




How to tackle standardization issues in the microbiome field

Cornelia Stumptner
 Medical University of Graz, BBMRI.at, Austria
Cornelia.stumptner@medunigraz.at

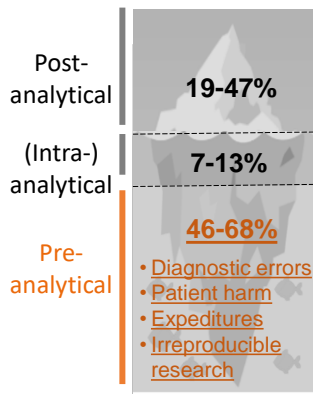


 This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101094353

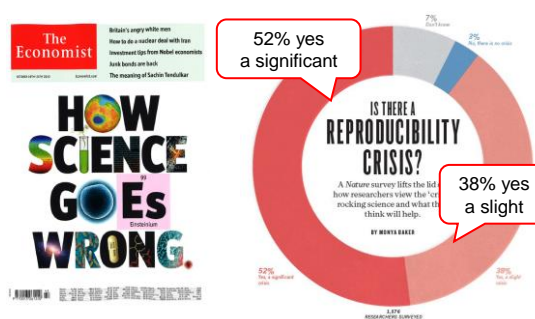


Why standardization & focus on pre-analytical phase?

Diagnostic testing process (%):



Research & development:



very critical but often neglected or underestimated

also in the microbiome field

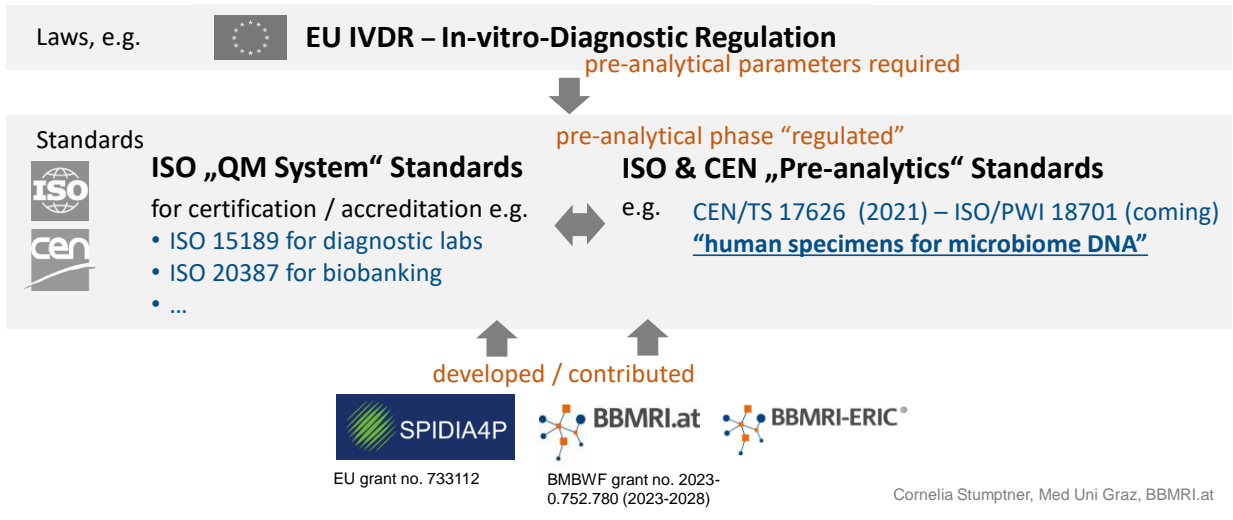
FREEDMAN LP et al, PLoS Biol 13(6): (2015) doi.org/10.1371/journal.pbio.1002165
 Economist, *How science goes wrong* (2013)
 BAKER M., Nature (2016) doi.org/10.1038/533452a

Lippi et al., CCLM (2014). [doi: 10.1515/cclm-2014-1051](https://doi.org/10.1515/cclm-2014-1051)
 Institute of Medicine (IOM) Report, Sep (2015) <https://doi.org/10.17226/21794>
 Green SF. Clin Biochem, (2013) <https://doi.org/10.1016/j.clinbiochem.2013.06.001>

