COMPARISON REPORT:

Final report

EURAMET Project 1508

Pilot study intercomparison of ultra-low liquid flow rates in range below 100 nL/min

February 2022

Contents

1 – Introduction

This report was written as part of activity A1.3.5 from the EMPIR Metrology for Drug Delivery (MeDD II) project. The three-year European project commenced on 1st June 2019 and focused on providing traceable measurements of volume, flow and pressure of existing drug delivery devices and mixing behaviour and occlusion phenomena in multi-infusion systems. For more details about this project, please visit www.drugmetrology.com.

The aim of this comparison is to validate the developed primary standards for flow rates below 1500 nL/min for steady flow and for fast changing flow. This will allow the participating laboratories to test the agreement of their results and uncertainties despite the use of different equipment and calibration methods for the calibration of two different flow meters and a syringe pump.

NEL acted as the pilot laboratory by analyzing the data and producing the final report. METAS performed the initial and final measurements in this inter-comparison to test the stability of the flow meters over time and IPQ tested the stability of the CETONI Nemesys pump over time.

2 – Participants

The following Institutes have participated in this comparison

no	Participant Type	Short Name	Organisation legal full name	Country
1	Internal Funded Partner	NEL	TÜV SÜD National Engineering Laboratory	United Kingdom
2	Internal Funded Partner	IPQ	Instituto Português da Qualidade, I.P.	Portugal
3	Internal Funded Partner	CMI	Cesky Metrologicky Institut	Czech Republic
4	Internal Funded Partner	METAS	Eidgenössisches Institut für Metrologie METAS	Switzerland
5	Internal Funded Partner	CETIAT	Centre Technique des Industries Aérauliques et Thermiques	France
6	Internal Funded Partner	RISE	RISE Research Institutes of Sweden AB	Sweden
7	Internal Funded Partner	DTI	Teknologisk Institut	Denmark
8	External Funded Partner	BHT	Bronkhorst High-Tech BV	Netherlands
9	External Funded Partner	HS	Hahn-Schickard-Gesellschaft für angewandte Forschung e.V.	Germany
10	External Funded Partner	THL	Technische Hochschule Lübeck	Germany
11	External Funded Partner	UoS	University of Strathclyde	United Kingdom

3 – Transfer standards

There were three transfer standards (TS) used in the inter-comparison exercise between the nine laboratories. Table 1 details the Transfer standard and flow range of the device during the comparison exercise.

Table 1 – Transfer standard details

	Transfer Standard 1 [TS1]	**Transfer Standard 2 [TS2]**	**Transfer Standard 3 [TS3]**
Manufacturer & model	Sensirion SLG64-0075	Bronkhorst L01	CETONI Nemesys
Type of device	Thermal flow meter	Thermal flow meter	Syringe pump
Flow range [nL/min]	1500, 1000, 500, 100, 70, 50 and 20	1500, 1000, 500, 100, 70, 50 and 20	100, 50, 20, 10 and 5

All three transfer standards were used in the static tests, but only the thermal flow meters were used for the dynamic tests (Table 2).

Table 2 – Type of comparison testing per device

	TS1	**TS2**	**TS3**
Static Tests			
Dynamic Tests			

4 – Comparison schedule

The inter-comparison started in January 2021. The two flow meters and the syringe pump were shipped in separate boxes to shorten the dead laboratory time of each instrument. Each laboratory had 2 weeks for each instrument with an estimated Shipping period of 1 week. Each laboratory had a couple of weeks between each transfer standard to perform daily business work or improve the analysis of the data.

The following diagram illustrates the comparison schedule (Figure 1). METAS performed the initial and final measurements of the flow meters to test the flow stability over time. IPQ tested the flow stability of the CETONI Nemesys pump.

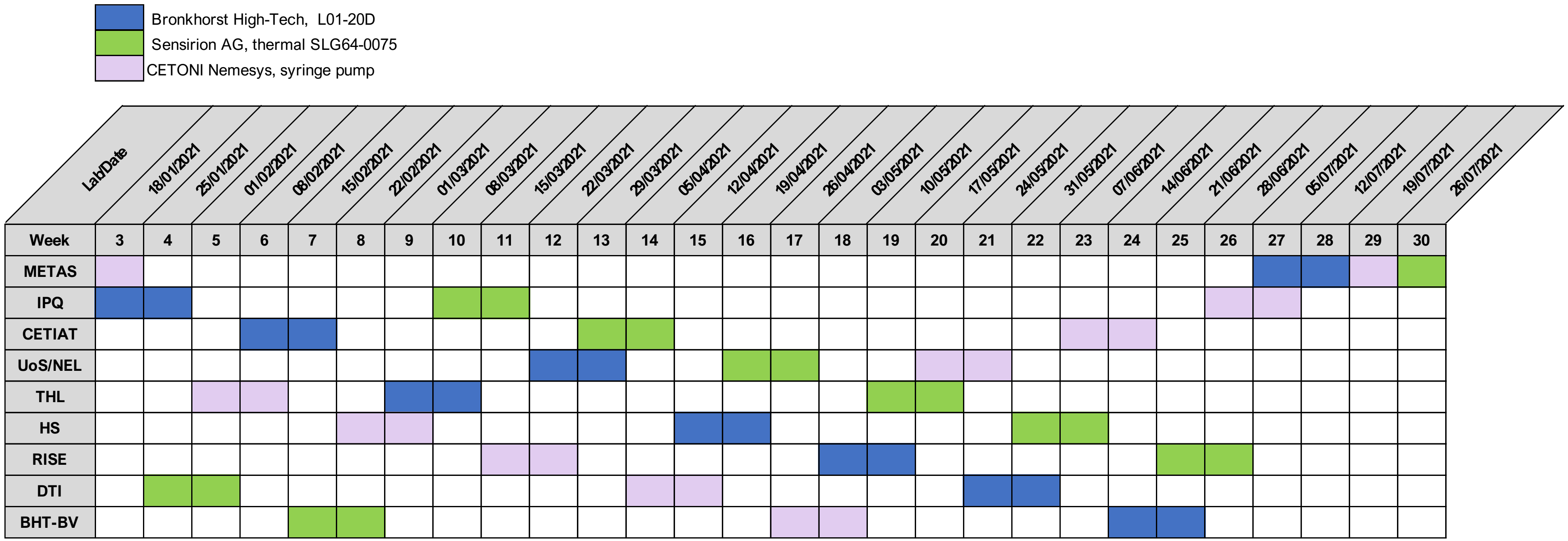

Figure 1 – Comparison Schedule by laboratory for each comparison device

5 – Facility descriptions

This section of the report describes the participating laboratories and includes details of the methodology, measurement principle, fluids, flow ranges, operating conditions, and measurement uncertainty [1].

5.1 – Instituto Português da Qualidade (IPQ)

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<td>Description of Facility</td>
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Interferometry

The Interferometric method uses an interferometer (Hewlett-Packard, model 5528A; it operates at 633 nm, and the signal is processed using a LabVIEW application) to monitor the distance travelled by a pusher block of the syringe pump in order to determine the flow rate. The use of interferometry for flow measurement involved the use of the following components: a laser unit (A) with a detector incorporated (an optical arrangement composed by two retroreflector cubes (C) (one of which with a beam splitter attached (B)), a Control Unit, a pusher block, the syringe pump Nexus 3000 (D) with the removable glass syringe (E), (Figure 2).

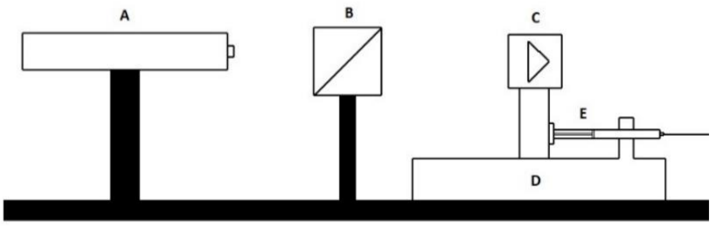

Figure 2 – Assembly of interferometric method setup

In practice, the generation of flow was accomplished by a stepper motor which drove a screw connected to a pusher block that itself pushed the syringe piston. Therefore, knowing the internal diameter of the syringe with very high precision, the travelled distance, and the time needed for that travelled distance, it was possible to calculate the flow rate of the fluid inside the syringe and its uncertainty. Three repetitions were performed for each flow rate in each instrument.


Figure 3 – Setup for the Sensirion meter calibration

The syringe used was of 0.1 ml and 1 ml depending on the flow rate.
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	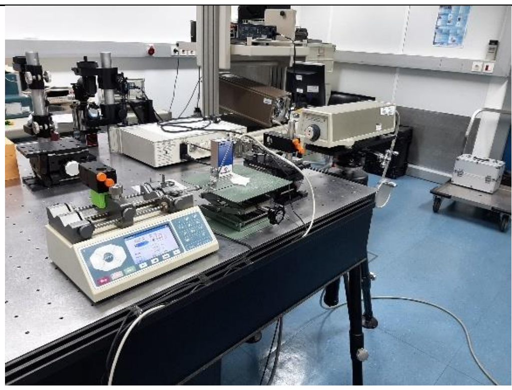 Figure 4 - Setup for the Bronkhorst meter calibration The syringe used was of 0.1 ml and 1 ml depending on the flow rate.  Figure 5 - Setup for the CETONI Nemesys pump calibration The syringe used was of 0.01 ml and 0.1 ml depending on the flow rate.
Description of Measurement Principle	**Interferometry** Laser interferometry is used to measure the intensity of a wave resulting from the overlapping of two or more waves that have travelled over different distances and are superimposed on a single point therefore this methodology can be applied to measure the distance of the pusher block of a flow generator connected to a glass syringe to determine the flow rate. Model: $Q = v \times A = \frac{x_2 - x_1}{\Delta t} \times \pi r^2 = \frac{d\pi r^2}{t}$
Facility Flow Ranges	**Interferometry** 1 mL/h to 0.0001 mL/h, *U*= 2% to 3 %
Temperature / Pressure Ranges	For all methods the temperature can go from 17 °C to 23 °C at atmospheric pressure.
Other Fluids	For all methods the primary fluid is water, but any other fluid can be used.

Uncertainty Budget	**Interferometry** The main standard uncertainties considered are: Distance (*d*), time (*t*), radius of the syringe (*r*), stability of the setup (δQ_{sta}), water temperature (*T*), time (*t*), expansion coefficient (*γ*), standard deviation of the measurements (δQ_{rep}) and repeatability of the 3 repetitions.

5.2 – Centre Technique des Industries Aérauliques et Thermiques (CETIAT)

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<td>Description of Facility</td>
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CETIAT’s optical measurement system consists of a JAI SP-12000M-CXP4-F camera (188 fps at 12MP), Qioptiq Optem FUSION Lens System 7:1 with a motorized zoom controller and a KL 2500 LED backlight for image acquisition, Quartz glass capillaries with inner diameters ranging from 250 µm to 1 mm to circulating water or water and oil, and 4 translation and 1 rotary stages (Figure 6)

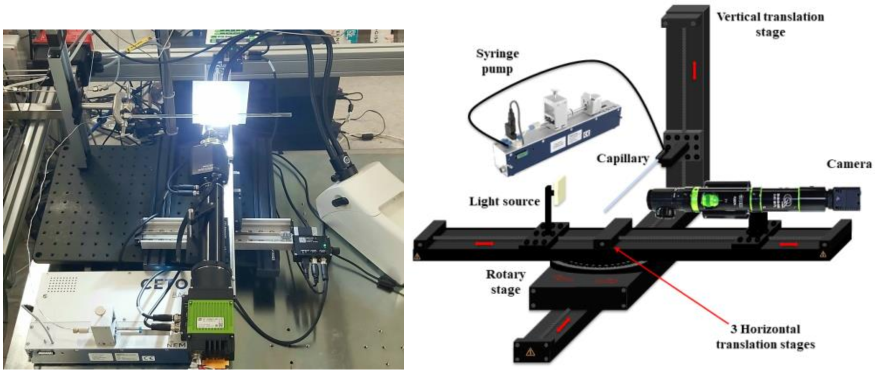

Figure 6 – CETIAT’s Interface tracking measurement system

Camera calibration is realized using a calibrated Olympus OB-M transmitted light objective micrometer. A signal generator that is calibrated against an atomic clock is used for triggering the camera to start image acquisition. Liquid flow is generated by CETIAT’s micro-flow bench for flow rates going down to 16 µL/min and by a CETONI Nemesys syringe pump (using 1mL to 10µl syringes) below that limit.
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<tr>
<td>Description of Measurement Principle</td>
<td>Meniscus tracking consists in measuring the displacement as a function of time of a liquid/air or liquid/liquid interface moving inside a glass capillary tube that is connected to a flow generating device. Images of the moving meniscus are acquired by a high-speed camera. A computer program written in Python language is used to measure the flow velocity from the frequency of the signal generator i.e., the frame rate and the interface displacements measured using Digital Image Correlation, and then to deduce the flow rate from the calculated velocity and the previously measured inner diameter of the capillary.</td>
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<tr>
<td>Can the Facility be used for Static / Dynamic or Both?</td>
<td>The nano-flow facility is used in both Static and Dynamic conditions. The dynamic flow profiles are generated using either Bronkhorst (ML120, BL 100, etc.) flow controllers or the Nemesys Syringe pump with dedicated software. Generated flow changes are comprised up to a factor ten of the flow rate value in less than one second. The meniscus tracking by image correlation in 100 µm inner diameter capillaries enables the measurement of flow rates down to 1nL/min within time intervals as small as 1s. For all flow conditions, a reference flow rate value over a time interval of one second is available. This allows the study of flow rate fluctuations and thus, a complete characterization of flow generating devices.</td>
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<tr>
<td>Facility Flow Ranges</td>
<td>The nano-flow facility can cover flow rates ranging from 1 nL/min to 16 µL/min. The micro-flow (gravimetric, developed during MeDD1) can cover flow rates ranging from 1 µL/min to 167 mL/min.</td>
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Temperature / Pressure Ranges	Liquid flow temperature is controlled at +/-1°C from 10°C to 50°C, as the device under test and/or the flow generator (depending on the calibration conditions needed) is placed inside a climatic chamber.
Other Fluids	Measurements are carried out using ultra-pure water flowing through the device under test. CETIAT degassed ultra-pure water properties are density of 998.2 kg/m3 at 20°C and conductivity of 0.06 µS/cm. Other liquids can be used if necessary.
Uncertainty Budget	The different measured quantities in our system are: Inner diameter of the capillary tube, positions of the interface inside the capillary, timestamps corresponding to each interface position and velocity of the interface, to which are associated the following uncertainty components : 1-camera calibration and specifications, pixel intensity profile and digital image correlation methods for the diameter and position measurements, 2-frame rate calibration and exposure time for time measurements and 3- linear regression parameters for velocity. Additionally, the uncertainty budget includes the system's drift and temperature effect as well as the uncertainties corresponding to the correction of physical phenomena e.g., evaporation, thermal expansion, and stick-slip effect. The current relative expanded (*k*=2) uncertainty is between 10% and 1% from 1 nL/min to 16 µL/min.

