Evaluation of flow-related quantities in microfluidic devices

EURAMET Technical Guide No. 4
Version 1.0 (05/2024)

Flow

Authorship and Imprint

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The guide was developed within the EMPIR Project 'Establishing metrology standards in microfluidic devices (20NRM02 MFMET) and in cooperation with members of the EURAMET Technical Committee for Flow (TC-F).

Version 1.0 (05/2024)

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ISBN 978-3-942992-84-8

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Guidance for Users

This technical guide was developed to provide information on the evaluation of flow quantities in microfluidic devices. In this guide, the measurement methods for the most important flow quantities in the microflow range are explained in detail. It is fundamental to develop guidelines as future microfluidic standards on measurement quantities traceability and accuracy that will contribute to a proper physical and chemical functionality of the device.

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Version 1.0 (05/2024)

Evaluation of flow-related quantities in microfluidic devices

Purpose

This document will act as a technical guide on the evaluation of flow quantities in microfluidic devices. In this guide, the measurement methods for the most important flow quantities in the microflow range are explained in detail. Finally, three selected examples will show how this guide can be implemented and how such measurements are carried out for microfluidic devices.

The current version reflects the actual practice applied in European National Metrology Institutes in terms of calibration procedures.

Content

1 INTRODUCTION

This technical guide was developed to act as a guide on the evaluation of flow quantities in microfluidic devices. In this guide, the measurement methods for the most important flow quantities in the microflow range are explained in detail. Finally, three selected examples presented in the guide shows how this guide can be implemented and how such measurements are carried out for microfluidic devices.
Microfluidics, concerned with fluid-handling in the millilitre to nanoliter scale, has major applications in biomedical and chemical analysis. In the first years after the millennium, 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. In order to understand the industry needs related to measurement quantities, an inventory was compiled based on interviews with microfluidics industrial experts and the results of the interview are available in Annex 1.
It is fundamental to develop guidelines as future standards in the areas of design, materials and test that are of direct relevance particularly to the industrial partners and also to other user communities. 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. Also, there will be a 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 specifications guidelines.

2 TERMINOLOGY AND SYMBOLS

Symbols, whose signification are not self-evident, will be described at their first appearance in the text.

The terminology used in this document is mainly based on existing documents e.g. VIM [1] and ISO 10991 [2].

3 MEASUREMENT OF FLOW QUANTITIES

3.1 Flow rate

Flow describes the unit quantity delivered over a unit time. Fluid flow related to the gravimetric method consists of mass flow rate $\left[\frac{kg}{s}\right]$ and volume flow rate $\left[\frac{m^3}{s}\right]$.
There are various methods for determining the flow rate, e.g. gravimetric method, front tracking, displacement methods (piston prover as flow generator, interferometry), µPIV and others. In addition, there are also secondary methods (e.g. flow meters) which are not included in this report. In the following, the gravimetric method will be presented in more detail, which is suitable for static (standing start stop method) and dynamic (flying start stop method) measurement of the flow rate.

3.1.1 Gravimetric method

The gravimetric method is used to determine a delivered mass of a liquid over a time interval. The method can be used for measurements of both inline flow sensors and flow generators. To achieve the measurements a weighing vessel, placed on a balance, collects the liquid to determine the mass delivered. While the delivery time interval for the collection of the mass is determined as well.
The mass flow rate relates the delivered mass to the delivery time and the volume flow rate relates the delivered mass in the mass flow rate to the delivered volume presented in Eq. (1) and Eq. (2) respectively:

$$q_m = \frac{m}{t} = \frac{I_L - I_E}{t}\left(\frac{1-\frac{\rho_0}{\rho_B}}{1-\frac{\rho_A}{\rho_L}}\right) \quad (1)$$

$$q_V = \frac{Q_m}{\rho_L t} = \frac{I_L - I_E}{\rho_L t}\left(\frac{1-\frac{\rho_0}{\rho_B}}{1-\frac{\rho_A}{\rho_L}}\right) \quad (2)$$

According to IEC 60601-2-24 [3], AAMI TIR 101 [4] and Bissig et al. [5] corrections for needle buoyancy, evaporation, and surface tension should be included, given by Eq. (3):

$$q_V = \frac{1}{\tau_f - \tau_i}\left[\left((I_L - I_E) \times \left(1 - \left(\frac{D_{tube}}{d_{tank}}\right)^2\right)\right) \times \frac{1}{\rho_L - \rho_A} \times \left(1 - \frac{\rho_0}{\rho_B}\right) \times [1 - \gamma(t - 20)]\right] + \delta q_{evp}, \quad (3)$$

where:

q_m Mass flow rate,
q_v Volume flow rate,
m Mass,
I_L Balance indication of the weighing vessel with final amount of liquid,
I_E Balance indication of the weighing vessel with initial amount of liquid,
τ Time,
ρ_L Density of liquid,
ρ_A Density of air,
ρ_0 Density at reference conditions for weighing 1.2 kg/m^3,
ρ_B Density of the mass pieces (8000 kg/m^3),
τ_I Start time,
τ_f End time,
D_{tube} External diameter of the tube,
d_{tank} Internal diameter of the weighing vessel,
t Temperature,
γ Coefficient of cubic thermal expansion of the material (tubes or syringe),
δq_{evap} Evaporation rate.

In a gravimetric microfluidic setup, an electronic balance is used in most cases to determine the delivered mass of a fluid from/through a test object (can be a flow generator, a microfluidic device, or a flow meter). The time interval in which the mass is delivered is determined by a timing module to derive the mass flow rate. The ambient conditions such as air temperature, relative humidity and pressure are determined to correct for buoyancy effects on the balance in AAMI TIR 101 [4].

The liquid temperature is measured to convert the mass flow rate into a volumetric flow rate, considering the density of the liquid used. Often a layer of oil is applied on top of the water surface in the weighing vessel (beaker) to reduce evaporation, especially at low flow rates.

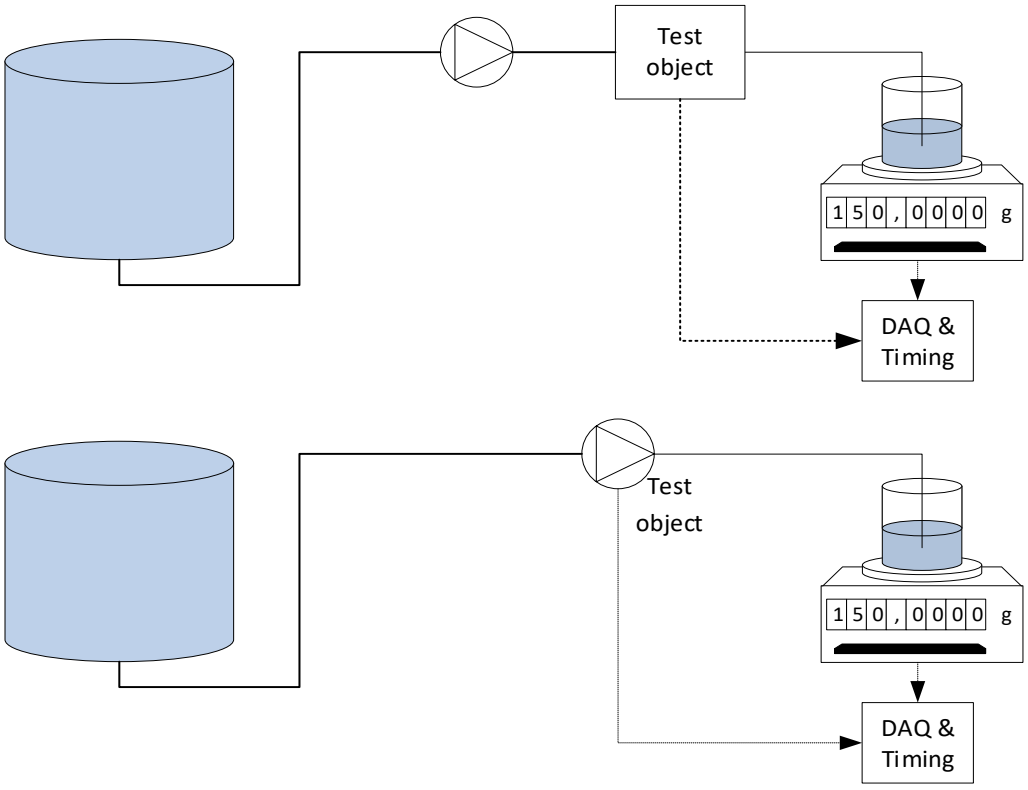


Figure 1. Example of a measurement setup for the gravimetric method

All instruments used should be calibrated by an accredited laboratory [6] or at a National Metrology Institute (NMI)/Designated Institute (DI).

