

start | 3 I | VP... | Ch... | Ch... | 3 I | Mi... | My Computer | 10:36 PM


Metabolomics Standards Initiative (MSI) - Chemical sample context WG - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Back Search Favorites Media

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<http://msi-workgroups.sourceforge.net/chemical-analysis/reporting/>

Biological sample context WG

This working workgroup have produced draft reporting standards, available in [MS Word](#) and also as [pdf](#).

PARTICIPANTS

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Chemical Analysis Working Group GOALS

- The scope of our efforts will be to identify, develop and disseminate best chemical analysis practices in all aspects of metabolomics.
- From best practices formulate a minimum set of reporting standards that ***describe*** the experiments



Chemical Analysis Working Group Specific Objectives

- work cooperatively on a consensus draft for a minimum core set of necessary data related to the chemical analyses associated with metabolomics experiments
- include key persons from the Group's specialist area to participate in the discussion in an inclusive manner.
- reach out and evaluate previous and relevant work in their specialist areas including similar work in transcriptomics and proteomics studies, and recent metabolomics standardization efforts.
- 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).
- respond to documents from the other groups and produce an advanced draft ready for discussion in February 2006
- respond to documents from the other groups and produce a final draft ready for discussion in June 2006
- Involve journal editorial boards of Metabolomics, Phytochemistry, Analytical Chemistry, analytical biochemistry, and relevant life sciences to review and advise on the practicality, acceptability, and support of standards.



Scope of Chemical Analysis WG

- *4.0 Proposed Minimum Metadata for Sample Preparation*
- *4.1 Proposed Minimum Metadata Relative to Chromatography*
- *4.2 Proposed Minimum Metadata Relative to Mass Spectrometry*
- *4.3 Proposed Minimum Metadata Relative to Metabolite Identification*
- *4.4 Proposed Minimum Metadata Relative to Nuclear Magnetic Resonance*
- *4.5 Proposed Minimum Metadata Relative to Stable Isotopes & Flux Analysis ?*

- *4.7 Proposed Minimum Metadata Relative to Capillary Electrophoresis*
- *4.8 Proposed Minimum Metadata Relative to Electrochemical Detection*
- *4.9 Proposed Minimum Metadata Relative to Infrared Spectroscopy*
- *4.10 Proposed Minimum Metadata for Data Export*
- *4.11 Proposed Minimum Metadata for Quantification*



4.0 Proposed Minimum Metadata for Sample Preparation

- Sample Processing protocol
 - *Harvesting method*: sample freezing method (e.g. LN2, dry ice acetone bath, freeze clamping), sample wash method for removing unwanted external components, time & duration, to include time (e.g. from tissue resection to LN2 freezing), temp, sample storage prior to further preparation (e.g. -80C for 2 weeks). All temperatures should be measured if possible; however temp setpoints are acceptable assuming quality monitoring was performed and no abnormalities recorded.
 - *Tissue processing*: lyophilization, fresh tissue processing, pulverization (e.g. . LN2 grinding, dry tissue homogenization, expected particle sizes), storage conditions (e.g. -80°C, duration, atmospheric or vacuum, dessication, preservatives), dilution...
- Extraction solvent and volume per quantity tissue, number of sequential/parallel extraction
 - 1ml MeOH per 6mg lyophilized tissue, twice extraction, CHCl₃/MeOH (2/1, v/v) followed by 10% trichloroacetic acid extraction



4.0 Proposed Minimum Metadata for Sample Preparation

- Extract concentration, and resuspension processes
 - Dried under nitrogen, resuspended in H₂O or pyridine,
- Sample enrichment (if relevant)
 - SPE (column, sorbent, manufacturer)
 - Desalting, MWCO etc.
- Derivatization
 - OMS/TMS (temperatures & duration)
- Extract cleanup & additional manipulation
 - e.g. Ultrafiltration, removal of paramagnetic ions, addition of metal chelators such as EDTA, citrate, pH buffer
- Extract storage
 - Storage conditions prior to analysis
- Ionic strength & conductivity ???



4.1 Proposed Minimum Metadata for Chromatography

- Chromatography Instrument
 - Manufacturer, model number, software package and version number or date,
- Auto-injector
 - Injector model/type, software version, method name, injection volume, wash cycles (volumes); solvent, split/splitless
- Separation column and pre/guard column
 - manufacturer, product #, stationary media composition (support and coating e.g. silica C8 etc) & physical parameters (i.e. coating thickness for GC/MS & particle size and pore size for LC/MS), internal diameter, length
- Separation parameters
 - Method name, injector temperature, split or splitless mode & ratio, mobile phase compositions, mobile phase flow rates, thermal/solvent/solute gradient profiles,
- Quality Control to validate chromatography performance
 - Minimum should include description whether or not QC was performed and how it was measured
 - Validation sample, internal standards, chromatographic resolution, cycles per column/injector/septum/blank, calibration standards
 - Data acquisition
 - SOP Protocol name, date, and publication reference (can be journal or website URL but should be publicly accessible and link should be stable.



