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Primary standards for measuring flow rates from 100 nl/min to 1 ml/min – gravimetric principle

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Abstract: Microflow and nanoflow rate calibrations are important in several applications such as liquid chromatography, (scaled-down) process technology, and special health-care applications. However, traceability in the microflow and nanoflow range does not go below 16 µl/min in Europe. Furthermore, the European metrology organization EURAMET did not yet validate this traceability by means of an intercomparison between different National Metrology Institutes (NMIs). The NMIs METAS, Centre Technique des Industries Aérauliques et Thermiques, IPQ, Danish Technological Institute, and VSL have therefore developed and validated primary standards to cover the flow rate range from 0.1 µl/min to at least 1 ml/min. In this article, we describe the different designs and methods of the primary standards of the gravimetric principle and the results obtained at the intercomparison for the upper flow rate range for the various NMIs and Bronkhorst High-Tech, the manufacturer of the transfer standards used.

Keywords: dynamic gravimetric calibration; intercomparison; liquid; metrology for drug delivery; microflow; primary standard; validation.

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Introduction

Microflow and nanoflow rate calibrations are important in several applications such as liquid chromatography, (scaled-down) process technology, and special health-care applications. Examples of applications that operate at low flow rates are pain treatment with Prialt® (maximum daily dose 21.6 µg [16]), implanted infusion device (down to 70 nl/min [1]), microreactors (down to 25 nl/min [27]), and liquid chromatography (down to 5 nl/min [28]).

Currently, commercial sensors based on the thermal dilution principle [18, 23] can measure flow rates down to 70 nl/min with a claimed uncertainty of 10% [12, 23]. Flow sensors based on the Coriolis principle [17, 24] are believed to yield a lower uncertainty; however, these sensors are yet in a developmental phase. Nevertheless, all common flow sensors and microfluidic applications require calibration because they do not establish a direct link to SI units. Calibration is required to achieve a low flow rate error (accuracy) or to verify the repeatability and reproducibility. Calibration is typically performed with a gravimetric set up, which is based on measuring the mass of liquid as a function of time on a sensitive balance. The flow rate is in general determined by the quotient of the mass difference and time difference including some corrections.

However, in Europe, at the time this project started (2010), the existing gravimetric methods did not go below 16 µl/min. Furthermore, the European metrology organization EURAMET did not validate these standards by means of an intercomparison. An intercomparison is typically performed between National Metrology Institutes (NMIs) and aims to confirm or reject the stated uncertainties for the flow rate range. The NMIs METAS (Switzerland), Centre Technique des Industries Aérauliques et Thermiques (CETIAT, France), IPQ (Portugal), Danish Technological Institute (DTI, Denmark), and VSL (the Netherlands) have therefore developed and validated primary standards to cover the flow rate range from 0.1 µl/min to at least 1 ml/min as part of the EMRP project “HLT07 Metrology for Drug Delivery – MeDD” [19]. Other approaches to measure and calibrate microfluidic

Table 1: Summary of the main characteristics of the facilities.

	Flow rate range	Uncertainty (k=2) (%)	Water temperature (°C)	Pressure range upstream DUT (bar)
METAS	0.1 µl/min–1 ml/min 0.006–60 g/h	0.6–0.1	Ambient	0–2.5
DTI	0.017 µl/min–10 ml/min 0.001–600 g/h	5–0.05	Ambient	0–5
CETIAT	16.6 µl/min–167 ml/min 0.001–5000 g/h	0.6–0.1	10–50	0–10
IPQ	0.05 µl/min–10 ml/min 0.003–600 g/h	6–0.15	Ambient	0–2
VSL	4.2 µl/min–83 ml/min 0.25–5000 g/h	1.0–0.05	Ambient	0–5
Bronkhorst	16.6 µl/min–3.33 ml/min 1–200 g/h	0.62–0.06	Ambient	0–6

Some of the facilities can operate with liquids of different viscosities.

applications are based on a volumetric expansion source, where the flow is produced by a controlled temperature gradient in a given volume similar to the case of a liquid thermometer [25] and front tracking of the meniscus in a capillary [1]. These approaches are, however, more suitable for even lower flow rates.

In this article, we describe the different designs and methods of the primary standards that are based on the gravimetric principle and the results obtained from an intercomparison. Table 1 shows the summary of the flow rates covered by each facility and the corresponding uncertainties. The indications of the flow rate range from mass flow are converted to volume flow by assuming a density of 1000 kg/m^3, which is close enough to the water density at ambient temperature, being 997.8 kg/m^3 for 22°C.

General measurement principle

The general working principal is the same for all gravimetric setups and consists of flow generation, connections to a device under test (DUT), and connections to a water collection on top of a precision balance or an analytical balance, as shown in Figure 1. This balance reads the mass, which can be combined with timing equipment to calculate the $\Delta m/\Delta t$ quotient.

Primary standard at METAS

METAS developed a primary standard to cover the flow rate range from 0.1 µl/min to 1 ml/min, with uncertainties

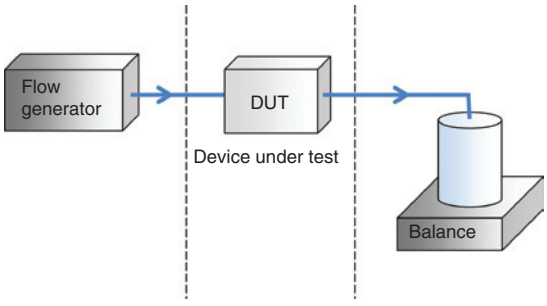

Figure 1: General working principle of a gravimetric setup.

in the range from 0.6% to 0.1% for steady flow rates, and calibration of pulsating flow rates from 1 µl/min to 1 ml/min, with measurement uncertainties from 2.7% to 0.2%. The measurement times for steady flow vary from 240 min for the lowest flow rate to 1 min for the highest flow rate.

Design of the facility

One of the main issues in the development of the facility is not only to generate a very low flow rate but also to ensure good flow rate stability in the case of steady flow. To realize this, METAS applies the principle of generating the flow by means of a constant pressure drop over a capillary tube according to the law of Hagen-Poiseuille. In Figures 2 and 3, the simplified working principle and a picture of the facility are shown. A metallic bellow is immerged into a water tank and separates the pressurized air from the pressurized water in the tank to avoid any air absorption in the degassed water. To control the water pressure in the water tank, the metallic bellow is

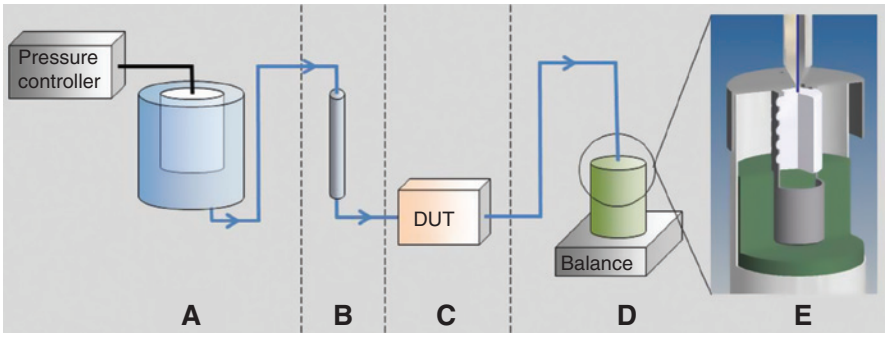

Figure 2: Simplified drawing of the working principle of the facility at METAS.
(A) Water tank with immerged metallic bellow and pressure controller, (B) capillary tube, (C) DUT, (D) measurement beaker on the balance, and (E) detailed cross section of the top part of the measurement beaker.