Balance

The balance must have the capacity to measure the desired mass to deliver, depended on flow rate, required stability time, calibration time, and repetitions. The resolution of the balance must be suitable to the desired flow rate to be measured.

Timing module

The timing module delivers a known accuracy of time synchronization related to the measured mass increase. Narrow calibration window or smaller time intervals places higher demands on the timing equipment.

Weighing vessel

Select a weighing vessel suitable to manage the amount of liquid dispensed during an entire calibration sequence, considering pre-fill, flow rate, stabilization time, etc.

Thermometer and ambient conditions

The thermometer is utilized for the liquid temperature measurements to determine the density of the liquid. Where the ambient conditions are applied to determine stability of the ambient conditions and air density.

Calibration liquid

According to ISO 3696 [7], the test solution should be either 0,9 % normal saline water or distilled water of minimum grade 3. If clinically relevant liquids or other liquids relevant to microfluidic applications are used, the cause and impact must be carefully considered.

When performing the gravimetric method, there are several important issues that should be considered that are described below.

Weighing vessel dimensions and needle

The weighing vessel placed on the balance, to collect the liquid, must have well known and uniform dimensions since these have impact on the uncertainty contribution of needle buoyancy effect. The weighing vessel and needle must be clean to avoid undesired changes in surface tension and needle roughness.

Stabilization time

The system requires time to settle and then stabilize after introducing a flow rate. To reduce the measurement fluctuations, it is necessary to exceed well past the settling time for the system, typically, 95 % of the set flow rate must be reached before starting the measurements [3].

Liquid and ambient condition stability

The stability of both the liquid and the ambient conditions has large impact on microfluidic measurements. Therefore, they must be reduced as much as possible. It is recommended to have 1 ºC maximum variation.

Evaporation

The amount of evaporation from the surface of the liquid can affect the measurements, making the flow appear lower than it really is. Therefore, it must be considered by either correcting for or reducing the evaporation, using for example an evaporation trap or an oil layer on top of the weighted liquid.

Degasification

If the calibration liquid does contain dissolved gas, it may cause measurement issues. Typically, the calibration liquid is degassed prior to calibration or inline during the calibration. Degasification can be performed using a commercial degasser, which is composed of a vacuum pump and a permeation membrane.

A. Test protocols

Preparation

Place all equipment in a controlled ambient temperature room, where the test is to be carried out, at least 24 hours before starting the measurement for thermal stabilization purposes.

Flushing the system

Dead volume in the system may cause entrapment of air and could compromise the integrity of the measurements. Therefore, the system must be flushed prior to calibration start up. Circulating the system with degassed liquid will in time absorb entrapped air. At low flow rates CO_2 and isopropanol alcohol (IPA) are recognized methods to flush air from fluid systems. When flushed, run the system continuously with the calibration liquid until no flushing media is left.

Calibration start-up

Carefully prepare a clean weighing vessel with enough liquid to insert the needle. During preparation no liquid must be present on the walls. Place the vessel on the balance.

Insert the needle into a liquid layer in the container, if applicable. The needle must be well below the water surface inside the vessel. However, the flow that exits the needle must not be located too close to the vessel's floor, since the inertia of the liquid is in risk of exerting an undesired force on the balance. There are other measurements methods where the needle is not inserted [5].

Measure the temperature at the liquid source reservoir and set the flow rate.

During the calibration

When the stabilisation time is reached (typically when the flow rate remains within ± 5 % of its target value [4]), acquire mass, time, and ambient condition data continuously. Check the ambient conditions for fluctuations.

End of calibration

When the calibration time is reached stop the acquisition and determine the temperature of the liquid in the weighing vessel.

The number of repetitions should be at least 3 per flow rate.

B. Uncertainty budget

An example of uncertainty components that can be considered in a gravimetric setup are: mass measurements $(I_L - I_E)$, density of the mass pieces (ρ_B), density of the liquid (ρ_L), density of the air (ρ_A), evaporation rate (δQ_{evap}), liquid temperature (t), time (τ), expansion coefficient (γ), standard deviation of the measurements (δQ_{rep}) and buoyancy of the immersed dispensing needle (δQ_{mbuoy})[8].

Table 1. Example of gravimetric uncertainty budget for a set flow rate of 1 ml/h

Uncertainty components	**Estimation**	$u(x_i)$	c_i	$(c_i x_i)^2$
Final mass indication (g)	3,51	7,18E-06	3,53E-03	6,43E-16
Density of the liquid (g/ml)	0,997747	6,16E-04	-2,76E-04	2,89E-14
Density of the air (g/ml)	0,001188	2,89E-06	2,42E-04	4,87E-19
Density of the mass pieces (g/ml)	8,00	2,50E-03	5,11E-09	1,63E-22
Liquid temperature (°C)	221	5,72E-01	-2,75E-09	2,47E-18
Expansion coefficient (/°C)	1,00E-05	2,89E-07	-5,74E-04	2,74E-20
Initial mass indication(g)	3,44E+00	7,18E-06	-3,53E-03	6,432E-16
Evaporation (g/ml)	3,74E-04	5,29E-05	3,60E+03	7,75E-13
Final time (s)	1,51E+02	7,00E-04	9,68E-07	4,59E-19
Initial time (s)	4,35E+02	7,00E-04	-9,68E-07	4,59E-19
Buoyancy (g)	0,0003	7,60-06	3,53E-03	7,20E-16
Repeatability (ml/h)	3,05E-03	6,35E-04	3,60E+03	1,12E-10
Flow rate (ml/h)	0,9907			
u_{comb} (ml/h)	8,98E-04			
v_{eff}	63			
k	2,04			
U_{exp} (ml/h)	0,0018			
U_{exp} (%)	**0,19**			

3.1.2 Front tracking method

The front tracking method for flow measurements [9], [10] is an optical method that consists of tracking the position of the meniscus of a liquid (liquid/air or liquid/liquid interface) inside a (typically) capillary tube over time. An optical image acquisition system and image processing software is used to achieve the position over time of the meniscus. Knowing the displacement of the meniscus over time and the cross-section area of the capillary, it is possible to calculate the flow rate. Alternatively, the front tracking method can be applied in a microfluidic channel if the inner dimensions of the channel are known along with their associated uncertainties.

The fluid flow rate related to the front tracking method relates the optically acquired velocity of the fluid $\left(\frac{x_2-x_1}{\Delta\tau}\right)$ to the dimensions of the capillary tube (πr^2). If the flow is measured inside a channel, replace (πr^2) by the channel's cross-section.

$$q_v = \frac{x_2-x_1}{\Delta t}\,\pi r^2, \tag{4}$$

where:

x_1 Initial position of the meniscus,
x_2 Final position of the meniscus,
$\Delta\tau$ Time interval between the positions,
r Capillary section radius.

The capillary tube's material expansion property as a function of temperature $[1-\gamma\,(t-20)]$ is included to take deviation of the dimensions into account.

$$q_v = \frac{x_2-x_1}{\Delta t}\,\pi r^2[1-\gamma\,(t-20)], \tag{5}$$

where:

q_v Volume flow rate,
γ Capillary material coefficient of cubic thermal expansion,
t Calibration liquid temperature.

Similarly, the liquid's thermal expansion during the measurement should be evaluated using Eq. (5).

The test method includes calibration of the camera (determining the pixel size), image processing, and determination of position of the meniscus.

- The calibration of the camera determines the relationship between dimensions of a known and calibrated object (for example the outer diameter of the capillary) and pixels of an image. Usually, the output of the camera's calibration is the pixel size in microns (µm).

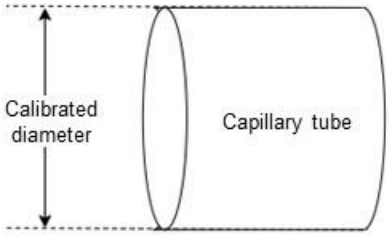


Figure 2. Camera calibration using capillary's outer diameter

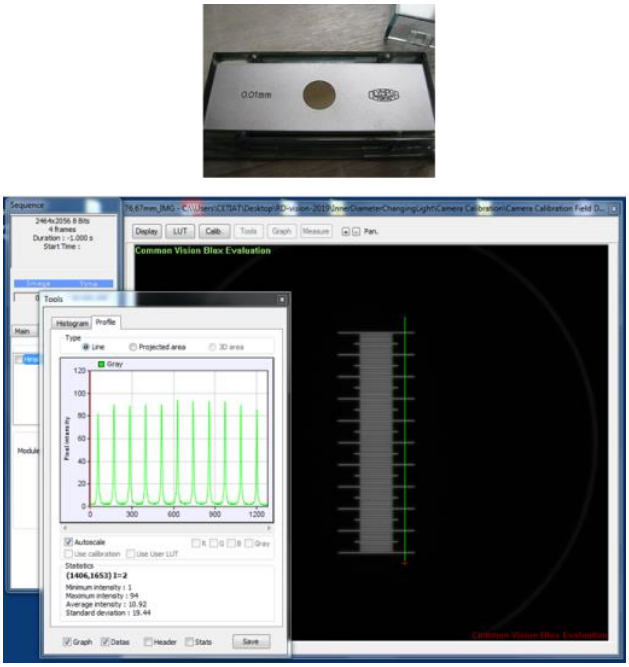


Figure 3. Camera calibration using a standard (micrometre object from Olympus)

- Image processing facilitates the identification of contours which makes the meniscus available for accurate positioning. Image processing includes image segmentation, digital image correlation, correction for misalignment, etc.

