CIMR: *In vitro* Biology/Microbiology Context

Metabolomics Standards Initiative (MSI)

Sponsor: Metabolomics Society http://www.metabolomicssociety.org/
Reference: http://msi-workgroups.sourceforge.net/bio-metadata/reporting/invitro/
Version: 2.0
Date: 2006/12/11 11:07:57
Authors: Mariët van der Werf, TNO, The Netherlands <vanderWerf@voeding.tno.nl>
Uwe Sauer, ETH, Switzerland <sauer@imsb.biol.ethz.ch>
Ralf Takors, Degussa, Germany <ralf.takors@degussa.com>
Jørn Smedsgaard, Technical University of Denmark <js@biocentrum.dtu.dk>
Tom Ferenci, University of Sydney, Australia <t.ferenci@microbio.usyd.edu.au>
Jean Charles Portais, CNRS, France <jean-charles.portais@insa-toulouse.fr>
Christoph Wittmann, University of Saarland, Germany <c.wittmann@mx.uni-saarland.de>
Mark Hooks, University of Wales, Bangor, UK <bss606@bangor.ac.uk>
Alberta Tomassini, University of Rome, Italy <alfredo.miccheli@uniroma1.it>
Greg Stephanopolous, MIT, USA <gregstep@mit.edu>
Marco Oldiges, Forschungszentrum Jülich, Germany <m.oldiges@fz-juelich.de>
Jennifer Fostel, National Center for Toxicogenomics, NC <Fostel@niehs.nih.gov>
Dalibor Stys, University of South Bohemia, Czeck Republic <stys@jcu.cz>

Edited by Mariët van der Werf
Credits: *Chair:*: Mariët van der Werf

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1. This document

This document forms part of the the standards for reporting metabolomics experiments developed under the Metabolomics Society (http://www.metabolomicssociety.org/ Metabolomics Standards Initiative (MSI). It should be read in the context of top level document for those standards.

2. Scope and Goals

2.1. Scope of the *in vitro* biology/microbiology sample context subgroup

The scope of our efforts will be to identify, develop and disseminate best reporting practices in all aspects of *in vitro*/microbial metabolomics that are related to describing the samples generated in *in vitro* or microbial studies to be analyzed by metabolomics. The focus of the efforts is on metabolomics-specific methodologies and/or technical information that are critical to metabolomics experiments.

The aim will not be to **prescribe** how to perform an *in vitro* biology or microbial metabolomics experiment, but to formulate a minimum set of reporting standards that **describe** the methods (what are the methods and how they were actually executed). Consequently, there will be no attempt to restrict or dictate specific practices, but to develop consistent and appropriate descriptors to allow the evaluation of the experiments performed and to support the dissemination and re-use of metabolomics data. Such reporting standards will specify the data identified as necessary for complete and comprehensive reporting in a range of identified contexts, such as submission to academic journals and public databases.

2.2. The Goals of the *in vitro* biology/microbiology Sample Context Group

1. To work cooperatively on a consensus draft for a **minimum core set** of necessary metadata needed to evaluate, understand, repeat, compare and re-investigate metabolomics data generated in *in vitro* or microbial studies.
2. To include key persons from the field of *in vitro* biology/microbiology to participate in the discussion in an inclusive manner.
3. To reach out and evaluate previous and relevant work in *in vitro* biology/microbiology biology including similar work in transcriptomics and proteomics studies, and recent metabolomics standardization efforts.
4. To pay careful attention to the distinction of best practice (which will change), reporting standards (which should have longer validity) and data exchange standards (which support reporting).