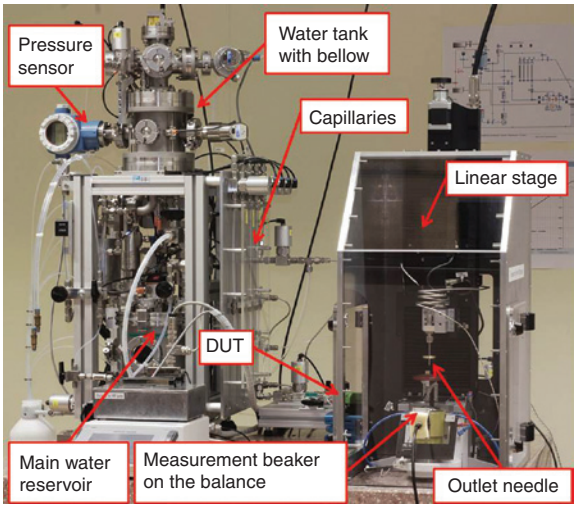

Figure 3: Photograph of the METAS facility with the main components: water tank with metallic bellow, capillary tubes, DUT, measurement beaker on the balance, main water reservoir.

expanded or compressed by adjusting the air pressure inside the bellow with a pressure controller. The air pressure inside the bellow is adjusted according to the signal of a pressure sensor inside the water tank in order to reach the desired relative water pressure in the tank. The stability of the water pressure is guaranteed by the fact that at a constant time interval of several tens of seconds, the target pressure is readjusted by the pressure controller by means of a regulation loop controlled by software. The constant pressure drop from the water tank to the atmospheric pressure at the outlet needle and the size of the capillary tube determine the flow rate and guarantee the steadiness of the flow rate once the stabilization time for a given flow rate is reached. Therefore, the flow rate can be set by increasing the water pressure in the water tank up to 2.5 bar and by selecting the appropriate capillary from the set of four capillaries with the inner diameters of 50, 80, 150, or 2000 µm and a length of 200 mm.

The installation is filled with degassed ultra pure water. To hamper growth of bacteria and algae, we added 50 mg of sodium azide to 2 l of ultrapure water, which does not lead to a measurable change in the water density. Additionally, we perform regularly flushing cycles through the various tubing and treat the water with UV light. To continuously degas the water in the piping, we use the flushing cycles where the water flows in closed loops from the water reservoir through the various piping before reaching the water reservoir again. In the water reservoir, a negative pressure of 160 mbar with respect to the average atmospheric pressure is maintained. The water is pumped through the piping and the pressure-reducing valve prior to the water reservoir allows increasing the water pressure in the piping up to 1 bar overpressure. As the water flows through the pressure-reducing valve and enters the water reservoir, the drop in pressure degasses the water.

Additionally, each time we connect a DUT to the facility, we have to make sure that the air inside the DUT is flushed out. Close to the connections of the DUT, we have a purging system with CO_2 where we first flush the DUT with CO_2 and direct the CO_2 out of the piping into the waste. We then flush this part of the piping with water and direct the water out of the piping into the waste. As the connections to the DUT are now full of water, we can switch to the bypass and flush the piping with flushing cycles to remove any residuals of CO_2 or air. The same procedure can be performed for the flushing of the outlet needle, which is not included in the flushing cycles. The outlet needle can be flushed with CO_2 first and then with water. To remove any residuals of CO_2 in the connections of the inlet pipe of CO_2, another flushing cycle can be done. The connections for the DUT offer also the possibility to connect a flow generator and to flush the piping in a similar way.

Control of evaporation

An important issue is the evaporation rate of the water in the beaker during the measurement. To control this process, we apply the conventional method of adding water in an evaporation trap in the weighing zone (blue-colored water in Figure 4) to saturate the air with humidity. To avoid any condensation on the outlet needle, the degree of humidity is regulated by two small holes at the top of the weighing zone housing, which are connected to tubing of 1 m length acting as humidity exchanger. Additionally, we have built a special measurement beaker as drawn in the detailed cross section in Figures 2E and 4. The outlet needle is generally positioned 50 µm above the glass filter (white cylinder) where the water enters the measurement beaker. The capillary force in the glass filters sucks the water in before any droplet can be formed at the surface. The water flows through the glass filter and continues in the green water-absorbing foam into the bulk. This prevents the water from being at the surface. With this setup, we get stable evaporation rates of 3.2±0.5 and 3.4±1.0 µg/min depending on the porosity of the glass filters used. These evaporation rates are larger than for the case where the water is covered by a thin layer of oil. However, the advantage is that the outlet needle is only connected to the measurement beaker *via* a water stream. The water stream is like a water bridge that induces a capillary force that remains nearly constant in time and is therefore only a minor influence on the relative increase of the measurement beaker's weight. Any sharp change in the capillary force is detected by the weighing data.

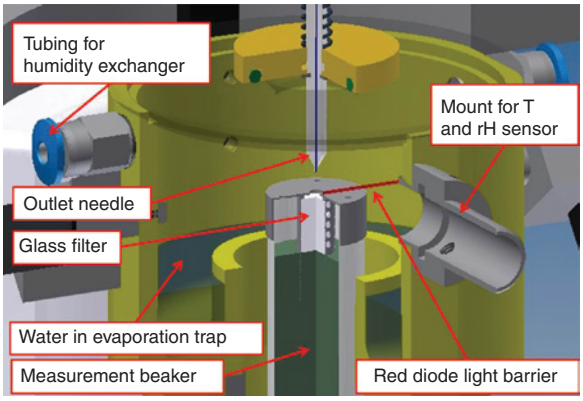

Figure 4: METAS facility.
Cross section of the weighing zone with its evaporation trap filled with water and the holes at the top of the weighing zone housing acting as humidity exchanger. A red diode light barrier is used to detect any water overflow caused by clogging in order to stop the measurement.

Dynamic gravimetric weighing

The measurements are performed by means of the gravimetric dynamic method. This means that the desired flow rate is set and data acquisition is only started once the flow rate has reached a steady state. Therefore, the measurement beaker is continuously filled with water and the weighing data are continuously collected with the time stamp of the balance at an acquisition rate of 10 Hz. The other sensor values such as water pressure upstream and downstream of the DUT, the water temperature at various positions, and the ambient conditions are recorded as well.

Determination of flow rate

The collected weighing data are then fitted by means of the "trust-region Levenberg-Marquardt algorithm" [26], where the uncertainties in the weighing data as well as the time measurements are taken into account. To perform the fit, we use the Software IGOR Pro and the fitting function "orthogonal distance regression (ODR)" [22, 26].

Flow rate determination is done by first choosing a fixed time window and performing an ODR linear fit on the collected data. The resulting mass flow rates are then converted to volume flow rates including several corrections. The time stamp of the determined volume flow rate of each fixed time window is the center time of these data. This guarantees that any strong change in the flow rate is detected at the occurring time independently on the chosen fixed time window. By increasing the starting time of this fixed time window by time steps corresponding to the acquisition rate of 10 Hz, we can follow the evolution of the flow rate in time. Applying this to the collected weighing data, we get the evolution in time of the volume flow rate and its stability over time [9, 10].

