FINAL PUBLISHABLE REPORT

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RMG: -		

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1 Overview

Microfluidics, concerned with fluid-handling in the nano-to-millilitre scale, has major applications in biomedical and chemical analysis however global standards are lacking. ISO/TC48/WG3 has been established to address the standardisation of microfluidic components, interfaces, protocols for associated testing and protocols for microflow control to be applied in the development and the fabrication processes (manufacturing, testing and assembly) of microfluidic devices. This project aimed to contribute to the development of globally accepted standards for microfluidics and disseminate them to end users in industry (health and pharmaceutical sectors) and academia.

In general, the project supported several ISO technical committees in the development and revision of standards related to microfluidic technology by incorporating the results obtained in the different activities of the project. It also contributed to the developed of roadmaps of ISO TC 48 and FGOOC. Several workshops for industry and academia and metrologist were performed with very good feedback from all of the attendees. A microfluidic components database was produced and a webinar on metrology standards for microfluidics was developed. All this information has been very well accepted by the microfluidic community and this can be observed by the more than 2500 downloads of documents on the project's Zenodo website.

2 Need

The increased technical capability required to miniaturise devices along with the growing need for faster, more accessible, and cost-effective solutions for precision analytical tools has led to the rapid and continuous growth of microfluidics in diverse sectors (e.g. pharmaceutical and biomedical industries). According to a recent study, the global microfluidics market size is expected to reach 44.0 billion Euros by 2025 from an estimated value of 15.7 billion Euros in 2020. However, microfluidics and specifically the control of fluids in microfluidic devices still lacks universal solutions and standards. Stakeholders from industry, academia and government have recognised the need for globally accepted metrology standards for microfluidic devices and as a result ISO/TC 48/WG 3 was established to address this underpinning requirement. Measurement accuracy and traceability of microfluidic devices is critical to improve healthcare, including medical diagnostics and drug development sectors. For example, enabling rapid prototyping of low-cost high-volume point-of-care tests that can be shipped to individuals for rapid in-situ detection of viruses is a critical step in tackling future healthcare crisis, as highlighted by COVID-19. Current on the spot diagnosis that involves clinical input is cumbersome and expensive – microfluidic devices for on the spot diagnosis (such as pregnancy, glucose and PH tests) can provide cheaper, simpler and faster results.

Standardisation of performance characteristics is needed for the different classes of microfluidic components, including test conditions, measurement protocols and guidelines. The increasing demand for passive flow devices has already led National Metrology Institutes (NMIs) to establish protocols and calibrations services for very low flow rates. Traceability to National Standards has been available since 2012 down to 0.1 µL/min through facilities developed under EMRP JRP HLT07 MeDD. Recently EMPIR JRP 18HLT08 MeDDII tackled microflow measurements down to 5 nL/min and introduced new facilities which are now under implementation. These new technologies can now be used to develop microfluidic measurement protocols, and the new microflow pump devised in MeDDII can be used as a traceable flow generator.

In 2016, a first step towards microfluidic standardisation was made through ISO IWA23. The document was created to facilitate the uptake of microfluidic devices by making them easier to use, reducing the cost for assembling and enabling plug-and-play functionality. Recently a new standard, ISO/CD 22916, is being established based on the information from ISO IWA 23 which it will replace; however, this new standard still lacks the metrological specifications required for accurate and reproducible manufacturing.

3 Objectives

The overall objective of this project was to contribute to the development of globally accepted standards for microfluidic devices used particularly in the health and pharmaceutical industry.

The specific objectives were:

1. To investigate, evaluate and formulate consensus-based flow control specifications, guidelines and protocols to enhance the manufacturing capability of the microfluidics industry supply chain through voluntary compliance.
2. To develop measurement protocols for different flow quantities and liquid properties, in different microfluidics devices to be used in pharmaceuticals, biomedical and mechanobiology applications. A EURAMET guide and a technical report on these measurement protocols will be developed.
3. To define consensus-based standards and guidelines for interfaces and connectivity between fluidic passages and optical/electrical connections of microfluidics components and corresponding measurement standards, from micro to macro size scales.
4. To define guidelines for the standardisation of dimensions and accuracy for modularity (either module-to-module or module-to-world) and sensor integration (combination of sensing elements/materials with microfluidic modules), in accordance with good practices in microfluidic component design and manufacturing.
5. To collaborate with ISO/TC48/WG3 and end users of the standards (e.g. health and pharmaceutical industry) to ensure that the outputs of the project are aligned with their needs and in a form that can be incorporated into standards (e.g. new technical guides, ISO 10991 and ISO/CD 22916) at the earliest opportunity.

4 Results

These are the results of joint collaborative work.

4.1 *Objective 1: To investigate, evaluate and formulate consensus-based flow control specifications, guidelines and protocols to enhance the manufacturing capability of the microfluidics industry supply chain through voluntary compliance.*

The work in this objective started with the partners conducted research to compile and classify flow control components, including reservoirs, valves, and tubing, by investigating definitions, characteristics, specifications, and applications.

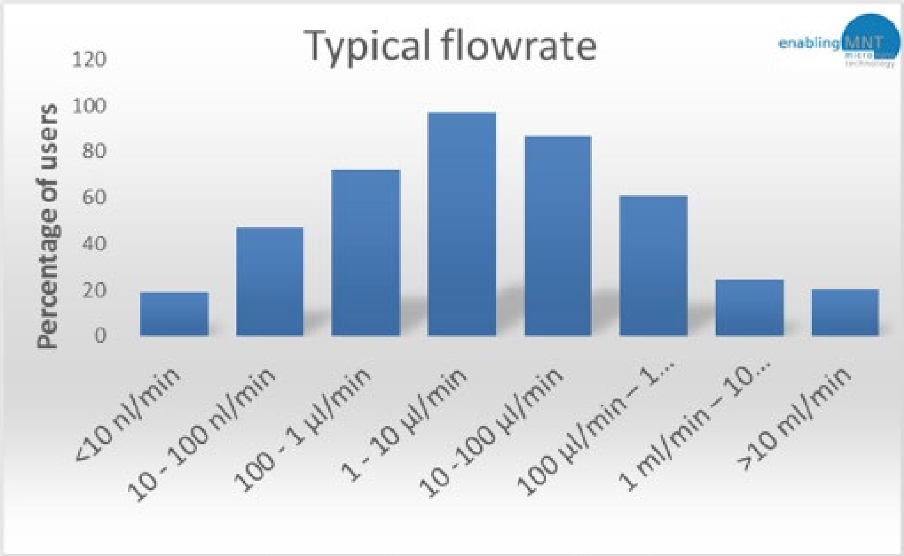
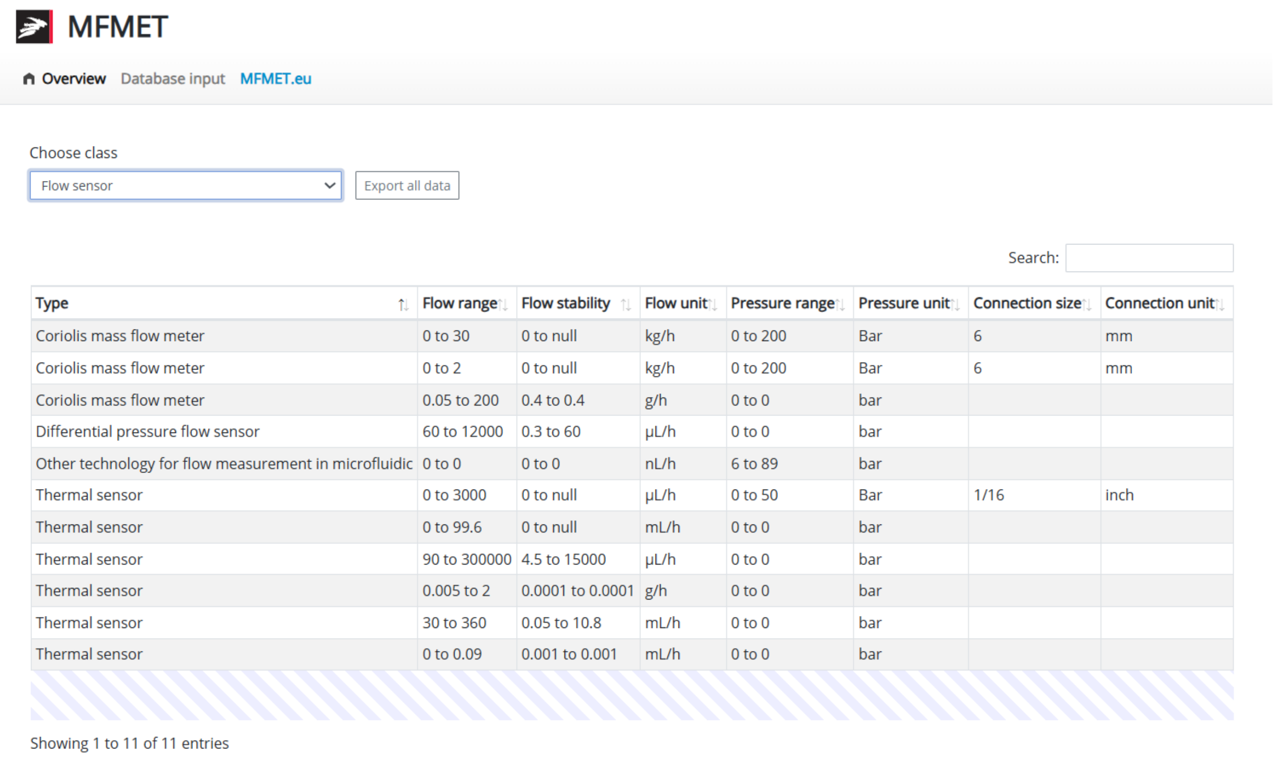


Figure 1. Typical flow rate used in microfluidic applications.

Definitions and symbols for basic flow control concepts were identified and harmonised and sent to ISO/TC 48/WG 3 for the development of a new Technical Standard ISO/TS 6417 Microfluidic pumps – Symbols and performance communication (https://www.iso.org/standard/82270.html). A vocabulary of flow control terms was prepared and sent to ISO/TC 48/WG 3 to be included in ISO 10991:2023 Microfluidics – Vocabulary (https://www.iso.org/standard/82146.html). The task resulted in the compilation of a comprehensive database detailing the flow control components used in microfluidics and is available online (https://zenodo.org/records/8336435). The database provides a clear understanding of the various types of components that can be selected for use in different microfluidic applications, based on their specific function and requirements that can be feed by any end user.



