



MICROBE
the Microbiome
Biobanking (RI)
Enabler

Let us pave the way
for microbiome
biobanking



MICROBE training sheet

Pre-analytical Phase

Lessons from the human microbiome field: Pre-analytical variables for reliable non-human biobanking and research

Introduction and summary.

The pre-analytical phase is a major - yet often underestimated - source of inaccurate or even false and irreproducible analysis. It consists of multiple steps, each associated with variables/factors that can alter sample quality and thus the outcome of analyses. Typically, not all process steps are in the hands of the related biobank or the principal investigator of a certain research study, but are performed by multiple involved parties. This increases the need for a coordinated and standardized process and the recording of relevant metadata to ensure fit-for-purpose samples of high and defined quality as well as FAIR data.

This guide raises awareness of critical variables across the pre-analytical phase in the plant microbiome field. It supports researchers and biobanks by highlighting key factors to consider when planning plant microbiome studies and drafting standard-operating procedures (SOPs).

Overview and background.

Over the past years, European CEN and international ISO standards have been developed for the human field including the human microbiome field. These standards are relevant for manufacturers of in vitro diagnostic devices (IVDs) and diagnostic laboratories, but also for biobanks and research laboratories. One key pre-analytical sample quality standard is [CEN/TS 17626:2021*](#) for human specimens intended for DNA-based microbiomes analysis. As the human microbiome research field is more advanced, it was selected as a best practices example for other (i.e., environmental) microbiome research fields. The existing SPIDIA4P-/BBMRI.at-contributed standard CEN/TS 17626:2021 was used as a template for the development of pre-analytical guidance documents for other sample types (i.e. plant, soil, and marine water) relevant to the MICROBE project.

Pre-analytical workflow and variables for plant microbiome samples.

1) Consider the pre-analytical workflow relevant to your plant microbiome study

The pre-analytical workflow of a plant microbiome study is a multistep process (Figure 1). It starts with the environment, habitat and host (e.g. plant), and includes the sample collection (e.g., agricultural land and wild ecosystem, greenhouse or laboratory), typically followed by sample stabilization/preservation (e.g., freezing, or fixation in stabilizer solutions), intermediate storage at the collection site, and transport to the laboratory (or biobank). After receipt in the laboratory/biobank there is often a sample processing step (e.g., aliquoting or pooling of samples, surface sterilization, and/or homogenization of the sample). Finally, analyte isolation (e.g., DNA or RNA extraction) and assessment of the yield and quality, or other types of preparation for analysis (such as cultivation/propagation) can take place, followed by storage until analysis.

(* <https://www.dinmedia.de/en/standard/pd-cen-ts-17626/342128726>)

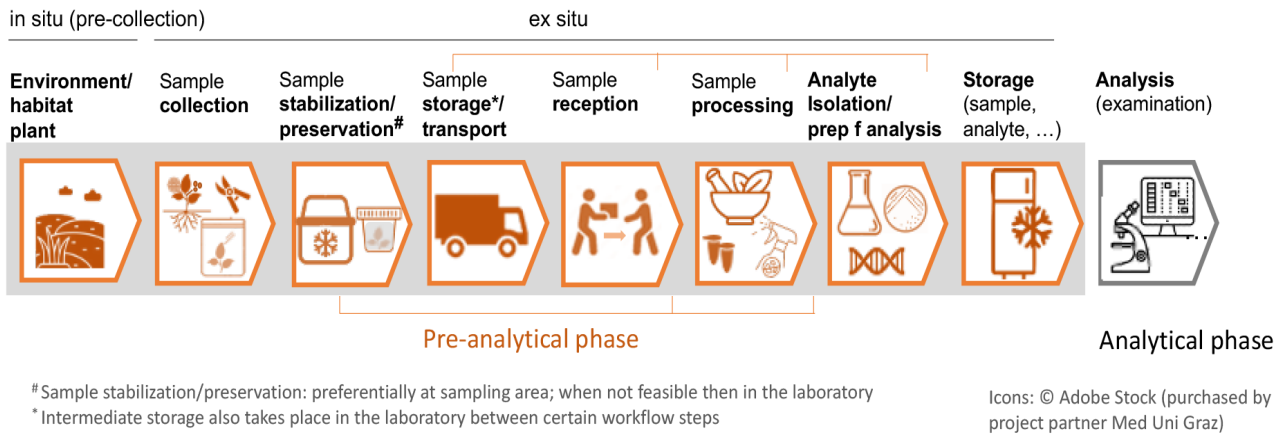


Figure 1: Classical (ideal) pre-analytical workflow of plant microbiome studies

2) Be aware of potential pre-analytical variables impairing sample quality and modifying your plant microbiome study data

Along the entire workflow multiple pre-analytical variables/factors exist. These differ to some extent depending on the plant study type (i.e. wild ecosystem, agricultural land, greenhouse with soil or substrate, in vitro plants) and the study purpose (intended use of the samples). Major critical variables are outlined in Figure 2. They can drastically change the plant microbiome composition and biomolecule (e.g., DNA or RNA) profiles in the sample and thus also the resulting data and interpretation. Where possible these variables need to be standardized and measures taken to avoid or minimize the artificial impact of the pre-analytical procedure on the sample. As not all of these variables can be influenced (e.g., weather conditions at the collection site in the field) they need at least to be documented for later use in the interpretation of results, comparability of studies, or reuse of samples and data.