5.3 – Eidgenössisches Institut für Metrologie (METAS)

Description of Facility

The METAS Microflow and Milliflow facilities consist of homemade piston provers to generate the flow and the gravimetric method to determine the flow rate and calibrate the volumes and the volume flow rates of the piston prover. The flow generator, is filled with water and the water is pressed at the desired flow rate through the DUT and collected in the beaker on the balance, as shown in Figure 7.

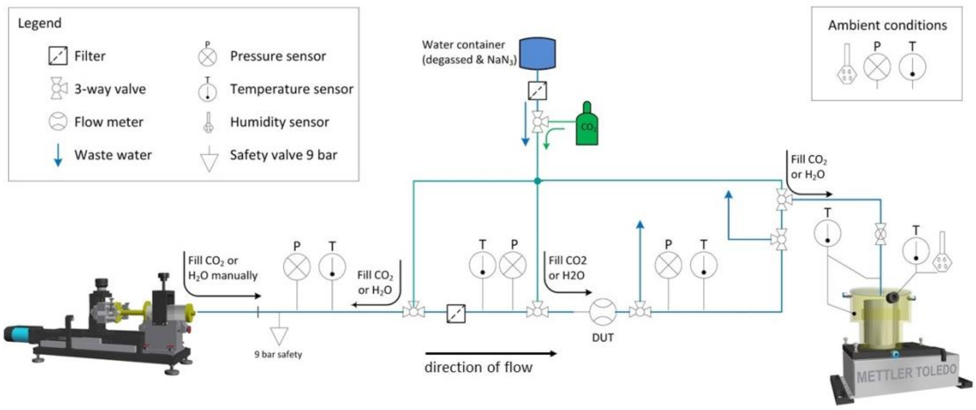

Figure 7 – Schematic of the updated Microflow and the developed Milliflow facility at METAS. The METAS piston prover presses the water through the DUT in the beaker on the balance, where it is continuously collected. Other components are the pressure sensors, temperature sensors, pressure relief valve and the water reservoir

Weighing data are continuously collected by a real time system (RT), which communicates with the balance at 20 Hz via RS232. The weight value is directly paired with the time stamp of the RT. 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 at a frequency of 1 Hz.

The position the piston prover is determined by counting the pulses sent by the linear measuring system by means of an FPGA, which is a Field Programmable Gate Array with hard coded program code running on a defined constant cycle time of the order of 25 ns (40 MHz). For each additional pulse in any direction, a time stamp of the FPGA is recorded and a pair with the position and the timestamp is formed. These pairs of values are then read from the main software at a lower frequency. The real time position is used to calculate the real time speed by means of linear least square fit over a time window that is adjustable. Multiplying the speed with the cross section of the piston gives the volume flow rate.

In the mode of fast changing flow rates, the data of the pressure sensors are collected with a FPGA and are paired with a timestamp. These pairs of data are also then read from the main software at a lower frequency. To synchronize the RT and the two FPGA-systems, a trigger signal is produced by one FPGA system and the other FPGA system is recording this trigger signal allowing a perfect match of the two timescales.

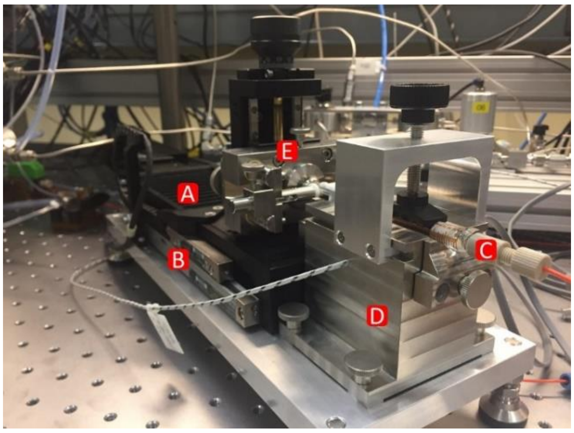

Figure 8 – METAS piston prover of the Microflow facility with a speed range from 0.1 mm/s to 0.1 µm/s. (A) high precision linear stage, (B) linear measuring system, (C) syringe, (D) mounting syringe body, (E) mounting and positioning for syringe plunger. The same design and components are used for the METAS piston prover of the Milliflow facility, but with a different speed range from 4 mm/s to 4 µm/s.

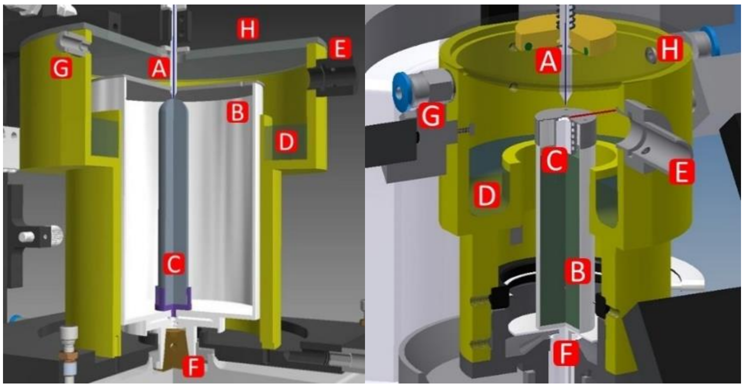

Figure 9 – Weighing zone on the balance of the Milliflow (left) and Microflow (right) facilities. (A) outlet needle, (B) beaker with cover, (C) glass filter, (D) water in evaporation trap, (E) mount for T and rH sensor, (F) balance, (G) tubing for humidity exchanger, (H) cover.

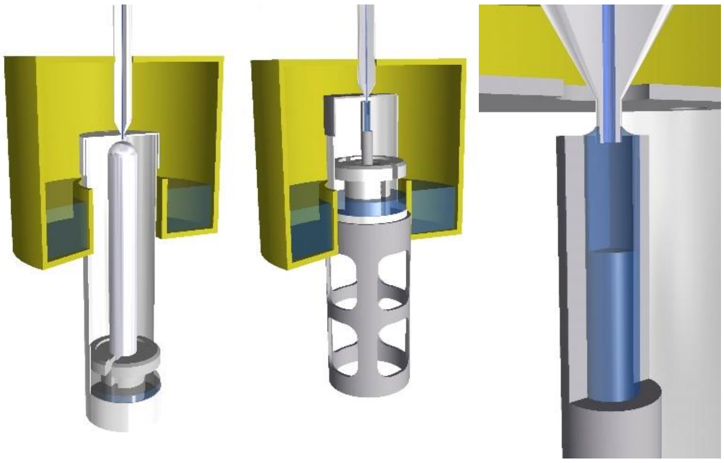

Figure 10 – Other measurement beakers for the Microflow facility. (left) beaker with glassfilter like the beaker for the Milliflow facility. (center and right) Capillary beaker, where the outlet needle is placed in the center of the capillary. Capillary beaker is suitable for the detection of fast changing flow rates.

Description of Measurement Principle	Flow is generated by the piston prover. The real time position is used to calculate the real time speed by means of linear least square fit over a time window that is adjustable. Multiplying the speed with the cross section of the piston gives the volume flow rate. The gravimetric method consists of weighing the collected water in a beaker and applying several corrections such as evaporation, buoyancy correction, etc. The ambient conditions must be well controlled and recorded to avoid any virtual flow incident due to temperature instabilities in the absolute temperature and temperature gradients.
Can the Facility be used for Static / Dynamic or Both?	The facilities operate in static and dynamic mode. The facilities can produce dynamic or transient conditions (fast flow changes). The changes occur within a time of less than 1 second and a ratio of 1:500 can be obtained. The range of flow rates is set by the choice of the piston used. The gravimetric methods can detect these fast flow changes in the range of 400 mL/min down to 50 nL/min. The piston provers can generate these fast flow changes in the range of 400 mL/min down to 20 nL/min. Steady flow rates are also generated in the range from 400 mL/min down to 20 nL/min and measured by the gravimetric method in the range from 400 mL/min down to 50 nL/min. Volumes of the delivered liquid are determined either by the piston prover or the gravimetric method.
Facility Flow Ranges	Dynamic mode: 20 nL/min - 400 mL/min (Piston Prover) Dynamic mode: 50 nL/min - 400 mL/min (Gravimetric Method) Steady mode: 20 nL/min - 400 mL/min (Piston Prover) Steady mode: 50 nL/min - 400 mL/min (Gravimetric Method)
Temperature / Pressure Ranges	Temperature: ambient Pressure: 0 – 10 bar
Other Fluids	Primary fluid: distilled water Additional fluids: any non-harmful fluid
Uncertainty Budget	Dynamic mode: 20 nL/min (50 nL/min) - 400 mL/min, 2.0 % - 0.2 % Steady mode: 20 nL/min (50 nL/min) - 400 mL/min, 1.0 % - 0.07 %

	Uncertainty components of the gravimetric method includes: • drift, calibration and reading uncertainty of the balance • uncertainty of linear least square fit • evaporation buoyancy correction • correction from conventional weight to real weight • stability of ambient temperature conditions • stability of water temperature and temperature gradients at different positions along the piping (piston, DUT, Balance) • repeatability and stability of the flow detection • stability of the capillary forces acting at the water bridge to the measurement beaker Uncertainty components of the gravimetric method includes: • Linearity of the linear stage • Repeatability and stability of the flow generation • Influence of torque and force on the accuracy of the position of the plunger

5.4 – RISE Research Institutes of Sweden AB (RISE)

Description of Facility

RISE operates two different flow facilities for measuring very small flow rates and quantities of liquids. The larger of the two flow facilities has a flow measuring range of 1 ml/h to 200 ml/h and is based on the gravimetric measuring principle. The flow facility is equipped with a 220 g scale from Mettler Toledo (XPR205) and has an extended measurement uncertainty of U(k=2) = 0.2% over the entire flow range. This test facility was not used for the measurements shown here in the report.

The second test facility is also based on the gravimetric weighing principle and is used in a flow rate range of 0.25 µL/h to 1 mL/h. The flow is generated by means of a high-precision syringe pump (CETONI Nemesys) with calibrated glass and stainless-steel syringes with different volumes. The syringe volumes range from 50 µl to 100 ml.

The traceability is primarily realized a weighing scale (10.1 mg) from Mettler Toledo (XPR10). The weighing scale is calibrated in regular intervals at the National Laboratory for Mass at RISE.

Data acquisition (DAQ) for the weighing scale is performed by a PC and LabView (as GUI) via USB. The scale is normally read out with a frequency of 1 Hz. For dynamic tests such as rapid flow changes and bolus tests, the scale can be read at a maximum frequency of about 10 Hz. The DUTs investigated in this report were connected to the same PC via serial interface or USB and configured and read out with the help of the software supplied.

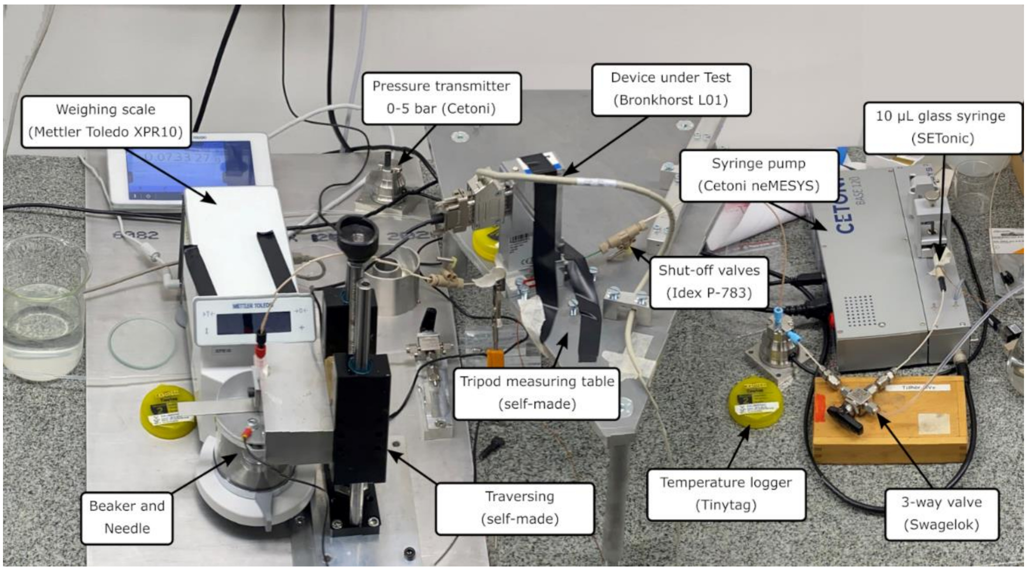

Figure 11 – Overview of the measurement setup at RISE

Data acquisition (dosed volume) of the syringe pump is performed via USB and the software (Qmix Elements) supplied by CETONI. Two external pressure sensors were used to measure the upstream and downstream pressure of the DUT. The two pressure sensors have a nominal pressure rating of 500 kPa (5.0 bar) and are directly connected to the CETONI Nemesys syringe pump by means of a CETONI I/O port splitter. Since the sensors are connected to the pump, the values of the two pressure sensors were also logged directly in the programme of the CETONI pump. The measurement data (absolute time stamps, dosed volume and both pressure values) can be exported as a single CSV file.

For further data acquisition a compactRIO DAQ system (cRIO-9040 with three expansion cards NI 9210, NI 9207 and NI 9216) from National Instruments is available.

For example, temperature is measured with the help of type K thermocouples with 2-pin mini-TC connectors at different points in the measurement set-up. The thermocouples are connected directly to the DAQ system via the NI 9210 expansion card.