Choose class: Flow sensor

Type	Flow range	Flow stability	Flow unit	Pressure range	Pressure unit	Connection size	Connection unit
Coriolis mass flow meter	0 to 30	0 to null	kg/h	0 to 200	Bar	6	mm
Coriolis mass flow meter	0 to 2	0 to null	kg/h	0 to 200	Bar	6	mm
Coriolis mass flow meter	0.05 to 200	0.4 to 0.4	g/h	0 to 0	bar		
Differential pressure flow sensor	60 to 12000	0.3 to 60	µL/h	0 to 0	bar		
Other technology for flow measurement in microfluidic	0 to 0	0 to 0	nL/h	6 to 89	bar		
Thermal sensor	0 to 3000	0 to null	µL/h	0 to 50	Bar	1/16	inch
Thermal sensor	0 to 99.6	0 to null	mL/h	0 to 0	bar		
Thermal sensor	90 to 300000	4.5 to 15000	µL/h	0 to 0	bar		
Thermal sensor	0.005 to 2	0.0001 to 0.0001	g/h	0 to 0	bar		
Thermal sensor	30 to 360	0.05 to 10.8	mL/h	0 to 0	bar		
Thermal sensor	0 to 0.09	0.001 to 0.001	mL/h	0 to 0	bar		

Showing 1 to 11 of 11 entries

Figure 2. Flow components data base layout.

Representative ranges and specifications for flow control devices were defined, together with a test protocol for leakage and burst pressure. The results were compiled in a peer-reviewed paper and a White Paper. Guidelines and a test protocol for flow control in microfluidic devices were developed and are available online. The test protocol will ensure traceability to national standards and conformity to existing normative standards and is being shared with relevant standardization groups. Deliverable D1: 'Guidelines and a test protocol for flow control evaluating leakage and burst pressure in microfluidic devices' gathers the information prepared (https://doi.org/10.5281/zenodo.7901265), this document was developed by DTI with the contributions from CETIAT, IPQ, INESC MN, CMI, BHT, HSG-IMIT, TUBITAK, RISE, NQIS and EnablingMNT. The guidelines and the protocol in this document aim to operate within the following parametric range: non-hazardous water-based flow media, temperature (4-50) °C, pressure below 200 kPa (2000 mbar), and flow rates between 1 and 100 µL/min.

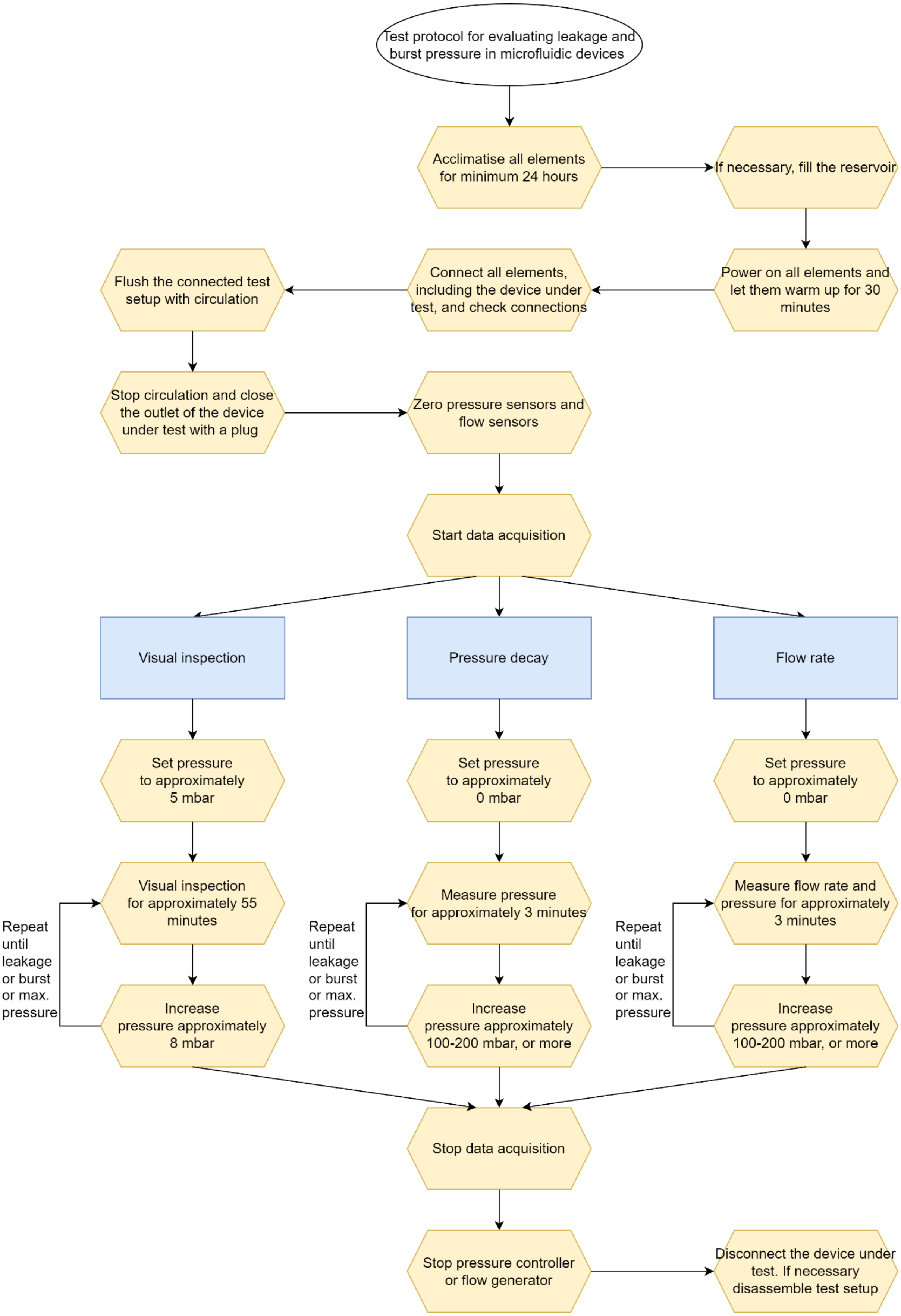

Test protocol for evaluating leakage and burst pressure in microfluidic devices
Acclimatise all elements for minimum 24 hours
If necessary, fill the reservoir
Power on all elements and let them warm up for 30 minutes
Connect all elements, including the device under test, and check connections
Flush the 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
Visual inspection
Pressure decay
Flow rate
Set pressure to approximately 5 mbar
Set pressure to approximately 0 mbar
Set pressure to approximately 0 mbar
Visual inspection for approximately 55 minutes
Measure pressure for approximately 3 minutes
Measure flow rate and pressure for approximately 3 minutes
Repeat until leakage or burst or max. pressure
Repeat until leakage or burst or max. pressure
Repeat until leakage or burst or max. pressure
Increase pressure approximately 8 mbar
Increase pressure approximately 100-200 mbar, or more
Increase pressure approximately 100-200 mbar, or more
Stop data acquisition
Stop pressure controller or flow generator
Disconnect the device under test. If necessary disassemble test setup

Figure 3. A process flow diagram summarising protocols for combined tests for leakage and burst pressure with visual inspection, pressure decay and flow rate.

Tests of leakage and burst pressure were conducted at CETIAT using the setup of figure 4 and results are presented in the following figure 5.

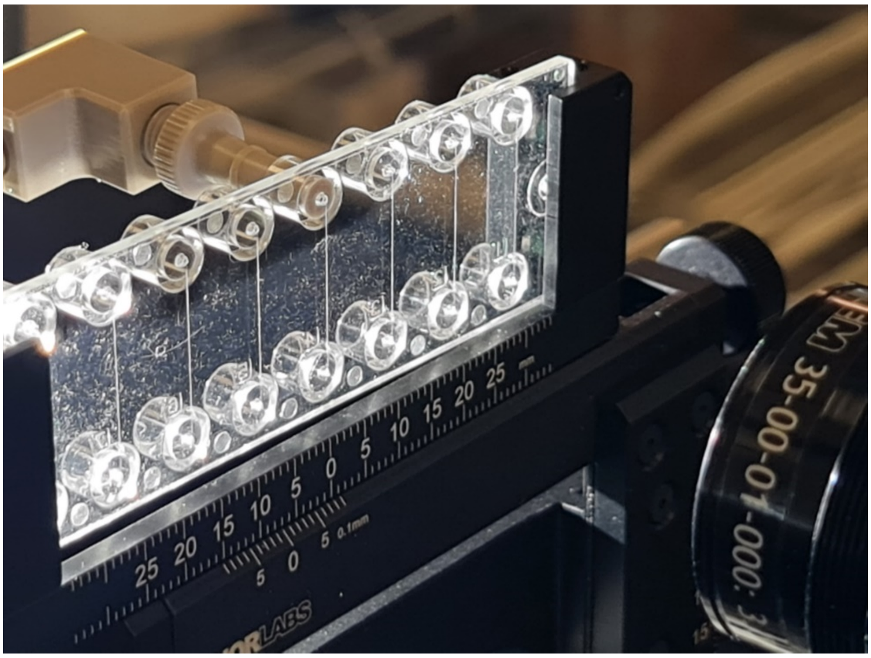


Figure 4. Burst pressure setup with microfluidic chip model Fluidic 157 at CETIAT.

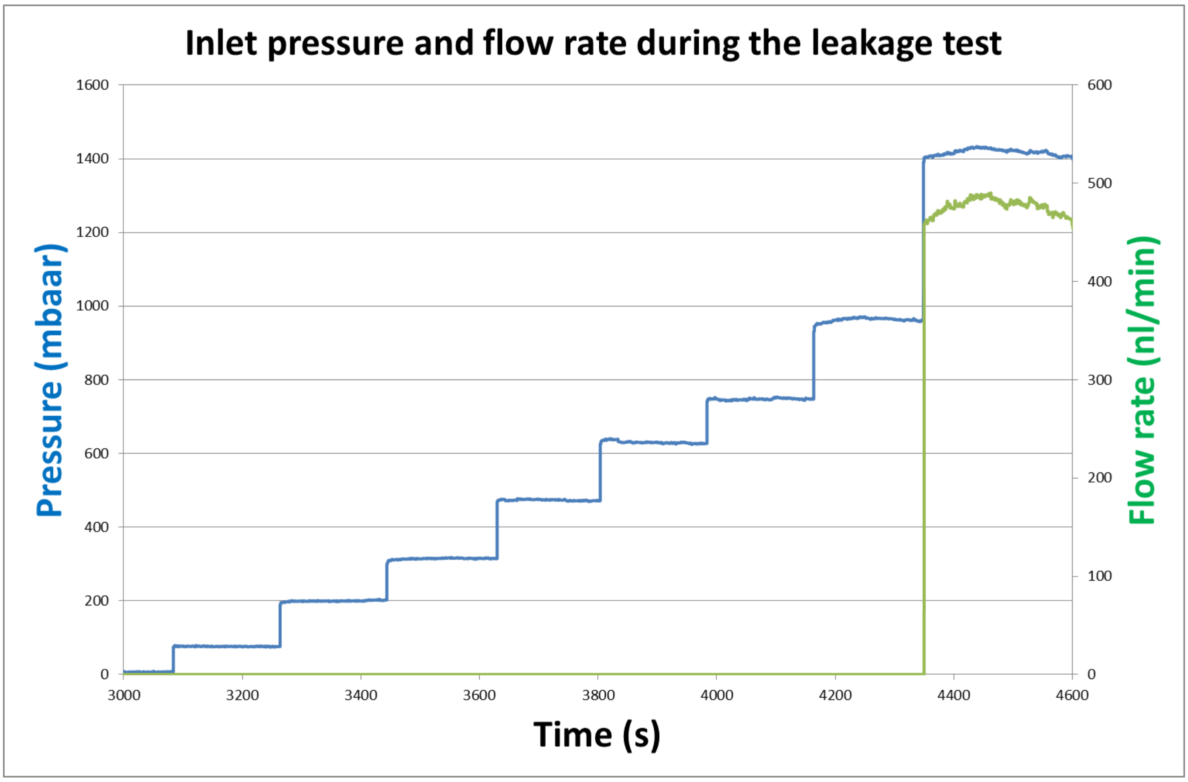


Figure 5. Pressure measured by the pressure sensor prior to the inlet of the device under test, during visual inspection for a combined leakage test and burst pressure test.