Primary standard at CETIAT

According to its mission of national reference laboratory, CETIAT (Villeurbanne, France) completed the construction and validation of a calibration laboratory based on the gravimetric method to cover the flow rate range from 17 to 167,000 µl/min, with uncertainties from 0.6% to 0.1%.

Design of the facility

A calibration bench at low liquid flow rates, such as CETIAT's facility, requires the control of both process and environnemental parameters. The water flowing through

the system is demineralized at first and degassed in a reservoir to avoid deposit formation and bubbles in the circuit. Water temperature can also be adjusted and stabilized from 10°C to 50°C. Then, the water is transferred to a second reservoir where nitrogen bubbles are blown to perform air stripping. Finally, the water is transferred to a third storage reservoir (Figure 5).

The measuring line is placed in a clean room to ensure stable environmental conditions (temperature and humidity) and the prevention of particulate pollution (Figure 6).

The flow is generated from a finely regulated tank pressure from 0 to 10 bar by a metal bellow. The water temperature is adjustable from 10°C to 50°C. The tank is placed in a thermostatic chamber to maintain the water at the chosen test temperature (Figure 7).

The device to be calibrated is also installed in a thermostatic chamber to maintain the environmental conditions close to working conditions (adjustable between 10°C and 50°C).

The flow is adjusted by a set of eight capillaries operating under laminar flow with a diameter between 100 and 500 µm. Only one capillary is chosen for a given flow point. The choice of the upstream pressure/capillary couple determines the flow point generated in the system. The stability of this flow depends on the temperature stability of the entire line, especially in the capillaries. The measurement line is kept at 20°C by heat exchangers, and capillaries are immersed in a bath thermostatically controlled at 20.00°C±0.01°C (Figure 8).

Figure 7: CETIAT facility.
The water tank in the thermostatic chamber.

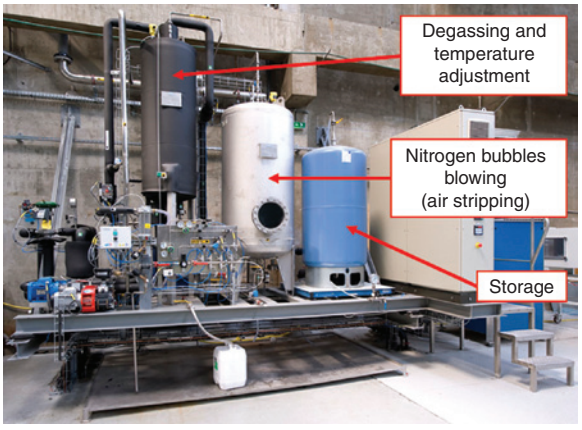

Figure 5: Photograph of the water preparation setup at CETIAT.

Figure 6: CETIAT clean room with the measuring line.

Gravimetric method and evaporation

The amount of water flowing through the meter during the test time is determined by four precision balances placed on a marble support structure (decoupling frequency at 3 Hz) with weighing ranges between 0.5 and 500 g depending on the flow rate (Figure 9). The weighing system was designed to remove the effect of falling droplet, which is very critical at small flow rates. A saturator was placed around the weighing tank to limit the effects of evaporation. A calibration point (mass over time measurement) is fully automated and the whole calibration process is software controlled.

Primary standard at IPQ

IPQ developed a primary standard to cover the flow rate range from 0.05 µl/min to 10 ml/min, with uncertainties from 0.6% to 0.15% [4]. The measurement times vary from 15 to 20 min.

Design of the facility

Two different systems were built depending on the measurement range. For flow rates from 0.05 µl/min up to 0.33 ml/min, the setup uses a balance with a maximum capacity of 20 g and a resolution of 0.001 mg (Figure 10).

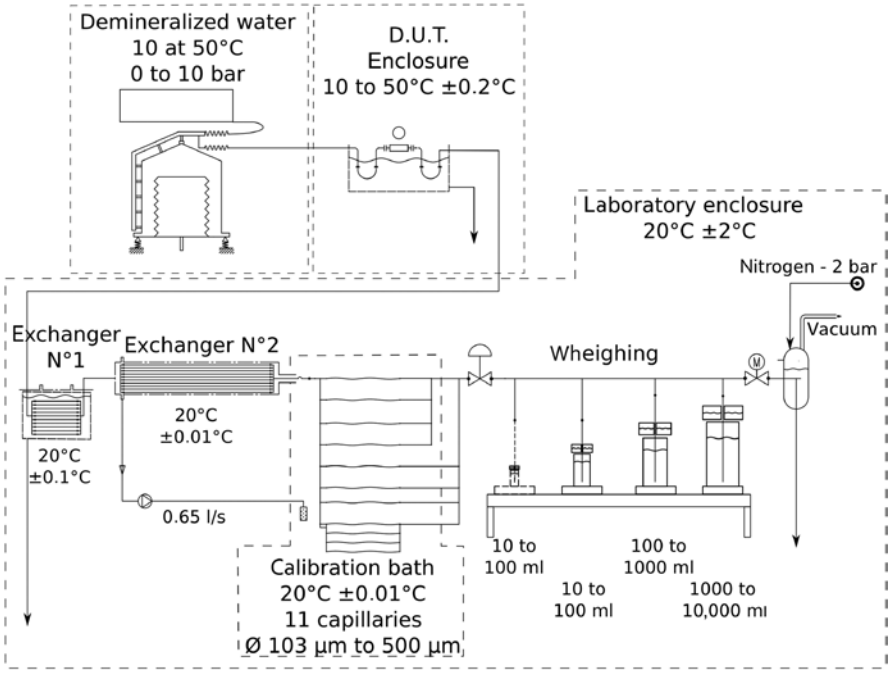

Figure 8: Schematic of the working principle of the facility at CETIAT.

Figure 9: The weighing section of the CETIAT facility inside the clean room.

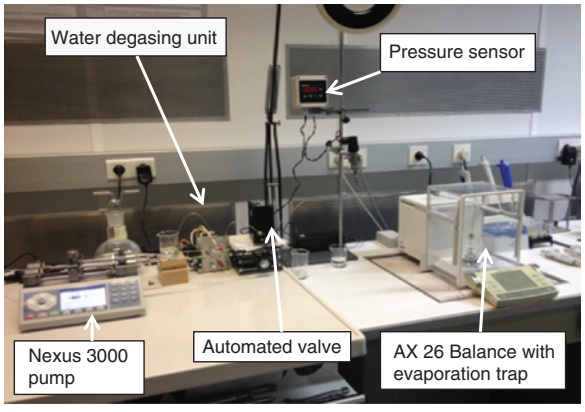

Figure 10: The IPQ facility using a balance with a 20-g maximum capacity for flow generator calibration.
All tubings are 1/16-in stainless steel.

For flow rates from 0.33 ml/min up to 10 ml/min, the setup uses a balance with maximum capacity of 300 g and a resolution of 0.1 mg (Figure 11). Both balances are installed in vibration-free tables, in the same ambient control room where the calibrations are performed at 20°C±3°C with a relative humidity above 50%. The pressure inside the tube is measured by an absolute pressure sensor from 0 up to 5 bar. The water temperature is measured in the beginning and at the end of each measurement. An average value and its uncertainty are used for flow determination.