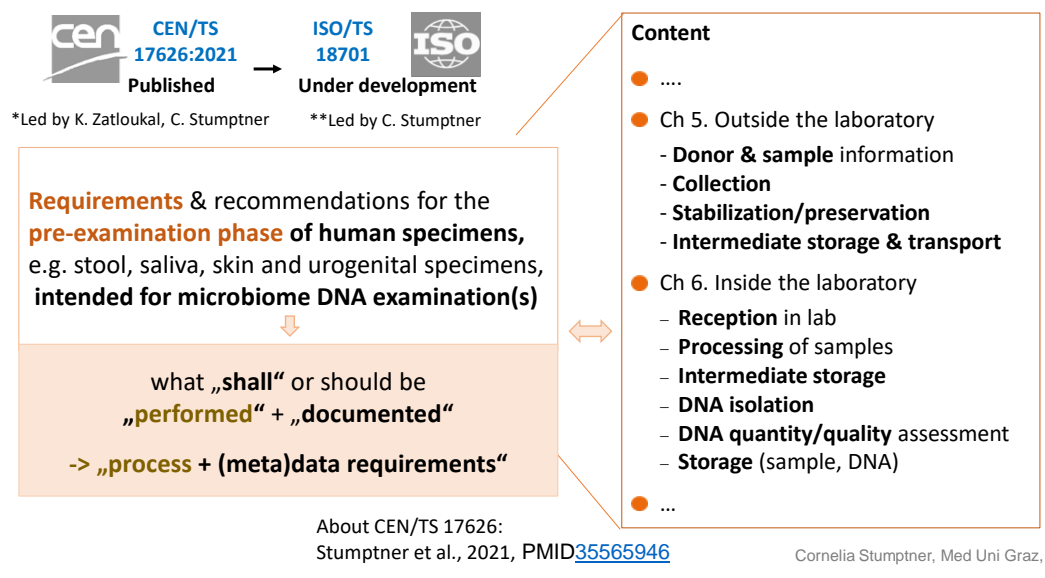
Cornelia Stumptner, Med Uni Graz, BBMRI.at



Reducing pre-analytical errors



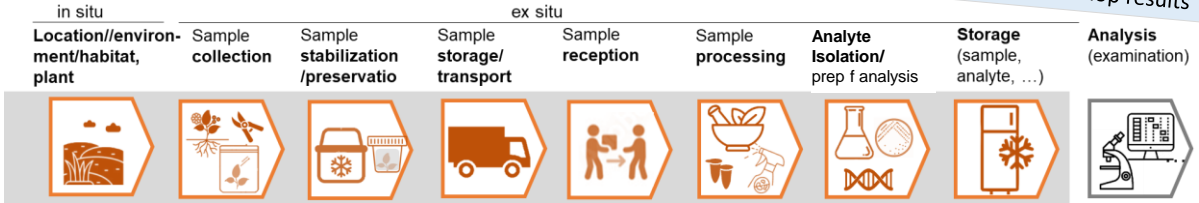
Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for human specimen — Isolated microbiome DNA



Pre-analytical phase & variables – plant microbiome field



Incl. MICROBE/EPSo workshop results



Major pre-analytical factors that can have a dramatic effect on the analysis result

- | | | | | | |
|---|---|--|---|--|---|
| Location/environment/habitat/plant <ul style="list-style-type: none"> • Geography (GPS) • Climate/weather • Agric./plant managem. • Former weather • Plant genetics, • Plant devel. stage • Plant health/disease • Soil • (Legal/regulatory) • ... | Collection <ul style="list-style-type: none"> • C. site heterogeneity • C. procedure • C. date/time • C. container • Plant heterogeneity • Weather during C. • Labelling • ... | Preservation <ul style="list-style-type: none"> • Time-point (in field or lab) • Method • Reagents • Temp. • ... | Storage/Transport <ul style="list-style-type: none"> • Duration • Condition (Temp., ...) | Processing <ul style="list-style-type: none"> • Homogenization • Sterilization, ... • Aliquotting • ... | DNA extraction <ul style="list-style-type: none"> • Method/Kit/reagents • Plant cell lysis • Microbiome lysis • Inhibitors • Host DNA • .. |
|---|---|--|---|--|---|
- (Cross-)contamination

Posters
546 (8C)
549 (3A)

Cornelia Stumpner, Med Uni Graz, MICROBE, BBMRI.at

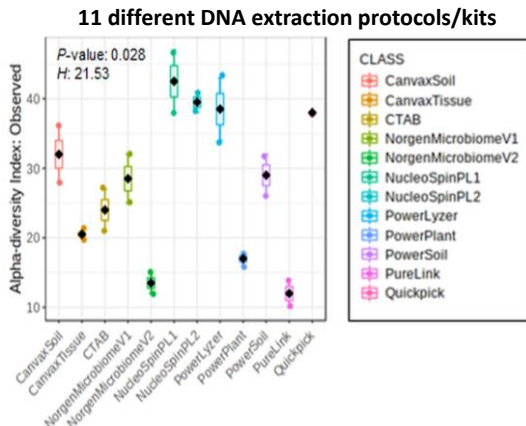
The importance of quality-controlled workflows & comprehensive metadata – plant microbiome field