- The determination of the position of the meniscus is identified by software tools able to find coordinates of the meniscus, either at reference point(s) along its contour or its average position along the flow direction. Meniscus positions are determined for each successive image. The meniscus velocity is calculated as the time derivative (global or on a defined sliding time window) of its successive timestamped positions.

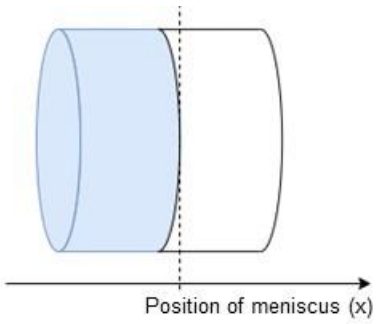
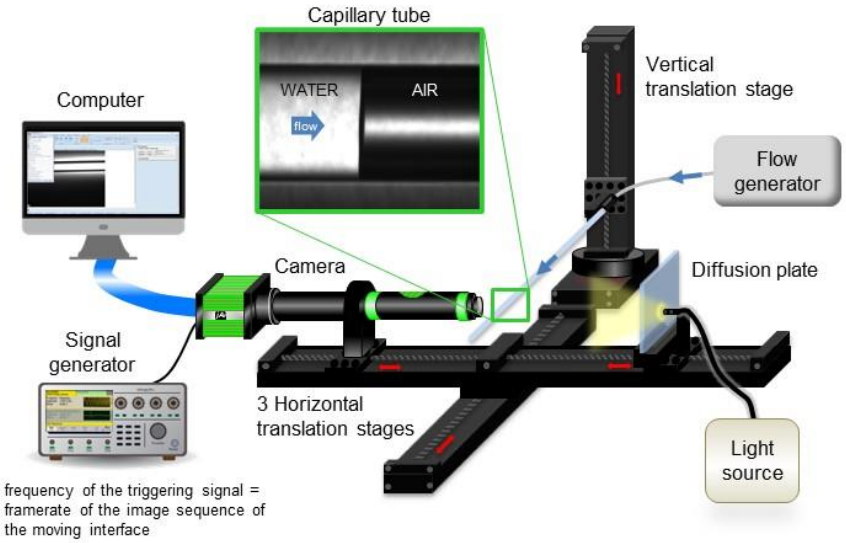


Figure 4. Determination of the position of the meniscus

All the instruments used should be calibrated by an accredited laboratory or an NMI/DI.

A typical setup may include:

- Capillary tube (or any other tubing or channel of known dimensions with associated uncertainties)
- High resolution camera
- Image processing software



$$Q_V = v.\pi R^2 = \frac{\Delta x}{\Delta t}\pi R^2$$

Q_V	flow rate
v	flow velocity
R	capillary's inner diameter
Δx	interface displacement
Δt	time interval between 2 images

Figure 5. Example of a front tracking setup for micro and nano flow measurements and calibrations [8]

A. Test protocols

Preparation

Place all equipment in the room where the test is to be carried out at least 24 hours before starting the measurement.

Flushing the system

Dead volume in the system may cause entrapment of air and could compromise the integrity of the measurements. Therefore, the system must be flushed prior to calibration start up. Circulating the system with degassed liquid will in time absorb entrapped air. At low flow rates CO_2 and isopropanol alcohol (IPA) are recognized methods to flush air from fluid systems. When flushed, run the system continuously with the calibration liquid until no flushing media is left. In the case of front track all the system in flushed except for the capillary.

Calibration start-up

Position the calibrated camera in front of the capillary's (or channel) section in which the meniscus will flow. Adjust the lighting, so that the image has the best contrast for the exposure time that will be used for the measurement. Make sure that the capillary is in focus and align it with the image bottom or top border so that it appears horizontal in the image.

Measure the temperature at the liquid source reservoir and set the flow rate.

During the calibration

When the stabilization time is reached (typically, if the flow rates remain at ±5 % of its target value [3]), acquire timestamped images, and ambient condition data continuously. Check the ambient conditions for fluctuations. If dynamic (fluctuating) flows are to be measured, make sure to choose a framerate sufficiently high (typically equal or superior to 1 frame per second - 1 fps).

End of calibration

When the calibration time is reached stop the image acquisition and ambient conditions recording.

Image processing and flow rate calculation

Apply image processing to determine the meniscus positions in each successive image and calculate the flow as the time derivative of the meniscus' positions.

The number of repetitions should be at least 3 per flow rate.

B. Uncertainty budget

The main uncertainty components to be considered for the front tracking method are: meniscus displacement, $u(\Delta x)$; time interval between frames, $u(t)$; capillary radius, $u(r)$; liquid temperature, $u(t_L)$; material expansion, $u(\gamma)$; stability, $u(\delta qsta)$; standard deviation of the measurements $u(\delta qrep)$ [11].

Table 2. The estimated contribution for the front tracking method

Uncertainty components	**Estimation**	$u(x_i)$	c_i	$(c_i x_i)^2$
Meniscus displacement (mm)	11,76	1,50E-+02	2,35E-02	1,25E-07
Radius (mm)	0,575	1,47E-03	9,63E-01	2,01E-06
Time (s)	44,107	2,97E-03	-6,28E-03	3,48E-10
Repeatability (µl/s)	0,0013	0,0013	1	1,80E-06
Stability (µl/s)	1.37E-4	1,37E-04	1	1,88E-08
Expansion coefficient (/°C)	0,00024	6,92E-06	-8,14E-01	3,18E-11
Liquid temperature (°C)	22,94	0,005	-2,77E-06	1,92E-16
Flow rate (µl/h)	997,1			
u_{comb} (µl/h)	7,2			
v_{eff}	100,15			
k	2,02			
U_{exp} (µl/h)	15			
U (%)	**1,5**			

3.2 Flow resistivity/resistance vs flow rate

Flow resistivity measurement is used to determine the necessary pressure to achieve the desired flow through a microfluidic circuit, or conversely, to determine the flow rate corresponding to a given inlet pressure. Flow resistivity measurement consists of measuring the differential pressure (or solely the inlet pressure if the outlet is at atmospheric pressure) of a given microfluidic circuit and measuring the flow passing through the same microfluidic circuit, either simultaneously or sequentially, for at least one (preferably several) couple of flow rates and pressure. The output of this test can be a table of results and/or a graphical representation of 'flow rate vs pressure'. The flow resistivity can also be expressed in $Pa \cdot s \cdot m^{-3}$.

Flow resistance, also called hydrodynamic resistance or just microfluidic resistance, corresponds to the opposition that a fluidic element, like a pipe, offers to a flow through itself. Each element of a microfluidic circuit offers some resistance to the flow, which is translated into a pressure drop, which can be calculated by using the Hagen-Poiseuille equation:

$$\Delta P = qR_H, \tag{6}$$

where ΔP is the pressure difference, or drop, between two points of the system, q is the flow rate and R_H is the hydraulic resistance.

Under the assumptions stated below, it can be demonstrated that the pressure drop associated with a microchannel depends on the flow rate, the dimensions of the channel and the dynamic viscosity of the fluid itself. The flow resistor expressions for the most typical channel cross sections are as follows. These are, respectively: circle, rectangle, and square shapes:

$$R_{Hc} = \frac{8\mu L}{\pi r^4} \qquad R_{H,rec} = \frac{12\mu L}{1-0{,}63\left(\frac{h}{w}\right)}\left(\frac{1}{h^3 w}\right) \qquad R_{H,sq} = \frac{12\mu L}{1-0{,}917\times 0{,}63}\left(\frac{1}{h^4}\right), \tag{7}$$

where:

r Radius of the circle,
μ Dynamic viscosity,
L Length of the channel,
h Height of the channel,
w Width of the channel.

The assumptions for this theoretical model are the following:

- Low Re number <2300,
- Almost incompressible fluid at calibration conditions,
- Unidirectional flow,
- Steady flow along the channel,
- Small fluid mass per distance unit, so gravity is negligible,
- Negligible surface tension forces,
- Negligible friction forces from the wall of the channel.