The flow generator is a syringe pump that has two syringes working in parallel. Several types of syringes (glass and stainless steel) with different capacities (0.1–100 ml) can be used depending on the flow rate and accuracy needed.

The system is prepared to use any type of liquid, but normally, the calibrations are performed with ultrapure, degassed water. An inline vacuum membrane degasser removes dissolved gasses from the water prior to the filling process of the flow generator. All parts of the system are connected with stainless steel tubes (1/16 or 1/8 in), and the flow direction is controlled by a distribution valve (automatic or manual). The tube is immersed into the water in the balance collecting glass vessel to avoid droplet formation

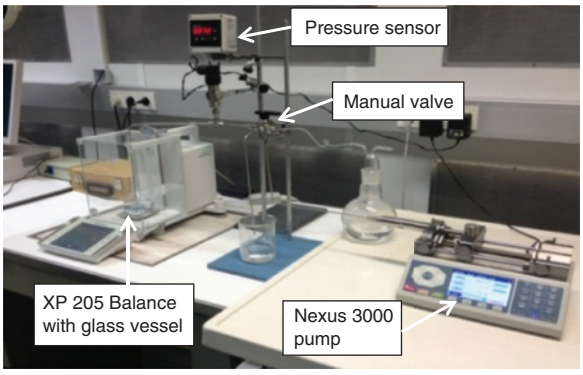

Figure 11: The IPQ facility using a balance with 300-g maximum capacity for flow generator calibration.
All tubings are 1/8-in stainless steel.

and allow a continuous reading of the flow rate in time. To remove any remaining air, the system is purged before starting the measurements. When the syringe and all tubes are full with the calibration liquid (water), the measurements can start. In case of flow meter calibration, the setup is changed to install the meter after the pressure sensor. The acquisition routine is started and the flow rate is directly calculated with the dedicated developed procedure.

Determination of flow rate

To minimize and control evaporation of water in the vessel, an evaporation trap was installed around the vessel on the balance. The measured evaporation rate (Q_{evap}) with its uncertainty is taken into account as a correction term in the volume flow rate equation (1). Other contributions to the model used to determine the volumetric flow rate (Q) are the density of the liquid (ρ_W), final time (t_f), initial time (t_i), final mass (I_f), initial mass (I_i), air density (ρ_A), weights density (ρ_B, corresponding to 8000 kg/m^3), expansion coefficient of the material used in case of syringes (γ), and water temperature (T).

$$Q=\frac{1}{t_f\text{-}t_i}\left[((I_f\text{-}I_i)\text{-}(Qm_{buoy}))\times\frac{1}{\rho_W\text{-}\rho_A}\times\left(1\text{-}\frac{\rho_A}{\rho_B}\right)\times[1\text{-}\gamma(T\text{-}20)]\right]+Q_{evap} \quad (1)$$

Data acquisition

A data acquisition system was developed using LabView. Different modules were developed to automate the acquisition, validation, and online visualization of measured data, statistical treatment, and uncertainty calculation. The data acquisition is done directly from the balance every 50 ms and the measurement of time is done simultaneously.

Uncertainty

The main standard uncertainties considered for IPQ's setup are mass measurements (m), density of the mass pieces (ρ_B), density of the water (ρ_W), density of the air (ρ_A), evaporation rate (δQ_{evap}), water temperature (T), time (t), expansion coefficient (γ), standard deviation of the measurements (δQ_{rep}), and buoyancy on the immersed dispensing needle (δQ_{mbuoy}). Detailed information regarding the uncertainty calculation and validation using the Monte Carlo method is described in [3].

Primary standard at DTI

At the Danish Technological Institute (DTI), the newly established primary standard covers a flow range from 0.017 µl/min to 1.6 ml/min, with uncertainties from 5% to 0.05% and calibration time from 75 to 10 min. The microflow laboratory at DTI is accredited to a flow rate from 17 µl/min to 10 ml/min, with uncertainties from 4% to 0.05%.

Design of the facility

The setup shown in Figure 12 covers a large range of flow rates, but the very-low-range gravimetric flow calibration has been considered too demanding due to the high evaporation of 2–3 mg/h/cm^2 and due to surface forces that become dominant in this flow rate range. Therefore, to be able to measure in the microflow range, e.g. below 1.6 ml/min, it is necessary to limit the evaporation first. At the lowest flow rate, the evaporation is two orders of magnitude higher than the flow rate itself if left untreated. At DTI, a liquid oil cover is used as an evaporation trap, which is very efficient, as only an evaporation rate of 0.009 µl/h is remaining. However, the water needs to be delivered below the oil surface through the outlet pipe, which leads to a number of challenges, e.g. capillary forces, buoyancy, inertia, stiction and friction, absorption and adsorption, stick and slip, and vibration transfer. To limit these effects, the outlet pipe is made of 1/32-in stainless steel tubing; the

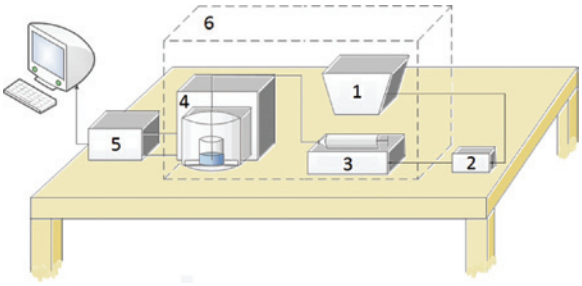

Figure 12: DTI facility.
Setup, where the DUT is a syringe pump. Water is led from the reservoir (1) through the degasser (2) and into the syringe pump (3). From the syringe pump, water is led to the balance (4) using stainless steel tubing. The outlet tube is traversed through the oil-based layer and into the water in the beaker. The balance is connected to balance electronics (5) and to a PC. The setup is enclosed in a chamber (6).

surface is coated to become oleophobic, and this tube is fixed above the beaker. To further equalize any temperature effects, a tube in shell heat exchanger is mounted as part of the piping. This enables a more stable flow as well as makes the density and buoyancy corrections more accurate. The measurement is dynamic, which means that each mass measurement (10 Hz) is time stamped and the increase in mass is fitted by means of Deming regression [21] (ODR linear fit) in order to get the actual flow rate as a function of time.

To get a stable foundation for the weighing, the balance is placed upon a vibration-free base. The measuring beaker used is thoroughly cleaned and acclimatized to the balance. The beaker is filled with demineralized water until a level of about 5 mm using a dispenser pipette. On top of the water, a 5-mm oil-based cover is laid to prevent or minimize evaporation. Demineralized water is filled into a reservoir (ultrasonic bath) and then led through an inline vacuum membrane degasser. If the DUT is a flow meter, then the demineralized and now degassed water runs through a pulsation-free pressure-driven pump and into the DUT. If the DUT is a syringe pump, the water is led directly to the syringe. The water exits the DUT and is lead to the scale *via* stainless steel tubing. The outlet tube is moved down and through the oil-based cover and into the water in the measuring beaker wherein the water is pumped.

The balance is placed in a custom-built isolation chamber. The goal is to keep both temperature and ambient pressure constant inside the chamber and thus minimize draft and convection effects. The chamber is made of aluminum and has a window front to get access to the chamber and to get a clear view during operation.