Figure 12 – Flow generator: Syringe pump CETONI Nemesys including glass syringe

Figure 13 – CETONI pressure sensor (pressure range 0-5 bar) and I/O-splitter

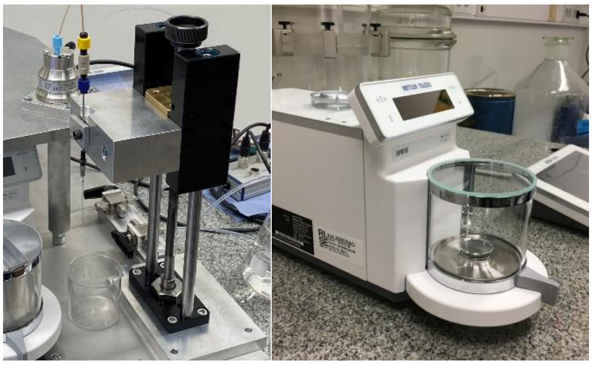

Figure 14 – Traversing including needle and weighing scale Mettler Toledo XPR10

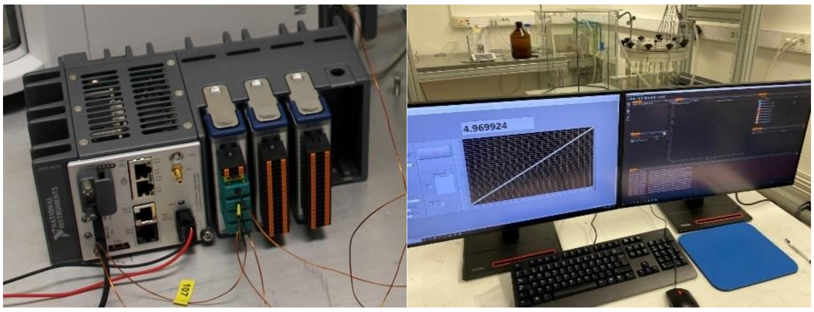

Figure 15 – NI compactRIO and control computer (LabView and Qmix Elements)

Description of Measurement Principle	Flow is generated by means of a syringe pump equipped with different syringes of various sizes. The desired flow rates can be adjusted by means of the syringe pump. The weighing method is performed by collecting the water (or another liquid) in a beaker. The weighing scale is continuously read-out. After the measurements several corrections for evaporation and buoyancy effects, etc. are applied. The measuring conditions in the laboratory (air pressure, room temperature and air humidity) are measured separately by means of a Vaisala PTU300 and various other (separate) sensors logged on RISE internal server (EXOscada). The Vaisala device has an internal data logger that can be read out via different interfaces. In addition, there is the possibility to connect the Vaisala device directly to the NI DAQ system compactRIO. The Vaisala device has current and voltage signal outputs for air temperature and air humidity.
Can the Facility be used for Static / Dynamic or Both?	The facility can be operated in static and dynamic mode. The facility can handle dynamic transient conditions (fast flow changes). The quantification of the change is mainly depended on the read-out of weighing scale used. In general, the weighing scale is read out at a higher frequency (up to 20 Hz) in the event of rapid changes.
Facility Flow Ranges	The flow rate range of the test facility is 0.25 µl/h to 1 ml/h.
Temperature / Pressure Ranges	Lab1: ambient temperature (around 23.5 ± 0.5 °C, climatic room) Lab2: ambient temperature (around 20.0 ± 0.5 °C, climatic room); limited access Pressure: 0 to 5 barg (upgradeable to max. 10 barg)
Other Fluids	Primary fluid: ultra-pure and degassed water Additional fluids: liquids other than water (non-toxic)
Uncertainty Budget	Steady (static) flow: Target uncertainties: 0.5% (higher flow rates) to 5.0% (5 nL/min); lower flow rates on request Unsteady (dynamic) flow: Target uncertainties: 0.5% (higher flow rates) to 5.0% (20 nL/min); lower flow rates on request

5.5 – Teknologisk Institut (DTI)

Description of Facility	At DTI the primary standard covers a flow range from 100 ml/h (1.6 ml/min) to 1 µl/h (17 nl/min) with uncertainties from 0.05 % to 5 % with calibration time from 10 - 75 min. The micro flow laboratory at DTI is accredited to a flow rate from 600 ml/h (10 ml/min) to 1 ml/h (17 µl/min) with uncertainties from 0.05 % to 4 %. <u>Design of the facility</u> The setup is based on gravimetric measurements, i.e., the flow is determined from $Q = \frac{\Delta V}{\Delta \mathrm{t}} = \frac{\Delta m/\rho_w}{\Delta \mathrm{t}}.$ The time, Δt, is determined from an oscillator in order to make traceability feasible. The water density, ρ_w, is determined using a formula from literature and checked by measurements. The measuring beaker used is thoroughly cleaned and acclimatized to the balance. Demineralized degassed water is used as fluid. The most difficult parameter to determine accurately for small flow rates is the mass change, Δm. It is based on measurements made with a laboratory scale with microgram resolution. Several corrections are required, e.g., for buoyancy by air, displacement by the inlet ("needle"), evaporation, capillary forces and sticking. To allow for accurate weighing the entire setup is place on a granite table, which provides an almost vibration-free base. Furthermore, the temperature of the setup is stabilized. Rapid temperature variations are prevented by placing the entire setup in a custom-built isolation chamber. This chamber also minimizes draft and convection effects. The chamber consists of metal box which is shielded from the surroundings by an isolating layer. Long-term stability is ensured by the laboratory's climate control. Evaporation is dealt with in different ways depending on the flow rate. In the case of the smallest flows the evaporation is almost completely eliminated by covering the water by an oil layer (see below). Alternatively, evaporation can be limited by increasing the relative humidity near the beaker and/or reducing the reducing the opening aperture of the beaker. For small flow rates it may be needed to measure the evaporation rate regularly in order to reduce the resulting uncertainty. The liquid oil cover used as evaporation trap is very efficient as only an evaporation rate of < 9 nl/h is remaining. However, the water needs to be delivered below the oil surface through the outlet pipe, which leads to several challenges e.g., capillary forces, buoyancy, inertia, stiction and friction, absorption, adsorption, stick/slip, and vibration transferal. To limit these effects, the outlet pipe is made of 0.4 mm stainless steel tubing. The operational volume of the beaker is < 5 ml, yet the setup includes a system for emptying the beaker automatically when it becomes full. This system provides significant benefits for measurements at relative high flow rates, by reducing the amount of manual handling required and increasing the thermal stability of the setup. If the Device under test (DUT) is a flow meter, then the demineralized and degassed water runs from pump and into the DUT. For that purpose, three syringe pumps are installed. Two Cavro XLP 6000 pumps are working in parallel and used for flow rates > 50 µl/min, whereas a Cavro Centris pump is used for smaller values; the minimum rate for continuous flow is 1 µl/hr ≈ 17 nl/min. In addition to steady flows the pumping system can also provide dynamic flow-rate profiles. If the DUT is a pump, the water exits the DUT and is lead to the scale via stainless steel tubing. This configuration has e.g., been used for some medical devices.

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<u>Dynamic gravimetric weighing</u>

The balance is connected to a computer, enabling measurements with a frequency of 10 Hz and traceable timestamps with a dedicated timing hardware. By having continuous read out 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.

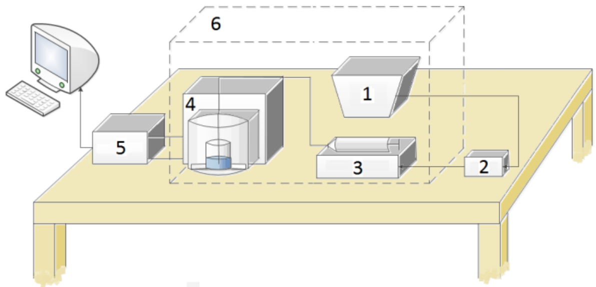

Figure 16 – Setup where the DUT is a syringe pump. Water is lead from the reservoir (1) through the degasser (2) and into the syringe pump (3). From the syringe pump water is lead to the balance (4) using stainless steel tubing. The outlet tube may be traversed through the oil-based layer and into the water in the beaker. The balance is connected to balance electronics (5) and to a PC. The setup is enclosed in a chamber (6). Flow meters are installed between pump (3) and scale (4).

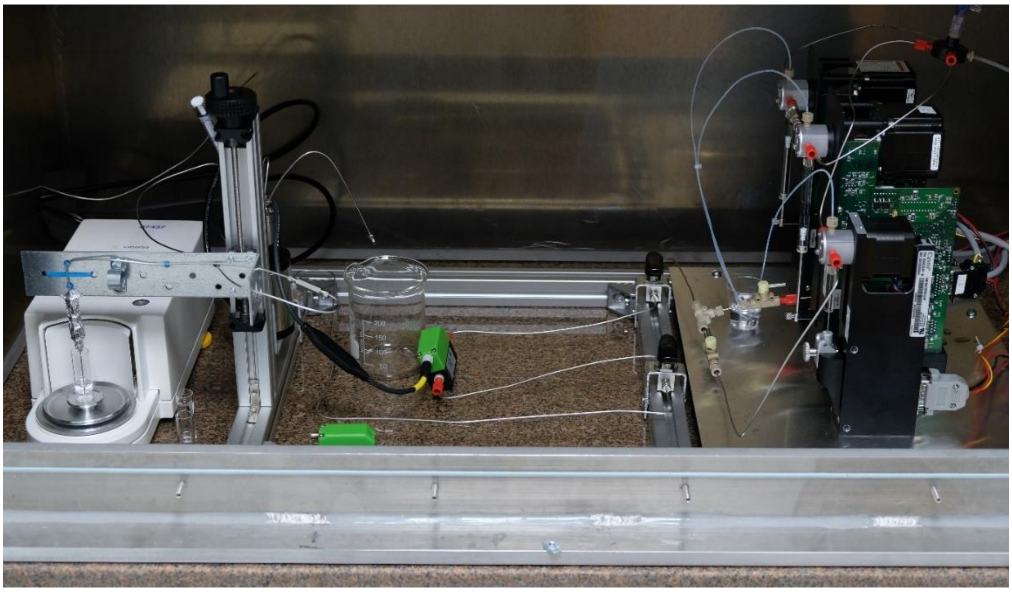

Figure 17 – The syringe pumps are visible to the right, the flow meter (green) in the center and the weighing system to the extreme left. The height-adjustment system for the inlet and the six-port valve for the emptying system are located towards the left side between scale and flow meter. Different beakers are available depending on the flow rate. For this photo, the draft screen of the scale has been removed and the insolating box around the setup is opened.

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<td>Description of Measurement Principle</td>
<td>The facility is based on the gravimetric principle with an optional oil layer in the beaker as evaporation trap.</td>
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Can the Facility be used for Static / Dynamic or Both?	The scale can deliver data with a frequency of 10 Hz – and it seem suitable for measuring dynamic and transient conditions. In MeDD I the dynamic behavior of a syringe pump was measured/check on this setup.
Facility Flow Ranges	The maximum flow rate is 60 mL/hr (1 mL/min), if the automatic system for emptying the beaker can be employed (e.g., if the DUT is a flow meter). Otherwise, the maximum flow rate may be limited to 10 mL/hour (0.167 mL/min). The facility is tested (with success) for 5 µL/hr (0.083 µL/min), however a realistic aim is 1 µL/hr (0.017 µL/min).
Temperature / Pressure Ranges	The facility can only operate at ambient conditions, meaning room temperature in the lab (18 °C – 25 °C)
Other Fluids	Normally degassed demineralized water is used. However, it is also possible to use other fluids as well. Prior to the project insulin mimicking fluid has been tested.
Uncertainty Budget	The dominant uncertainty contribution (>90 %) for the setup comes from the uncertainties of the corrections for the forces between the outlet pipe, the water and oil in the measurement beaker. The measurement uncertainty is approx. 0.5 % (*k*=2) at the flow rate of 10 ml/h. Below flow rates of 300 µl/h the balance uncertainty and uncertainty of the correction for buoyancy become dominant (90 % at 5 µl/h), while 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.

5.6 – Hahn-Schickard-Gesellschaft für angewandte Forschung e.V. (HS)

<table>
<tr>
<td>Description of Facility</td>
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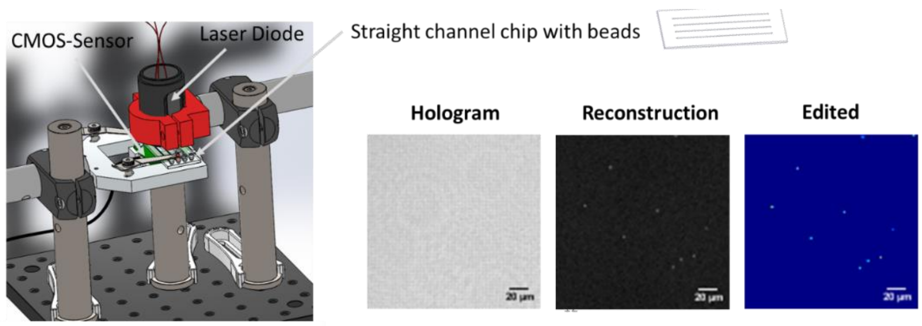
The “Hahn-Schickard-Gesellschaft für angewandte Forschung e.V.” (HS) was founded in 1955 on the initiative of the watch industry and ranks in Baden-Württemberg among the leading research and development service providers of microsystems technology. The main core competences of the institutes in Freiburg, Stuttgart and Villingen-Schweningen are: Integrated Microsystems; Information Technology - Industry 4.0; Sensors (flow, pressure, dew point, gas, etc.); Actuators and dosing technology; Microelectronics; Lab on a chip and microanalysis systems and Measurement technology (volume and flow). Hahn-Schickard is therefore very well positioned to handle the project. The selected project officer (Dr. Sabrina Kartmann) has many years of expertise in the field of flow sensor development, especially for low flow rates and the measurement of low volumes and flow and is a member of the standards committee "Volumenmessgeräte" of the German Institute for Standardization e. V. (DIN) and nominated expert for the working group ISO/TC 48/WG 5 Liquid Handling Devices- Automatic.
<u>Holographic Micro-PTV Flow sensor</u>
Hahn-Schickard (HS) – has a test stand for measuring small flow rates (10 – 500 nL/min) based on the micro-PTV principle. Particle Tracking Velocimetry (PTV) is a non-contact optical method for determining velocity fields in fluid mechanics. A flow of seeded water is induced in a microfluidic transparent channel. This channel is imaged at short time intervals through a holography setup (Figure 18). The direction and speed of movement can be determined from the particle positions. Currently the images are post-processed using different algorithms. As medium for the Holo-PTV set-up, HS use water with polyethylene beads (density: 0.989 g/cm³, diameter: 1-10 µm). The sensor in the Holo-Setup has a pixel size of 1.67 µm and a frame rate of 3.2 fps.


Test setup for holographic imaging. Holographic analysis of silica beads (d_{bead}= 1 µm) on a microscope slide.
Figure 18 – shows the test setup for the holographic imaging method on the left. The setup consists of a laser diode, a hole, the straight channel chip, and a CMOS sensor to capture the images. On the right, you can see a typical holographic analysis consisting of the recorded hologram, the reconstructed image and the post-processed one. (The imaged on the right was taken from a microscope slide, not from the chip).
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<tr>
<td>Description of Measurement Principle</td>
<td>
Hahn-Schickard has developed a flow rate measurement device based on micro particle tracking velocimetry (micro-PTV) method to address flow rates below 100 nL/min. Shear rate, maximum velocity, velocity profile shape, and flow rate can be derived from this nonintrusive method for the flow rate measurement at microscales.
Micro-PTV is an optical measurement technique for flow velocity determination. It is based in on the displacement of tracer particles between two points in time. The experimental set-up of a PTV system consists of several subsystems: a transparent micro channel with seeded tracer
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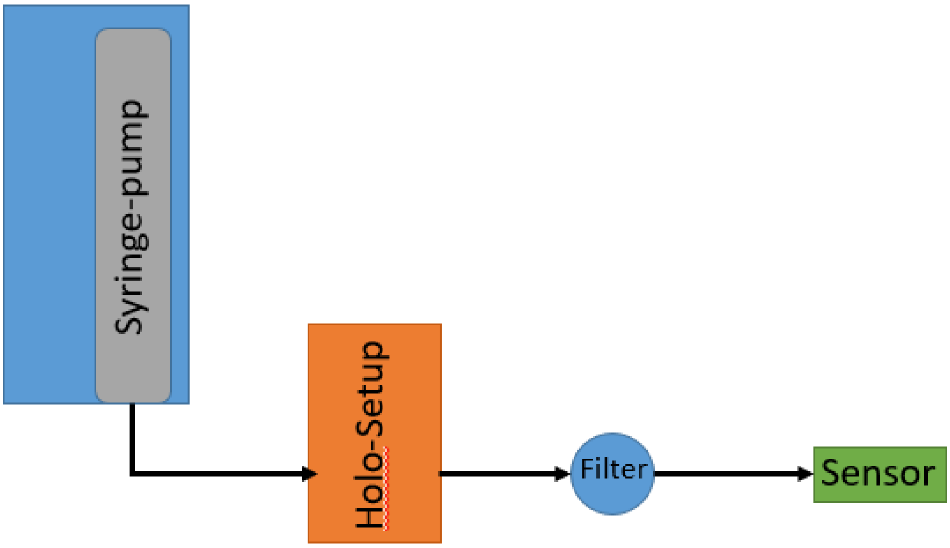
<td>particles, a light source, a camera to record a sequence of frames and a software to determine the flow velocity.