See Silverio & Cardoso [12] for a discussion of surface tension forces and friction forces from the wall of the channel. Their work explains that surface tension forces become increasingly dominant for smaller scales. Consequently, there is a case when a scale too small for the above assumption of negligible surface tension forces to be valid, for example capillary devices. Moreover, if the

scale is too large the assumption of low Re number will be violated, for example macro scale pipes. It is worth noting that the description of pressure drop stated by Eq. (6) and Eq. (7) is applicable for an intermediate range of scales for microchannels.

Measuring flow resistivity across a microfluidic device is performed using at least a pressure source (pressure controller in the figures below, which includes a pressure source an element uses to stabilize and control the pressure, such as a control valve), a flow sensor, and either:

- one pressure sensor at the device's inlet, the outlet being at atmospheric pressure, as shown in the following schematic:

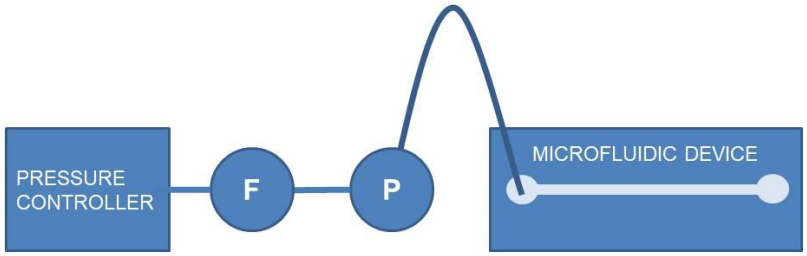


Figure 6. One pressure sensor

- one differential pressure sensor in parallel of the microfluidic device, as shown in the following schematic:

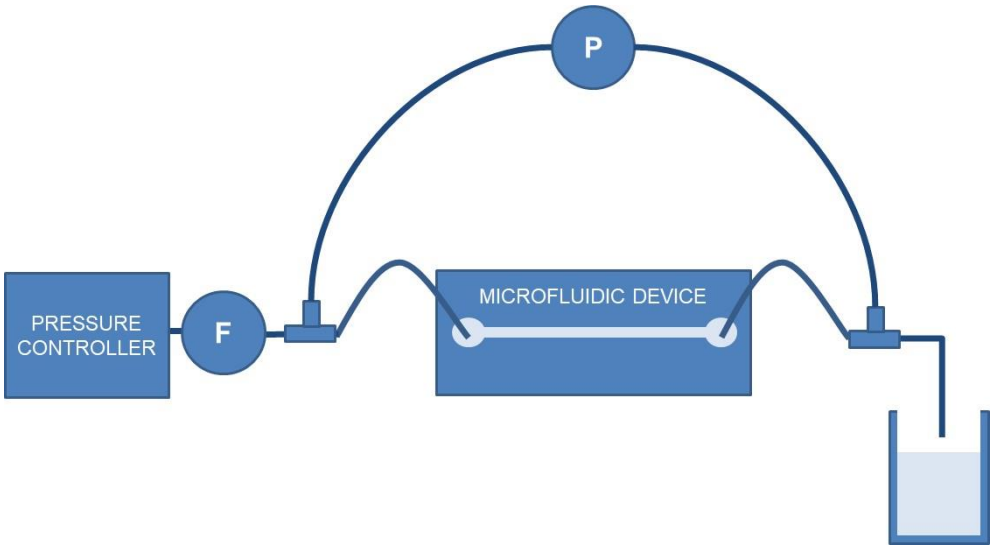


Figure 7.One differential sensor

- two pressure sensors at inlet and outlet of the microfluidic device, as shown in the following schematic:

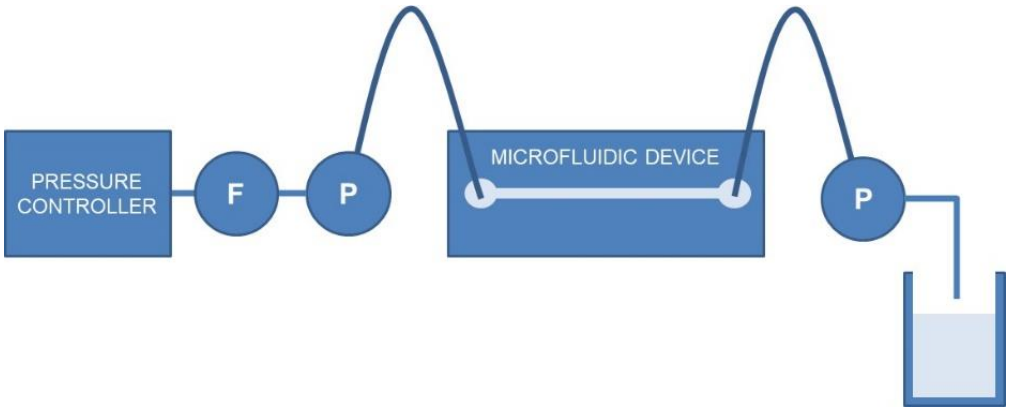


Figure 8.Two pressure sensors

In the three schematic drawings above, the specifications are:

Pressure controller

- It must be chosen according to the admissible pressure range in the microfluidic device.
- Minimum pressure at the outlet of the pressure controller is generally around 100 mbar to ensure good pressure control and stability.
- The stability of the pressure controller must be in accordance with the accuracy required for the pressure drop / flow resistivity measurement. This information can be found in the datasheet of the pressure controller. Note that the stability can be degraded due to the compliance (elasticity) of the entire setup and dead volumes which can entrap air (in case of a liquid as the test media).
- A sufficient pressure level at the pressure source (inlet) of the pressure controller should be provided in order to perform in a normal way. The minimum and maximum pressure level can be found in the datasheet of the pressure controller. The same apply for the required electrical power supply.
- The pressure controller should be at the same altitude as the microfluidic device.

Pressure sensor(s), differential pressure sensors

- It must be chosen according to the pressure range (or pressure drop range in case of a differential pressure sensor) to be measured in the microfluidic device. Generally, a pressure sensor should be used between 10 % and 100 % of its pressure range capability.
- The accuracy of the pressure sensor must be in accordance with the accuracy required for the pressure drop / flow resistivity measurement. This information can be found in the datasheet of the pressure sensor. Note that this accuracy can be degraded due to the compliance (elasticity) of the entire setup and dead volumes which can entrap air (in case of a liquid as the test media).
- The pressure sensor(s) should be at the same altitude as the microfluidic device.
- All pressure sensors should be calibrated before any pressure measurements. The calibration should be performed by an accredited laboratory or NMI/DI.

- In case of a liquid as the test media, the tubing connecting the pressure sensor(s) to the pressure controller and microfluidic device should be set downward so that air bubbles are expelled (purged) downstream of the setup.
- In case of a gas as the test media, the tubing connecting the pressure sensor(s) to the pressure controller and microfluidic device should be set upward so that liquid droplets (due for example to condensation of humidity) are expelled (purged) downstream of the setup.

Flow sensor

- It must be chosen according to the flow range to be measured in the microfluidic device.
- The accuracy of the flow sensor must be in accordance with the accuracy required for the pressure drop / flow resistivity measurement. This information can be found in the datasheet of the flow sensor. Note that this accuracy can be degraded due to the compliance (elasticity) of the entire setup and dead volumes which can entrap air (in case of a liquid as the test media).
- All flow sensors should be calibrated before any pressure measurements. The calibration should be performed by an accredited laboratory or NMI/DI.
- In the case of a liquid as the test medium, the tubing connecting the flow sensor(s) to the pressure controller and microfluidic device should be directed downwards so that any air bubbles are expelled (purged) downstream of the setup.
- In the case of a gas as the test medium, the tubing connecting the flow sensor(s) to the pressure controller and microfluidic device should be directed upwards so that liquid droplets (e.g. due to moisture condensation) are expelled (purged) downstream of the setup.

Fittings/connectors

- All fittings and connectors should be chosen and set keeping in mind that any leakage will cause measurement error and will degrade the flow resistivity accuracy.
- All fittings and connectors should be chosen as to minimize their dead volume.

Measurements should be performed in a temperature and humidity-controlled room. Temperature, ambient pressure, and humidity fluctuations range should be known, either by measurement (using calibrated sensors) or by knowledge of the control ranges, ensuring that the climate-control system (air-conditioning for example) is performing normally. Ideal conditions are (23 ± 2) °C and (55 ± 5) %R_H.
All elements of the setup (pressure controller, measurements sensors, test media, and microfluidic devices) should be left in the room where the tests are to be performed at least 24 hours before the test, to ensure thermal equilibrium. Electrically powered elements such as the pressure controller and the pressure sensor(s) should be powered on at least 30 minutes before beginning of any measurement or otherwise stated in manual of manufacturer. For pressure controllers requiring the filling of a reservoir, the latter should be filled beforehand, at least 30 minutes before beginning of any measurement. The entire system, including pressure taps in case of differential pressure sensor, should be purged before beginning of any measurement. Purge can be performed using the test media at the maximum admissible pressure of the pressure controller/pressure sensor(s)/microfluidic device (whichever has the lesser maximum admissible pressure).

