MFMET project - Establishing metrology standards in microfluidic devices

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Abstract

This paper presents the objectives and initial outcomes of EMPIR Project 20NRM02 MFMET - Establishing metrology standards in microfluidic devices, funded under the EURAMET EMPIR program of the European Commission, that intends to tackle the lack of metrological specifications for microfluidics. It started in June 2021 with the involvement of 15 partners and will have a duration of three years. The main goal of this project is to contribute to the development of globally accepted standards for microfluidics, with a focus in metrology for the methodologies and fabrication processes that are essential to ensure measurement accuracy and traceability of microfluidic devices and their dissemination to end users in industry (health, pharmaceutical) and academia. This project is expected to create impact, as new calibration guidelines for microfluidics and microfluidic devices will be developed, that are of direct relevance particularly to the industrial partners in the project but also to other user communities. An experimental setup and results from the characterization of the flow rate in a microfluidic device are also given in this work.

1. Introduction

Microfluidics, which deals with the handling of fluids in the nano-to-millilitre scale, has major applications in biomedical and chemical analysis, however global standards are still lacking. Stakeholders from industry, academia and government have recognised these needs and as a result ISO/TC48/WG3 has been set up to develop microfluidic standards covering metrology for the methodologies and fabrication processes that are essential to ensure measurement accuracy and traceability of devices. Standardisation of performance characteristics is needed for the different classes of components, including test conditions, measurement protocols and guidelines. 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 growth of microfluidics in diverse sectors (e.g. pharmaceutical and biomedical industries). However, due to this rapid growth, microfluidics and specifically the control of fluids in microfluidic devices still lack universal solutions and standards. The project MFMET - Establishing metrology standards in microfluidic devices aims 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 [1].

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 USD 44.0 billion by 2025 from an estimated value of USD 15.7 billion in 2020. However, microfluidics and specifically the control of fluids in microfluidic devices still lack 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/TC48/WG3 was established to address this underpinning requirement [2]. 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. Figure 1 shows an overview of some microfluidic devices and applications.
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 [3]. Recently EMPIR JRP 18HLT08 MeDDII tackled microflow measurements down to 5 nL/min and introduced new facilities which are now under implementation [4]. These new technologies can now be used to develop microfluidic measurement protocols, and the new microflow pump developed in MeDDII can be used as a traceable flow generator [5].

Figure 1. Microfluidic devices and applications.

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 22916:2022 - Microfluidic devices — Interoperability requirements for dimensions, connections and initial device classification was published, and the metrological specifications required for accurate and reproducible manufacturing was supplied by the project partners. A new standard ISO/DIS 10991 - Microfluidics — Vocabulary is almost completed and will also be published in 2022 with several contributions from MFMET partners.

3. Objectives

The overall objective of this project is to contribute to the development of globally accepted standards for microfluidic devices used particularly in the health and pharmaceutical industry.
The specific objectives are:
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 (WP1).
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 (WP2).
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 (WP3).
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 (WP4).
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 (WP5).

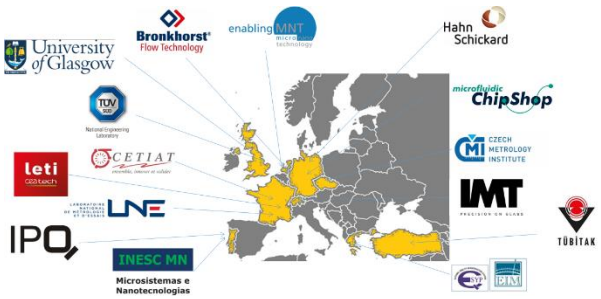

Figure 2. Map of the MFMET project consortium

The MFMET project consortium consist of seven National Metrology Institutes and Designated Institutes, four research institutions and universities, and four companies, coming from ten countries around Europe (Figure 2).

4. Progress beyond the state of the art and results

4.1 Consensus-based flow control specifications for microfluidics

By developing a consensus-based harmonisation of the metrological criteria for the design, qualification, and use of flow control devices such as pumps and valves, this project will provide guidelines and standardised protocols and methodologies beyond the state of the art.

It started with a literature review and market search to compile and classify flow control components, followed by definitions, symbols and vocabulary of flow control. Both documents are already available on the project website [1] and Zenodo platform [6] and delivered to ISO/TC 48/WG 3. A database inventory for flow control components containing base operating principles, family of components and types and subtypes of components is being developed,and will be available on the project website [1] to be explored and filled by any microfluidics user. The definition of a specifications list for comparison of flow control components is being developed, to make easier the choice between various components in the market.