Dynamic gravimetric weighing

The balance is connected to a computer, enabling measurements with a frequency of 10 Hz and traceable time stamps with a dedicated timing card. By having continuous readout, it becomes possible to detect the actual flow rate as a function of time in contrast to the case of static weighing where the delivered mass is measured and divided by elapsed time. The resolution of the balance is 1 µg, the output stability is below 10 µg, and the 99.9999% step response time is 0.5 s.

Uncertainty

The dominant uncertainty contribution (>90%) for the setup comes from the uncertainties of the corrections for the forces between the outlet pipe and the two liquid layers (water as well as oil) in the measurement beaker. The measurement uncertainty at the flow rate of 10 ml/h is approximately 0.5% (k=2). Below flow rates of 300 µl/h, the balance uncertainty and the uncertainty of the correction for buoyancy become dominant (90% at 5 µl/h), whereas the level of water rise within the beaker becomes insignificant (about 20 µm). The measurement uncertainty rises to about 5% (k=2) at the lowest flow rate of 1 µl/h.

Primary standard at VSL

VSL, the Dutch National Institute, developed a water flow facility to complement the nanoflow generator as described in [20, 25]. The facility described here is named µFlow and is a dynamic gravimetric flow standard. It was built to cover a flow range of 1–1000 g/h with an uncertainty goal of 0.1% for the highest flow rates and up to 0.2% at the lowest flow rate. After completion of the setup and the accompanying uncertainty budget, it was found the flow rate range can be expanded down to 4.2 µl/min and up to 83.3 ml/min with uncertainties of 0.05–0.15% (333 µl/min–83 ml/min) and 0.15–1.0% (4.2–333 µl/min).

Design of the facility

The flow is controlled by means of Coriolis-based mass flow controllers (CMFCs). These CMFCs receive a digital set point from the software and then generate this flow rate by adjusting the regulating valve using piping and instrumentation diagram (PID) algorithms. At the output, the

CMFC is fitted with an additional shutoff valve because a regulating valve cannot be considered leak-free. From this shutoff valve, numbered 1 and 4 in Figure 13, a connection is made to a DUT (see Figure 14). At the end of the DUT, temperature and pressure are measured before the flow is directed to the three-way ball valves. Per balance, there are 2 three-way ball valves. The first valves (numbered 2 and 5 in Figure 13) direct either toward the reservoir for flushing and priming or toward the second three-way ball valve (numbered 3 and 6). This second three-way ball valve selects the function for the balance, which can be either measuring by connecting the immersion tube to the output from the DUT or draining the measurement beaker on the balance by connecting it *via* the return line to the vacuum reservoir. All valves are pneumatically actuated to avoid heating effects usually associated with electrically driven valve actuators.

The flow rate is generated from a supply of demineralized degassed pressurized water. Demineralized water is prepared by means of a standard industrial resin ion exchange cartridge to a conductivity of <50 µS/cm. This water is stored in a stainless steel tank where a vacuum of 23 mbar is applied to bring the water near the boiling point at room temperature and thereby remove any dissolved gas from the water. A small gear pump increases the pressure up to 5 bar into an elastomeric bladder inside a pressurized vessel. An electronic pressure controller regulates this pressure, thereby creating a similar and constant pressure inside the bladder. Further treating of the water is limited to filtering the water before the CMFCs and refreshing the entire volume of water regularly.

Evaporation

Evaporation is handled by covering the measurement beakers with a lid with a hole just large enough for the immersion tube, without touching the lid or the beaker at

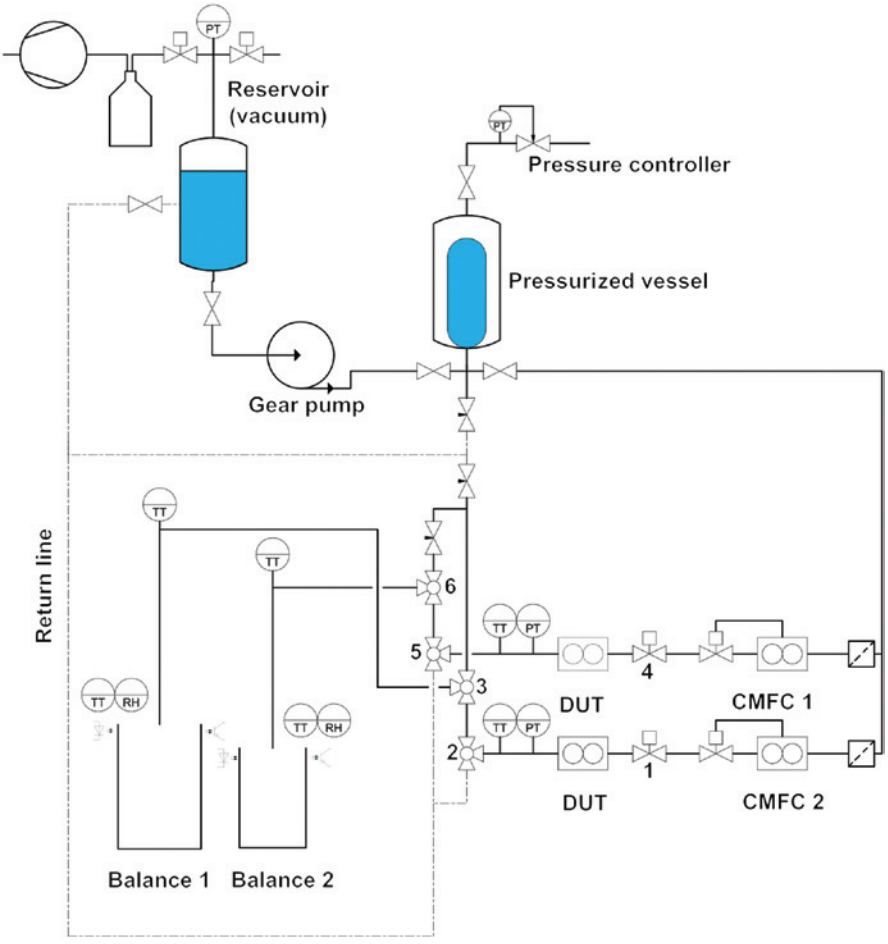

Figure 13: Simplified piping and instrumentation diagram of the VSL µFlow facility.
(Top) Water preparation with the vacuum degasser, pump, and pressurized vessel. (Bottom, right to left) CMFCs, location for a DUT, switching three-way valves, and the two balances.

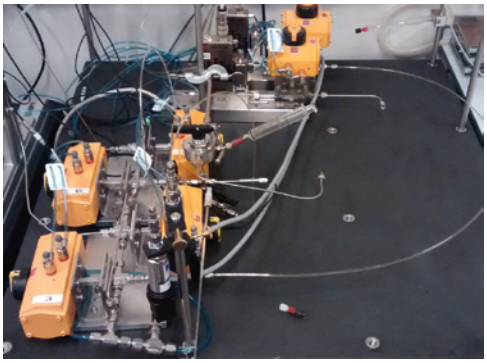

Figure 14: VSL facility.
The support table for the DUT along with the CMFCs (top) and the three-way valves (left).

any point. For the small balance, this is a machined PVC lid with an elastomeric seal between the lid and the beaker, whereas for the larger balance, this is a PMMA plate with a hole and three bolts to prevent it from sliding off. These lids greatly reduce the ventilation of the beakers, which results in an atmosphere with very high humidity above the water, which will reduce evaporation. The measured humidity exceeds 98% RH, and at various places, condensation is observed. Locally, however, the humidity can be lower, such as near any opening in the lid. At these places, condensation will not occur, and hence, no water bridge will be formed between the lid and the immersion tube, as can be seen on the left balance in Figure 15. Such a water bridge would exert a force of unknown magnitude, direction, and stability. The result of these evaporation lids are evaporation rates of 0.53±0.005 µl/min for the large balance and 0.02±0.0003 µl/min for the small balance.