A. Test protocol

1. Place of all setup elements in the room where the tests are to be performed, for at least 24 hours before beginning of any measurement.
2. Connect all elements together to mount the setup, making sure that all fittings are tightly connected.
3. If necessary, fill the pressure controller reservoir at least 30 minutes before beginning any measurement.
4. Connect all required electrical power supplies to the pressure controller and pressure sensor(s), and power on at least 30 minutes before beginning any measurement.
5. Purge the setup using the test media at the maximum admissible pressure of the pressure controller/pressure sensor(s)/microfluidic device (whichever has the lesser maximum admissible pressure).
6. Zero the pressure and flow sensors to minimize any measurement offset.
7. Set a target pressure to the pressure controller and monitor the evolution of the pressure using the pressure sensor(s).
8. After reaching a stable pressure (not drifting upwards of downwards), record the measurement result.
9. Repeat step 7 and 8 for all required target pressure.
10. In case of a differential pressure sensor or two pressure sensors, remove the microfluidic device and connect directly both pressure taps to measure the pressure drop. Repeat step 7 and 8 for all measured flows in the previous steps.
11. Calculate each flow resistivity according to Eq. 6. In case of a differential pressure sensor or two pressure sensors, subtract the pressure taps pressure drops to the calculated device's pressure drop.

3.3 Volume of a microfluidic channel

The volume of a microfluidic channel can be determined gravimetrically according to the formula described in ISO 4787 [13]:

$$V_{20} = (I_L - I_E) \times \frac{1}{\rho_L - \rho_A} \times \left(1 - \frac{\rho_0}{\rho_B}\right) \times [1 - \gamma(t - 20)], \quad (8)$$

where:

V_{20} Volume at a reference temperature of 20 ºC,
I_L Balance indication of the vessel with the contained liquid,
I_E Balance indication of the vessel with empty vessel,
ρ_L Density of liquid,
ρ_A Density of air,
ρ_0 Density at reference conditions for weighing, 1,2 kg/m^3,
ρ_B Density of the mass pieces (8000 kg/m^3),
t Temperature,
γ is the coefficient of cubical thermal expansion of the material of which the chip tested is made.

Each channel is tested separately. The difference obtained in the weighing measurements gives the mass of the liquid contained in a particular channel. At least 5 tests should be performed in order to obtain the measurement repeatability.

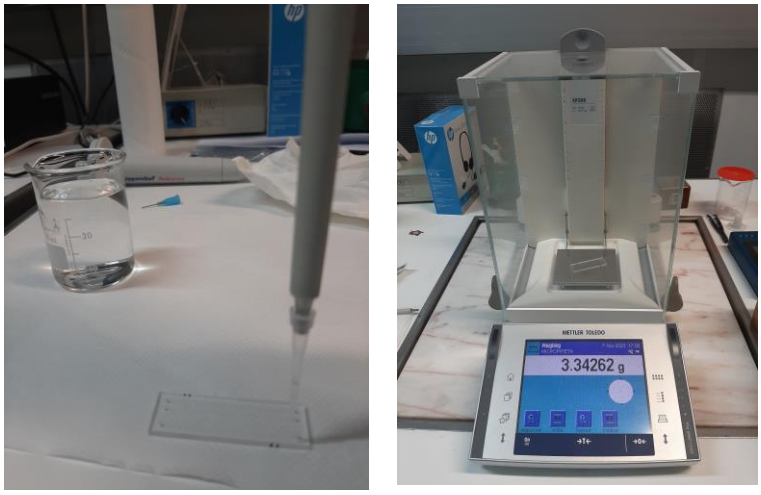


Figure 9. Volume measurement setup

3.3.1 Internal contained volume of a microfluidic channel

The internal volume of a microfluidic channel is determined gravimetrically by weighing the chip to be calibrated when empty and when a microfluidic channel to be tested is filled with a suitable liquid, normally distilled water.

Preparation

The tests using the gravimetric method for internal volume determination are performed under the following conditions:

a) Acclimatization time of 24 h, all the instruments and the water stood in the same room for 24 h;
b) Controlled room temperature equal to (20 ± 3) °C;
c) Room temperature variation during the measurements not more than 1 °C during the tests;
d) Humidity room above 50 %rh.

A. Test protocol

1. Measure the water temperature in the water container;
2. Weight the empty and dry chip;
3. Fill the microfluidic channel to be tested with water ensuring no bubbles are inside (a syringe or a micropipette can be used);
4. Weight the chip full of water.

During the tests, the temperature of the water and the air, the relative humidity and the atmospheric pressure need to be continually measured and recorded. It should be noted that the placeholders for the plugs should remain dry. Only the tested channel inside should be filled with water.

B. Uncertainty budget

The uncertainty calculation on the internal volume can be performed according to EURAMET Calibration Guide No. 19 [14].

3.3.2 Residual volume of a microfluidic channel

In order to determine the residual volume of each microfluidic channel the preparation and calculations will be similar to the described in 3.3.1, this includes the uncertainty calculation.

A. Test protocol

1. Weight the empty and dry chip;
2. Measure the water temperature;
3. Fill the microfluidic channel to be tested with water ensuring no bubbles are inside (a syringe or a micropipette can be used);
4. Remove the water from the microfluidic chip by aspiration, using a pipette or a syringe;
5. Weight the chip.

4 Example of application

Examples how this guide could be used for specific microfluidic devices are presented in Annex 2.

5 REFERENCES

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15. BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP, and OIML. Evaluation of measurement data | Guide to the expression of uncertainty in measurement. Joint Committee for Guides in Metrology, JCGM 100:2008.
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ANNEX 1. SURVEY OF FLOW-RELATED METROLOGY NEEDS OF THE MICROFLUIDICS INDUSTRY

In order to understand the industry needs related to measurement quantities, an inventory was compiled based on interviews with microfluidics industrial experts performed under the project MFMET – Establishing Metrology Standards in Microfluidic Devices, the results of the interview are available on the project website, https://mfmet.eu.

The priorities identified by industry experts for measurements during manufacturing of microfluidic devices and components are listed in order of their importance:

1. Factors affecting pressure drop and flow resistance in the device (e.g. wettability or deviation from ideal dimensions);
2. Factors affecting the bonding quality of polymeric components (glass transition temperature, melting temperature, molecular weight number and distribution, etc.);
3. Leakage, burst pressure and maximum working pressure;
4. Consensus on the information of materials used, such as optical transmission, autofluorescence of polymeric materials, thickness of glass substrates, etc.

Other topics of interest are:

- Measurement of rapidly changing flow rates.
- Verification of biological viability after dispensing biomaterial on chip / in device.

From this survey, two categories (flow quantities and liquid properties) were identified.

A1.1 - Flow quantities

The flow quantities are presented in order of priority:

1. Flow rate (static, dynamic)
2. Flow resistivity/resistance vs flow rate (Reynolds (Re) numbers, drag force)
3. Internal volume
4. Dead volume
5. Droplet size/volume variation
6. Flow pressure (inline pressure)

Note: Leakage rate (non-destructive/non-intrusive and fast test – use pressure drop with air; air/liquid ratio) is dealt within Work Package 1 of MFMET project.

A1.2 - Liquid properties

The liquid properties are presented according to their priority:

1. Viscosity
2. Density
3. Contact angle
4. Refractive index (optical methods)

Note: Surface tension and wettability are dealt within Work Package 3 of MFMET project.

In the context of this work, a comprehensive literature review was conducted regarding normative flow property standards and terminology in the field of microflow. The report, A2.2.1: “A literature review of existing metrology and normative standards related to the flow properties and microfluidic devices”, can be found on the MFMET project’s website https://mfmet.eu.

ANNEX 2. EXAMPLE OF THREE INDUSTRIAL APPLICATIONS

The Annex shows how this guide can be used by means of three specific examples of microfluidic devices:

a) Thermoplastic polymer (TOPAS®) microfluidic chips
b) Silicone polymer (PDMS, polydimethylsiloxane) microfluid chip
c) Microfluidic pump (developed within the EMPIR 18 HLT08 MeDD2 project, task 3.2)

A2.1 TOPAS® microfluidic chips

At CETIAT a "Fluidic 157" microfluidic chip provided by Microfluidic ChipShop (https://www.microfluidic-chipshop.com/catalogue/microfluidic-chips/polymer-chips/straight-channel-chips-microscopy-slide-format/straight-channel-chips-with-eight-parallel-channels-fluidic-157/) with the following characteristics was investigated:

- 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, similar to "female mini luer port" integrated directly on the chip. Those ports can be found as "stand alone" ports, such as https://darwin-microfluidics.com/products/stand-alone-female-mini-luer-ports-pack-of-10

The following figure shows a schematic of the device under test (DUT), TOPAS® Fluidic 157 chip.