4.2 Proposed Minimum Metadata for Mass Spectrometry

- Instrument
 - manufacturer, model #, operational software name & version
- Ionization source
 - Ionization mode (EI, APCI, ESI....), polarity, vacuum pressure, skimmer/focusing lens voltages (e.g. capillary voltage etc.), gas flows (e.g. nebulization gas, cone gas etc., source temperature)
- Mass Analyzer
 - Type, m/z range, calibration, resolution, mass accuracy, logic program for data acquisition, spectral acquisition rate, vacuum pressure, lock spray (concentration, lock mass, flow rate, frequency tec)
- Quality Control
 - Tune, sensitivity, mass accuracy, and resolution
- Data acquisition
 - SOP Protocol name, date, operator, data acquisition rate



4.3 Proposed Minimum Metadata for Metabolite Identification

- Most metabolites are not novel identifications (*i.e. previously characterized & identified using high rigor*)
- Propose a minimum of two independent domains relative to an authentic compound for metabolite identifications (retention time & mass spectrum- unit res molecular ion, or high res. Mass or fragmentation pattern?), (retention time & chemical shifts) , (accurate mass & tandem MS), (retention time & UV spectrum)
- More than two adds additional confidence (Rt, m/z, UV, chemical shift- one chemical shift is not very good- needs more detail such as multiplicity, 2D connectivity, etc)
- Reporting of chemical identifiers & nomenclature:
INCHE, SMILES, IUPAC name, Common names
Unknown identifiers & nomenclature?



4.4 Proposed Minimum Metadata for NMR

- Instrument
 - manufacturer, model #, magnetic field strength in Tesla {example 14.1 T Varian Inova ; 18.8 T Bruker Avance}
- Hardware
- VT control, pulsed field gradients (z or x,y,z) and max gradient strength; no. shims, no channels;
- Probe type (e.g. 10 mm 31P, 5 mm HCN coldprobe, 3 mm flow-probe, etc.), solution or solid-state, automation or manual operation, autotune or manual tune. LC-NMR: sample handler, injection volumes, wash cycles & solvent
- Sample property
- Temperature, Volume, extract/powder/intact organisms, tissue or cells, type of NMR tube (e.g. conventional, Shigemi,, mircocell etc.), pH, solvent (D2O, CD3OD, CDCl3, etc)
- Acquisition & Data Processing Parameter
- For 1-D NMR: observed nucleus, pulse sequence name, pulse sequence implementation (e.g. gradient selection, sensitivity enhancement), spinning rate, solvent saturation or decoupling method, excitation pulse width, spectral width, acquisition time, interpulse delay (or recycle time), digitization parameter, number of transients For solvent suppression, technique, excitation maximum and bandwidth should be stated.
- Additional parameters for 2-D NMR: observed nucleus in F2 and F1, pulse sequence, excitation pulse widths for relevant nuclei, spectral width in F2 and F1, solvent saturation method, number of transients in t2 and number of increments in t1, acquisition times for t2 and t1 ; phase sensitive or magnitude detection



4.4 *Proposed Minimum Metadata for NMR*

- For homonuclear NMR, presence or absence of heteronuclear decoupling (e.g. isotope-enriched samples), decoupling mode and bandwidth; spin lock field strength (in Hz) and duration (in sec); mixing time (NOESY, ROESY etc.)
- For heteronuclear NMR: direct or indirect detection; proton decoupling mode (Waltz, Garp, Wurst, Stud etc.) and effective band width ; evolution time for constant time experiments; editing mode (cf. INEPT-based experiments); heteronuclear spin lock strength and mixing time (e.g. HCCH-TOCSY)
- Data Processing: Degree of zero filling, degree of linear prediction; apodization parameters and window function in all dimensions (exponential, Gaussian, sine bell etc). Baseline corrections (dc offset, linear or non-linear corrections), first point multipliers, any shifting of the fids.
- Quality Control
- Calibration (chemical shift & concentration) standard used (e.g. DSS, TMS), duplications, standard error/deviation of quantification. State the 50% and 1% line widths on the reference (cf. DSS, TSP or TMS methyl peak) or residual water, pH marker used & shift correction. For X nuclei, external or internal reference and conditions, correction made for susceptibility effects.
- Shift referencing method for indirect dimension in 2D experiments (direct or indirect based on g ratios).
- For quantitation, state method used (peak integration or deconvolution method, spiking with substance x at relative concentration y; intensity normalized to reference line- correction for saturation effects- T1 values measured?). Any relaxation agents added (type, amount). For direct X-detection (esp. ^{13}C or ^{31}P), correction for NOEs as well as saturation? For light water samples, what corrections are made for non-linear excitation profile and method?



4.5 Proposed Minimum Metadata for Stable Isotopes & Flux Analysis

- Labeled precursors used including element/isotope, position(s) labeled, % e.g. [13C-1]-D-glucose 98%, [15N2]-L-glutamine (99%), chemical purity of the labeled compound(s), and concentration of the compound used in the experiment; fraction of total present (requires detailed breakdown of media composition for cell and tissue studies, including analysis of any added FCS or other growth supplements; labeling scheme (duration, pulse or continuous addition; top up etc.)
- Total no. moles isotope added during the experiment.
- Data analysis: method for determining positional and fractional labeling, standard error of the estimates; estimated isotope recovery in observable fractions (and fraction of total isotope supplied)



Additional topics under consideration

- 4.7 Proposed Minimum Metadata for Capillary Electrophoresis ?*
- 4.8 Proposed Minimum Metadata for Electrochemical Detection ?*
- 4.9 Proposed Minimum Metadata for Infrared Spectroscopy ?*
- 4.10 Proposed Minimum Metadata for Data Export?*
- 4.11 Proposed Minimum Metadata for Quantification?*