Three test methods were investigated: visual inspection, pressure decay and flow rate. The tests were carried out as combined tests of leakage test and burst pressure test. During the tests, leakages were identified.

However, it proved difficult to burst the device under test, as the chip would leak rather than burst. Hence, no results from an actual burst were presented in this protocol. The leak, which prevented a potential burst, happened between the inlet port and the connector. This is probably due to inadequate matching of connection tolerances between the inlet port inner diameter and the outer diameter of the connector. This test protocol made a total of three burst pressure tests, which was most likely too few for a statistically rigorous evaluation the burst pressure test.

The state of the art of flow control methodologies for nano/micro/meso/macro fluidics was investigated, identifying existing operational conditions, protocols, and needs. Flow rate uncertainty ranges for accuracy, were collected considering existing standards and metrology specifications. The consortium additionally identified gaps in microfluidic control flow methodologies and assessed the need for new guidelines for implementing existing protocols and standards. Guidelines for the implementation of consensus-based flow control specifications in microfluidics were prepared and shared with relevant standardisation groups. Deliverable D2: 'Guidelines for the implementation of consensus-based flow control specifications in the microfluidics industry supply chain' compiles the information collected (https://doi.org/10.5281/zenodo.11394489) and it was developed by INESC MN with contributions from, CETIAT, IPQ, DTI, UofG, CMI, HSG-IMIT, TUBITAK, NQIS and EnablingMNT.

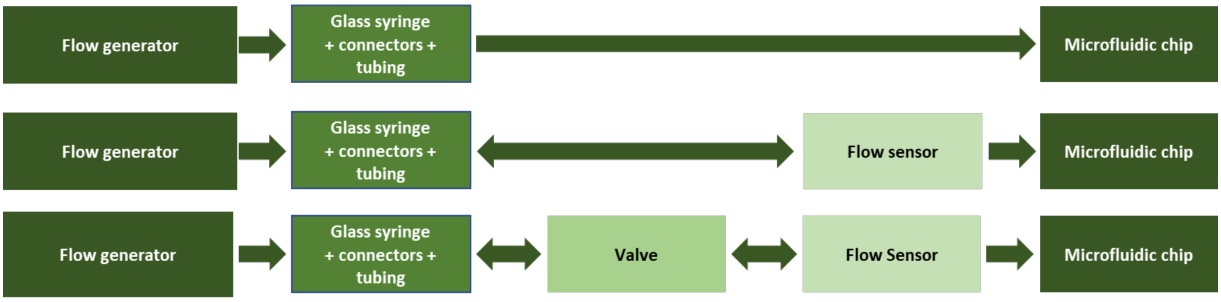


Figure 6. Flow components data base layout.

The increasing complexity of microfluidic systems has led to the need for consensus-based flow control specifications to ensure reliable and optimal operation. However, the implementation of these specifications requires careful consideration of various factors, including system architecture, sensor and actuator selection, and control algorithms. Without clear guidelines, the implementation of consensus-based flow control specifications in microfluidics may lead to inconsistent results, reduced reliability, and increased risk of errors.

Summary

A vocabulary of flow control terms was prepared and submitted to ISO/TC 48/WG 3 for inclusion in ISO 10991:2023 Microfluidics – Vocabulary. This standardisation effort aims to establish consistent terminology across the field. In addition to the vocabulary standardisation efforts, the task also led to the creation of a comprehensive database. This database details the various flow control components used in microfluidics, and it is accessible online. Researchers and practitioners can refer to this resource to explore and understand the intricacies of flow control mechanisms within microfluidic devices.

Additionally, guidelines related to leakage and pressure were developed, likely to ensure reliable and safe microfluidic operation. CETIAT conducted tests on a microfluidic chip to identify leakages. However, during these tests, the chip would leak rather than burst when subjected to pressure. This behaviour highlights the challenges in achieving burst-proof designs.

Guidelines for implementing consensus-based flow control specifications were prepared and shared with relevant standardisation groups. These guidelines aim to create a standardised approach for flow control in microfluidics. The focus is on efficient and reliable fluid flow control within microfluidic devices, including lab-on-a-chip (LOC) devices, micro-total analysis systems (μTAS), and other platforms.

This objective was achieved.

4.2 Objective 2: To develop measurement protocols for different flow quantities and liquid properties, in different microfluidics devices to be used in pharmaceuticals, biomedical and mechanobiology applications. A EURAMET guide and a technical report on these measurement protocols will be developed.

The quantities and properties of interest for the stakeholders in the microfluidic industry have been identified by a survey. Interviews were also conducted to get the best understanding of the needs and challenges associated with flow-related metrology in microfluidics. This work was the ground for the development of two important protocols, which were the main deliverables expected from WP2.

Deliverable D3 is a ‘Calibration guide for the evaluation of flow-related quantities in microfluidic devices’ (https://doi.org/10.5281/zenodo.11164417) that was developed by RISE with the cooperation of INESC MN, DTI, CETIAT, IPQ, CMI, TUBITAK and HSG-IMIT. It covers the development of test protocols for the most critical quantities in a microfluidic chip identified in the survey: flow rate, hydrodynamic resistance (also known as flow resistivity) and internal volume. The deliverable presents industrial applications based on gravimetric and optical methods, and how they are applied in metrology laboratories. This document was published as a EURAMET technical guide nº4 - Evaluation of flow-related quantities in microfluidic devices.

Flow rate determination

Two different TOPAS® chips with 16 parallel channels from Microfluidic Chip shop were tested at IPQ using the gravimetric method and front track method. The setups are presented in figure 7.

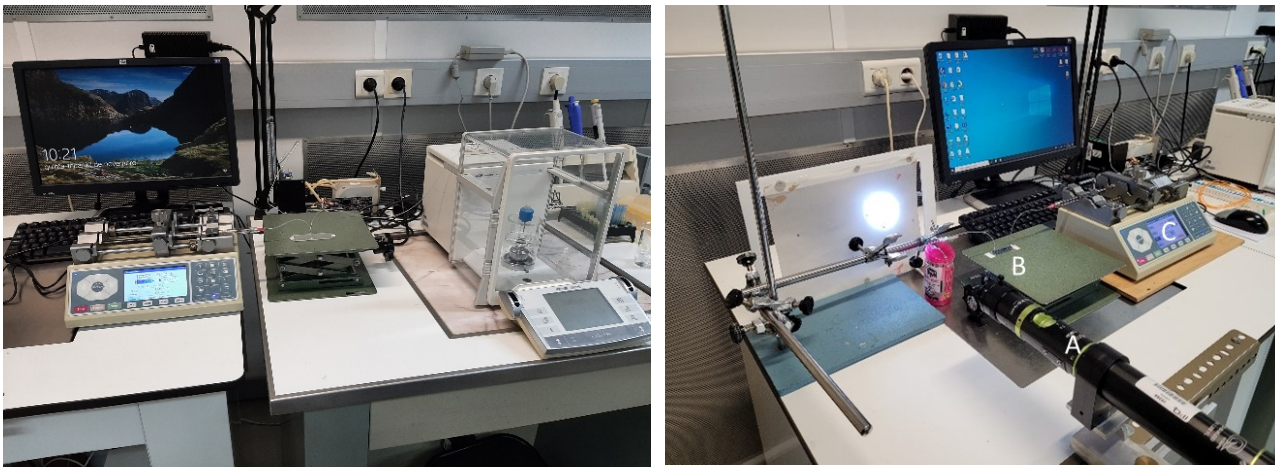


Figure 7. Assembly of the gravimetric method setup at IPQ on the left and on the right the front track setup.

The following tables shows the experimental results obtained for the measurements of flow rate using the gravimetric method and the front track method for Chip A and Chip C.

Table 1. Results of the gravimetric flow measurements at IPQ (Chip A and Chip C).

Chip	Generated Flow (ml/h)	Flow without the chip	Error (%)	*U* (%)	Flow with the chip (ml/h)	Error (%)	*U* (%)
A	0,1	0,09991	0,09	3,3	0,0983	1,7	3,8
	1	1,00356	-0,36	2,2	0,9907	0,9	3,5
C	0,1	0,09991	0,09	3,3	0,097	2,6	3,4
	1	1,00356	-0,36	2,2	1,017	-1,7	3,0

Table 2. Results of the front tracking measurements at IPQ (Chip A and Chip C).

Chip	Generated Flow (ml/h)	Flow without the chip	Error (%)	*U* (%)	Flow with chip (ml/h)	Error (%)	*U* (%)
A	0,1	0,09961	0,39	2,1	0,0967	3,4	2,8
	1	0,99706	0,02	1,5	0,9819	1,8	6,2
C	0,1	0,09961	0,39	2,4	0,0996	0,5	1,9
	1	0,99706	0,02	2,4	1,0259	-2,5	4,0

Flow tests were performed in each chip assembly using the front track method and the gravimetric method. In the majority of the cases for both methods the error is larger when adding the chip to the system and the uncertainty increases.

Flow resistivity

CETIAT performed flow resistivity test on the TOPAS® "Fluidic 157" chip obtained from microfluidic-chipshop with 8 parallel channels of 100 µm width, 100 µm depth, 18 mm length.