As a flow generation source, we used a syringe pump (CETONI Nemesys Low pressure). As medium for our Holo-PIV set-up, we use water with polyethylene beads (density: 0.989 g/cm^3, diameter: 1-10 µm). The channel's dimensions were 100 x 300 µm (height x width). The sensor in the Holo-Setup has a pixel size of 1.67 µm and a frame rate of 3.2 fps. To prevent beads from getting into the device under test (sensor), we have inserted a filter (pore size: 0.45 µm) in between. The filter was exchanged between each measurement to prevent clogging of the filter.



Measurement Protocol:
Prior each measurement the setup was set to rest 30-60 minutes to determine the zero-flow value
Each flow rate was set for 30-60 minutes before the measurement was recorded
During the measurement 200 images were acquired
During each measurement the temperature of the reservoir was determined
The measurement points for each flow rate were repeated on different days at different times</td>
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<tr>
<td>Can the Facility be used for Static / Dynamic or Both?</td>
<td>The described method could be used for static and dynamic measurements because it only depends on the framerate of the chosen camera. In this case, a camera with a framerate of 3.2 fps is used. Therefore, in theory dynamic changes in the range of 312.5 ms can be detected.</td>
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<tr>
<td>Facility Flow Ranges</td>
<td>Flow range: 5 to 500 nl/min (planned)

Flow range: 60 to 6000 µl/min (current status)</td>
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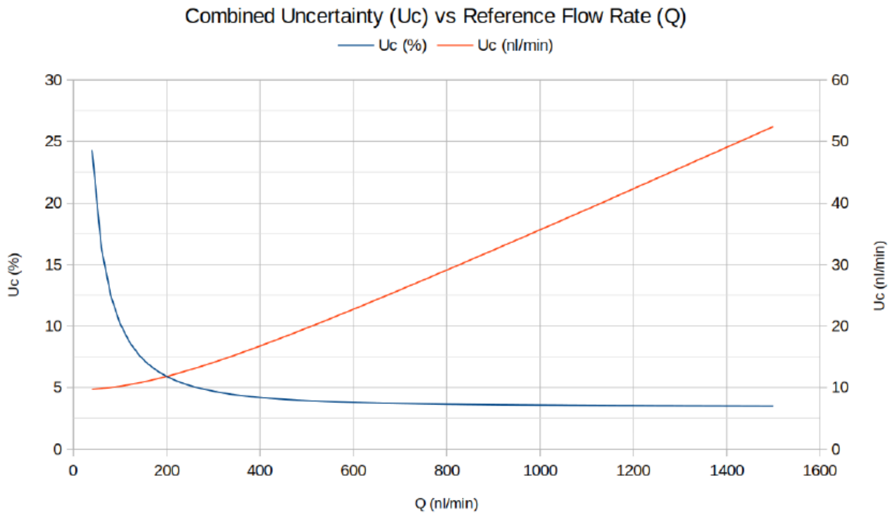
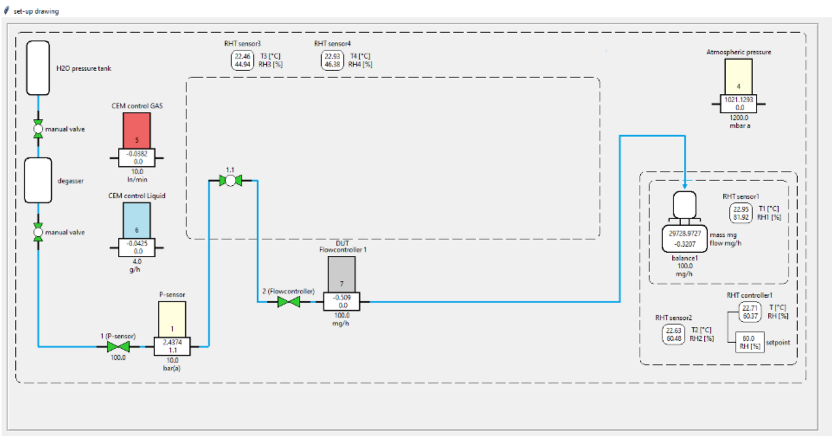
Temperature / Pressure Ranges	Pressure 0 to 2 bar
Other Fluids	If other fluids are used, the density of the beads has to be adapted (should be identical to prevent sedimentation).
Uncertainty Budget	A detailed description of the uncertainty budget can be found in "2020-07-29-MeDD_II A1.2.1 Report Template_microPIV". The main components of the facility uncertainty are: • Effective resolution of optical setup • Angular deviation of camera and flow channel (width b and height a) from 90° • Channel fabrication errors The combined uncertainty can be calculated as: $$\frac{u_c(Q)}{Q} = \sqrt{\left(\frac{1.67\mu m}{\Delta x}\right)^2 + (1.37 \times 10^{-3})^2 + \left(\frac{3\mu m}{a}\right)^2 + \left(\frac{5\mu m}{b}\right)^2}$$ Given that: $$\Delta x = \frac{Q \times \Delta t}{a \times b}$$ With: $$\mathrm{a} = 100\mu m$$ $$\mathrm{b} = 300\mu m$$ $$\Delta \mathrm{t} = \frac{1}{3.2s}$$ This enables the calculation of the combined uncertainty as a function of the reference flow rate:  Figure 19 – HS facility uncertainty with respect to reference flow rate

5.7 – Bronkhorst High-Tech BV (BHT)

Description of Facility	At the Bronkhorst facility the primary reference for mass flow is a high precision balance. The primary standard covers flow calibrations in 2 ranges: (1) 1–2000 [mg/h] mass flow of water (20 [nl/min] – 33.3 [µl/min]) with an uncertainty between 8.8% and 0.25% (*k*=2). Measurement times range from 2 hours to 2 minutes, respectively. (2) 0.3 mg/h–1.2 mg/h mass flow of water (5–20 [nl/min]) with an uncertainty between 29.2% and 8.8% (*k*=2). The measurement time for this range is 2 hours. The setup consists of a pressurized liquid tank, filter, degasser, pressure sensor, DUT with control valve, balance, several temperature, and humidity sensors, and shut-off and control valves with small internal volume. All parts in the setup are connected by 1/16" OD, 0.25[mm] ID tubes. In Figure 20 a schematic of the setup is shown. It is placed on a granite table with shock absorbing blocks to reduce vibration interference from the environment. The complete setup with table is installed in a box to reduce fast temperature and humidity changes. During the measurements different temperature and humidity sensors monitor the stability of these changes. The humidity inside the box is controlled to minimize and stabilize the evaporation rate.  Figure 20 – Schematic of Bronkhorst facility
Description of Measurement Principle	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 20 provides a schematic overview of the setup, and in Figure 21, 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 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 [bar] using helium. Before a calibration or measurement is started, the setup is prepared to ensure a stable and pure liquid flow. "Pure liquid flow" refers to 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 shut-off valves used in this setup are pneumatic with small internal volume and do not generate heat.

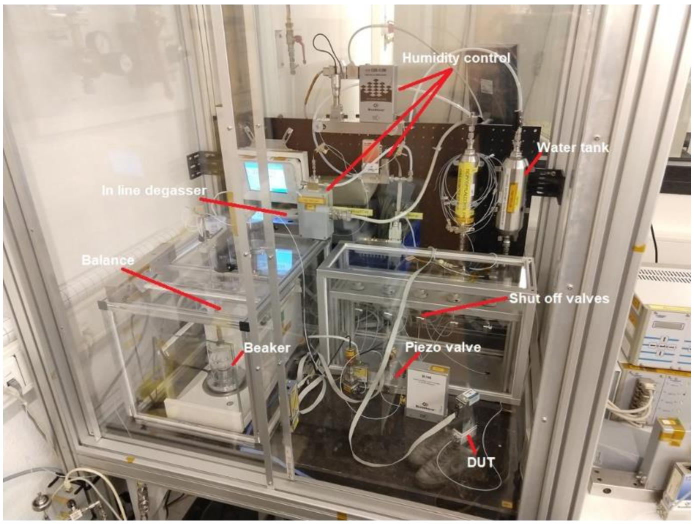


Figure 21 – Photograph of the primary standard at the Bronkhorst calibration facility.

The water that flows towards the balance is collected in a beaker via a small glass tube with its outflow positioned above a glass filter that is placed in the beaker. A constant liquid "bridge" is created during the measurements, Figure 22.

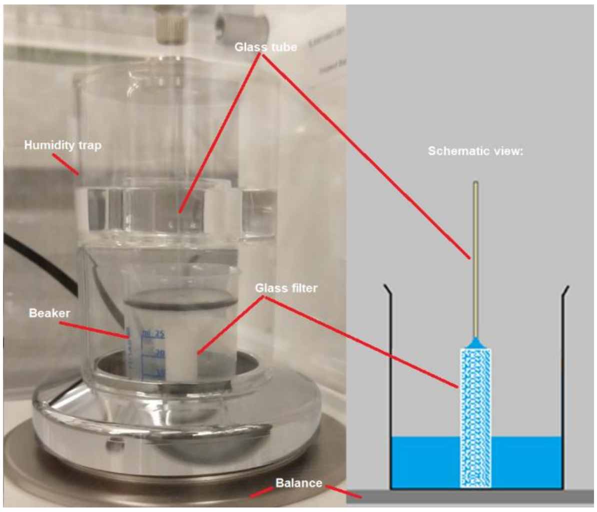


Figure 22– Weighing zone on the balance in an environment with humidity control.

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:

$$m^{\cdot} = lim_{\Delta t \to 0} \frac{\Delta m}{\Delta t} = \frac{m_r}{t_r}$$

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, which we call the “flying” method. 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.
Can the Facility be used for Static / Dynamic or Both?	The facility can be used for static and dynamic measurements. The main focus of the facility was to improve static measurements by improving the setup and the uncertainty budget of the gravimetric method. The dynamic measurement is based upon this method. The balance is read out at a frequency of 10 [Hz]. The largest uncertainties of low flow rate beneath 2000 [mg/h] (33.3 [µl/min]) when measured within 60[sec] are the balance calibration and linearity uncertainties. The balance calibration uncertainty provides a worst-case approach during a dynamic measurement and will be much better in practice. To prove this, further investigation is necessary.
Facility Flow Ranges	For liquids: • 1 g/h – 30 kg/h under ISO17025 accreditation. • < 1 g/h down to 1 mg/h or less under investigation. • High flows up to 1000 kg/h.
Temperature / Pressure Ranges	Pressure: 1 – 9 bar absolute; (for liquids) Temperature: 19 – 24 °C
Other Fluids	Primary fluid: ultra-pure water
Uncertainty Budget	Static measurements: The primary standard in the range of 1–2000 [mg/h] mass flow of water (20 [nl/min] – 33.3 [µl/min]) has an uncertainty between 0.25% and 8.8%. This setup can also be used for calibrations in the range of 0.3–1.2 [mg/h] mass flow (5–20 [nl/min]) with an uncertainty between 8.8% and 29.2%. Dynamic measurements: 1.8–6 [mg/h] mass flow of water (30–100 [nl/min]) has an uncertainty between 163% and 543%.

5.8 – Technische Hochschule Lübeck (THL)

<table>
<tr>
<td>Description of Facility</td>
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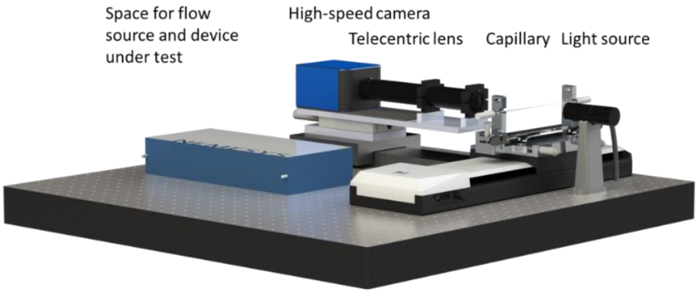
Camera-Front-Tracking-System: High precision capillaries (0.15 mm to 1 mm inner diameter) in combination with telecentric lenses and a high-speed camera were used to examine flows between 50 nl/min and 500 µl/min at sample rates up to 5 kHz (5000 fps) (Figure 23). Acquisition times between 2 s and 60 s are possible. The acquisition time and resolution can be adjusted via different capillary diameters (150 µm, 300 µm, 600µm, 1000µm), different magnifications (2x, 4x, 5x) of the measuring lenses and via the recording speed (Table 3). The camera is mounted on a linear stage to adjust the distance after a lens change. The capillary is mounted with an adjustable holder on a linear stage (Figure 24).
Calibration method: The inner diameters of the capillaries are determined with a Keyence digital microscope VHX600. The conversion factor (pixel to µm) is determined with a glass scale from Leica.
The resolution also depends on the resolution of the camera used (SpeedCam MiniVis-Eco2, 1280 x 1024 px).
The facility is primarily designed to characterize the dynamic properties of various medical sensors or devices. Different flow sources, syringe pumps (CETONI Nemesys; Nexus 3000), pressure sources Fluigent MFCS-EZ (2 channels 0-1 bar), valves (Bürkert, Giga, Asco) or sensors, pressure sensors (Wika), flow sensors (Sensirion, Bronkhorst) can be used.
Data acquisition is realised either via the software of the respective device manufacturers or in LabView with NiDAQ cards.


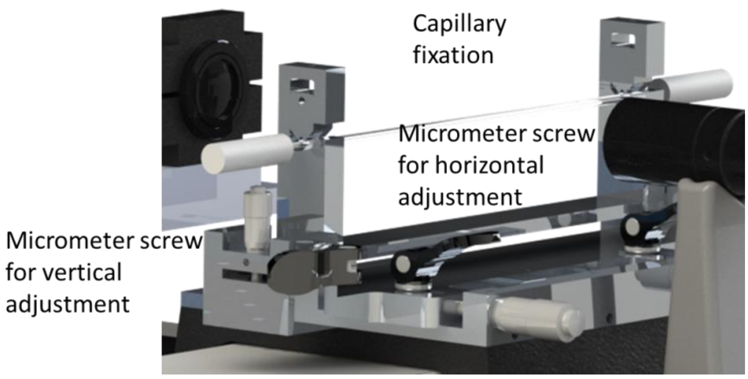
Figure 23 – Camera-Front-Tracking-System. High precision capillary in combination with telecentric lenses, a collimated LED light source and a high-speed camera.