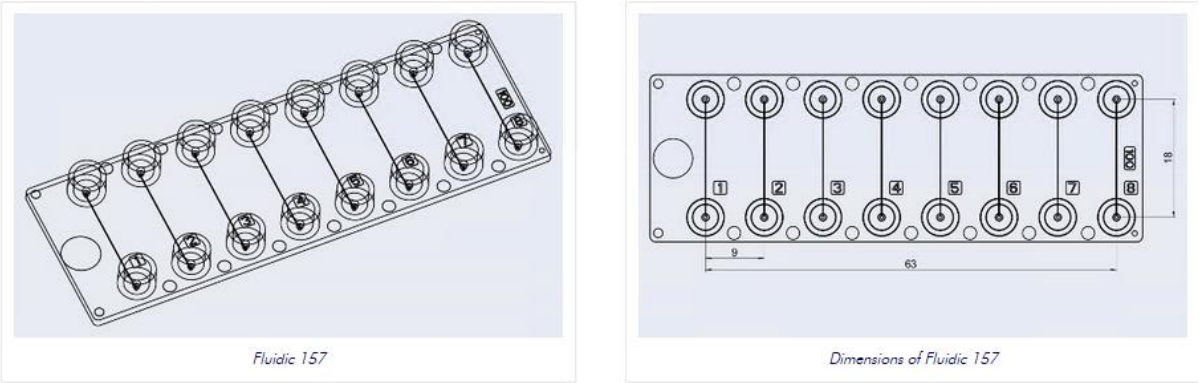

Figure A2.1. Schematic of the TOPAS® "Fluidic 157" chip obtained from microfluidic-chipshop.com

The following picture shows the DUT.

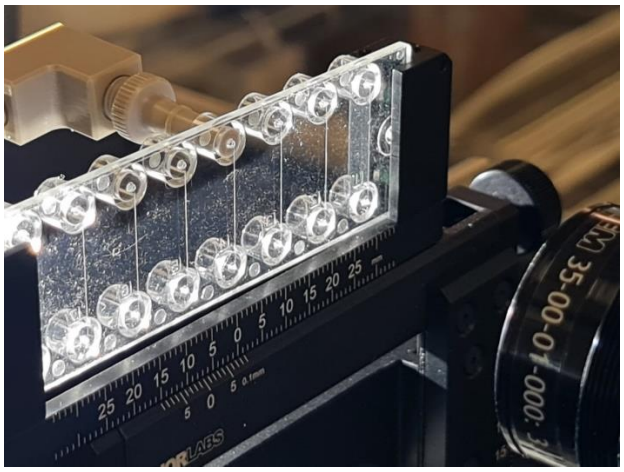

Figure A2.2. TOPAS® “fluidic 157” chip during testing

IPQ performed the volume and flow measurements on two different TOPAS® chips provided by Microfluidic ChipShop with the following characteristics:

Chip A - 16 parallel channels with fluid interface holes, Material: TOPAS®, Dimensions: 75,5 mm x 25,5 mm x 1,5 mm, model 10000198, batch Z1112070.

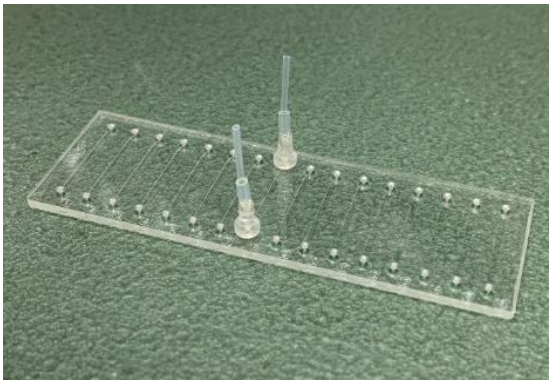

Figure A2.3. TOPAS® Chip A (IPQ)

Chip C - 16 parallel channels with mini luer fluidic interface, Material: TOPAS® (polymer for medical use), Dimensions: 75,5 mm x 25,5 mm x 4 mm, model 10000168, batch JI125176

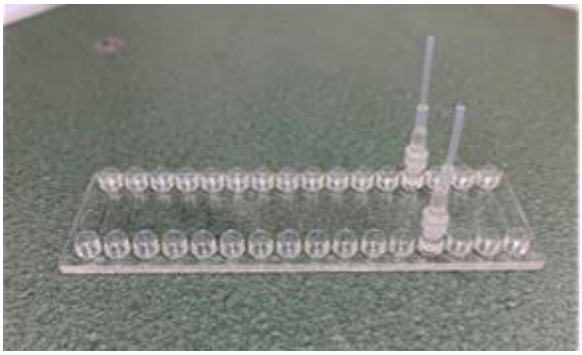

Figure A2.4. TOPAS® Chip C (IPQ)

A2.1.1 Flow rate determination at IPQ

The following figure presents the test setup used for the flow test at IPQ using the gravimetric method.

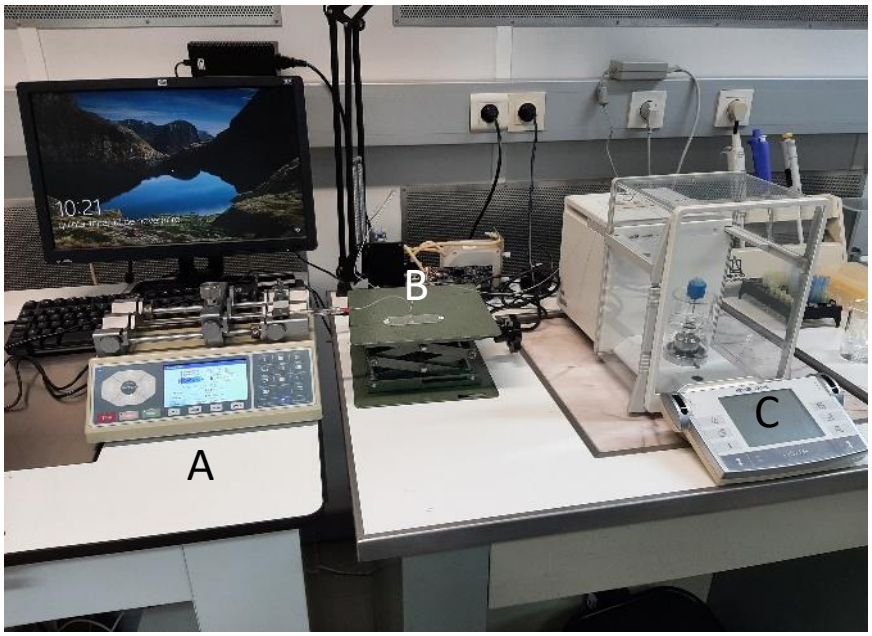

Figure A2.5. Gravimetric flow setup, where A is the syringe pump, B is the chip and C is the balance

The flow is generated by a programmable syringe pump with flow measurement range from 2 pl/min to 500 ml/min (± <0,35 %, 0,1 % reproducibility, according to manufacturer specification), Nexus 3000 infuse/withdraw system, Chemyx Precision Syringe Pumps, using a 1 ml ILS glass syringe. PE tube of 1,26 mm (0,05") inner diameter was used in the flow path. Tests were performed with and without the chips connected to the system, using a AX26 Mettler Toledo balance with 0,001 mg resolution and an evaporation trap.

The fluid used for the tests is ultra-pure water.

The following figure presents the test setup used for the flow test at IPQ using the front track method.