The need for guidelines and test protocols to qualify leakage and burst pressure in microfluidic devices has led the partners to develop, together with The Microfluidics Association and other Microfluidics players, a White Paper for the Microfluidics Industry [7]. A test protocol for leakage and burst pressure is being developed following the work prepared for the White Paper.

The documents elaborated in this WP are aiming to be applicable throughout the entire microfluidics industry supply chain, from the manufacturer to the end user with the guarantee of traceability to the SI.

4.2 Measurement protocols for different flow quantities and liquid properties

A literature review of existing metrology and normative standards related to the flow properties and microfluidic devices, along with a general metrology methodology describing recommendations and good practices on writing a measurement or test protocol, have been published and are available on the project website [1] and Zenodo platform [6]. A EURAMET guide based on the measurement protocol for different flow-related quantities will be developed. Test protocols for flow and liquid properties including documented examples will be produced, as well as a technical report for the manufacturing of transfer standards for microfluidic components, representative of the diversity of the applications, to be used to calibrate testing equipment of end users and industrials. Two microfluidic transfer standards will be manufactured in order to test the protocols developed through WP1 to WP4. These transfer standards, also called golden samples, will be characterized in NMIs and available for the microfluidics industry to compare and qualify their measurement capabilities, in particular regarding the following quantities:

- Flow rate vs. inlet pressure
- Flow resistivity
- Internal volume
- Internal dimensions of channels

4.3 General standards and guidelines for interfaces and connectivity

This project will develop harmonised metrological specifications (such as a measurement protocols, guidelines) for the dimensions, positions, physical and material compatibility of the connections in microfluidics components and operational functionality, (such as dimensioning tolerances, leakage and burst pressure) from micro to macro size scales, focusing on fluidic passages and electrical/optical connections of components.

4.4 Guidelines for the standardisation of dimensions and accuracy for modularity and sensor integration

This project will develop a landscape document on component design and manufacturing for interoperability and heterogeneous integration, and measurement protocols for dimensional characterisation, ensuring integrity, functionality and metrological compliance of related devices.

5. Impact

5.1 Impact on industrial and other user communities

During the last decade, microfluidics has shown a phenomenal growth. So far, quality production of microfluidic devices has been established mainly based on the manufacturer's expertise, without reliance on well-established calibration procedures or standards that could have streamlined and accelerated production. Despite the expected impact of microfluidics (societal, health, well-being, environment), success stories are rare in comparison with the number of laboratory developments. The main reason is the gap between laboratory microfluidic devices (home-made chips and connections, customised test protocols, materials not compatible with high volume production, etc.) and a reliable and reproducible product. This project is crucial to bridge this gap by providing guidelines as future standards in the areas of design, materials and test. This will enable more reliable products, which is critical in healthcare

(e.g. point-of care solutions), enabling the manufacturer to reduce the number of references, cost and ultimately increase its sales. The project will support manufacturers to establish robust quality control and provide product datasheets with standardised terminology for comparison with other products. Laboratories will have more confidence to use commercial products and to make comparisons of components as to the suitability for applications and connection to in-house fabricated devices, reducing costs and downtime. Complete integrated microfluidics systems will be tested with the standardised test protocols as used by manufacturers, increasing the success rate of a technological transfer.

This project will create impact by developing new calibration guidelines for microfluidics and microfluidic devices that are of direct relevance particularly to the industrial partners in the project but also to other user communities. These new calibration normative standards will include flow, volume, dimensional, optical and material related testing, which will be of benefit for the characterisation of microfluidics devices, for the accuracy of the physical and chemical functionality of the device and all metrological operations involved in the lifetime of microfluidic devices, from manufacturing to its application by the end user. These characteristics will be traceable and will enable the comparison of characteristics of different products (using datasheets) using harmonised specification and guidelines developed within the project. Two microfluidic transfer standards will be manufactured to test the protocols developed through WP1 to WP4. These transfer standards can later be used by manufacturers and accredited laboratories in order to validate their instrumental facilities and protocols.

Consumer protection and economic development are directly linked to the credibility of goods and processes. In particular, the global growth of microfluidics requires the provision of agreed specification guidelines for independent quality control. Interchangeable microfluidic parts fabricated in mass production must be gaged accurately to fit together, enabling plug-and-play functionality, and to function as a fully integrated product. Harmonised and standardised guidelines and design rules for connectivity (electrical, fluidic, and optical) of microfluidic devices, such as spacing and sizing of connectors and portholes will benefit the end users by providing compatibility independent of manufacturer and simplification of microfluidics use and by guaranteeing standard quality rules such as leakage rate and maximum pressure resistance. This project will also help the industry by providing simple sensor integration and modularity design rules specified as metrological tolerances and testing guidelines. New methodologies developed within the project will present test protocols ensuring traceability to national standards and relevant accuracy.