Figure 24 – Adjustable holder for the capillary.
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	Table 3 – Acquisition resolution **Capillary ID** | **resolution [nl/px] 5.0L / 4.0L / 2.0L** | **Volume [µl]** **150** | 0.04 / 0.05 / 0.10 | 0.05 / 0.07 / 0.13 **300** | 0.17 / 0.21 / 0.42 | 0.21 / 0.27 / 0.54 **600** | 0.66 / 0.84 / 1.68 | 0.85 / 1.07 / 2.15 **1000** | 1.84 / 2.33 / 4.66 | 2.35 / 2.98 / 5.57
Description of Measurement Principle	The measurement principle is optical front tracking. The volumetric change inside a cylindrical geometry is measured with high precision by monitoring the displacement of the liquid front and the related time. The volumetric flow rate Q is determined by the displacement Δx of the liquid front during a time interval Δt and the radius R of the capillary or of the syringe. $Q = (\Delta x/ \Delta t) \pi R^2$
Can the Facility be used for Static / Dynamic or Both?	The facility can handle dynamic conditions. The volume can be determined every ms. The camera can sample 1000 frames per second.
Facility Flow Ranges	The facility has been used between 50 nl/min and 500 µl/min.
Temperature / Pressure Ranges	The facility can cover only room temperature. Pressure range is limited between ambient pressure and 6 bar
Other Fluids	Primary fluid is water. Water based solutions could also be used.
Uncertainty Budget	Uncertainty is 4% for flow rates higher than 50 nl/ min.

Table 3 – Acquisition resolution

Capillary ID	resolution [nl/px] 5.0L / 4.0L / 2.0L	Volume [µl]
150	0.04 / 0.05 / 0.10	0.05 / 0.07 / 0.13
300	0.17 / 0.21 / 0.42	0.21 / 0.27 / 0.54
600	0.66 / 0.84 / 1.68	0.85 / 1.07 / 2.15
1000	1.84 / 2.33 / 4.66	2.35 / 2.98 / 5.57

5.9 – TÜV SÜD National Engineering Laboratory (NEL) / University of Strathclyde (UoS)

The original plan was for NEL to complete the work in their own facility. However, the decision was made to complete the test work at University of Strathclyde's facility.

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<td>Description of Facility</td>
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The facility includes a system comprising five main parts (Figure 25): a microfluidic chip, a pressure system, a fluorescent microscope, a CCD camera, and a software interface.

Fluids with a known concentration of fluorescent beads (1 µm diameter) are introduced into the microfluidic chip from a fluid reservoir under adjustable positive pressure (ranging from 50mbar to 200 mbar). The geometry of the microfluidic channel in the chip determines the resulting flow rate according to the pressure value applied. A fluorescent microscope is used to illuminate the flow behavior inside the chip. A camera (1-500 Hz acquisition rates) is used to record images of beads moving in the flow using a LabView interface. An *in house* developed software routine (Matlab) is then used to calculate the velocity of the beads and extrapolate the flow rate according to the geometry of the microfluidic channel used.

The microfluidic chips are made with polydimethylsiloxane (PDMS), enabling dimensions down to a few microns to be obtained. The chips are transparent.

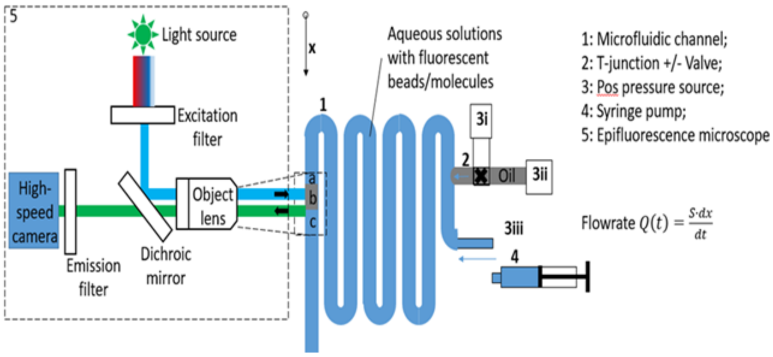

Figure 25 – Flow rate measurement facility. It includes a microfluidic chip (1) with a T-junction (+/- PDMS Quake valve), a positive pressure source or syringe pump for fluid actuation, an epifluorescence microscope, and a CCD camera. Images are recorded and analysed to extract fluid velocity.

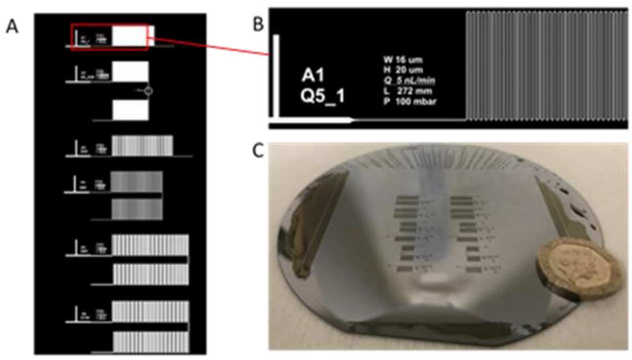

Figure 26 – Designs and fabrication of the microfluidic chips. (A-B) Examples of microfluidic serpentine channels. (C) Microfluidic master obtained from photolithographic processes for casting microfluidic chip in PDMS.

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Description of Measurement Principle	The measurement relies on tracking fluorescent beads suspended in water-based fluids at approx. 21 ° C flowing into a microfluidic serpentine of known dimensions. To measure different flow rates at approximately the same flow velocity and applied pressure values, the fluidic resistance of the microfluidic serpentine is varied by changing the length and width of the microfluidic channel while fixing its height. The cross section of the channel is rectangular. The fluidic resistance is derived from Hagen-Poiseuille's law: $$R_h = \frac{12\mu L}{wh^3(1-0.63h/w)}.$$ where R_h is the fluidic resistance, μ is the dynamic viscosity, *L* is the length of the channel, *w* is the width and *h* is the height of the channel. Different values of R_h will determine the flow rate achieved inside the serpentine. Other options for flow measurements are available which include the use of immiscible phases. The flow rate measurements will rely on tracking a moving water/oil interface.
Can the Facility be used for Static / Dynamic or Both?	The facility can handle both steady-state and transient flow rate measurements. To achieve this, the applied pressures must be programmed to the appropriate transient values (through a software interface controlling the pressure system) or connecting a second devices which creates intermittent flow. Prior to each measurement, fluids are degassed to reduce air bubbles in the liquids to minimize the fluidic compliance. The walls of the microfluidic channels can be treated to become hydrophilic and low adhesion to prime water fluids, avoid air bubbles and beads adhesion to channel walls.
Facility Flow Ranges	The minimum flow rate the facility is expected to be able to measure is 5 nL/min. The maximum flow rate that has been designed to be measured, according to project specification, is at 100 nL/min.
Temperature / Pressure Ranges	The applicable full pressure range of the facility is from 30 to 1000 mbar. However, pressure values from 50 to 200 mbar will be used. Currently, the facility will be operated at room temperature (~21.7 °C). However, further measures can be implemented (such as a Peltier chamber and/or a humidity chamber) to maintain temperature and compensate liquid evaporation.
Other Fluids	The primary fluid used is distilled water (density 0.9982 g/mL at 20 °C). Any fluids (Newtonian or Non-Newtonian fluids) that are in aqueous phase can be used within the facility, e.g., blood.
Uncertainty Budget	The overall uncertainty of the flow rate measurement is calculated based on the variation in dimension of the microfluidic channel and the uncertainty of the position of the bead in each frame depending on the frame rate used. Because of the fabrication processes, there are variations on the dimensions of the channels. The channels will be characterized using a scanning electron microscope (SEM) and an Alpha-Step surface profiler.

6 – Test procedures

The procedures detailed in *Protocol Comparison: microflow* (Activity A1.3.2) were followed for both the Static and Dynamic calibrations at each laboratory [2]. The following sub-sections provide a summary of the two calibration types.

6.1 – Static calibration

The calibration conditions were specified as follows:

- Upstream pressure: 0.5 bar to 2.5 bar
- Water temperature: Between 17 °C and 23 °C
- Minimum measurement time was dependent upon the test set up, but stable flow must be achieved before logging

For each test run, at least three repetitions per point were performed. Each laboratory decided whether to perform three single measurement test points or analyse three independent sections of a single longer measurement test point.

Table 4,

Table 5 and Table 6 summarise the test points completed at each laboratory using each of the applicable Transfer Standard devices respectively. Green means that the TS was calibrated at that test condition. Red means the TS was not calibrated at that test condition.

Table 4 – Flow rates completed by each flow laboratory for TS1

Flow rate [nL/min]	IPQ	CETIAT	METAS	RISE	DTI	HS	BHT-BV	THL	UoS/NEL
1500	Green	Green	Green	Green	Green	Red	Green	Green	Red
1000	Green	Green	Green	Green	Green	Red	Green	Green	Red
500	Green	Green	Green	Green	Green	Green	Green	Green	Red
100	Green	Green	Green	Green	Green	Green	Green	Green	Green
70	Green	Green	Green	Green	Green	Green	Green	Green	Green
50	Green	Green	Green	Green	Green	Red	Green	Green	Green
20	Green	Green	Green	Green	Green	Red	Green	Green	Green

Table 5 – Flow rates completed by each flow laboratory for TS2

Flow rate [nL/min]	IPQ	CETIAT	METAS	RISE	DTI	HS	BHT-BV	THL	UoS/NEL
1500									
1000									
500									
100									
70									
50									
20									

Table 6 – Flow rates completed by each flow laboratory for TS3

Flow rate [nL/min]	IPQ	CETIAT	METAS	RISE	DTI	HS	BHT-BV	THL	UoS/NEL
100									
50									
20									
10									
5									

6.2 – Dynamic calibration

The calibration conditions were specified as follows:

- Upstream pressure: 0.5 bar to 2.5 bar
- Water temperature: Between 17 °C and 23 °C
- Minimal measurement time was dependent upon the test set up

For each test run, at least three repetitions per point were performed. The flow profile start times are shown in Table 7 below. The flow profile trend is shown in Figure 27.

Table 7 – Dynamic flow profile for 3 minute log time

Start time [s]	Log time [s]	Volume flow [nL/min]	Volume [nL]	Total Volume [nL]
0	30	50	25	25
30	30	80	40	65
60	30	100	50	115
90	30	50	25	140
120	30	30	15	155
150	30	50	25	180

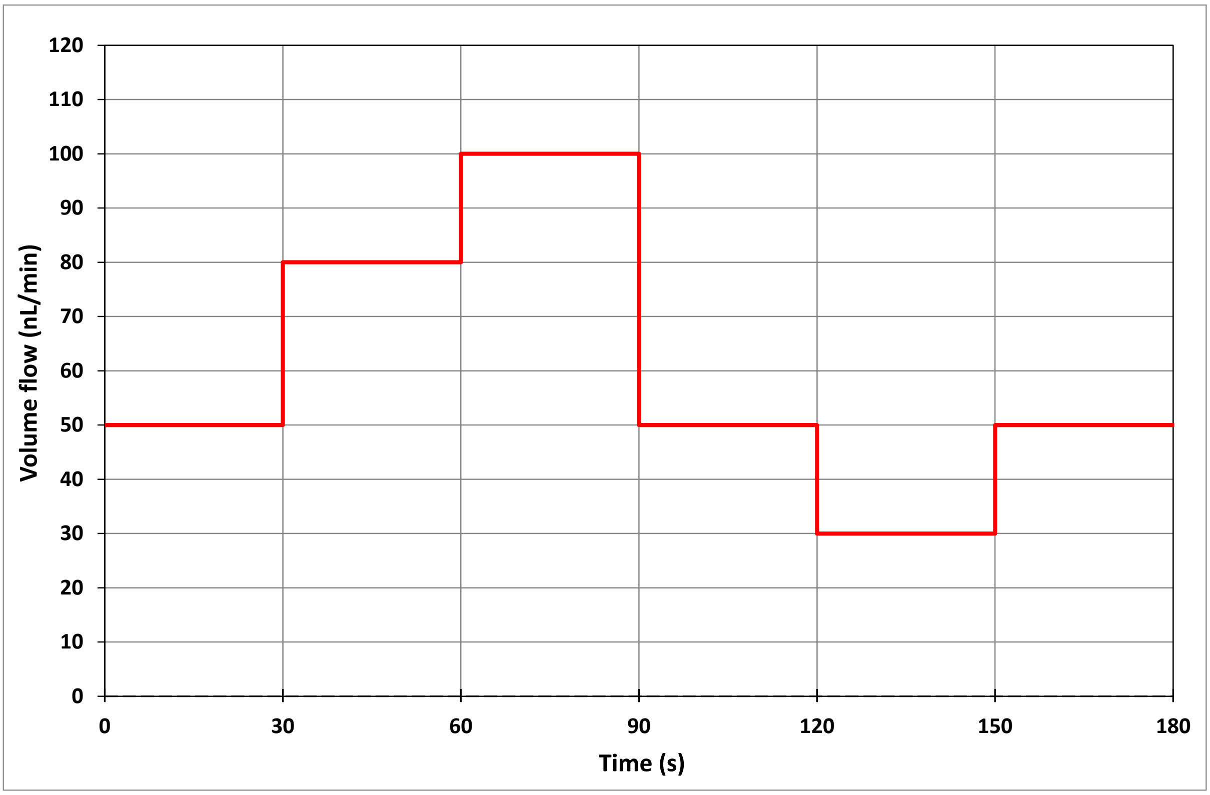

Figure 27 – Dynamic flow profile for 3 minute log time

7 – Organization of the comparison and reference value determination

Each laboratory evaluated their own measurement uncertainty for each calibration data point. The calculations were based on standardised methods as found in the Guide to the Expression of Uncertainty in Measurement [3]. The measured data and calculated measurement uncertainties were then provided to the Pilot laboratory for the Equivalence analysis and reporting. The uncertainty procedures detailed in *Uncertainty components and methods of their evaluation* (Activity A1.2.1) were meant to be followed for both the Static and Dynamic calibrations at each laboratory [4].

In this intercomparison exercise, the individual laboratories each had different practices for estimating their measurement uncertainty. Each partner provided information on the facility measurement uncertainty over a defined range in Section 3. However, in some instances different flow ranges were covered, and the values did not include all of the potential measurement uncertainty sources. Another potential difference between the laboratories was whether the calculation included stability and repeatability or just repeatability. By incorporating different methodologies and definitions, the magnitude of measurement uncertainty for each laboratory could vary significantly.

A summary of key components of each laboratory's uncertainty calculation method is shown in Table 8 below. This table is not exhaustive and only includes three uncertainty sources. Indeed, the various components that contribute to the reference flow rate uncertainty have not been detailed and will be facility methodology dependent. The colour code for Table 8 is as follows:

Green indicates that the budget captures that uncertainty source separately. Red indicates the that the uncertainty source is not considered exclusively. For example, IPQ considers the *Repeatability* uncertainty to be captured as part of the *Reference flow rate* uncertainty.