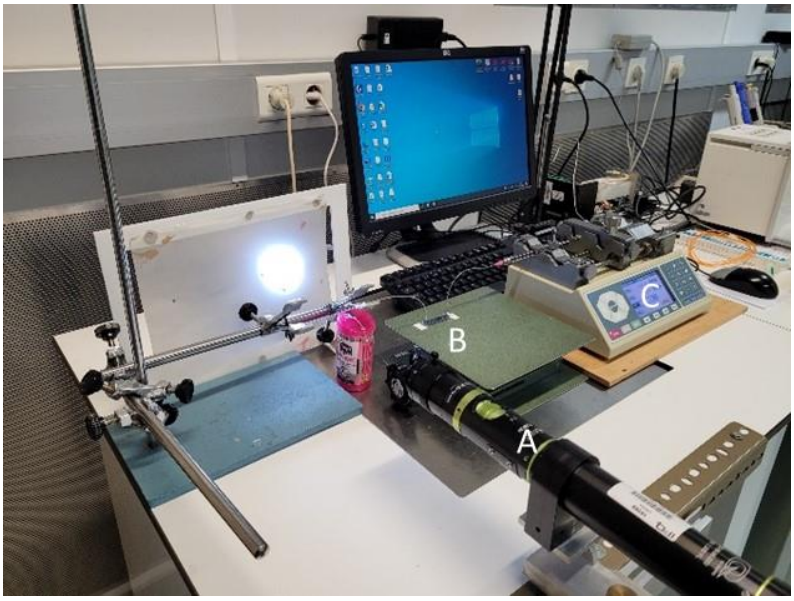

Figure A2.6. Front track flow setup, where A is the camera, B is the chip and C is the syringe pump

A high-resolution Alvium 1800 U-1240 camera with a resolution of 12 MP and a Qioptic Optem 7:1 telecentric zoom lens was used. The camera is connected to a computer and uses Python as the programming language that identifies the meniscus and calculates its position. The flow is generated by a programmable syringe pump with a flow measurement range from 2 pl/min to 500 mL/min (± <0,35 %, 0,1% reproducibility, according to manufacturer specification), Nexus 3000 infuse/withdraw system, Chemyx Precision Syringe Pumps. For the measurements, a PE tube of 1,26 mm (0,05 in) inner diameter was used along with a using a 1 ml ILS glass syringe, and a 1,6 mm glass capillary.

The fluid used for the tests is ultra-pure water.

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

Table A2.1. 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

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

Table A2.2. 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.

A2.1.2 Flow resistivity determination at CETIAT

The following figure presents the test setup used for the flow resistivity test.

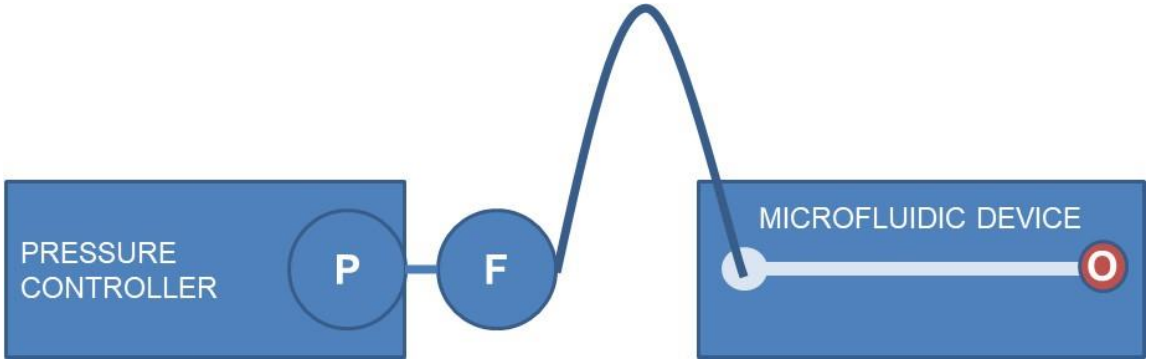

Figure A2.7. Schematic of the flow resistivity test setup. (F) flow sensor, (P) pressure sensor, (O) outlet of the microfluidic chip.

The following picture shows photographs of the test setup.

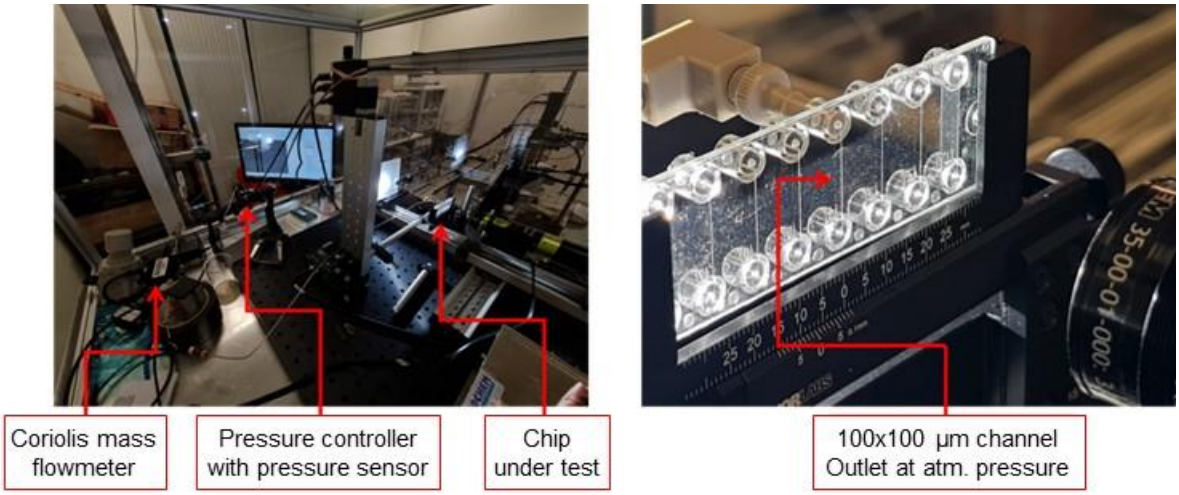

Figure A2.8. Photographs of the test setup

The test setup used the following equipment:

- Pressure controller: Elveflow OB1 MkII, used on its 0 to 200 mbar or 0 to 2000 mbar channels. The 0 to 200 mbar channel, used for this test, has been calibrated before the test and used for the pressure readings.
- Flow sensor:
  - Bronkhorst ML120 (83 µl/min full range, readings acquired using the FlowPlot software)
  - Elveflow MFS 2 (7 µl/min full range, reading acquired using the ESI software)
- Thermo-hygro-barometer ROTRONIC LOG-HC2-P1/HC2-C05 for recording of temperature, humidity, and ambient pressure at the device under test location.

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

The fluid used for the tests was ultra-pure degassed water (conductivity inferior to 0,06 µS/cm, filtered at 0,2 µm).

All pipes used to make the fluidic connection between the different items of the setup, from the pressure controller to the device under test, were stainless steel 1/16” pipes.

At the inlet port of the device under test, a luer male to threaded Upchurch adapter (reference P-683-01), with an additional threaded female-female Upchurch elbow, has been used to connect the fluidic path to the device under test, as shown in the next figure.

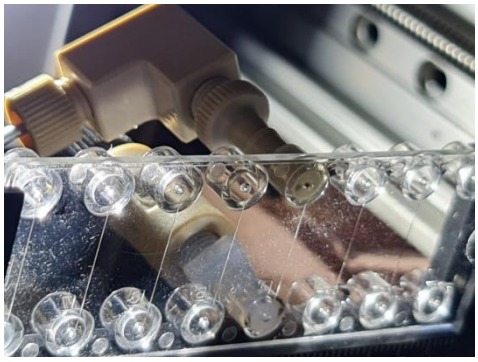

Figure A2.9. Adapter used to connect the fluidic setup to the microfluidic chip

The reference of three assembly components (from the device under test to the 1/16” stainless steel pipe) are:

- Luer male to threaded Upchurch adapter (reference P-683-01)
- Threaded female-female Upchurch 1/16” OD PEEK elbow (reference P-430)
- Upchurch Flangeless Nut for 1/16” OD (reference P-235)

Table A2.3 presents the experimental results obtained for the measurement with the full setup (see Figure A2.10) for flow resistivity:

Table A2.3. 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]	[$Pa \cdot s \cdot m^{-3}$]
39	18000	3900	3,00 E-10	1,30 E+13
32	14000	3200	2,33 E-10	1,37 E+13
25	9000	2500	1,50 E-10	1,67 E+13
20,5	6000	2050	1,00 E-10	2,05 E+13
15	2500	1500	4,17 E-11	3,60 E+13

Figure A2.10 shows the pressure and flow readings during the test of the full setup.