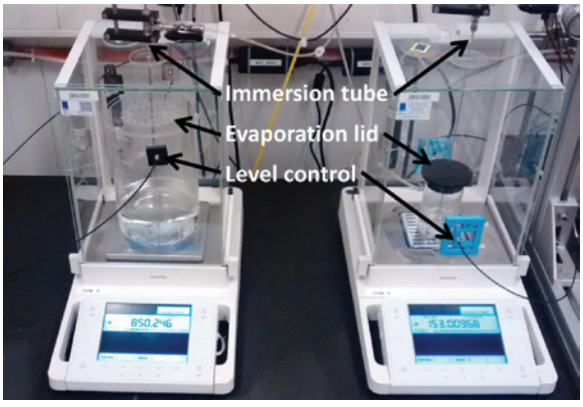

Figure 15: VSL facility.
Two precision balances on the granite support table. The level control is an optical detection of the water level to avoid overflowing of the beakers.

Immersion tube and corrections

Owing to the immersion of the immersion tube, a number of forces will act on the immersion tube, most notably the buoyancy force and a surface tension force. Most models that cover these forces are unclear on limitations, scale, and uncertainty of the model; therefore, we decided to abandon theoretical models and rather measure the combined forces on the immersion tube. This is done by mounting the immersion tube on a vertical translation stage and increase its immersion depth in repeated steps, applying a waiting time after each step. For example, for the large balance, this was done in steps of 25 mm with a speed of 0.026 mm/s over a length of 150 mm. After each step, the tube is held stationary for 30 min. This speed corresponds to a flow rate of approximately 1000 g/h. We get a step function where each upward step of the weight corresponds to the increase of the immersion depth by 25 mm. During the waiting time, a slow decrease due to evaporation is observed. By means of this step function, we can determine the tube immersion correction factor in units of mass over length. To get a completely representative correction with accompanying uncertainty, these measurements are performed for both balances at three flow rates with 10 repetitions for each flow rate. This results in correction factors for the immersion tubes K_{tube}, which are equal to $K_{tube,225s}$=2.4±1.1 g/m and $K_{tube,2203s}$=7.9±1.1 g/m. The magnitude of this correction only depends on the submerged depth of the tube. The dependency on immersion speed (proportional to the flow rate) and the repeatability are, among other things, included in the uncertainty.

Dynamic gravimetric weighing

VSL performs a dynamic gravimetric measurement with request-send balance readout. This means that we measure changes in the flow rate at a sample rate of up to 10 Hz. However, in the case of long measurement times, often in the order of hours, it makes little sense to gather tens of thousands of data points per hour. Instead, in such cases, the software will only request a readout when needed, while maintaining the timing uncertainty associated with a 10-Hz sampling rate. This is done by measuring the time difference between requesting the reading and receiving the reading. This delay is typically 77 or 96 ms.

The collected weighing data are fitted by means of linear least squares following the procedures described in [2]. The fit window is chosen based on visual inspection of the flow rate stability and is typically in the order

of 5–10 min. A standard low-order polynomial (order 2 or maximum 3) is used to fit the data. Standard Matlab routines are used to perform the fitting. Corrections for evaporation, immersion tube forces, etc. are applied to the result of the fit to obtain the correct flow rate.

Primary standard at Bronkhorst

The company Bronkhorst developed a primary standard to cover calibrations in the range of 1–200 g/h mass flow of water (17 µl/min–3.3 ml/min) with an uncertainty between 0.62% and 0.06%. Measurement times range from 20 to 2 min, respectively. This setup can also be used for calibrations in the range of 125 mg/h–1 g/h mass flow (2–17 µl/min) with an uncertainty between 5% and 0.62%. The measurement time for this range is 20 min. All uncertainties are only valid for stable flow. By "stable flow", we mean a mass flow that is constant within a predefined criterion for several minutes or more. This criterion equals 0.2% relative deviation (RD) or the combined uncertainty of the setup and the DUT at the actual flow rate, whichever is larger. The stability of the flow is constantly checked using this stability criterion. If the stability criterion is not met during the calibration cycle, calibration is automatically stopped and restarted when the stability is restored.

Design of the facility

The method is gravimetric and mass flow is determined using the flying method, which is explained below. The most important feature of the setup is that a stable flow is generated using the DUT as the flow controller, which controls a piezoelectric valve.

Figure 16 gives a schematic overview of the setup, and in Figure 17, a photograph of the setup is given. The piezoelectric valve is placed in front of the DUT to have a flow path between the DUT and the balance without active

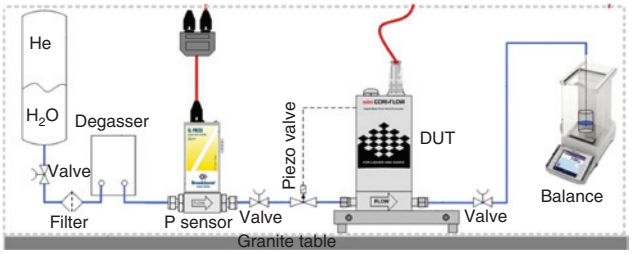

Figure 16: Schematic overview of the gravimetric standard at Bronkhorst.

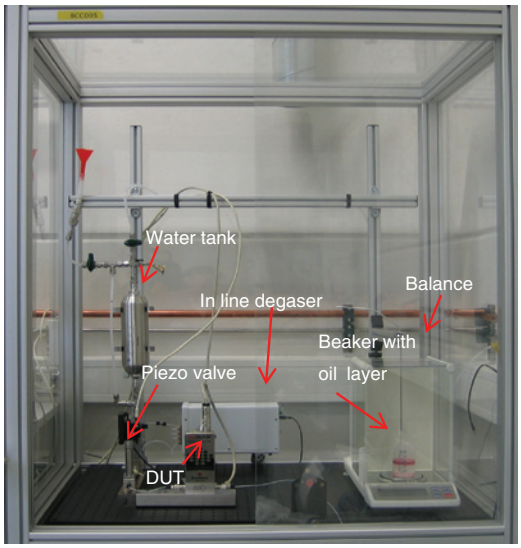

Figure 17: Photograph of the primary standard at the Bronkhorst calibration facility.

elements that might introduce discrepancies between the DUT and the balance. The medium that is used is extra pure deionized water that is subsequently filtered (0.5 µm pore size) and degassed. The water is placed inside a 750-ml stainless steel tank THAT is pressurized at 5.8 bar using helium.