Table 8 – Facility measurement uncertainty source

Uncertainty Source		IPQ	CETIAT	METAS	RISE	DTI	HS	BHT-BV	THL	UoS/NEL
A	**Reference flow rate**									
B	**Repeatability**									
C	**Stability**									

To determine the reference value the formula of the weighted mean was used, using the inverses of the squares of the associated standard uncertainty as the weights [5]:

$$y = \frac{x_1 / u^2(x_1) + ... + x_n / u^2(x_n)}{1/u^2(x_1) + ... + 1/u^2(x_n)} \qquad (1)$$

To determine the standard deviation *u(y)* associated with *y*:

$$u(y)=\sqrt{\frac{1}{1/u^2(x_1)+...+1/u^2(x_n)}} \tag{2}$$

To identify the overall consistency of the results a chi-square test was applied to all *n* calibration results.

$$\chi^2_{obs}=\frac{(x_1-y)^2}{u^2(x_1)}+\cdots\frac{(x_n-y)^2}{u^2(x_n)} \tag{3}$$

where the degrees of freedom are defined as:

$$\nu = n-1 \tag{4}$$

The consistency check was deemed to be a pass when $\Pr\,(\chi^2(\nu) > \chi^2_{obs}) < 0.05$

where *Pr* is the probability. The calculations were performed in Microsoft Excel using the function CHIINV (probability, degrees, of freedom-1):

$$\chi^2_{obs} < CHIINV(0.05;\nu) \tag{5}$$

If the consistency check passed, then *y* was accepted. If it failed, then the results from the laboratory with the largest contribution to χ^2_{obs} were omitted from the evaluation and the consistency check repeated.

This enabled the comparison of the mean results of each laboratories to the weighted mean value (*REF2*), using each laboratory's uncertainty values. The equivalence of the references can be quantified by calculating the normalised deviation, or equivalence, in the form of the *En* value (*En2*) [6] [7] [8].

$$En2=\frac{LAB-REF2}{\sqrt{(U_{95}LAB)^2+(U_{95}DRIFT)^2-(U_{95}REF2)^2}} \tag{6}$$

where *REF2*, $U_{95}REF2$ and $U_{95}DRIFT$ are defined as:

$$U_{95}REF2=\frac{1}{\sqrt{\sum\left(\frac{1}{(U_{95}LAB)^2}\right)}} \tag{7}$$

$$REF2=\frac{\sum(LAB/(U_{95}LAB)^2)}{\sum(1/(U_{95}LAB)^2)} \tag{8}$$

$$U_{95}DRIFT=\frac{\Delta\varepsilon}{\sqrt{3}} \tag{9}$$

The Epsilon was calculated as the maximum deviation per flow rate at separate calibration dates. From Report *Calibration of Transfer Standards* (Activity A1.3.1), METAS performed characterisation measurements including reproducibility tests on the Sensirion SLG64-0075 [TS1] and Bronkhorst L01 [TS2] flow meters [9].

The Sensirion SLG64-0075 [TS1] was calibrated at METAS on 28.08.2020, 07.01.2021 and 14.09.2021. The maximum deviation (*Epsilon*) was 0.82 % at 20 nL/min and this value was used for the calculation of $U_{95}DRIFT$ for TS1. The resulting $U_{95}DRIFT$ value for TS1 was 0.47 % (*k*=2).

The Bronkhorst L01 [TS2] was calibrated at METAS on 09.12.2020, 11.12.2020 and 15.07.2021. The maximum deviation (*Epsilon*) was 0.71 % at 20 nL/min and this value was used for the calculation of $U_{95}DRIFT$ for TS2. The resulting $U_{95}DRIFT$ value for TS2 was 0.41 % (k=2).

No variability was found in the CETONI Nemesys pump [TS3], therefore the drift uncertainty was not added to the reference value for the results of the CETONI Nemesys pump [TS3].

To establish equivalence between the different laboratories, the *En* value must meet the condition of being less than 1. A warning was issued when $1 > En \leq 1.2$. If the *En* value was greater than 1.2 then the results were considered inconsistent.

8 – Results of the test measurements

To avoid overwhelming the reader with numerous tables and charts, the static calibration data for Transfer Standard 1, Transfer Standard 2 and Transfer Standard 3 are presented in Appendix A, B and C respectively. The dynamic calibration data for Transfer Standard 1 and Transfer Standard 2 are presented in Appendix D and E respectively.

The following results for each Transfer Standard for each calibration method have been presented in the sub-section 6.1 and 6.2. The population size, observed chi-squared value and calculated threshold for each of the comparisons have been included.

The results have all been plotted with an artificial offset to the flow rate to aid visibility for the reader. For example, the 1500 nL/min data points were presented for each laboratory with a 45 nL/min offset ranging from 1275 nL/min to 1725 nL/min.

The uncertainty bands for each result are based upon the supplied measurement uncertainty for that calibration point and include the sources as described in Section 3 and Section 5.

The calculated Equivalence values for each laboratory are also displayed in the following sub-sections. In the Equivalence value tables, the following grading scheme was used:

- $En < 1$ = pass
- $1 \geq En \leq 1.2$ = warning and cell highlighted ***Amber***
- $En > 1.2$ = fail and cell highlighted ***Red***
- *No En value* = Laboratory did not conduct the experiment and cell highlighted ***Dark Grey***
- *(-) En value* = Laboratory excluded after the chi-square test and cell marked with a "-" and highlighted ***Light Grey***

8.1 – Static calibration

The static calibration results are presented below along with the weighted mean error, *REF2* below. Figure 28 displays the Sensirion flow meter [TS1] static results. To improve the resolution, Figure 29 shows the same set of data but with the chart scaled for the 20 nL/min – 100 nL/min range. Table 9 displays the Equivalence values for each laboratory for the Sensirion flow meter [TS1] static results. Table 10 displays the weighted mean error and weighted uncertainty. Table 11 displays the population size, observed chi-squared value and calculated threshold.

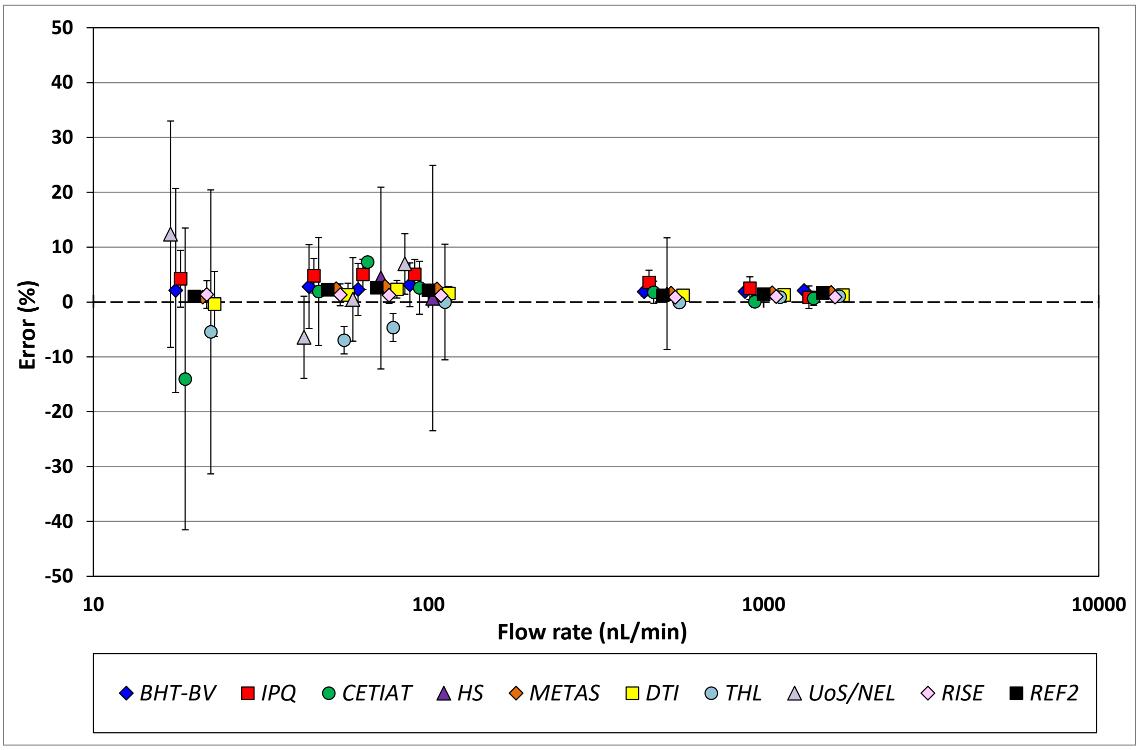

Figure 28 – Sensirion flow meter [TS1] static results

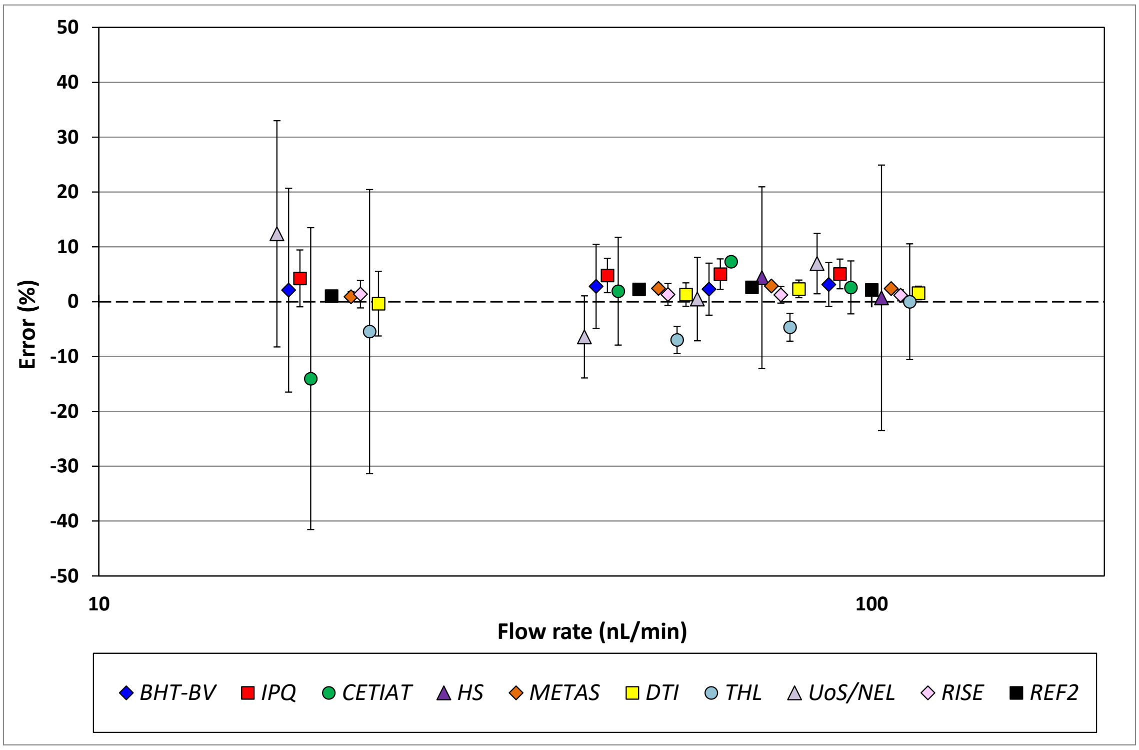

Figure 29 – Sensirion flow meter [TS1] static results from 20 nL/min – 100 nL/min

Table 9 – Laboratory *En* values for the Sensirion flow meter [TS1] static results

Flow rate [nL/min]	IPQ	CETIAT	METAS	RISE	DTI	HS	THL	UoS/NEL	BHT-BV
	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2
1500	0.39	0.74	0.12	1.16	0.62		0.54		0.83
1000	0.51	1.21	0.40	0.70	0.21		0.58		0.79
500	1.03	0.27	0.61	0.59	0.01	0.03	1.42		0.73
100	1.08	0.10	0.41	0.96	0.43	0.06	0.20	0.87	0.25
70	0.86	-	0.37	0.95	0.20	0.10	-	0.28	0.07
50	0.81	0.03	0.31	0.49	0.45		-	-	0.07
20	0.63	-	0.22	0.14	0.24		0.25	0.55	0.06

Table 10 – Weighted mean error and weighted uncertainty for the Sensirion flow meter [TS1] static results

Flow rate [nL/min]	Weighted mean error	Weighted uncertainty
	REF2	$U_{95}REF2$
1500	1.67	0.18
1000	1.45	0.21
500	1.25	0.25
100	2.14	0.46
70	2.64	0.57
50	2.25	0.65
20	1.04	0.84

Table 11 – Population size, observed chi-squared value and calculated threshold for the Sensirion flow meter [TS1] static results

Flow rate [nL/min]	Population size	Observed chi-squared value	Calculated threshold
	n-1	χ^2_{obs}	$\chi^2(n-1)$
1500	6	3.66	12.59
1000	6	3.84	12.59
500	7	5.79	14.07
100	8	9.45	15.51
70	6	4.29	12.59
50	5	3.00	11.07
20	6	10.33	12.59

Figure 30 displays the Bronkhorst flow meter [TS2] static results. To improve the resolution, Figure 31 shows the same set of data but with the chart scaled for the 20 nL/min – 100 nL/min range. Table 12 displays the Equivalence values for each laboratory for the Bronkhorst flow meter [TS2] static results. Table 13 displays the weighted mean error and weighted uncertainty. Table 14 displays the population size, observed chi-squared value and calculated threshold.