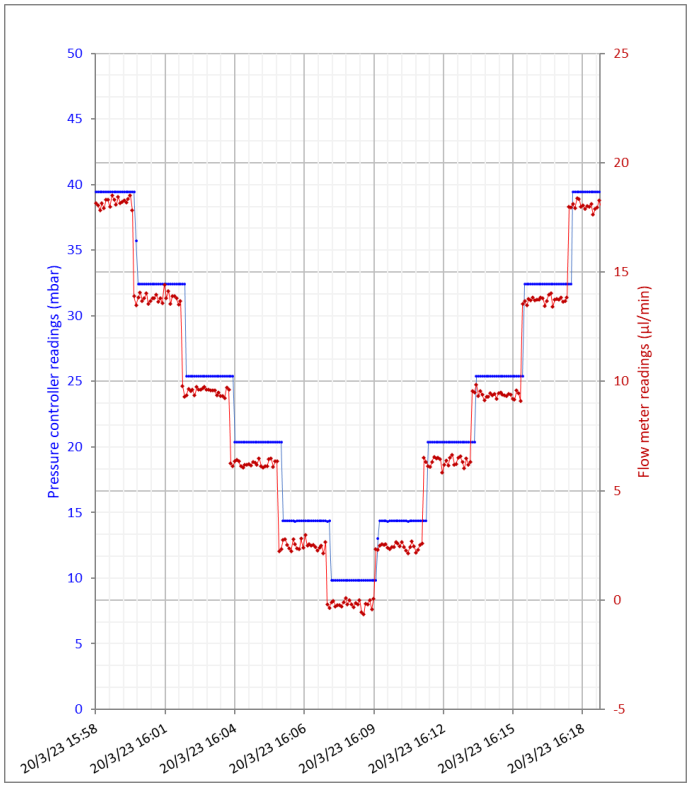

Figure A2.10. Pressure (blue) and flow (red) readings during the flow resistivity test with chip

A2.1.3 Internal volume determination at IPQ

The internal volume of the two TOPAS® chips A and C were determined using the gravimetric method, a XP205 Mettler balance with 0,01 mg resolution was used. The reference liquid is ultrapure water. The liquid temperature was measured with a PT100 sensor with 0,01 °C resolution.

The following table shows the experimental results obtained for the measurements of internal volume using the gravimetric method for Chip A channel 1 and 8 and Chip C, channels 1 and 13. The volume values given in Table represent the average of 10 repeated measurements.

Table A2.3. Results of the internal volume determination at IPQ (Chip A and Chip C)

Chip	Channel	Volume (ml)	*U* (ml)
A	1	0,00390	0,00010
	8	0,00426	0,00014
C	1	0,00387	0,00016
	13	0,00385	0,00007

The internal volume of the microfluidic channels measured in the TOPAS® chips is similar.

A2.1.3 Residual volume determination at IPQ

The residual volume of the two TOPAS® chips A and C were determined using the gravimetric method, a XP205 Mettler balance with 0,01 mg resolution was used. The reference liquid is ultrapure water. The liquid temperature was measured with a PT100 sensor with 0,01 °C resolution.

The following table shows the experimental results obtained for the measurements of residual volume using the gravimetric method for Chip A and Chip C, channel 1. The volume values given in table A.2.4 represent the average of 10 repeated measurements.

Table A2.4. Results of the residual volume determination at IPQ (Chip A and Chip C)

Chip	Channel	Volume (ml)	*U* (ml)
A	1	0,00001	0,00002
C	1	0,00004	0,00005

The residual volume and uncertainty of the of the Chip A are smaller than the chip C due to the characteristics of the filling hole.

A2.2 PDMS microfluidic chip

IPQ performed the volume and flow measurements on a PDMS chip with 1 channel of 100 μm width and 50 μm depth, with two 0,9 mm inlet holes, Material: PDMS, Dimensions: 40 mm x 10 mm, manufactured by INESC MN.

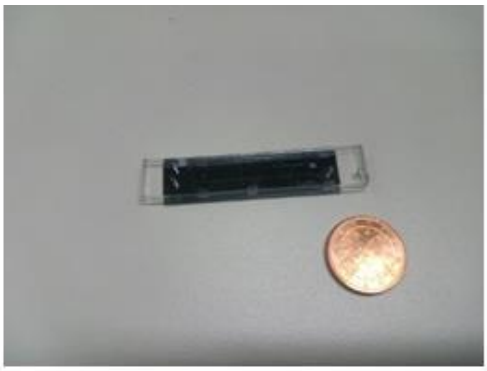

Figure 9. PDMS Chip

A2.2.1 Flow rate determination at IPQ

The following measurements were carried out at IPQ with the same test setup as described in section 3.1.1.

The following table shows the experimental results obtained for the measurements of flow rate using the gravimetric method for the PDMS chip.

Table A2.5. Results of the gravimetric flow measurements at IPQ (PDMS Chip)

Generated Flow (ml/h)	Flow without the chip	Error (%)	*U* (%)	Out Flow of chip (ml/h)	Error (%)	*U* (%)
0,1	0,09991	0,09	3,3	0,0918	8,2	2,5
1	1,00356	-0,36	2,2	0,9174	8,3	2,4

The following table shows the experimental results obtained for the measurements of flow rate using the front tracking method for the PDMS chip.

Table A2.6. Results of the front tracking flow measurements at IPQ (PDMS Chip)

Generated Flow (ml/h)	Flow without the chip	Error (%)	*U* (%)	Out Flow of chip (ml/h)	Error (%)	*U* (%)
0,1	0,09961	0,39	2,4	0,0961	4,0	2,6
1	0,99706	0,02	2,4	1,0257	-2,5	4,0

In general, similar to the results of the TOPAS® chips, in the majority of the cases for both methods the error is larger when adding the chip to the system and the uncertainty increases. Also, the PDMS chip has larger errors than the TOPAS® chips due to material proprieties retention.

A2.2.2 Internal volume determination at IPQ

The internal volume of the PDMS channel was determined using the gravimetric method, a XP205 Mettler balance with 0,01 mg resolution was used. The reference liquid is ultrapure water. The liquid temperature was measured with a PT100 sensor with 0,01 °C resolution.
The values given in Table A2.7represents the average of 10 repeated measurements.

Table A2.7. Results of the volume determination at IPQ

Chip	Channel	Volume (ml)	*U* (ml)
PDMS	1	0,00277	0,00004

A2.2.3 Residual volume determination at IPQ

The residual volume of the PDMS channel was determined using the gravimetric method, a XP205 Mettler balance with 0,01 mg resolution was used. The reference liquid is ultrapure water. The liquid temperature was measured with a PT100 sensor with 0,01 °C resolution.
The values given in Table 8 represents the average of 10 repeated measurements.

Table A2.8. Results of the PDMS residual channel volume determination at IPQ

Chip	Channel	Volume (ml)	*U* (ml)
PDMS	1	0,00023	0,00017

The residual volume in the PDMS channel chip is much larger than for the TOPAS® chips due to the type of material and its wettability characteristics.

A2.3 Microfluidic pump

IPQ performed flow measurements with a microfluidic pump which was developed and fabricated by INESC MN within the EMPIR project 18HLT08 Metrology for Drug Delivery (18HLT08 MeDD II). The operating principle of the pump is described in more detail in the MeDD2 Report A3.2.5 (Deliverable D5).

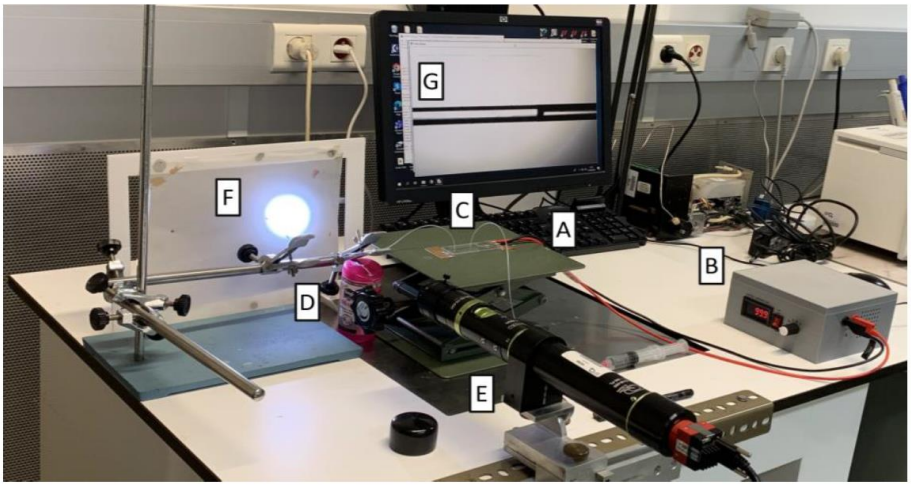

Figure A2.12. Microfluidic pump flow test using front track method

A – Microfluidic pump
B – Power source
C – Tubes BTPE 90
D – Capillary tube
E – Hight resolution camera
F – Paper for light diffusion and LED
G – Computer programme

As shown in Figure , due to the low flow rates of the pump (close to 0,005 ml/h), the flow determination of the microfluidic pump was carried using the front track method.

The following table shows the experimental results obtained for the measurements of volume rate using the front track method.

Table A2.9. Results of the front track flow measurements at IPQ (microfluidic pump)

	Set Flow (ml/h)	**Measured Flow (ml/h)**	**Error (%)**	***U* (%)**
Microfluidic pump	0,005	0,00504	-0,8	4,8

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