3. Related Work

Related Literature

[1] Lindon, J. C. and Nicholson, J. K. and Holmes, E. and Keun, H. C. and Craig, A. and Pearce, J. T. M. and Bruce, S. J. and Hardy, N. and Sansone, S. A. and Antti, H. and Jonsson, P. and Daykin, C. and Navarange, M. and Beger, R. D. and Verheij, E. R. and Amberg, A. and Baunsgaard, D. and Cantor, G. H. and Lehman-McKeeman, L. and Earll, M. and Wold, S. and Johansson, E. and Haselden, J. N. and Kramer, K. and Thomas, C. and Lindberg, J. and Schuppe-Koistinen, I. and Wilson, I. D. and Reily, M. D. and Robertson, D. G. and Senn, H. and Krotzky, A. and Kochhar, S. and Powell, J. and van der Ouderaa, F. and Plumb, R. and Schaefer, H. and Spraul, M.. *Summary recommendations for standardization and reporting of metabolic analyses*. *Nature Biotechnology*. 23. 7. 833-838. 2005.

[2] Orchard, S. and Hermjakob, H. and Apweiler, R.. *The proteomics standards initiative*. *Proteomics*. 3. 7. 1374-1376. 2003.

[3] Quackenbush, John. *Data standards for 'omic' science*. 22. 5. 613. 2004.

[4] Brazma, A. and Hingamp, P. and Quackenbush, J. and Sherlock, G. and Spellman, P. and Stoeckert, C. and Aach, J. and Ansorge, W. and Ball, C. A. and Causton, H. C. and Gaasterland, T. and Glenisson, P. and Holstege, F. C. P. and Kim, I. F. and Markowitz, V. and Matese, J. C. and Parkinson, H. and Robinson, A. and Sarkans, U. and Schulze-Kremer, S. and Stewart, J. and Taylor, R. and Vilo, J. and Vingron, M.. *Minimum information about a microarray experiment (MIAME) - toward standards for microarray data*. *Nature Genetics*. 29. 4. 365-371. 2001.

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[6] Jenkins, H., Hardy, N., Beckmann, M., Draper, J., Smith, A.R., Taylor, J., Fiehn, O., Goodacre, R., Bino, R.J., Hall, R., Kopka, J., Lane, G.A., Lange, B.M., Liu, J.R., Mendes, P., Nikolau, B.J., Oliver. S.G., Paton, N.W., Rhee, S., Roessner-Tunali, U., Saito, K., Smedsgaard, J., Sumner, L.W., Wang, T., Walsh, S., Wurtele, E.S. and D.B. Kell (2004) Nat. Biotechnol. 22:1601-1606

[7] Jenkins, H., Johnson, H., Kular, B., Wang, T. and N. Hardy (2005) Toward supportive data collecton tools for plant metabolomics. Plant Physiol. 138:67-77

4. Proposed Minimum Information Set for Reporting the sample context of *in vitro* biology/microbiology on Sample context ('context metadata')

The minimal information set for reporting on samples in *in vitro* biology or microbial metabolomics experiments builds upon the general biological practice of reporting biological experiments in scientific journals in a way that the materials and methods section should include 'sufficient, but brief, technical information to allow the experiments to be repeated' (as described in the instructions to authors, of journals like the Journal of Biological Chemistry [http://www.jbc.org], Microbiology-UK [http://mic.sgmjournals.org] or the journals of the American Society of Microbiology [http://www.journals.asm.org/ ASM]). These include aspects like:

- Species/strains/bioresource
- Source of the strains and substrates
- Experimental design
- Inoculation procedure
- Growth and/or treatment conditions
- Time of sampling

These aspects are considered to be general aspects that are reported in every biological scientific paper/experiment, and are not a part of the **minimal set of reporting standards** (paragraph 4.1) as identified by this subgroup. However, this does not mean that these factors are not critically to the outcome of a metabolomics experiment, and therefore also a **best practice set of reporting standards** for reporting *in vitro* biology or microbial metabolomics experiments is defined (paragraph 4.2)

Based on these considerations the minimal and best practice set of reporting standards are described in paragraph 4.1 and 4.2.

4.1 Minimal information for reporting *in vitro*

biology or microbial metabolomics experiments

The minimal set of reporting standards for *in vitro* biology or microbial metabolomics experiments contains those factors included that are **specific** for metabolomics experiments and that critically determine the outcome of the experiments.

Since metabolite data are extremely sensitive to environmental conditions, please report cultivation conditions and harvesting time points as accurately as possible.