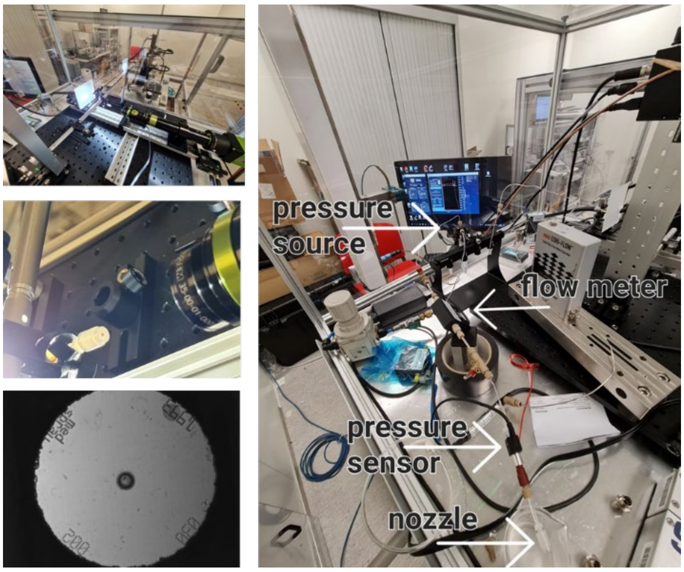


Figure 8. Assembly of the flow resistivity tests at CETIAT.

To calculate the flow resistivity of the device under test alone, and the tests with and without chip being done at constant pressure, one subtracts the flow resistivity obtained without the chip to the one obtained with the chip. The following table shows the flow resistivity calculation of the chip alone. The experimental results obtained for the measurement of CETIAT for flow resistivity are presented in table 3.

Table 3. Flow resistivity results.

Measurement results as acquired by the instruments		**Measurement results in SI Units**		**Calculation result**
Inlet pressure	Volume flow	Inlet pressure	Volume flow	Flow resistivity
[mbar]	[nl/min]	[Pa]	[m^3/s]	[$Pa.s/m^3$]
39	39000	3900	6,50E-10	6,00E+12
32	34000	3200	5,67E-10	5,65E+12
25	28500	2500	4,75E-10	5,26E+12
20,5	22000	2050	3,67E-10	5,59E+12
15	15500	1500	2,58E-10	5,81E+12
			average	**5,66E+12**

An average experimental value of $5.66.10^{12}$ $Pa.s/m^3$ is obtained for the device under test under the given test condition.

Liquid proprieties

In microfluidics the used liquids range from oil, water to blood but most liquids that are used are aqueous. The characteristics of the used medium can influence the response of the microfluidic device, such as the hydraulic resistance, in a capillary (passive) filling device therefore a test protocol for liquid properties in microfluidic devices for use in pharmaceuticals, biomedical and mechanobiology applications was also developed as Deliverable D4 ‘Report on test protocols for liquid properties in microfluidic devices for use in pharmaceuticals, biomedical and mechanobiology applications’ (https://doi.org/10.5281/zenodo.11164544). This document was elaborated by IPQ with contributions from RISE, CETIAT, CMI, HSG-IMIT and TUBITAK. Of the properties identified in the survey, the most important are density, viscosity, refractive index and contact angle. The deliverable describes the associated methods to measure these properties, illustrated with examples performed in the metrology laboratories participating in the project. The liquid properties, density, viscosity and refractive index of 2 different liquids with microfluidic medical applications (simulated body fluid and phosphate buffer saline) were determined. The value of water was also presented based on the given references. Simulated body fluid (SBF) was purchased from BioChemazone, Canada and phosphate buffer saline solution (PBS) was purchased from Sigma, USA.

For contact angle determinations, ultrapure water (type I) produced by the Milli Q Advantage water system from Merck Millipore, ethylene glycol p.a. from Merk and glycerol 99.5 %, reagent grade from Scharlau were used. The results are presented in the following tables.

Table 4. Density results.

	Water	PBS	SBF	*U*
Density, *D* / (kg/m^3) at 20 ˚C	998.203*	1005.225	1007.523	0.033

Table 5. Viscosity results.

	Viscosity, μ / (mm^2/s) at 20 ˚C	U / (mm^2/s)
Water	1,0034*	0,0017*
PBS	1,0386	0,0055

Table 6. Refractive index results.

	Water	PBS	SBF	*U*
Refractive index at 20 ˚C	1,332 986*	1,334 656	1,335 886	0,000 009

Two different chips, PDMS and TOPAS were tested with 3 different liquids, water, PBS and SBF at 0,025 mL/h, 0,1 mL/h and 0,4 mL/h using the gravimetric method.

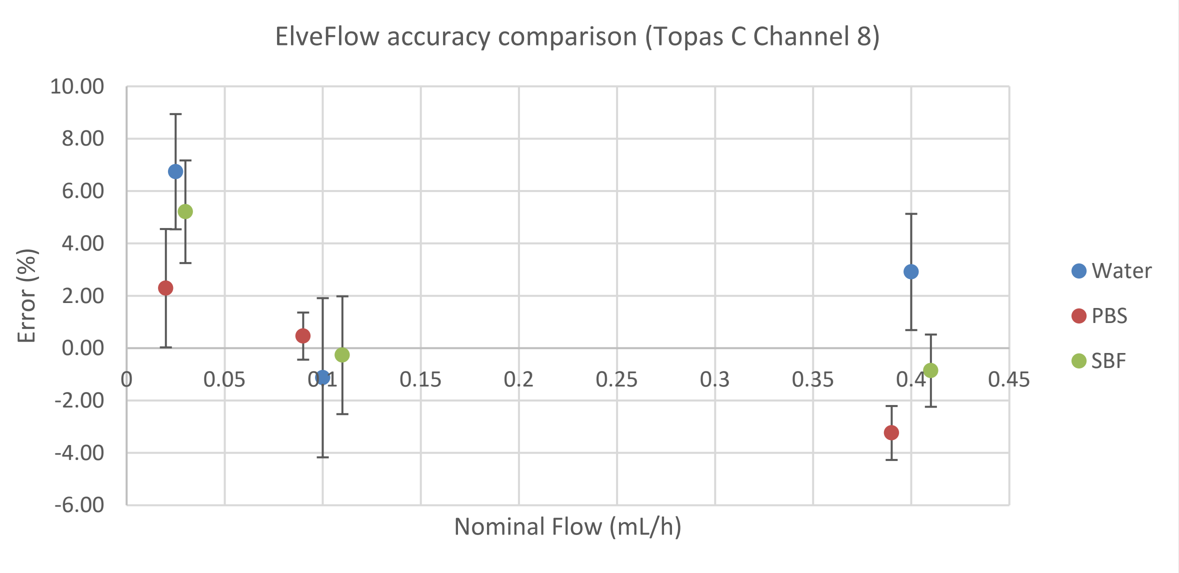


Figure 9. Flow results TOPAS Chip.

The results obtained highlight how these liquid properties impact the performance of microfluidic chips when using liquids other than water. The flow rates and volumes within microfluidic channels depend on the type of liquid being used and its interaction with the chip material. Overall, this D4 report sheds light on the importance of considering liquid properties when designing and using microfluidic systems.

Transfer standards

In addition to these deliverables, effort was put in the design, manufacturing and characterisation of transfer standards. The partnership with the industrial members of the consortium succeeded in producing glass and polymer microfluidic chips, which comply with the standards for interfaces and connectivity developed in WP3.

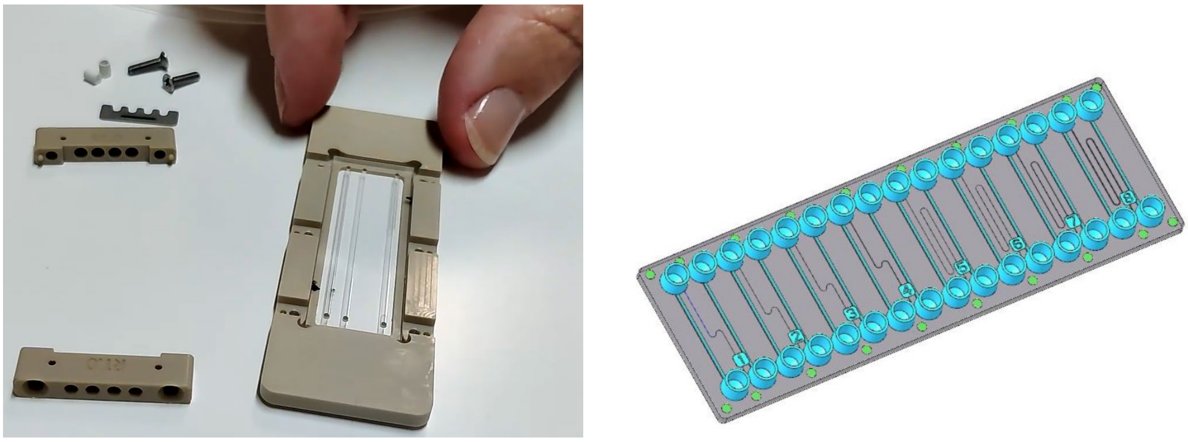


Figure 10. Transfer standards, on the left the glass chip and on the right the polymer chip.

The standard chips were then circulated between eight international laboratories, from Turkey to the USA and across Europe. This interlaboratory comparison aimed at characterising the dimensions of the chips and the influence of various configurations of leakage channels on the established flow. This is the subject of two reports.

The protocol for the comparison is developed in the report A2.4.3 ‘Documented example of leakage transfer standards test’ (https://doi.org/10.5281/zenodo.11403006). It describes the quantities to be measured, the methods to perform the tests, and the planned schedule.

The report A2.4.4 is the ‘technical report describing the design, fabrication, and calibration process of the transfer standards’ (https://doi.org/10.5281/zenodo.11402853). It contains information from the manufacturers about their respective chips and the many results obtained by the metrology laboratories to characterise the transfer standards. It also describes the challenges encountered during the measurements, sharing how the future protocols could be further improved for the benefit of the community in microfluidics.

Measurements were performed for flow, flow resistivity, volume, channel dimensions, roughness, and surface energy for both chips.

Glass chip results

Some results, for the glass chips, can be found below.

Table 7. Dimensions measured at CEA.

Design01 - serie 01	***Hole 01***		***Hole 02***		***width***		***Length***	
	Diam.	σ	*Diam.*	σ	Meas.	σ	Meas.	σ
Channel 01	**813,14**	*11,37*	**829,02**	*1,61*	**998,10**	*1,78*	40121,78	*802*
Channel 02	**822,85**	*3,55*	**821,10**	*0,82*	**1001,59**	*2,93*	40136,43	*803*
Channel 03	**822,20**	*3,93*	**825,52**	*0,17*	**999,26**	*1,35*	40078,11	*802*
Leakage Channel					**152,60**	*0,35*	6002,60	6

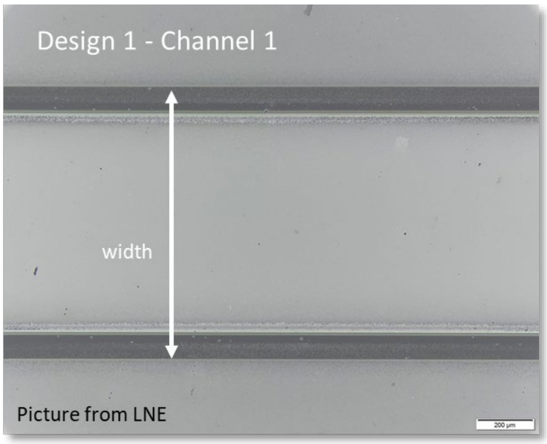


Figure 11. Glass chip channel picture at LNE.

