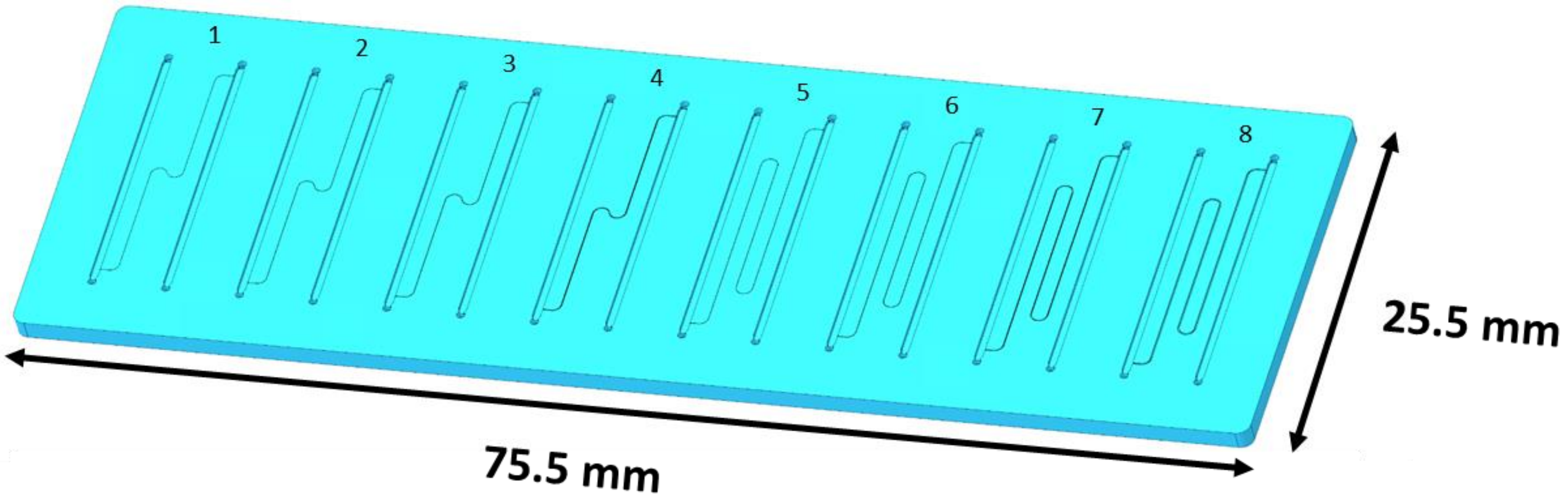
Side view



Transfer standards for leakage

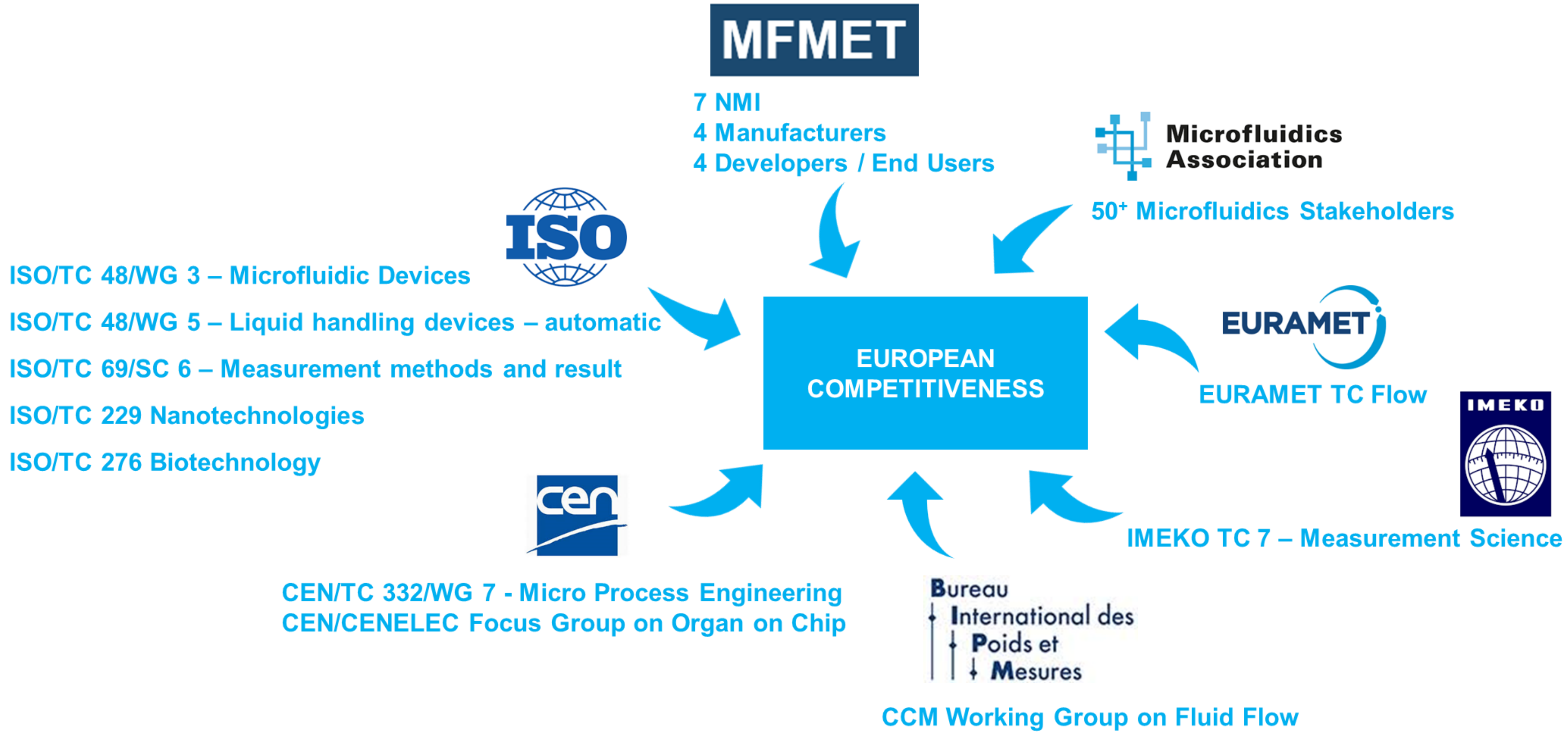
PLASTIC

Design nr.	Inlet/ outlet	1	2	3	4	5	6	7	8
Height (µm)		5	10	20	50	10	20	50	100
Width (µm)		5	10	20	50	10	20	50	100
Length (mm)		20	20	20	20	40	40	40	40
Diameter (µm)	500								




Waiting for results

EU infrastructure



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The knowledge gained from this project

Is being transferred to

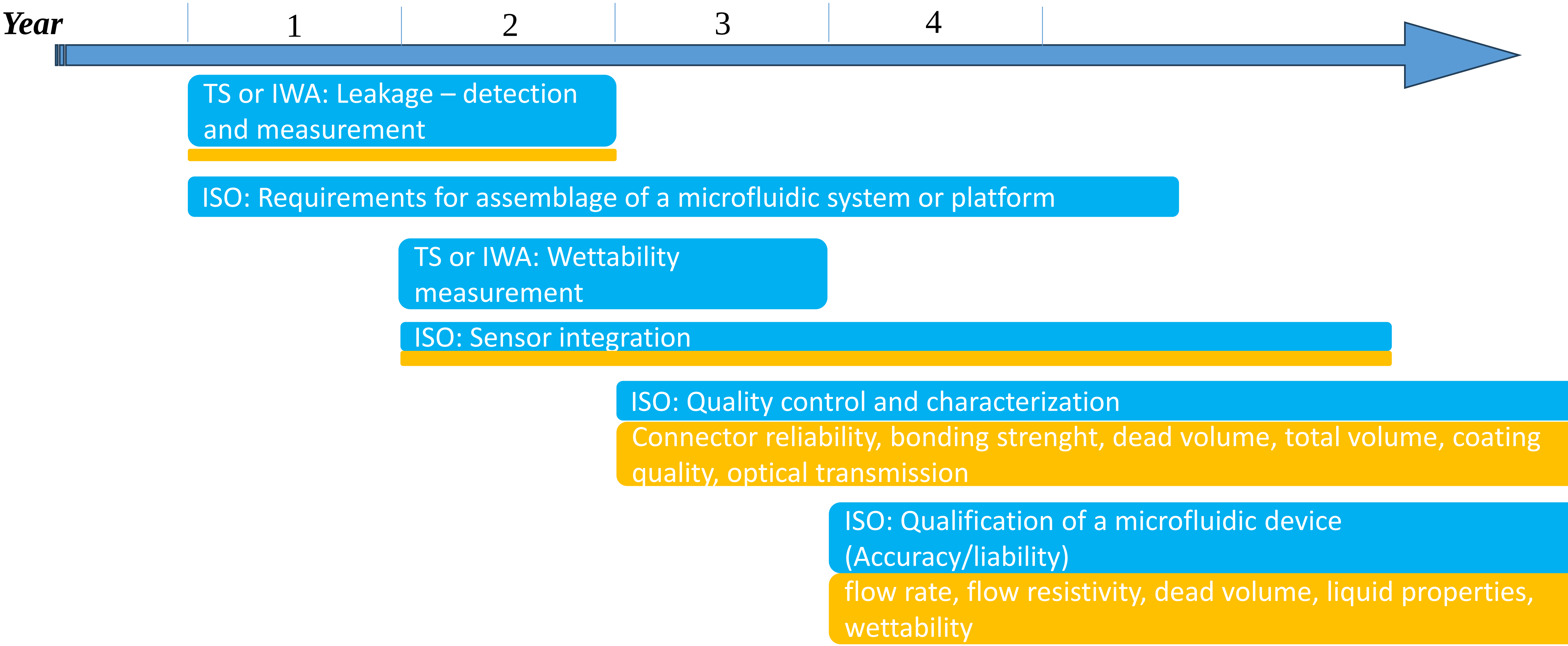
ISO TC 48/WG 3

and applied in the revision and development of new standards

ISO International Organization for Standardization

Timeline for working items ISO/TC 48/WG 3

From MFMET project
From surveys



Project Team

TÜBİTAK

enabling MNT micro nano technology

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IPQ

microfluidic ChipShop

CZECH METROLOGY INSTITUTE

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ESYP

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National Engineering Laboratory

leti ceatech

inesc mn

Microssistemas e Nanotecnologias

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ensemble, innover et valider

DANISH TECHNOLOGICAL INSTITUTE

Bronkhorst® Flow Technology

THANK YOU

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https://mfmet.eu

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