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) will further intensify the early adoption of the practices developed within this project.

5.2 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. The current issue to tackle is to establish and maintain the measurement infrastructure needed in the production and use of microfluidic devices and to improve the precision and accuracy of measurements. 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.

The collaboration between academia, industry, NMIs and microfluidics users in this project will accelerate the development of robust new EURAMET guidelines, which will help NMIs to extend their calibration and measurement capabilities towards microfluidic devices and microfluidics-related instruments.

5.3 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 will be developed. The consortium will create impact by supplying this information to the relevant ISO technical committees (TC) and will make efforts to ensure that these results are incorporated in any updates to standards (e.g., ISO 22916, ISO/DIS 10991) or guidelines. For example, the current version of ISO 10991:2009, which is used by manufacturers as it provides terms and definitions for micron sized process engineering applied in chemistry, pharmacy, biotechnology and food technology, lacks metrological guidelines and is now under revision. Measurement methods well established in the macro scale are often not suitable for the specific accuracy and ranges (for example flow rate ranges, size ranges of channels, etc) encountered in microfluidics applications. Thus, this project will adapt existing measurement procedures and define new measurement procedures for different types of devices and instruments used by the microfluidics industry.

It is expected that the outcomes of this project will directly impact the work being developed in:

- ISO/TC48, specifically ISO TC48/WG3 (Microfluidic Devices) which already has a liaison with EURAMET TC-FLOW,
- ISO/TC69 (Applications of Statistical Methods),
- ISO/TC229 (Nanotechnologies),
- ISO/TC276 (Biotechnology),
- CEN/TC332/WG7 (Micro Process Engineering), this is the European mirror committee of ISO/TC48/WG3

The following figure presents a schematic the MFMET impact and collaboration with relevant standardization working groups.

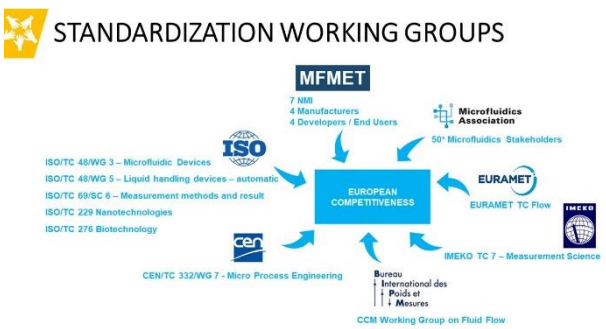

Figure 3. Schematic of MFMET impact and collaboration with relevant standardization working groups.

5.4 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. As a side effect, the COVID-19 pandemic accelerated the development of novel testing kits using microfluidics with integrated sensing components. 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. Results

Tests were performed by IPQ to assess the capability to test microfluidic devices using two different methods, gravimetric and front track. The chip chosen for test is a microfluidic Lab-On-a-Chip device for passive mixing and magnetic separation of bioanalytes with square channel cross-section and obstacles that promotes mixture of components for sample preparation (Figure 4).

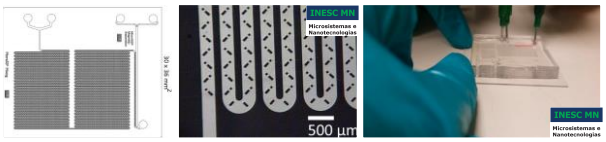

Figure 4. Microfluidic Lab-On-a-Chip device for passive mixing and magnetic separation of bioanalytes.