For the measurement of the depth of internal geometry, difficulty was encountered. The only protocol able to provide a measurement of the internal channel depth, optical profilometry, gave results that are highly inconsistent with their nominal values. Different avenues of future work are proposed including a study of how to apply the refractive index for deformation compensation when challenged with multiple refraction interfaces. Another possible method that was shown to merit further investigation is confocal fluorescence microscopy, which showed promising preliminary results.

Table 8. Channel volume measured at IPQ.

Chip	Channel	Volume (mL)	Uncertainty (mL)
1-1	1	0,004266	0,000088
	1+2	0,00788	0,00018
	1+2+3	0,012255	0,000064
	Residual volume	0,000346	0,000076

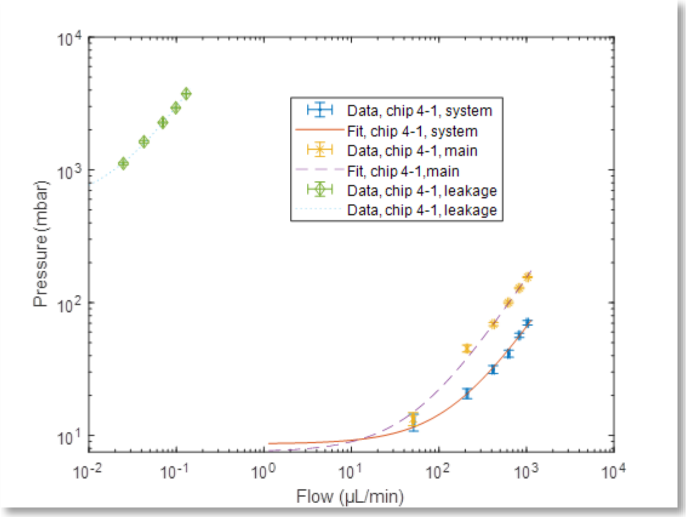


Figure 12. Flow measured at DTI.

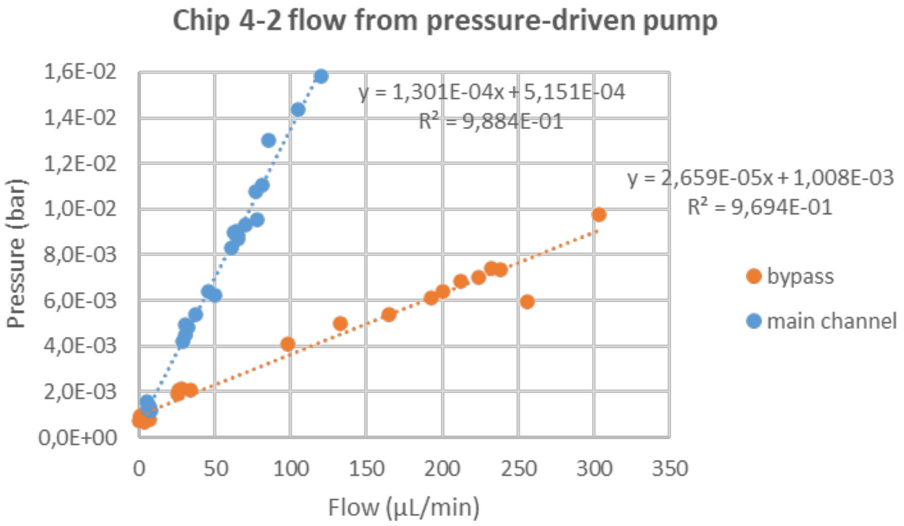


Figure 13. Flow measured at CETIAT.

For measurements of flow and hydrodynamic resistance, difficulties were found by all partners in driving and measuring flow through the leakage channels of the glass chips. This might be caused by the very small size of the leakage channels (150 µm x 2 µm) or partial to total blockage of the channels by small particles. Checking the chips with air at least confirmed the leakage channels were not totally blocked, and water was even observed flowing through for some chips (Figure 10). However, at least for the glass chip 1-1, the flow within the leakage channel was seen disturbed by small particles (Figure 11). This subject would deserve to be investigated in future works and protocols, and several partners already possess the tools to observe the behaviour of flows in such microchannels. The surface state inside the leakage channels might also play a more predominant role than inside the main channels.

Table 9. Flow resistivity measured at UofG.

Chip	Setup	Resistivity (mbar/µL/min)	Resistivity Unc (mbar/µL/min)	Intercept (mbar)	Intercept Unc (mbar)
1-1	system	0,0620	0,0052	13,5188	3,5039
1-1	main	0,1513	0,0047	17,1500	3,2093
2-1	system	0,0704	0,0017	7,3426	1,1589
2-1	main	0,1760	0,0003	8,6931	0,2388
3-1	main	0,1803	0,0039	6,1080	2,6879
4-1	main	0,1750	0,0038	8,3180	2,5325
5-1	main	0,1721	0,0025	9,8927	1,6673
6-2	main	0,1780	0,0021	9,4403	1,3862
7-1	main	0.1865	0,0066	-2,1220	4,3975
8-1	main	0,1475	0,0143	-20,8700	8,2837

When characterising hydrodynamic resistance of small channels that are associated with similarly high pressure and small volumetric flow, it may be very worth to consider a procedure like the one proposed by the UofG. Here the fluidic path upstream of the leakage channel was pressurised and then the pump was stopped, allowing a slowly decaying pressure and flow through the leakage channel. For very small channels, such a decay might reach conditions much closer to steady state laminar flow than a flow influenced by a pump. Such a protocol could also improve the efficiency of the plan of experiments, as it might allow for quicker measurements. It would however require an accurate description of the protocol and a test setup capable of recording the pressure and flow rate synchronously.

Polymer chip results

Some results, for the polymer chips, can be found below.

Table 10. Flow measured at IPQ.

Chip (+ flow generator)	Nominal Flow [mL/h]	Measured Flow [mL/h]	Error [%]	U (%)	Variation with and without chip [%]
Channel 5 big (ElveFlow)	0,025	0,0265	-6,00	1,92	0,75
	0,1	0,1078	-7,80	0,79	-1,32
	0,4	0,5215	-30,38	3,24	1,40
Channel 5 small (ElveFlow)	0,025	0,0237	5,20	4,64	11,24
	0,1	0,1036	-3,60	4,34	2,63
	0,4	0,5398	-34,95	2,76	-2,06
Channel 8 big (ElveFlow)	0,025	0,024	4,00	1,80	10,11
	0,1	0,1011	-1,10	3,51	4,98
	0,4	0,513	-28,25	3,19	3,01
Channel 8 big (Nexus)	0,025	0,021	16,00	5,56	18,29
	0,1	0,0987	1,30	3,25	1,21
	0,4	0,39	2,50	3,45	2,13
Channel 5 big (Nexus)	0,025	0,0253	-1,20	13,62	1,56
	0,1	0,0985	1,50	5,03	1,41
	0,4	0,3877	3,08	2,83	2,71

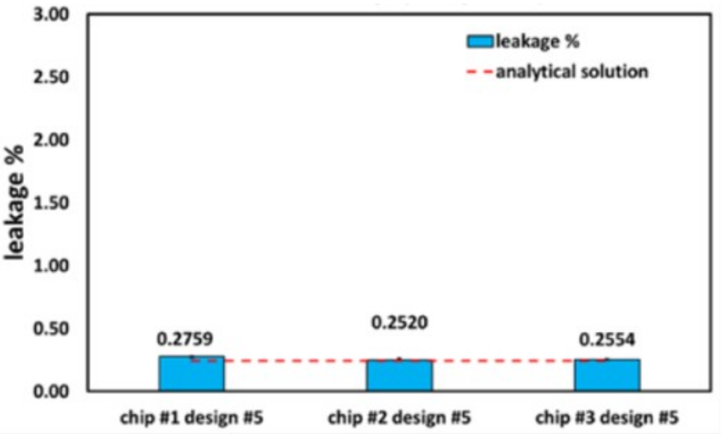


Figure 14. Leakage measured at FDA.

Table 11. Dimensions measured at CEA.

	Width		Depth	
	Average measured	Standard deviation	Average measured	Standard deviation
Channel 01	503,1 µm	0,1 µm (0,0 %)	358,0 µm	1,7 µm (0,5 %)
Leakage Channel 01	108,5 µm	0,1 µm (0,1 %)	123,6 µm	1,8 µm (1,4 %)
Channel 02	508,1 µm	0,1 µm (0,0 %)	372,9 µm	2,7 µm (0,7 %)

TOPAS© COC chips did not give significantly worse or better results than the D263©bio chips. Based on which the conclusion can be drawn that the materials are equivalently compatible with optical profilometry. Here too, the depth measurements form an exception. Paradoxically the channels with worse surface roughness were better measurable with the optical profilometer. Despite that, depth measurements in the TOPAS© COC chips were not satisfactory either. Further research comparing other common materials in microfluidics and measurement techniques is recommended.

The results of this work directly address the current lack of measurement methods for internal microfluidic structures by providing a comprehensive comparison of different protocols, ultimately suggesting a preferred option for immediate application within the microfluidic industry. Additionally, this study offers valuable directions for future research, serving as an initial step in overcoming a significant challenge that impedes the microfluidic industry from realising its revolutionary potential.

Summary

In this work package, researchers developed measurement protocols for various flow quantities and liquid properties within different microfluidic devices. These protocols were specifically designed for applications in pharmaceuticals, biomedical research, and mechanobiology.

