



Proposals for NORMAN Joint Programme of Activities 2025

Title	Intercomparison study on flow cytometry
Type of activity	Intercomparison study
Leader	IWW Institute for Water Research, Mülheim an der Ruhr, Germany
Topic activities	<p>Background / Justification for the proposed activity:</p> <p>Flow cytometry is not only used in the medical field but is also successfully applied for the microbiological analysis of drinking- and surface water. This applies to academic research and increasingly to drinking water companies. The technology is based on quantification of cells after staining with fluorescent dyes and subsequent measurement. This allows for a rapid quantification of these cells (mainly bacterial concentrations with approx. 15 min in an automated fashion. Measured parameters specifically include total and intact cell counts, the bacterial regrowth potential and the assessment of specific bacterial clusters (such as HNA (high nucleic acid) and LNA (low nucleic acid) bacteria. Together these data provide important information about the microbiological water quality and even enable the identification of a characteristic microbial fingerprint of a particular water. However, unlike for medical applications, there are no standard procedures, norms, or interlaboratory comparisons for the drinking water sector. Although many publications use similar methods, the measurements are subject to variations. These include the presence of solvents in dye solutions and their concentrations, incubation times with fluorescent dyes, incubation temperature, the manner of how samples are heated (e.g. in heat blocks of different sizes or air chambers) etc.. Additionally, the measurement results are influenced by flow cytometer devices from different manufacturers. Furthermore, parameters such as the sample storage time and temperature prior to measurement during sample transport can significantly influence the obtained values. Consequently, there is no guarantee of the accuracy of the acquired results as small differences in sample transport, handling and processing can result in substantial deviations of results.</p> <p>Initial comparative measurements of the total cell count in ten samples (unpublished data) between our and another laboratory resulted in minor deviations within our lab but significant deviations between the labs, ranging from 2% to 66%. Therefore, we believe that the implementation of a interlaboratory comparison for drinking- and surface water is necessary. Despite the huge potential of flow cytometry for rapid water quality assessment and process control, standardization is crucial to obtain comparable and reliable data.</p> <p>Project aim: With this JPA we aim to implement an intercomparison study for the field of drinking- and surface water flow cytometry. Therefore, we aim to produce standardized and stable samples for comparative measurements between laboratories all over Europe. The measurement of standardized samples will provide quality assurance for flow cytometry and the comparability of results between different laboratories.</p> <p>Description of the proposed activity and expected outcomes for 2025:</p> <p>Start of phase 1 in 2024: Testing of sample preparation for shipping We are planning a series of experiments in preparation of the intercomparison study. <i>E. coli</i> will serve as a model organism, since it is already successfully used as an internal standard for quality assurance in our laboratories with</p>

	<p>reproducible results.</p> <ul style="list-style-type: none"> • Development and testing of a procedure for sample homogenization • Standardization of a measurement procedure • Sample preservation for multi-day transport to participants. Since sample fixation with glutaraldehyde can interfere with fluorescent staining, alternatively the shipping of frozen samples is considered. The impact of the following parameters are addressed: <ul style="list-style-type: none"> - Freezing samples with glycerol as a cryoprotectant in different concentrations and its impact on measurement/devices - Shock freezing of samples using liquid nitrogen with and without glycerol - Impact of thawing samples on ice or at room temperature - Testing the reproducibility of the developed procedure through multiple repetitions • Optimization of the shipping procedure regarding material and sample volume • External testing of the shipping procedure and conducting first preliminary tests of comparative measurements with a small group of selected laboratories in Germany • Data evaluation and method improvement <p>In 2025 we will start with phase 2: Sending standardized samples to participating laboratories for comparative measurements</p> <ul style="list-style-type: none"> • Start of the main comparative measurements. Sending multiple rounds of standardized samples to all participation laboratories across Europe • Data evaluation from comparative measurements • Establishment of a standardized QA procedure for comparative measurements in flow cytometry <p><i>Added value / Link with other NORMAN activities and / or other projects</i></p> <p>The new Interlaboratory comparison will enable flow cytometry quality assurance of the participating laboratories. Standardization as key for obtaining reliable data will contribute to a more widespread application of flow cytometry in the water sector and its consideration as a standard method by regulatory authorities.</p>
Participants	Any interested laboratories (academia, R&D, water companies). The ILS testing will be carried out first with laboratories in Germany first during the testing process. The final intercomparison with the adjusted method will be extended towards non-German members (NORMAN members and interested laboratories) across Europe.
Proposed in-kind contribution	Planning of the experimental setup, provision of equipment, coordination with other laboratories and as well as the establishment of an evaluation template by members of the IWW Institute for Water Research.
Contribution needed from NORMAN	Total project associated costs of 10,000,- €. This will include all consumables, working hours of a technician performing the experimental part, sample preparation and shipping costs in phase 1 (within Germany) and phase 2 (within Europe), and the overall data evaluation



Association¹

¹ Please, provide here a transparent justification of the requested resources and of the in-kind contribution, thereby distinguishing between the costs associated with “person-months” for the organisation, the “travelling costs” for invited speakers and the costs for the logistics (e.g. meals, room rental etc.)