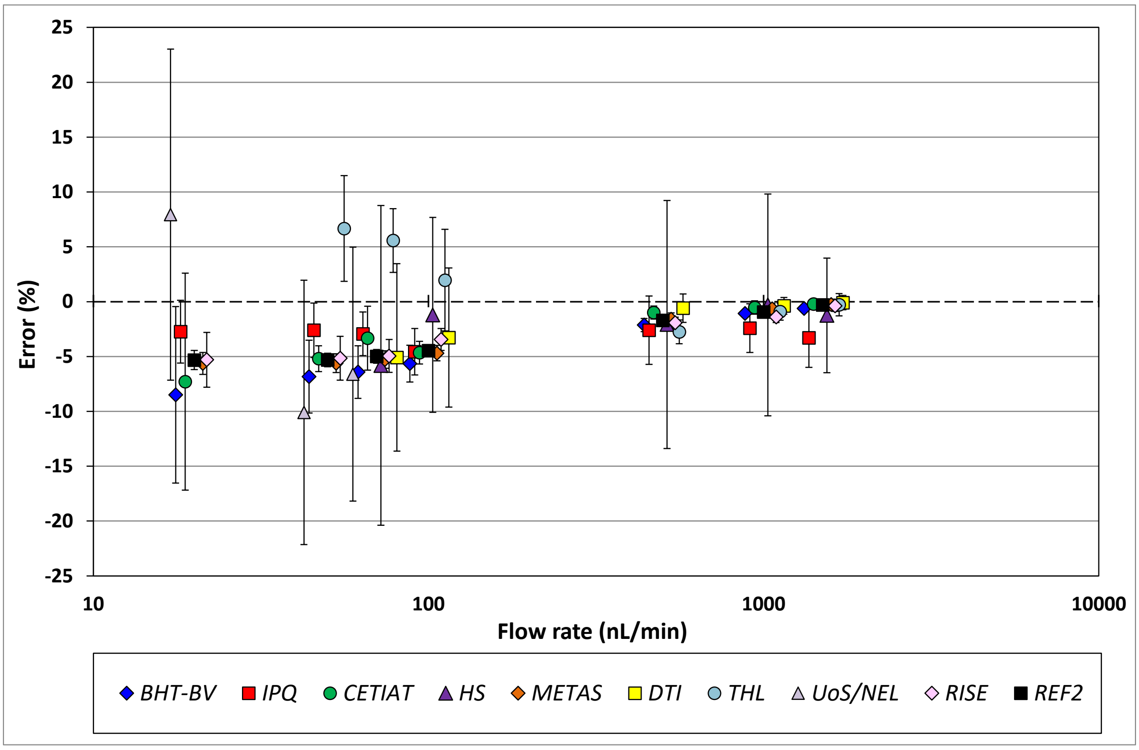

Figure 30 – Bronkhorst flow meter [TS2] static results

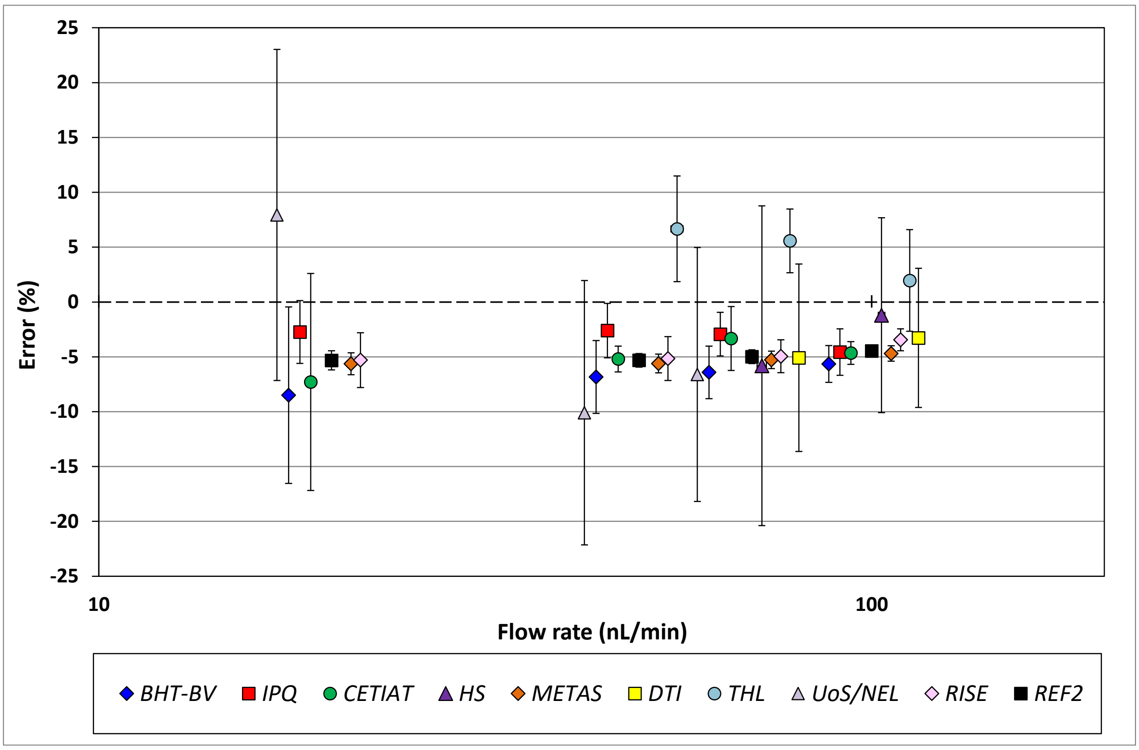

Figure 31 – Bronkhorst flow meter [TS2] static results from 20 nL/min – 100 nL/min

Table 12 – Laboratory *En* values for the Bronkhorst flow meter [TS2] static results

Flow rate [nL/min]	IPQ	CETIAT	METAS	RISE	DTI	HS	THL	UoS/NEL	BHT-BV
	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2
1500	1.11	0.18	0.06	0.14	0.27	0.18	0.02		0.65
1000	0.65	0.59	0.47	0.74	0.67	0.06	0.04		0.27
500	0.28	1.06	0.17	0.35	0.84	0.03	0.93		0.63
100	0.05	0.19	0.34	1.04	0.19	0.37	1.39		0.72
70	1.06	0.58	0.46	0.02	0.01	0.06	-	0.14	0.61
50	1.11	0.11	0.40	0.08			-	0.40	0.46
20	0.94	0.20	0.48	0.01			-	-	0.40

Table 13 – Weighted mean error and weighted uncertainty for the Bronkhorst flow meter [TS2] static results

Flow rate [nL/min]	Weighted mean error	Weighted uncertainty
	REF2	***U_{95}REF2***
1500	-0.29	0.12
1000	-0.94	0.21
500	-1.71	0.27
100	-4.46	0.47
70	-4.98	0.62
50	-5.31	0.62
20	-5.32	0.87

Table 14 – Population size, observed chi-squared value and calculated threshold for the Bronkhorst flow meter [TS2] static results

Flow rate [nL/min]	Population size	Observed chi-squared value	Calculated threshold
	n-1	χ^2_{obs}	$\chi^2(n-1)$
1500	7	3.99	14.07
1000	7	2.34	14.07
500	7	3.50	14.07
100	7	12.31	14.07
70	7	4.29	14.07
50	5	5.68	11.07
20	4	4.07	9.49

Figure 32 displays the CETONI Nemesys syringe pump [TS3] static results.

Table 15 displays the Equivalence values for each laboratory for the CETONI Nemesys syringe pump [TS3] static results. Table 16 displays the weighted mean error and weighted uncertainty. Table 17 displays the population size, observed chi-squared value and calculated threshold.

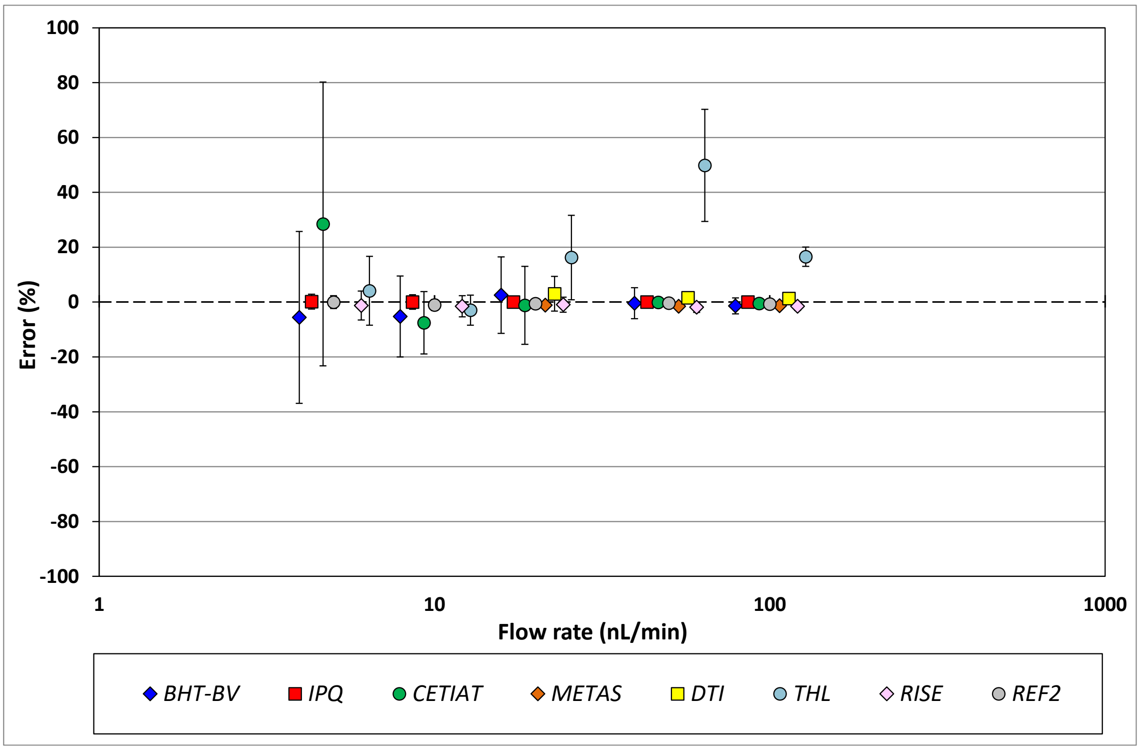

Figure 32 – CETONI Nemesys Syringe pump [TS3] static results

Table 15 – Laboratory *En* values for the CETONI Nemesys Syringe pump [TS3] static results

Flow rate [nL/min]	**IPQ**	**CETIAT**	**METAS**	**RISE**	**DTI**	**HS**	**THL**	**UoS/NEL**	**BHT-BV**
	E_n2	**E_n2**	**E_n2**	**E_n2**	**E_n2**	**E_n2**	**E_n2**	**E_n2**	**E_n2**
100	0.64	0.31	**1.00**	0.57	**1.25**		-		0.23
50	0.37	0.70	**1.31**	0.82	**1.13**		-		0.001
20	0.64	0.04	0.70	0.13	0.58		-		0.22
10	0.64	0.58		0.13			0.36		0.28
5	0.13	-		0.26			0.34		0.18

Table 16 – Weighted mean error and weighted uncertainty for the CETONI Nemesys syringe pump [TS3] static results

Flow rate [nL/min]	Weighted mean error	Weighted uncertainty
	REF2	*U_{95}REF2*
100	-0.71	0.42
50	-0.41	0.45
20	-0.61	0.75
10	-1.09	1.98
5	-0.03	2.35

Table 17 – Population size, observed chi-squared value and calculated threshold for the CETONI Nemesys syringe pump [TS3] static results

Flow rate [nL/min]	Population size	Observed chi-squared value	Calculated threshold
	n-1	χ^2_{obs}	$\chi^2(n-1)$
100	5	3.97	11.07
50	5	4.95	11.07
20	5	3.40	11.07
10	4	5.99	9.49
5	3	2.64	7.81

8.2 – Dynamic calibration

The dynamic calibration results are presented below along with the weighted mean error, *REF2* below. Figure 33 displays the Sensirion flow meter [TS1] dynamic results. To improve the resolution, Figure 34 shows the same set of data but with the chart scaled for the -25 % to +25% Error range. Table 18 displays the Equivalence values for each laboratory for the Sensirion flow meter [TS1] dynamic results. Table 19 displays the weighted mean error and weighted uncertainty. Table 20 displays the population size, observed chi-squared value and calculated threshold.

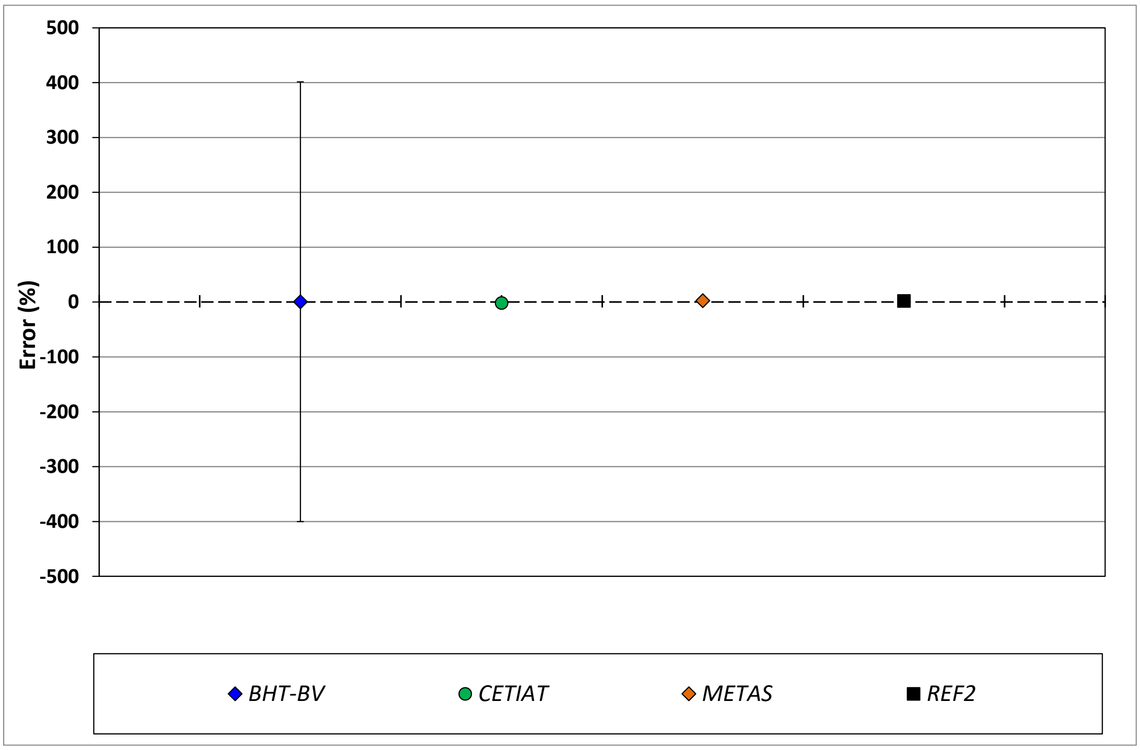

Figure 33 – Sensirion flow meter [TS1] dynamic results

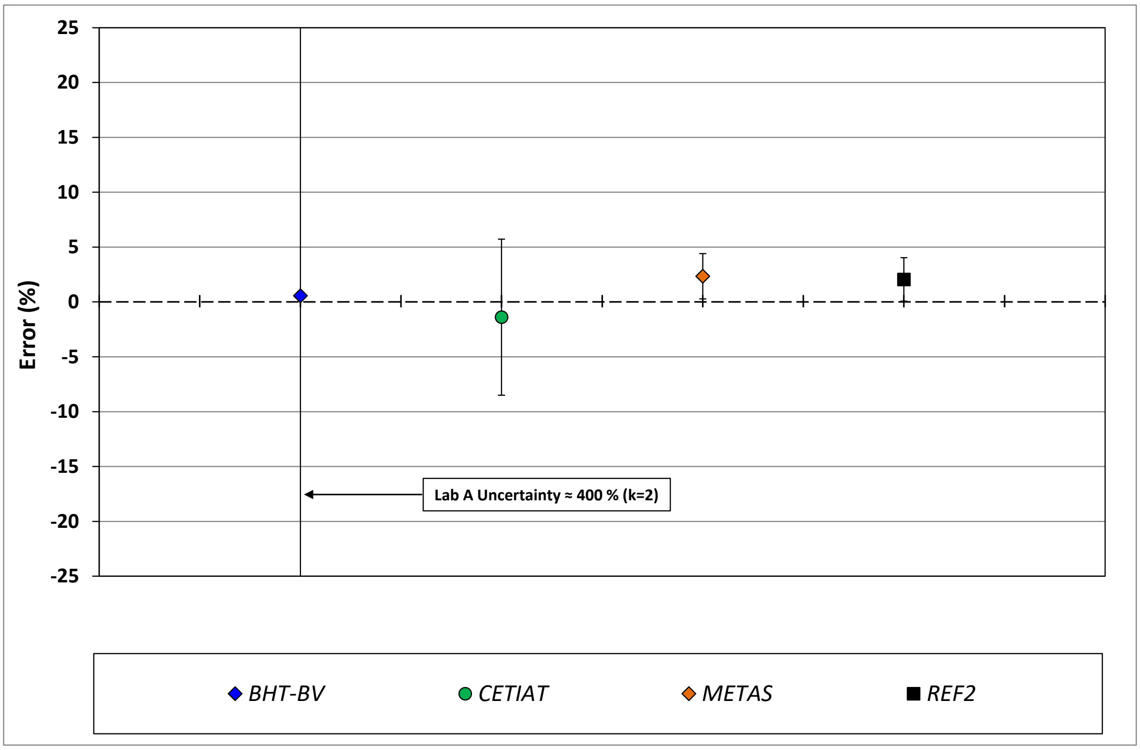

Figure 34 – Sensirion flow meter [TS1] dynamic results with reduced scale Y axis

Table 18 – Laboratory *En* values for the Sensirion flow meter [TS1] dynamic results