Selected examples from literature

The effect of microbiome DNA extraction protocols/kits

⇒ DNA extraction protocol significantly affected bacterial community assessment



Richness alpha diversity index showed significant differences ($P < 0.044$) among the different DNA extraction methods applied to Olive xylem sap (i.e. internal fluid in olive tree tissue)

Part of fig. 1 from Haro et al., 2021 doi.org/10.3389/fpls.2021.640829

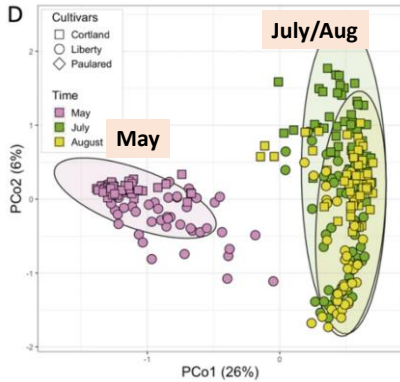
Cornelia Stumpner, Med Uni Graz, MICROBE, BBMRI.at



The importance of quality-controlled workflows & comprehensive metadata – plant microbiome field

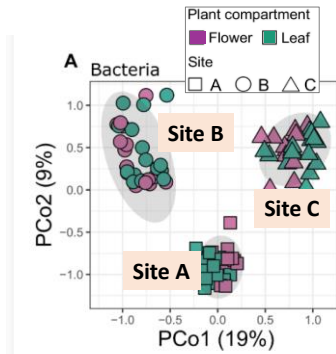
Effect of sampling time

⇒ Time and site are strong drivers of bacterial community structure

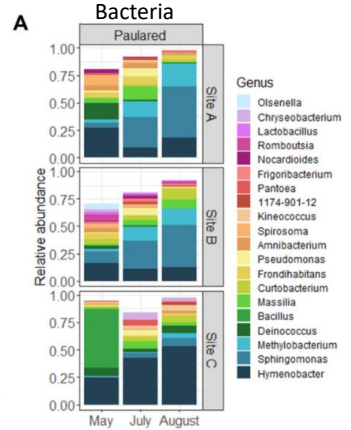


(D) PCoA on leaf samples from apple trees ($n=292$) showing variation through time

Effect of site



(PCoA) on apple tree flower and leaf samples collected in May (45 trees) at different sites A,B,C



Leaf microbial community composition (A Bacteria); (May) same cultivar - different sites A,B,C

Parts of Fig. 1 from Boutin, 2025 (doi.org/10.1038/s41598-025-10729-0); & Fig. 1+3 from Boutin, 2022 (doi.org/10.1139/cjm-2023-0215)

C Stumpfner

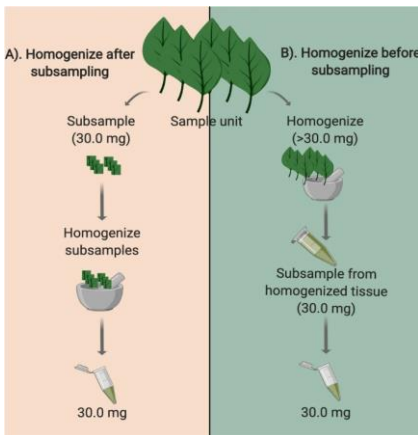
The importance of quality-controlled workflows & comprehensive metadata – plant microbiome field



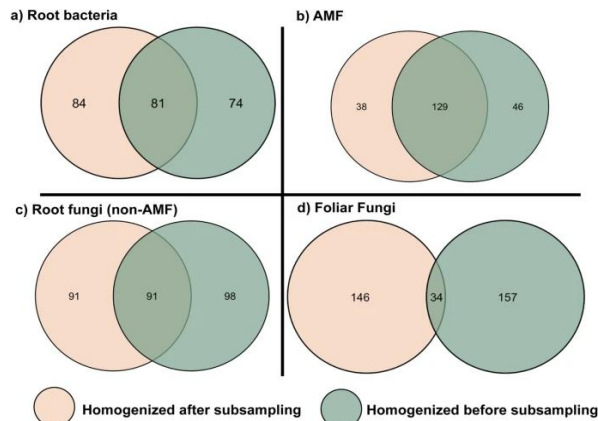
Selected examples from literature

The effect of plant sample processing

⇒ Different sample processing strategies yield different microbial communities



Bullington/Lekberg/Larkin, 2021 (doi.org/10.1038/s41598-021-83153-9)



Overlap of sequence variants (SVs) recovered from milkweed plants at a single site, when subsampling microbial communities using two different strategies.