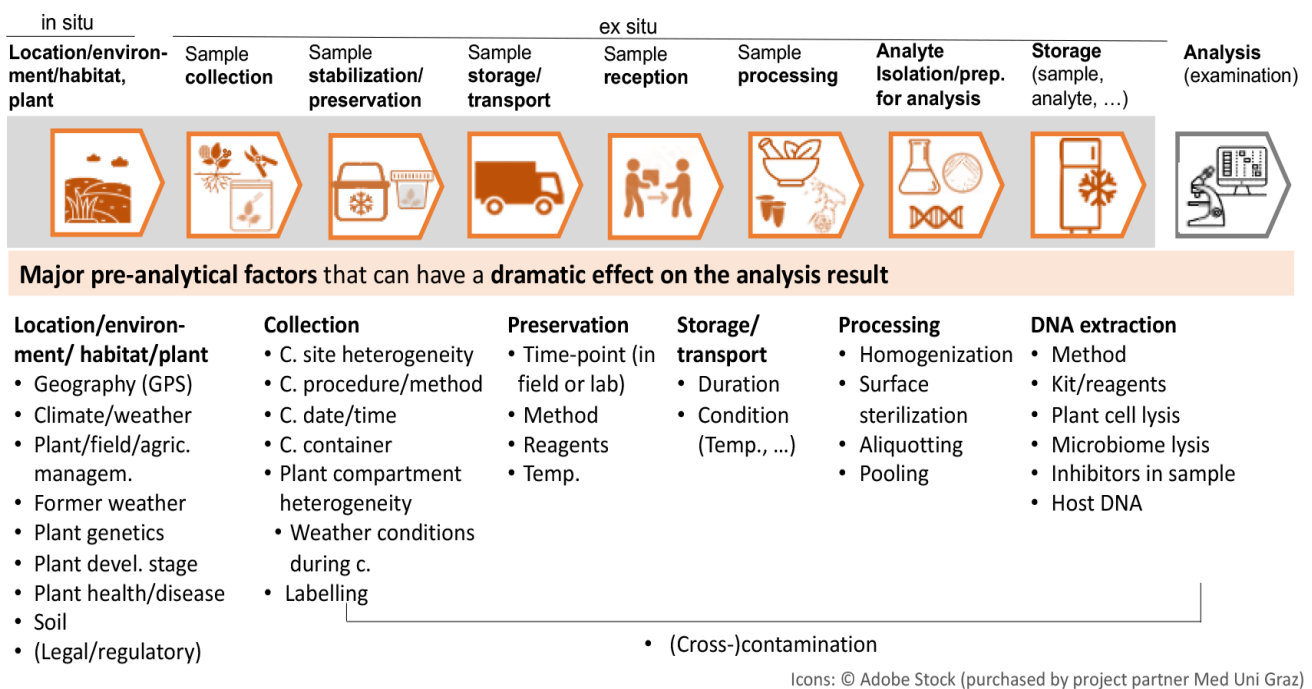


Figure 2: Potential critical pre-analytical variables along the workflow of plant microbiome studies

3) Reflect major requirements/recommendations for the pre-analytical phase and (meta)data

To support standardization across the pre-analytical workflow, special measures have to be taken. These should secure the stability of the microbiome and its biomolecule profiles once the plant sample has been removed from its natural habitat, and ensure the availability of relevant metadata.

A list of requirements/recommendations can be found in the [MICROBE D4.2 Guidance Document](https://shorturl.at/NsVlx). (<https://shorturl.at/NsVlx>)

Advantages for users and stakeholders

The main users of this guidance are researchers, scientists, plant experts and many more. Examples from user fields: Agriculture, forestry/ecology, environmental health, and One Health.

Benefits from reading this guide:

- Rapid orientation: Overview of the pre-analytical workflow and its critical variables.
- Raised awareness: Highlights for where errors and bias most often occur.

Benefits when applying pre-analytical guidance:

- Systematic and faster planning: clear, step-by-step pre-analytical workflow considerations save time when designing studies, writing a study plan and SOPs;
- Improved reproducibility and quality of samples through standardized handling;
- Better data comparability and FAIR-compliant metadata capture;
- Reduced risk, bias, and downstream costs via consistent pre-analytical procedures.
- Greater credibility with reviewers, funders, and regulators.



Involved experts (partners)

Medical University of Graz

MICROBE Consortium

EPSO WG Plants and Microbiomes

Links & further information

- [Guidance document for the implementation of a standardized pre-analytical workflow in selected fields](https://shorturl.at/4gtlK) (MICROBE Deliverable D4.2) (<https://shorturl.at/4gtlK>)
- Stumtner C, et al., [The Pre-Analytical CEN/TS Standard for Microbiome Diagnostics - How Can Research and Development Benefit?](https://shorturl.at/SbzB5) PMID: PMC9104691 (<https://shorturl.at/SbzB5>)
- [Preanalytical Quality Standards for Microbiome Samples](https://shorturl.at/2lncu) (BBMRI.at poster at EBW 2024 conference) (<https://shorturl.at/2lncu>)
- [Quality management and relevant standards for biobanking](https://shorturl.at/9gogh) (<https://shorturl.at/9gogh>)



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