Before a calibration or measurement is started, the setup is prepared to ensure a stable and pure liquid flow. By "pure liquid flow", we mean a water flow without particles, i.e. larger than 0.5 µm, and with a minimal amount of gas. The preparation is done by flushing the setup for several minutes by fully opening the piezoelectric valve and all other valves in the system before the DUT. Then the DUT is connected and flushed together with the tubing between the DUT and the balance by again fully opening the piezoelectric valve. The stability of both the DUT and the balance is checked manually, and when a stable flow is reached, the calibration or measurement can start.

The water that flows toward the balance is collected in a beaker *via* a small stainless steel tube with its outflow always under the water surface. To minimize evaporation of water, a layer of oil is placed on top of the water.

Dynamic gravimetric weighing

The primary standard at Bronkhorst uses a high-precision balance as mass flow reference. This is implemented by differentiating its measured mass (Δm) to measured time (Δt). The sample time equals 100 ms and the calculated

mass flow is filtered using a 60-s moving average. The resulting output is mass flow ($\dot{m}$), as shown in Eq. 2.

$$\dot{m}=\lim_{\Delta t\to 0}\frac{\Delta m}{\Delta t}=\frac{m_r}{t_r}, \quad (2)$$

where m_r and t_r are the reference mass and reference time, respectively. An RS-232 data interface is used between the balance and data acquisition. This interface combines each mass sample with the correct time sample, resulting in a mass flow. This way, the flow of the DUT can be directly compared to the flow indicated by the balance. The uncertainties on mass and time of the balance are known and, together with the uncertainty in the applied corrections, are used to determine the total uncertainty of the setup.

Summary of the different experimental facilities

All the gravimetric primary standards described above are different in their construction. To illustrate this, we have summarized the flow generator, the water collection methods in Figure 18, and the characteristics in Table 1.

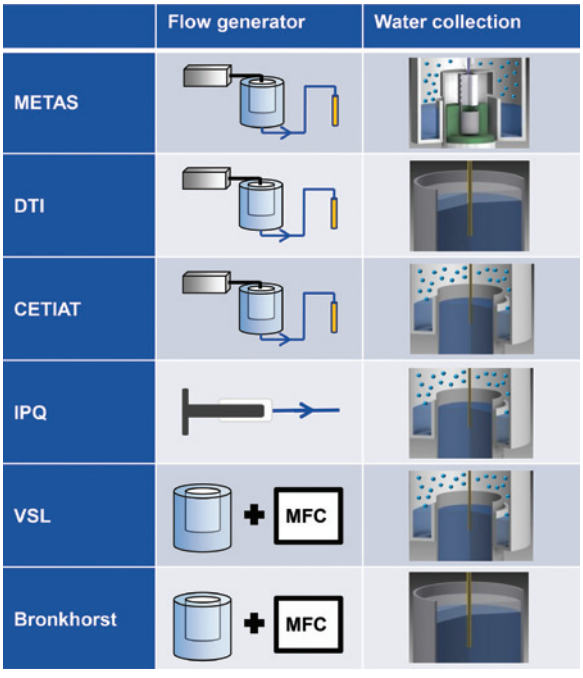

Figure 18: Summary of the main technical components at the introduced facilities.
Three different flow generators and three different water collection methods are used. Details are described in the text.

There are three different flow generators. The first is pressurized water in a vessel where the flow is generated and controlled by means of a pressure drop over a capillary (METAS, DTI, and CETIAT). The second flow generator used is a commercially available syringe pump (IPQ). The third uses pressurized water in a vessel where the flow is controlled by a mass flow controller and the corresponding pressure reduction valve (VSL, Bronkhorst).

The flow rate is determined by collecting the water in a beaker on the balance where the increase of mass is recorded as a function of time and several corrections are applied. To avoid droplet formation and to control the evaporation rate of the water, which is an important issue at very low flow rates, three different methods are applied. METAS uses the water bridge from the outlet pipe to water-absorbing materials in a beaker, which is surrounded by saturated air. DTI and Bronkhorst immerse the outlet pipe into the water in the beaker and covering the water with an oil layer to prevent the water from evaporating. CETIAT, IPQ, and VSL immerse the outlet pipe into the water in the beaker, which is surrounded by saturated air.

Main sources of uncertainties

A detailed analysis of the total expanded uncertainty of each facility is beyond the scope of this article. However, we can list the main influence factors as follows:

- mass measurement for the flow rate determination
- environmental temperature, pressure, and relative humidity
- temperature and pressure variations in buffer volume between DUT and outlet needle
- water density
- air density
- buoyancy correction factor of the beaker and the water filled into the beaker
- buoyancy correction factor of the effect of the immersed outflow pipe into the water, if applicable
- convection
- evaporation of water through the tubing, if plastic tubing is used instead of stainless steel tubing
- evaporation from the measurement beaker
- time measurement
- flow rate stability
- presence or formation of air bubbles in the system and their change in size over time

Additionally, there are a number of minor uncertainty components that should be determined but are usually not

dominant on the overall measurement uncertainty when handling flow rates described in this paper:changing buoyancy corrections due to changing air density

- jet force: the resulting force due to the speed at which the water leaves the outflow pipe
- Lastly, for the determination of the deviation of a DUT, there are a number of contributions that are specific for the DUT:
- resolution of the set point or output signal
- repeatability

The mass measurement using weighing cells or microbalances have, aside from a calibration uncertainty, further uncertainties from the resolution, nonlinearity, repeatability, reproducibility, sensitivity to eccentric loading, zero point stability, response time, and to a lower extent, density of the mass standards and drift since last calibration.

The environment of the calibration setup should be controlled or at least the values recorded to correct for any considerable change in the environmental conditions. The following parameters can cause errors in the mass determination: temperature, humidity, pressure, air density, and spatial or temporal environmental gradients.

The medium (calibration liquid) is typically (pure) water but can also be another fluid. The medium density and temperature should be considered as well as its evaporation rate. The fluid temperature affects the density and viscosity of the liquid. The fluid density is required to determine the buoyancy as well as to convert from mass flow to volume flow. It is a good practice to measure the density of the liquid. A temperature difference between the liquid and the surrounding air will also produce a convection draft.

Changes in buoyancy during calibration of both the offset masses (e.g. the vessel or internal moving parts of the balance) as well as of the medium will affect the mass measurement.

The outflow pipe connection to the balance should be considered. Capillary forces, intermediate interaction, and buoyancy correction due to tube contact or tube immersion into the measurement beaker need to be properly taken into account. Also, the increasing water level causes an increasing hydrostatic pressure, which affects the stability of the flow rate.

The evaporation rate should be determined to make the appropriate corrections. The uncertainty associated with this evaporation correction should be taken into account.

The calibration time with respect to the beginning and the end of the flow measurement for the reference and the DUT needs to be simultaneous. The determination of the time stamp that goes with each individual mass reading is also a cause for uncertainty. Without detailed knowledge of the inner workings of the balance, it is not possible to know how much delay there is between the measurement of the balance and the output of the reading. In a similar way, it is not possible to know how much this delay changes.

The flow generator might produce flow pulsations. Furthermore, the flow rate may drift over time. This should be taken into account when the effect on the DUT is not known.

The buoyancy correction of the vessel with the liquid inside might become more important at very low flow rates and if the air density is changing a lot over the measurement period. This will occur if the weight of the vessel with the liquid inside is much larger than the collected water. In this case, an online correction is recommended.

The jet force due to Newton's third law will get dominant at higher flow rates and the contribution has to be evaluated. There are ongoing projects at some microflow laboratories to experimentally quantify this effect.