Cornelia Stumpfner, Med Uni Graz, MICROBE, BBMRI.at



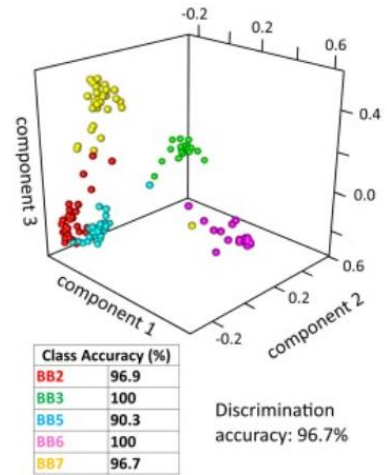
Pre-analytical effects – multi-centre study (human)



- Same sample types (blood -> **serum**)
- Collected & processed at different sites
- Metabolome analyzed at 1 site

Inter-centre variation significantly greater than inter-individual variations

Serum metabolomics RF spectra



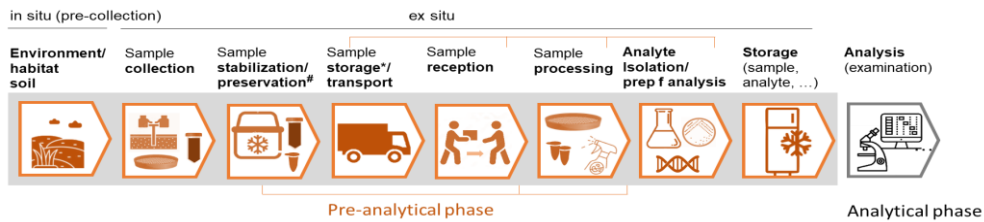
V Ghini (2022) DOI [10.1016/j.jnbt.2022.01.006](https://doi.org/10.1016/j.jnbt.2022.01.006) BBMRI-LPC project

Cornelia Stumpfner, Med Uni Graz, MICROBE, BBMRI.at



Pre-analytical workflow – ideal & reality

Ideal - simplified



Reality –

- Many pre-analytical steps
- In different order
- Several intermediate storage steps (different conditions/durations)
- Different methods
- + procedures
- + reagents (types, age)
- + handling
- ..
- Analysis

Workflows need to be harmonized/standardized, documented, and transparent

Cornelia Stumpfner, Med Uni Graz MICROBE, BBMRI.at



(Pre-)analytical effects – multi-centre study (soil microbiome)

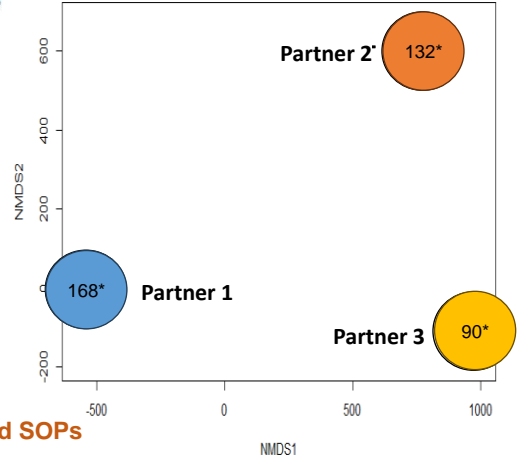


- **Same sample** (soil)
homogenized aliquots shipped to 3 site
- Different **preservation methods + storage durations**
tested at different sites
- **Analyzed at different sites** (same community analysis
method, same primers)

Inter-laboratory variation significantly greater than
different preservation effects