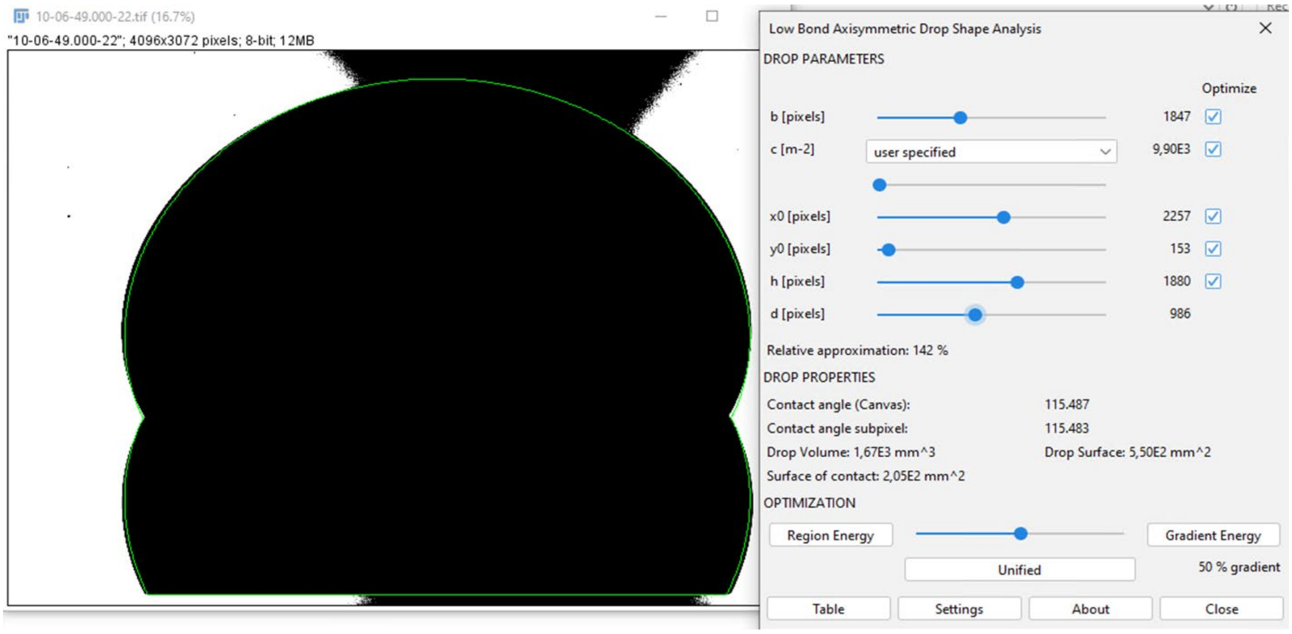
The next step involved applying these measurement protocols to transfer standards. These standards were designed and manufactured by project partners using materials such as glass and Topas. However, participants faced challenges during the measurements, likely due to a lack of practical experience in microfluidic measurement techniques. To address this, ongoing efforts are necessary to enhance the skills and setup of the national metrology institutes and industrial partners involved in microfluidics research. By improving their expertise, they can overcome measurement difficulties and contribute to the advancement of microfluidic technologies.

This objective was achieved.

4.3 Objective 3: To define consensus-based standards and guidelines for interfaces and connectivity between fluidic passages and optical/electrical connections of microfluidics components and corresponding measurement standards, from micro to macro size scales.

During this project, significant strides have been made in the field of microfluidics fabrication and testing. One of the most notable achievements is the identification of the most important and commonly used materials for microfluidics fabrication. By conducting thorough surveys and investigating suppliers, key performance parameters, types of connectors, and fabrication methods have been precisely identified, laying a strong foundation for future developments in microfluidics.

A pivotal milestone was the development and conclusion of task A3.2.2. - Test protocol for hydrophobicity, hydrophilicity and wettability. An exemplary test protocol was meticulously documented, performed at CETIAT's liquid flow laboratory in task A3.2.5 - [MFMET A3.2.5. Documented example of wettability test protocol (zenodo.org)](). This documentation detailed the measurand, specifically the contact angle, and the assessed property, wettability, quantified by the surface energy of materials such as glass slides (D263® bio) provided by IMTAG.



10-06-49.000-22.tif (16.7%)

"10-06-49.000-22"; 4096x3072 pixels; 8-bit; 12MB

Low Bond Axisymmetric Drop Shape Analysis

DROP PARAMETERS

		Optimize
b [pixels]	1847	☑
c [m-2] user specified	9,90E3	☑
x0 [pixels]	2257	☑
y0 [pixels]	153	☑
h [pixels]	1880	☑
d [pixels]	986	

Relative approximation: 142 %

DROP PROPERTIES

Contact angle (Canvas): 115.487

Contact angle subpixel: 115.483

Drop Volume: 1,67E3 mm^3

Drop Surface: 5,50E2 mm^2

Surface of contact: 2,05E2 mm^2

OPTIMIZATION

50 % gradient

Figure 15. LB-ADSA (Low Bond Axisymetric Drop Shape Analysis) contact angle measurement of a 120° angle standard.

Also, the partners performed an experimental assessment of the surface energy of a D263bio glass sample using the OWRK (Owens-Wendt-Rabel & Kaelble method) model tested with ultra-pure water, di-iodomethane and ethylene glycol. The measurement system for contact angle was calibrated using traceable angle standards and a comparison of the accuracy of contact angle software tools is also provided in the report.

In addition, the compatibility of at least three microfluidic components was rigorously tested and documented, with ongoing tests planned for the golden standards of glass and polymer. These efforts will provide a robust framework for understanding and enhancing material compatibility in microfluidic applications.

Significant progress has also been made in developing guidelines for optical interfaces of microfluidic devices, published as a White Paper. Further guidelines focusing on standardised methods for microfluidic components, particularly on port connections from microscale to macroscale, were developed by IMTAG with the cooperation of INESC MN, TUBITAK, EnablingMNT, CEA, CETIAT and LNE and were published as a Deliverable D6 - MFMET Deliverable 6 - Guidelines for the implementation of standardised methods of microfluidic components focusing on port connection from microscale fluidic channels to the macroscale world and associated changes in flow and pressure .

Dimensional measurements of all connectors in several microfluidic systems were performed using different methods.

	Mensurand	Value	*U* /mm
PE Tube	Inner diameter	1,26 mm	0,040

	Mensurand	Value	*U* /mm
Stainless steel catheter	External diameter	0,912 mm	0,010

	Mensurand	Value	SD/mm
Hole in the chip	Inner diameter	0,710 mm	0,003

Figure 16. Dimensions measured using the interferometer for the PDMS chip.

A comprehensive database for the surface roughness of commonly used microfluidic materials (COC/COP, glass, PMMA) has been established. Test protocols for AFM, confocal microscopy, and stylus profilometry were developed and exemplified on glass specimens (D263® bio). These protocols and documented examples provide accurate estimates of surface roughness in both bonding and channel areas of glass microfluidic devices, more information is described in MFMET A3.2.7 Documented example of surface roughness measurements (zenodo.org).

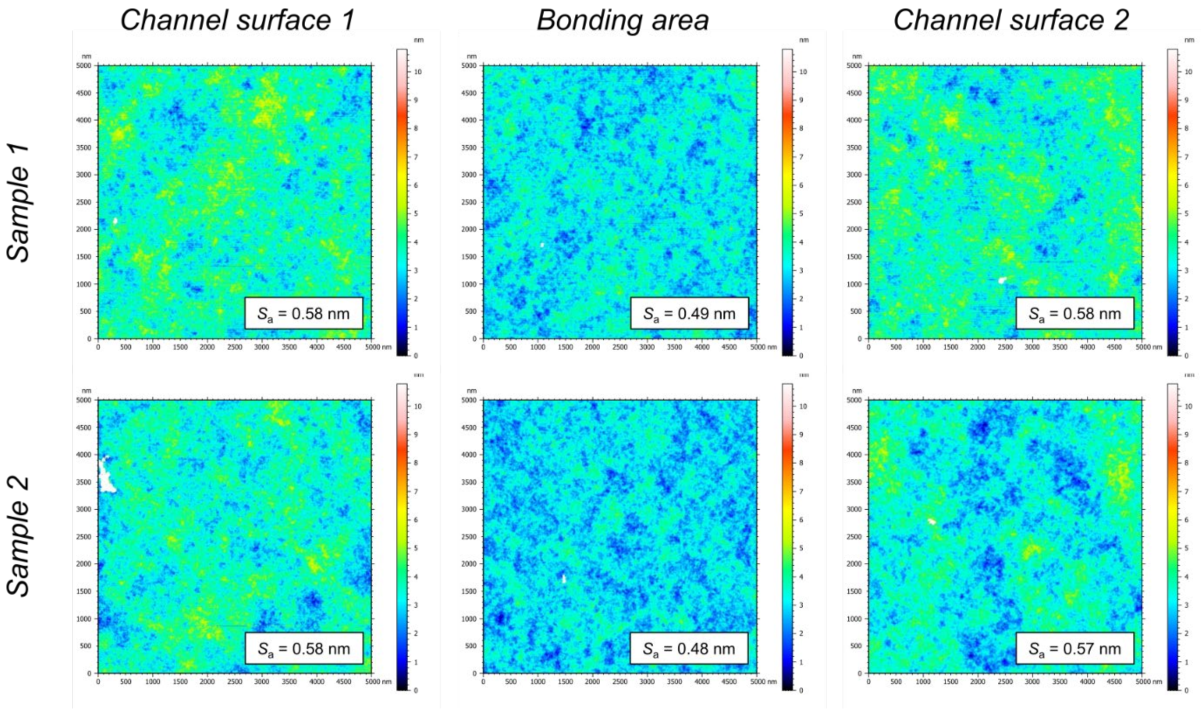


Figure 17. Topographic surfaces of selected measurements with Atomic Force Microscopy.

Lastly, guidelines for measuring key performance parameters of microfluidic connections have been developed, based on the key properties of microfluidic interfaces. These guidelines have been published as a Deliverable D5 - MFMET Deliverable 5 - Guidelines for the measurement of key performance parameters of microfluidic connections including the identification of key properties in an interface (zenodo.org), developed by IMTAG in cooperation with from LNE, INESC MN, UofG, microfluidic, CEA, IPQ, CETIAT and CMI contributing valuable standards for the field.