It has to be noted that the rank of each contribution varies considerably depending on the flow rate and the details of each laboratory. For example, the presence of the saturator on the beaker highly reduces the contribution of evaporation in the uncertainty budget. Likewise, the configuration of the capillary in the beaker (immersed or not) affects the buoyancy corrections and associated uncertainties.

Validation of the measurement uncertainties

After evaluating these uncertainty sources and defining the measurement uncertainty according to the *Guide to the Expression of Uncertainty in Measurement* [8] for the whole flow rate range, it is of common metrological practice to verify the stated measurement uncertainties. This is done by means of an intercomparison with other flow standards from several NMIs. A well-characterized flow meter is used as a transfer standard. All participants calibrate the flow meter. These intercomparisons are organized within the regional metrology organizations (RMOs) [6], and one of the main roles is to support mutual confidence in calibration and measurement capabilities (CMCs) of the participating NMIs (CIPM-MRA) [5]. The validated CMCs, which consist of the flow rate range with the

Table 2: The degree of equivalence of each laboratory for the calibrations of the flow meters (FM) and the syringe pump (SP).

	Flow rate	BHT		CETIAT		DTI		IPQ		VSL		METAS	
FM (g/h)	SP (µl/min)	FM	SP	FM	SP	FM	SP	FM	SP	FM	SP	FM	SP
(0.12)	2		0.07		0.83		0.20		0.72		0.05		0.36
^{1}0.5	8	0.43		0.86		0.75		0.09		0.57			
(0.6)	10		0.06		0.32		0.08		0.69		0.01		0.31
^{1}2	33	0.51	0.01	0.68	0.45	0.60	0.09	0.70	0.89	0.34	0.74	0.15	0.92
^{1}6	100	0.48	0.08	0.47	0.27	0.30	0.03	0.68	0.08	0.29	0.49	0.40	0.47
^{1}20	333	0.30	0.04	0.74	0.87	0.14	0.03	0.61	0.08	0.26	0.16	0.31	0.81
^{1}60	(1000)	0.57		1.07		0.45		0.21		0.06		0.53	
^{1}200	(3333)	0.61		2.63		0.92		0.29		1.01			
^{2}200	(3333)	1.08		0.67		0.42		0.73		0.44			
^{2}600	(10,000)			1.07		0.39		0.99		0.29			

In the column of the flow meter, index 1 refers to the first flow meter and index 2 refers to the second flow meter. The flow rates without parentheses are performed points; flow rates between parentheses only give the equivalent of the volumetric or mass flow rate.

corresponding uncertainties, are then published in the BIPM key comparison database [7].

Research intercomparison results

To validate the gravimetric setup, a research intercomparison (EURAMET project 1291 [15]) was organized within the EMRP project HLT07-MeDD [19]. The aim was to validate the results and uncertainties stated by the different participants for the flow rate range from 2 µl/min to 10 ml/min. According to a technical protocol, two transfer standards [11] have been calibrated by each laboratory. The flow meters were sent around with 1/8-in stainless steel pipe connection fixed upstream and downstream to the flow meter, where fast connecting valves were used to easily plug or unplug 1/8- or 1/16-in tubing. The flow meters were fixed on a mass block to reduce the influence of vibrations.

The first Coriolis flow meter has been calibrated at 2, 6, 20, 60, and 200 g/h (33.3, 100, 333, 1000, and 3333 µl/min). The second Coriolis flow meter was calibrated at 200 g/h (3.3 ml/min) and 600 g/h (10 ml/min). Calibrations were performed at ambient conditions using the individual procedures and the flow generators of each participant. Each participant performed the calibrations of the flow rates, which are covered by their facility according to the Table 1. In a second part of the intercomparison, a commercially available syringe pump has been calibrated in the flow rate range from 2 to 333 µl/min. These data are not shown in this report, but all the results are reported in EURAMET project 1291 [15]. Nevertheless, the degree of equivalence is also shown in Table 2.

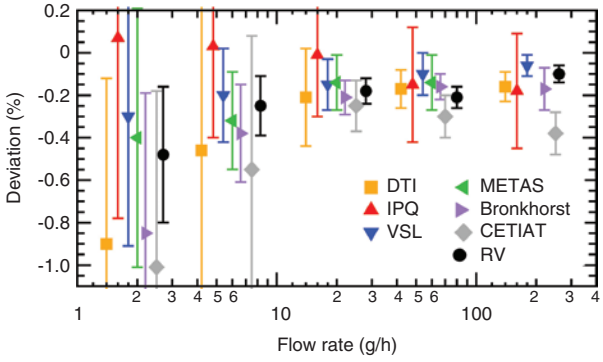

Figure 19: Calibration results of the first transfer standard from each participant.
For each flow rate, the results of the several laboratories are drawn with an artificial offset in the flow rate value for a better readability. Calibrated flow points are 2, 6, 20, 60, and 200 g/h (33.3, 100, 333, 1000, and 3333 µl/min, respectively).

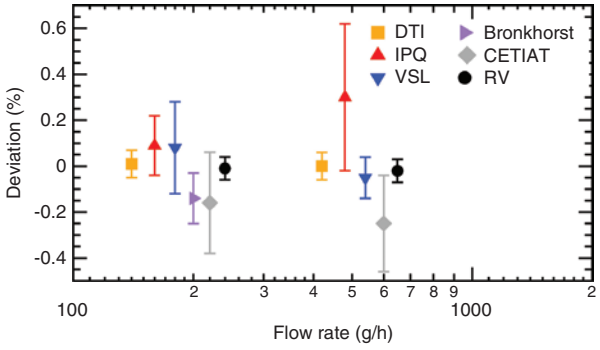

Figure 20: Calibration results of the second transfer standard from each participant.
For each flow rate, the results of the several laboratories are drawn with an artificial offset in the flow rate value for a better readability. Calibrated flow points are 200 g/h (3.3 ml/min) and 600 g/h (10 ml/min).

To evaluate all the results, the reference value and standard uncertainty are determined according to the procedure published by Cox [13, 14]. The reference value labeled as "RV" and the calibration results of all the participants are shown in Figures 19 and 20. Most of these calibration results are in agreement with the reference value as can be seen in Table 2, where the degree of equivalence is reported. However, the detailed discussion on the degree of equivalence is beyond the scope of this article and can be read in the report of EURAMET project 1291 [15].

This means that the validation procedure of the facilities was successfully performed for flow rates from 2 µl/min to 10 ml/min.

Further research intercomparisons are planned to validate flow rates from 0.1 to 2 µl/min.

Conclusion

The calibration capabilities and the corresponding uncertainties of the primary standards described above have been validated by means of the research intercomparison organized within EMRP project HLT07-MeDD [19] for the flow rate range from 2 µl/min to 10 ml/min. The calibration facilities are operational and the various NMIs will submit their CMCs to EURAMET (RMO of Europe). If applicable, the NMIs will also get the accreditation according to ISO 17025:2005 for declared CMCs. The validation of the lower flow rate range from 0.1 to 2 µl/min is still under investigation. Therefore, international traceability in the microflow and nanoflow is validated up to date down to 2 µl/min in Europe and will be soon extended to flow rates down to 0.1 µl/min. All the facilities of the NMIs are designed to calibrate flow meters using their flow generator as well as external flow generators, including drug delivery devices.

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