Metabolomics-specific factors critical to the outcome of metabolomics experiments:

Sampling	What is the time between the removal of the samples from their environment until metabolic activity is truly stopped?
Quenching	How was the metabolism of the samples shut down?
Extracellular metabolites	How were intracellular metabolites discriminated from extracellular metabolites?
Extraction of metabolites from the cells?	How were the (intracellular) metabolites extracted from the cells?
Normalization of the metabolome data	How were the metabolome data normalized? Specifically: how were the data normalized with respect to the amount of cells (no., mg) that they were obtained from?
Sample clean-up/work-up	How were the samples cleaned-up with respect to compounds that interfere with analysis?
Sample storage	How and how long were the samples stored after collection, during work-up and prior to analysis?
Quality control steps	How was verified that no biotic or a-biotic changes occurred during the complete sample collection and work-up phase?
Detection level	What is the detection limit of the metabolites for the samples analyzed in the study?

In view of the many steps in a metabolomics experiment, we advise to include a full schedule of the sample processing and analysis protocol.

4.2 Best-practice for reporting *in vitro* biology or microbial metabolomics experiments

The best practice set of reporting standards for *in vitro* biology or microbial metabolomics experiments contains not only those factors that are **specific** for metabolomics experiments (see minimal set of reporting standards) but **also the general aspects** that critically determine the outcome of any *in vitro* biological or microbiological experiments. The best practice set of reporting standards aims to support the scientific community in reporting their *in vitro* biology or microbial metabolomics data.

General aspects critical to the outcome of metabolomics experiments:

Experimental design	• The goal of the experiment • Experimental design – relationships between samples, treatments/growth conditions, extracts, repeats, and metabolome samples (e.g. diagram or table)
Biosource	• Source/supplier of the cell line/strain (e.g. ATCC,…..) • In case of natural isolates. If at all possible, cultures should be deposited in an international culture collection with an accession number and relevant details

	• In case of natural isolates. As micro-organisms are often identified incorrectly: What taxonomic system was used to identify the (micro-)organism? • In case of mutant strains. From which wild-type were they obtained and how? • In case of (higher) eukaryotes: Cell type, organ derivation, grade of differentiation, subcellular location • Cell storage
Growth environment	• Growth container: Type, supplier, geometry of the fermenter/bioreactor, (shake) flask or microtiter plates ○ Growth supports (type and supplier) in case of cells cultured in adherence • Growth configuration (monolayer, double layer, sandwich, spheroids, batch, fed-batch, perfusion, continuous fermentation,…..) • Inoculation procedure ○ Subculturing and splitting protocols ○ Inoculation size, seedling density (volume % [v/v], n° of cells/ml for suspension cultures; n° of cells/cm^2 for cells in adherence, subconfluence or confluence, • Medium/substrates (type and supplier) - including additions and supplementations (antibiotics, growth factors, serum type and batches,….) • Environmental conditions: Temperature, pH, gas composition, humidity, % CO_2,……, pO2, ○ Which of the environmental conditions were controlled and which could alter (freely) during growth? • If the cells were grown at a set fixed growth rate: which was the growth rate?
Treatment/incubation conditions	• Treatment factors ○ Biotic (e.g. competition with or infection by other organisms) ○ Abiotic (e.g. physical stresses, chemical substances, …..) ○ Intervention, pertubation • Treatment dose, vehicle • Pre-treatment and/or treatment time and intervals • In case of use/incubation with labeled substrates: ^{13}C compounds used (% enrichment, purity), labeling protocols
Harvesting	• Biotic characteristics of moment of harvesting (growth phase/stage [logarithmic, stationair, steady state, cytostatic phase, cell cycle phase, …], no. of generations in case continuous cultures, stabilization time/phase before experiment, number of culture passages) • A-biotic characteristics at time of harvesting (cell density [OD, DWT, counts], depletion of nutrients, treatment time) • Aspects mentioned under minimal reporting standards (paragraph 4.1)
Biotic factors related to sample work up	•Aspects mentioned under minimal reporting standards (paragraph 4.1)