Dynamic Flow	IPQ	CETIAT	METAS	RISE	DTI	HS	THL	UoS/NEL	BHT-BV
	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2
		0.50	0.50						0.004

Table 19 – Weighted mean error and weighted uncertainty for the Sensirion flow meter [TS1] dynamic results

Dynamic Flow	Weighted mean error	Weighted uncertainty
	REF2	$U_{95}REF2$
	2.06	1.99

Table 20 – Population size, observed chi-squared value and calculated threshold for the Sensirion flow meter [TS1] dynamic results

Dynamic Flow	Population size	Observed chi-squared value	Calculated threshold
	n-1	χ^2_{obs}	$\chi^2(n-1)$
	2	1.79	5.99

Figure 35 displays the Bronkhorst flow meter [TS2] dynamic results. To improve the resolution, Figure 36 shows the same set of data but with the chart scaled for the -25 % to +25% Error range. Table 21 displays the Equivalence values for each laboratory for the Bronkhorst flow meter [TS2] dynamic results. Table 22 displays the weighted mean error and weighted uncertainty. Table 23 displays the population size, observed chi-squared value and calculated threshold.

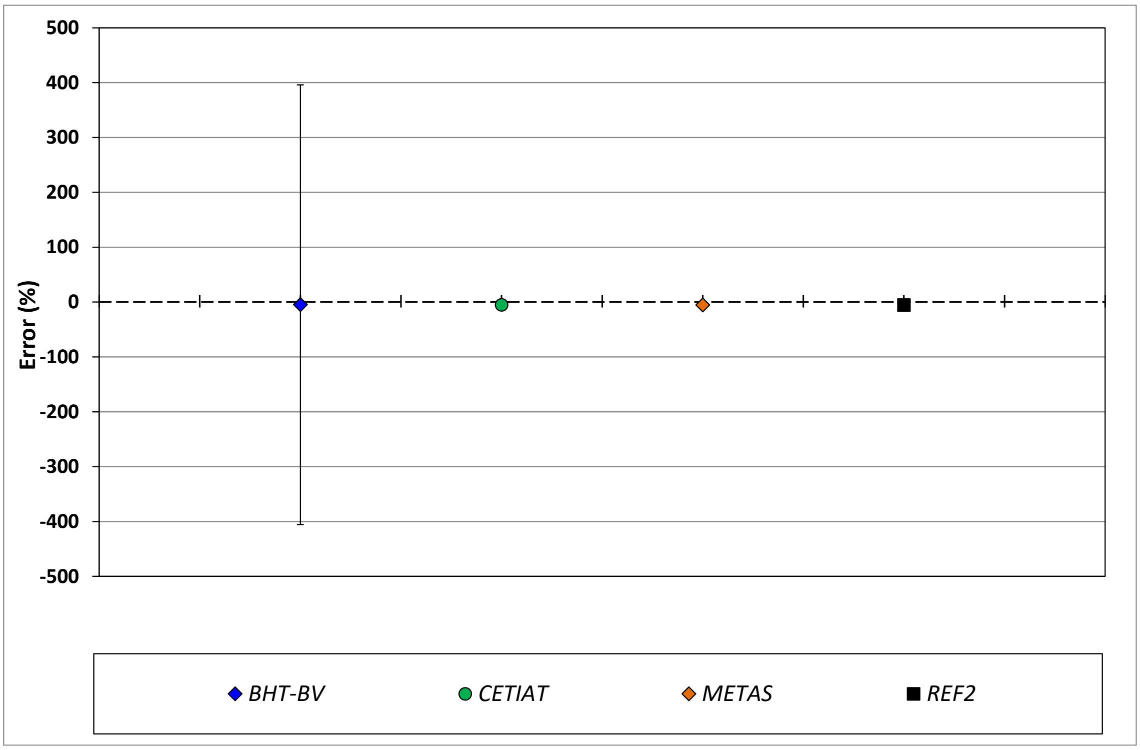

Figure 35 – Bronkhorst flow meter [TS2] dynamic results

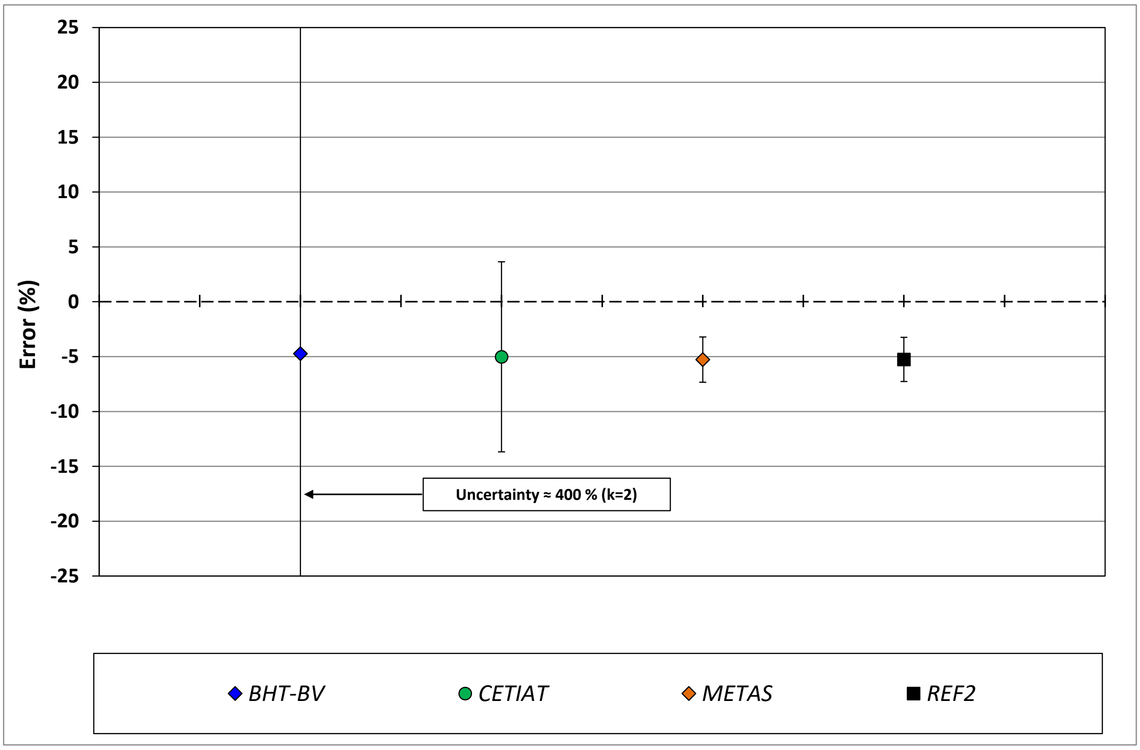

Figure 36 – Bronkhorst flow meter [TS2] dynamic results with reduced scale Y axis

Table 21 – Laboratory *En* values for the Bronkhorst flow meter [TS2] dynamic results

Dynamic Flow	IPQ	CETIAT	METAS	RISE	DTI	HS	THL	UoS/NEL	BHT-BV
	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2	E_n2
		0.03	0.03						0.001

Table 22 – Weighted mean error and weighted uncertainty for the Bronkhorst flow meter [TS2] dynamic results

Dynamic Flow	Weighted mean error	Weighted uncertainty
	REF2	***U_{95}REF2***
	-5.25	2.00

Table 23 – Population size, observed chi-squared value and calculated threshold for the Bronkhorst flow meter [TS2] dynamic results

Dynamic Flow	Population size	Observed chi-squared value	Calculated threshold
	n-1	χ^2_{obs}	$\chi^2(n-1)$
	2	0.01	5.99

9 – Conclusions

The overall conclusion from this comparison exercise was that it was extremely successful and enabled the partners to share knowledge, improve their methodologies, evaluate their measurement uncertainties, and enhance their calibrations facilities. All participants succeeded in performing the evaluation with at least one Transfer Standard at several flow rates and to obtain results in line with the agreed protocol.

The following two sub-sections provide the overall conclusions for the static calibration comparison and the dynamic calibration comparison.

9.1 – Static calibration

The results from the static calibrations were extremely promising and several laboratories achieved equivalency for at least two of the flow rates conducted with each of the three transfer standards. Specifically:

- The transfer standards used were found to demonstrate the required stability and repeatability
- 79% of calibration points with TS1 were recorded by the laboratories. 90% of the recorded calibration points with TS1 had an *En* value less than 1.0
- 81% of calibration points with TS2 were recorded by the laboratories. 88% of the recorded calibration points with TS2 had an *En* value less than 1.0
- 60% of calibration points with TS3 were recorded by the laboratories. 85% of the recorded calibration points with TS3 had an *En* value less than 1.0
- Some of the measurement uncertainty values claimed by the laboratories at certain flow rates might require further investigation
- It was possible to measure flow rates down to 5 nL/min with 3 % uncertainty.

9.2 – Dynamic calibration

The results from the dynamic calibrations validate the traceability of the three laboratories that participated in the comparison. Whilst only three laboratories conducted the comparison for dynamic flow with both of the Transfer Standards, the results were extremely positive and further underpin the laboratory measurement uncertainty. All of the calculated *En* values were less than 1.0 for the dynamic flow calibrations.

References

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Appendix A – Static Results Sensirion flow meter [TS1]

Table 24 – Measurement data for each flow laboratory for TS1

Flow rate [nL/min]	IPQ		CETIAT		METAS		RISE		DTI		HS		BHT-BV		THL		UoS/NEL	
	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)
1500	0.85	2.07	0.67	1.29	1.74	0.38	0.90	0.50	1.26	0.51			2.10	0.28	1.08	1.00		
1000	2.53	2.07	0.07	1.06	1.69	0.40	0.99	0.50	1.31	0.51			1.92	0.42	0.91	0.84		
500	3.58	2.23	1.79	2.01	1.63	0.48	0.87	0.50	1.24	0.54	1.52	10.19	1.88	0.77	-0.08	0.85		
100	5.06	2.70	2.61	4.82	2.39	0.60	1.17	1.00	1.59	1.25	0.71	24.21	3.14	4.01	-0.01	10.55	6.95	5.51
70	5.03	2.78	7.29	0.92	2.87	0.69	1.25	1.50	2.33	1.61	4.38	16.57	2.29	4.75	-4.67	2.54	0.48	7.60
50	4.78	3.14	1.91	9.84	2.44	0.75	1.30	2.00	1.31	2.14			2.80	7.65	-6.97	2.48	-6.42	7.50
20	4.27	5.18	-14.03	27.52	0.91	0.92	1.38	2.50	-0.34	5.89			2.11	18.59	-5.44	25.89	12.38	20.63

Appendix B – Static Results Bronkhorst flow meter [TS2]

Table 25 – Measurement data for each flow laboratory for TS2

Flow rate [nL/min]	IPQ		CETIAT		METAS		RISE		DTI		HS		BHT-BV		THL		UoS/NEL	
	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)
1500	-3.3	2.7	-0.22	0.15	-0.26	0.48	-0.38	0.50	-0.09	0.65	-1.25	5.22	-0.62	0.32	-0.28	1.02		
1000	-2.4	2.2	-0.53	0.61	-0.65	0.51	-1.40	0.50	-0.38	0.77	-0.30	10.12	-1.08	0.34	-0.91	0.78		
500	-2.6	3.1	-0.99	0.60	-1.60	0.58	-1.91	0.50	-0.59	1.29	-2.08	11.31	-2.13	0.60	-2.75	1.09		
100	-4.6	2.1	-4.65	1.03	-4.69	0.71	-3.44	1.00	-3.27	6.34	-1.20	8.88	-5.65	1.67	1.96	4.63		
70	-2.9	2.0	-3.33	2.91	-5.28	0.80	-4.95	1.50	-5.08	8.54	-5.81	14.57	-6.41	2.40	5.58	2.91	-6.61	11.58
50	-2.6	2.5	-5.20	1.18	-5.61	0.86	-5.16	2.00					-6.83	3.32	6.67	4.82	-10.09	12.05
20	-2.7	2.9	-7.29	9.89	-5.63	1.00	-5.30	2.50					-8.49	8.05	34.52	9.23	7.94	15.09

Appendix C – Static Results CETONI Nemesys syringe pump [TS3]

Table 26 – Measurement data for each flow laboratory for TS3

Flow rate [nL/min]	IPQ		CETIAT		METAS		RISE		DTI		HS		BHT-BV		THL		UoS/NEL	
	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)
100	0.02	1.21	-0.53	0.70	-1.29	0.72	-1.52	1.48	1.30	1.66			-1.37	2.9	16.54	3.52		
50	0.01	1.21	-0.09	0.64	-1.51	0.95	-1.88	1.86	1.62	1.85			-0.41	5.6	49.83	20.42		
20	0.00	1.22	-1.21	14.21	-1.11	1.04	-0.95	2.73	3.03	6.35			2.52	13.9	16.24	15.38		
10	0.04	2.65	-7.53	11.35			-1.53	3.86					-5.24	14.8	-2.94	5.47		
5	0.15	2.69	28.48	51.73			-1.25	5.26					-5.57	31.3	4.11	12.53		

Appendix D – Dynamic Results Sensirion flow meter [TS1]

Table 27 – Dynamic test completed by each flow laboratory for TS1

Dynamic Flow	IPQ		CETIAT		METAS		RISE		DTI		HS		BHT-BV		THL		UoS/NEL	
	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)
			-1.39	7.11	2.35	2.1							0.57	400.76				

Appendix E – Dynamic Results Bronkhorst flow meter [TS2]

Table 28 – Dynamic test completed by each flow laboratory for TS2

Dynamic Flow	IPQ		CETIAT		METAS		RISE		DTI		HS		BHT-BV		THL		UoS/NEL	
	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)	Error (%)	Uncertainty (%)
			-5.02	8.66	-5.27	2.06							-4.74	400.77				