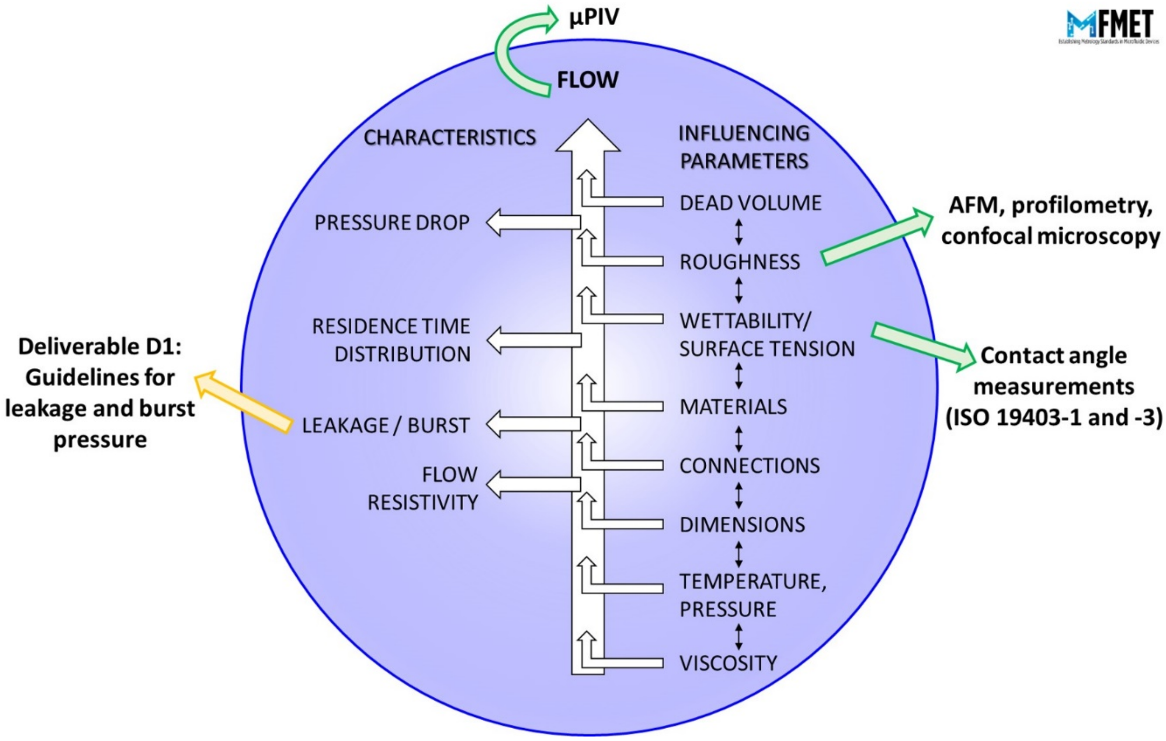


Figure 18. Overview of influencing parameters, characteristics and measurement methods concerning the flow in microfluidic devices.

Summary

In this work package, researchers focused on identifying and measuring key parameters related to microfluidic devices and their components. Specifically, they examined roughness, wettability, and dimensions. The impact of these parameters on the overall performance of the microfluidic system was thoroughly investigated and documented in various reports and two deliverables.

The outcomes of this work package mark a substantial step forward in both standardising and improving microfluidics fabrication and testing methodologies. By understanding and optimising these critical parameters, researchers can enhance the reliability and efficiency of microfluidic devices.

This objective was achieved.

4.4 *Objective 4: To define guidelines for the standardisation of dimensions and accuracy for modularity (either module-to-module or module-to-world) and sensor integration (combination of sensing elements/materials with microfluidic modules), in accordance with good practices in microfluidic component design and manufacturing.*

The aim of this work package was to establish guidelines for standardising the external dimensions and accuracy of modular microfluidic systems, focusing on module-to-module and module-to-world interfaces, as well as sensor integration with microfluidic modules. This was to ensure successful interaction between various components, including sensors made from different materials and manufactured using distinct methods.

A survey was conducted to gather feedback from 40+ stakeholders in the microfluidic industry on solutions for assembling modular microfluidic systems and components. The results were combined with input from various sources, including scientific literature reviews, expert interviews, end-users and advisory board feedback. Five experts were interviewed to gather insights on the current state of the art regarding modularity and heterogeneous integration. The project produced a report on the current state of the art of modularity and heterogeneous integration of microfluidic systems and components, which was shared with relevant standardisation groups such as ISO/TC 48/WG 3.

The consortium produced a comprehensive landscape document Deliverable D7: MFMET Deliverable 7 - Landscape document identifying standardization requirements for microfluidic component design and manufacturing with respect to modularity and heterogenous integration (zenodo.org). This document was developed by microfluidic in cooperation with INESC MN, CEA, EnablingMNT, IMTAG, UofG and LNE and it includes microfluidic component's external dimensions, orientation, and interfaces, as well as the integration of heterogeneous components such as sensors. This document was developed based on input from various sources, including internal expertise, end-user advisory board, and relevant standardization groups. The document focuses on key aspects for designing modular microfluidic components and integrating heterogeneous components, considering substrate materials such as polymers, glass, and Si, and related manufacturing method constraints, identifying existing gaps and issues in current developments to determine areas suited for standardization. The landscape document will be disseminated to various standardization organizations and was submitted to EURAMET as a deliverable (D7), providing a comprehensive overview of standardisation requirements for microfluidic component design and manufacturing with respect to modularity and heterogeneous sensor integration.

Table 12. Requirements hotspot for microfluidics interfaces

Requirement	Needs	Wants (might be covered in the roadmap)
Reliability	Leak tight.	
Pressure	Maximum 2 bar	Maximum 10 bar
Temperature	(4-50) °C.	(4-120) °C.
Flow rate	1 µL/min to 100 µL/min.	(1-1000) µL/min.
Medium	Water based fluids containing biomolecular matter	Also gasses
Reliability	Leak tight	
Materials	"Biocompatible" materials as wetted materials, materials used should be affordable and the supply chain sufficiently covered	Fluor free
Availability	Design is freely available but fixed in a standard	
Connectivity	Compatible with microfluidic chips	

A list of physical parameters relevant for microfluidic components, including dimensions, width, and length, as well as material-specific influences such as elasticity, flexibility, tensile stress, and deformation was prepared. Additionally, a list of measurement methods for these parameters, including 3DCMM, microscopy, cameras, and interferometry, was set up, while considering limitations of instrument capabilities. Measurement protocols for dimension characteristics and accuracy determination were developed. These protocols were used for building and measuring the transfer standards developed in WP2. The protocols were merged into Deliverable D8: MFMET Deliverable 8: Measurement protocols for dimensional characterisation of microfluidic components (zenodo.org), developed by RISE in cooperation with IMTAG, LNE and microfluidic, which will serve as guidelines for NMIs/DIs introducing new calibration services such as inner dimensions of channels (diameter/length/width) and which were distributed to the relevant standardisation committee.

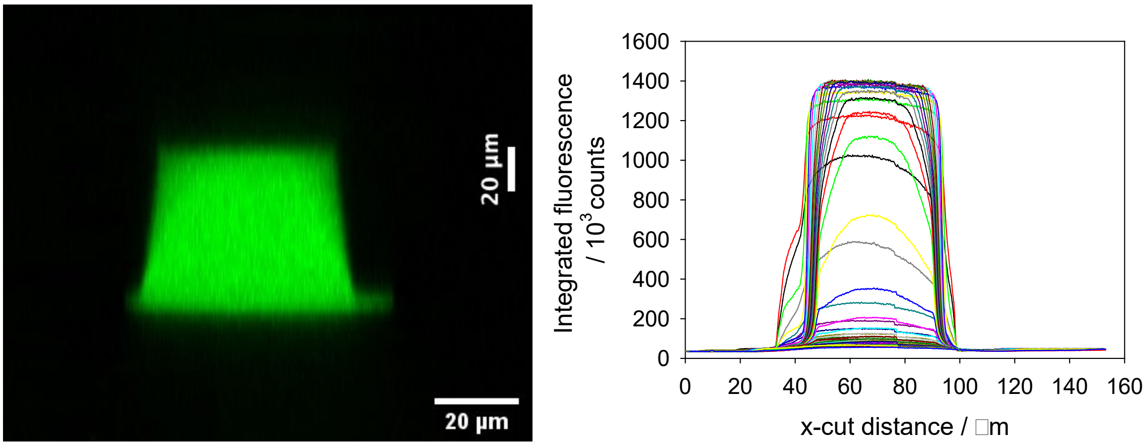


Figure 19. Integrated fluorescence intensity from z-stack of 50 confocal slices (20x). Left: cross-section of channel height. Right image: intensity profile of z-series images. Each optical section (i.e. z-step) was set for 2.07 µm.

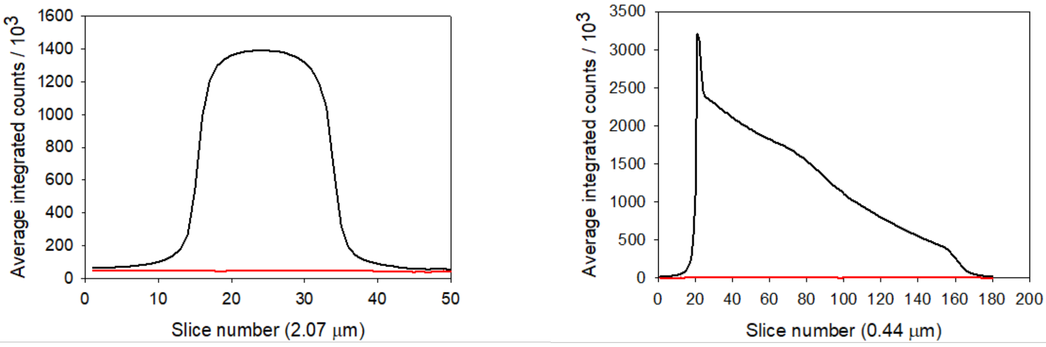


Figure 20. Average fluorescence intensity for successive z slices measured in the centre of the channel (black) and to the side of the channel (red).

Summary

In this work package standardisation requirements for microfluidic component design and manufacturing with respect to modularity and heterogenous integration were identified. Also, some consensus is still to be found among manufacturers in order to achieve standardisation in modularity; this was something observed with the project industrial partners.

Researchers tested various measurement methods for assessing dimensions in microfluidic chips made of glass and polymer. Unfortunately, the results from these different methods were inconsistent. As a result, further studies are necessary to determine the most accurate and reliable dimensional measurement approach.

This objective was achieved.

5 Impact

The project webpage (https://mfmet.eu) was regularly updated during the project lifetime with news and information such as project reports, and details of project meetings. Since the project's start, the website has been viewed over 53000 times from 68 countries, with 1438 views per month on average. In collaboration with the Microfluidic Association several surveys have been developed and four White Papers have been published namely on Leakage testing, on Optical Interfaces of Microfluidic Devices, on Flow resistivity and another on Hydrophobicity, hydrophilicity, and wettability. Six MFMET Newsletters are now available on the project's webpage. The project is also advertised at the EURAMET page. A news stories were published by EURAMET regarding the MFMET project: in July 2023: EMPIR project on microfluidic devices presents at major international conference, in September 2023: EMPIR project supports the development of metrological network for microfluidic devices and in May 2024: Resource management in microfluidic measurements. Three articles were published in regular press, 30 technical reports/protocols/guidelines have been produced by the consortium and are available on the webpage. So far, more than 2300 downloads of these documents have been done. The project was presented at EURAMET TC FLOW, EURAMET TC M and at BIPM WGFF. The partners have given 3 poster presentations to the scientific community i.e.., at CIM2021, at EUROoCS 2022 and MPS2023. 28 oral presentations have been given at metrology and microfluidic conferences, such as: Flomeko 2022, INO4VAC 2023, RIQUAL 2023 (Portugal), microTAS 2023, VI Congreso de Microfluidica Argentina 2022, Conference Polymer Replication Nanoscale 2022, Labsummit 2024 and many more. Five open access publication have been published in international journals and five are undergoing revision process.