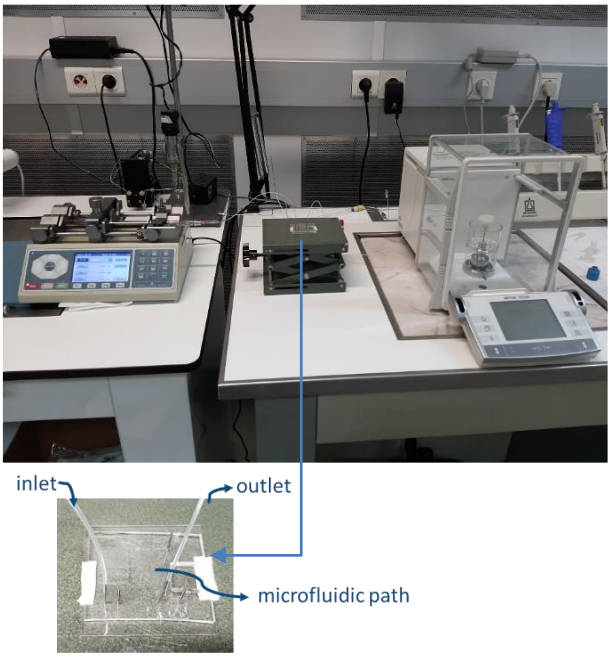

Figure 5. Gravimetric setup for microchip characterisation

The gravimetric setup (Figure 5) was composed of a Nexus 3000 syringe pump as a flow generator, a 1 mL removable syringe of glass (ILS, Luer lock, 4,61 mm internal diameter), a Polyethylene tube of 59 cm long and a AX26 Mettler balance. The set flow rate tested was 600 μL/h because this is the flow rate at which this chip is used. Water was used as a calibration liquid. The total acquisition time was 15 minutes, with one data point obtained every 30 s. Tests were performed with and without the chip. The results are presented in Table 1.

Table 1: Results of the microchips characterization using the gravimetric method.

Test	Reference flow (μL/h)	Uncertainty (μL/h)	Error (%)	Uncertainty (%)
1	595	13	0.84	2.1
2	595.5	5.9	0.76	1.0
3	596.1	7.1	0.65	1.2
Average	595.4	8.6	0.77	1.4
Without Chip	595.9	5.2	0.69	0.9

The chip was also characterized with the front track method using a 1 mL removable glass syringe. The setup is presented in Figure 6. The data acquisition was made every 1 s for a total of 70 s. Tests were performed with and without the chip.

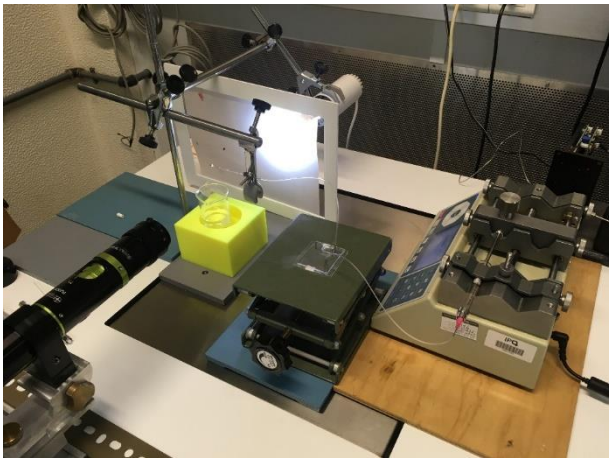

Figure 6. Front track setup. The experimental setup consists of using a high-resolution camera and an image processing software to track the distance travelled by the meniscus of a liquid in a capillary tube and calculate the flow rate. The displacement of the meniscus is calculated between frames.

The results are presented in Table 2.

Table 2: Results of the microchips characterization using the front track method.

Test	Reference flow (μL/h)	Uncertainty (μL/h)	Error (%)	Uncertainty (%)
1	594	16	1.0	2.7
2	601	16	-0.1	2.6
3	589	10	1.9	1.8
Average	595	14	0.8	2.3
Without Chip	611	12	-1.8	2.0

A comparison of results obtained for each method can be found in Figure 7.

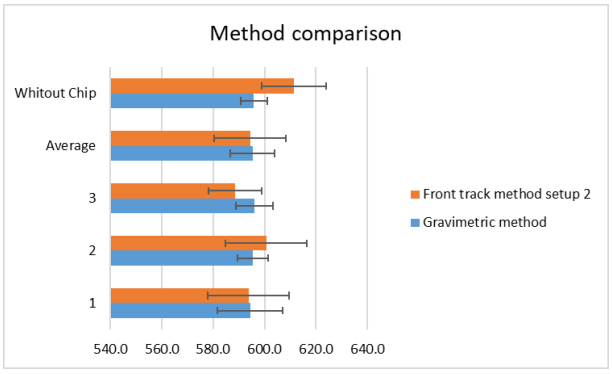

Figure 7. Comparison methods for microchips characterization.

The results obtained were consistent for the two methods used. The uncertainties values were very similar for the two methods, being higher for the front track method, probably due the small acquisition time arising from the limitations of the capillary used.

The uncertainty obtained without the chip was smaller in both cases due to better flow stability. The obstacles inside the channels cause flow disturbances, pressure losses and internal constraints, which will impact the overall flow uncertainty.

7. Conclusions

This paper presents the objectives and initial outcomes of EMPIR Project 20NRM02 MFMET - Establishing metrology standards in microfluidic devices, funded under the EURAMET EMPIR program of the European Commission for each work package, including the impact. This project intends to tackle the lack of metrological specifications for microfluidics, which are now being addressed in technical WP1 to WP4. A practical example of the calibration of flow in a microchip illustrates measurement protocols defined within the project.

Acknowledgements

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