⇒ **Rough harmonization might not enough => better shared SOPs**

16s rRNA microbiome community analysis

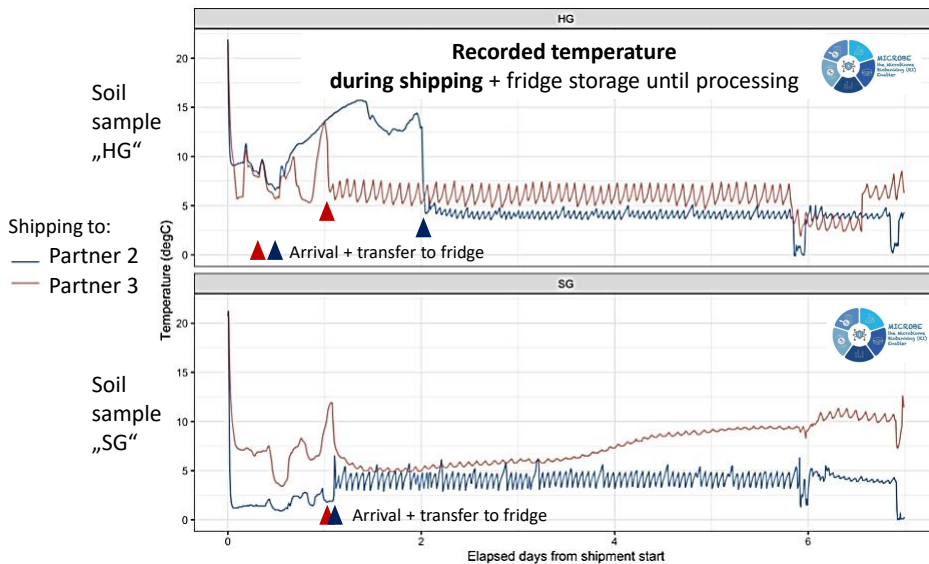


*Number of samples analysed

Cornelia Stumptner, Med Uni Graz, MICROBE, BMMRI.at



Not every pre-analytical variable can be controlled ...

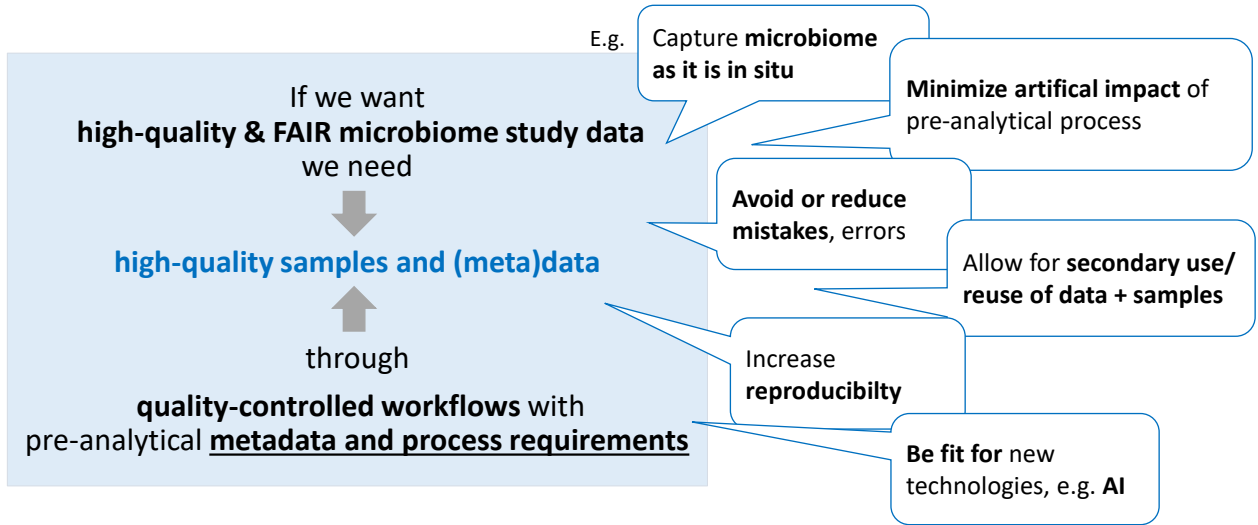


... but at least
"documented"





The need for pre-analytical metadata & process requirements



Thank you to all workshop participants!



Guidance document for sample pre-analytics for plant microbiome studies



Cornelia Stumptner
 Medical University of Graz,
 Neue Stiftingtalstrasse 2/B/6,
 8010 Graz, AUSTRIA
cornelia.stumptner@medunigraz.at

Acknowledgements:

MICROBE project	Others

Image: <https://www.gutmicrobiotaforhealth.com/meet-your-microbiome-when-science-comes-to-youtube/rencontrez-votre-microbiome-quand-la-science-debarque-sur-youtube/>



What to do?



Study Design

Detailed study design

- aim
- entire workflow
- (pre-)analytical variables
- standards/guidelines



RISK Assessment

Risk assessment

- likelihood of impact
- + mitigation



Validated

- Use validated methods/ reagents/kits**
- (where available)



SOPs

SOPs

- for all workflow steps;
- serve as **instructions** for use



(Pre-) Tests

Test

- where effects are unclear
- make upfront smaller tests



(Meta) Data

Meta(data)

- documentation



Edu & training

- Education, staff training and supervision** of junior staff by seniors



Workflow, Standards

Consider the entire workflow and variables;

- Follow **standards/ guidelines**