Elsa Batista from IPQ was invited by the European Commission to present the works of MFMET project 'A success story from a pre-standardisation research project' in the Workshop 'The Future of Metrology' held in Brussels, Belgium, in November 2023.

Impact on industrial and other user communities

This project is crucial to bridge this gap by providing guidelines as future standards in the areas of design, materials and test. This is expected to enable more reliable products, which is critical in healthcare (e.g. point-of care solutions), supporting the manufacturer to reduce the number of references, cost and ultimately increase its sales. Overall, the outcomes of this project will potentiate testing and improvement or development of new microfluidic devices with increased accuracy and quality, and their joint dissemination with The Microfluidic Association (MFA) has further intensified the early adoption of the practices developed within this project. Three workshops were organised in cooperation with the MFA.

On May 5th, 2022, the work "Metrology supports microfluidic fabrication and testing" developed under MFMET, was presented at the MFA Webinar "The road to user friendly integration of microfluidic components and devices".

Elsa Batista was invited to be a board member of the MFA in January 2024.

Contacts have been made with experts from the American Food and Drug Administration (FDA) and the American Institute of Standards and Technology (NIST) who are much interested in the outcomes of this project. FDA has tested the transfer standard and the results will be published in a cooperation report. The partners are also working together with other experts from outside the MFMET consortium ensuring meeting the expectations of the community and faster adoption of the project outcomes, e.g., Elveflow is now a collaborator of the project and lent a pressure pump for performing tests under WP2 and for the characterisation of the golden samples.

Three workshops were prepared specially for industrial and user microfluidic communities. The workshop "**On the road to standardization in Microfluidics and Organ-on-Chip**" organised between the MFMET project and The Microfluidics Association was hosted by CETIAT, the National Metrology Institute for Flow in France, from the 13th to the 14th of November 2023. This workshop was attended by 45 participants from 10 countries and 3 continents (Europe, North America and Asia). Fourteen oral presentations were given by experts from organisations working on metrology, regulation, and microfluidics and Organ-on-Chip product development, as well as from the semiconductor industry. On Thursday, March 7th, and Friday, March 8th, 2024, the Microfluidic Association (MFA) in cooperation with the MFMET project consortium held the workshop "**Integration of sensors and electronics in microfluidics: Challenges and opportunities**", to discuss the challenges and opportunities around the integration of sensors and electronics in microfluidics. The workshop was hosted and supported by the Belgium Research Institute IMEC (Interuniversity Microelectronics Centre). The workshop, with over 100 attendees mostly from the industry sector, was held with the aim to provide input for a shared technology roadmap for the microfluidics industry. Microfluidic organised a one-day Workshop "**UNLOOC Standardization Workshop**" on standardisation in microfluidics in Jena, Germany on May 16th, 2024. The event aimed to address the importance of standardisation in microfluidics and was attended by MFMET, UNLOOC, and AGRARSENSE partners. There were more than 30 participants from industry, online and onsite. In total the workshops for industrial partners and end users done by this project had more than 200 participants. The feedback from these workshops allowed the consortium to conclude that the impact was very good.

A webinar on metrology standards for microfluidics was developed by the consortium where the crucial aspects of ensuring accurate measurements in this rapidly evolving field are addressed. This Webinar features several informative videos of 10 minutes demonstrating best practices and techniques:

- MFMET webinar – 01. The role of Metrology and Standardization in microfluidic technology development.
- MFMET webinar – 02. Flow in microfluidics.
- MFMET webinar – 03. Wettability and surface roughness.
- MFMET webinar – 04. Leakage in Microfluidic Devices – detection and quantification.
- MFMET webinar – 05. Interfacing of microfluidic devices.
- MFMET webinar – 06. Measuring the dimensions of microfluidic devices using optical methods.

Deriving from needs identified by the microfluidics supply chain the consortium produced a database with an inventory for flow control components. The database creates an interactive and efficient overview of information on flow control components.

Impact on the metrology and scientific communities

The importance of quantitative measurements with a suitable degree of precision constitutes a basic underpinning framework for the scientific research and technological development. This project will create an early impact as it will allow NMIs to upgrade and adapt their existing facilities for the calibration of microfluidic devices and instruments. By developing transfer standards dedicated to microfluidics applications, the project will allow NMIs to disseminate the traceability chain towards both the manufacturers and end users.

It is generally acknowledged that there is still a lack of understanding of the importance of precision and standards, more so if standards and calibration methods are not available. New calibration methods and microfluidic transfer standards will be developed in the scope of this project, and impact will be created as these methods will be disseminated to the scientific community in relevant publications and EURAMET guidelines.

The final workshop on "**Standardization of test methods in microfluidics**" organised by the MFMET project and The Microfluidics Association was hosted by IPQ, the National Metrology Institute for Flow in Portugal on May 22nd, 2024, in close cooperation with INESC MN. This workshop aimed to present the final scientific outcomes of the project MFMET and the research made in microfluidics related to standardisation. The workshop was attended by 60 participants from 10 countries. Fourteen oral presentations were given by experts from organisations working on metrology, universities, and microfluidics industry. In addition, 5 industrial partners were invited to exhibit their products. It was a very successful workshop with very high-level presentations. The discussion was also very fruitful focussing on how standardisation can help the development and research in microfluidic activities.

IPQ send a new CMC for publication during 2024 following the work developed in this project on volume measurement of microfluidic channels. CETIAT, DTI, LNE and RISE also developed new measurement protocols for flow, and flow resistivity in microfluidic devices and participated in the EURAMET pilot study 1613 – 'Pilot study for comparison of flow quantities on microfluidic transfer standards', therefore new CMCs submissions are expected by the end of the year.

A technical guide on "**Evaluation of flow-related quantities in microfluidic devices**", was sent on September 2023 to EURAMET and was approved by the BoD in May 2024. It will be published as EURAMET technical guide nº 4.

The transfer standard chips (golden samples), 8 made of glass and 1 made of polymer, were developed, manufactured and tested by the project partners regarding flow and dimensions quantities. These artifacts are held by CEA for a period of at least three years under cleanroom conditions and are available for loan free of charge. However, anyone who wishes to borrow the chips must pay for shipping/transport.

A report highlighting how and why microfluidic devices fail was produced including a checklist that can be used by the full microfluidic supply chain https://doi.org/10.5281/zenodo.11357125.

Impact on relevant standards

In this project, procedures and methods for the calibration of microfluidics devices and microfluidics-related instruments that are already on the market were developed. The consortium created impact by supplying this information to the relevant ISO technical committees (TC) and made efforts to ensure that these results were incorporated in any updates to standards. This project adapted existing measurement procedures and defined new measurement procedures for different types of devices and instruments used by the microfluidics industry.

The consortium has engaged in several standardisation activities. Within ISO/TC 48/WG 3 Microfluidic Devices contributions were given to ISO 22916 – Microfluidic devices – Interoperability requirements for dimensions, connections and initial device classification, this document was published during 2022. Comments were sent by the consortium on ISO 10991 Microfluidics – Vocabulary, the final version of the document was published in September 2023. A new ISO TS: ISO/TS 6417 Microfluidic pumps — Symbols and performance communication was finalised and awaits publication during 2024. In November 2023 a new convenor was elected for the ISO/TC 48/WG 3, Vania Silverio from INESC MN, one of the project partners. In addition, the secretariat of this WG was attributed to IPQ, a coordinating partner of this project. Likewise, Vania Silverio was elected in May 2024 as the new convenor of CEN/TC 332/WG 7 Microfluidic Devices with the support from IPQ in the Secretariat. A roadmap was developed by the new team that considered the work done under the MFMET project. Further, in ISO/TC 48/WG 5 Liquid Handling Devices - Automatic the development of ISO/DIS 23783- 1, 2 and 3 were followed by IPQ and HSG-IMIT; these documents were published in 2022. IPQ and HSG-IMIT were also involved in the development of ISO/TR 6037 - Automated liquid handling systems – Uncertainty of the measurement procedures; this was published in May 2024. Within ISO/TC 84/WG 10 and WG 11 IPQ participated in the initial revision of ISO 7886-1:2017 - Sterile hypodermic syringes for single use and ISO 7864:2016 - Sterile hypodermic needles for single use — Requirements and test methods. Several partners were engaged on the work of the new CEN/CENELEC Focus group on Organ-on-Chip that started in 2022, mainly in WG1 – Terminology and WG3 - Engineering. A roadmap was developed and will be published in July 2024.

The project coordinator also engaged in contacts with ISO/TC 276/WG 4 and was invited to present the results of the project in the meeting of 2024 that will take place after the project completion.

Longer-term economic, social and environmental impacts

This project will directly benefit society because it will accelerate innovation, by allowing academia, end users in industry (health, pharmaceutical) and microfluidics devices manufacturers to develop and/or use standardised products with clear, traceable and controlled specifications. The rapid production of low-cost high-volume point-of-care tests that can be distributed to patients for swift detection of viruses is a good example of the importance of microfluidics in tackling future healthcare crisis.

Improvements in the accuracy of instruments and devices will reduce manufacturing costs while improving quality and usability. This will be achieved through the wider uptake of traceable calibrations & test protocols and by improved knowledge of how to calibrate instruments involved in the whole manufacturing process of microfluidic devices, from the early stages of chips designs to end-user tests in the laboratory.

6 List of publications

Batista, E. et al (2024) 'The Importance of Dimensional Traceability in Microfluidic Systems', *Metrology*, 4(2) p. 240-253. Available at https://doi.org/10.3390/metrology4020015

Gil, J.F. et al (2023) 'Cancer Models on Chip: Paving the Way to Large-Scale Trial Applications', *Advanced Materials*. Available at https://doi.org/10.1002/adma.202300692

Lima, João et al (2024) 'Determining Liquid Properties for Application in Microfluidic Devices', *TQM Journal*, 15 p. 147. Available at https://publicacoes.riqual.org/ed15-147-165/

van Heeren, H. et al (2022) 'Metrology challenges for microfluidics', *CMM Magazine*, 15 p. 20-25. Available at http://www.cmmmagazine.com/cmm-articles/metrology-challenges-for-microfluidics/

van Heeren, H. et al (2022) 'Overcoming technological barriers in microfluidics: Leakage testing', *Frontiers in Bioengineering and Biotechnology*, 10 p. 10.3389/fbioe.2022.958582. Available at https://doi.org/10.3389/fbioe.2022.958582

This list is also available here: https://www.euramet.org/repository/research-publications-repository-link/