Biomarkers Guidelines

- Based on the studies performed in CFS/ME to date and taking into consideration not to overlap other CDE subgroup topics, the CDE Biomarkers subgroup reviewed five categories of biomarkers:
  1. Microbiome/Microorganisms
  2. Proteome/Proteins
  3. Metabolome/Metabolism
  4. Genome/Epigenome
  5. Gene expression/Transcriptome

All potential CFS/ME biomarkers identified to date under these five biomarker categories, per available literature, should be classified as EXPLORATORY.

- As the only publication studying minimum data elements for research reports on CFS (Jason et al., 2012)


  Focuses on demographic and diagnostic elements, recommendations from alternative initiatives aimed at harmonizing data reporting and facilitating study replication in biomedical and biomarker research are adopted.

**BIOMARKER GENERAL CDE RECOMMENDATIONS**


  It is important to collect information on dosage of medication(s) used prior to biomarker sampling.

**EQUATOR Network** (Enhancing the QUALity and Transparency Of health Research; [http://www.equator-network.org/](http://www.equator-network.org/)


**MIBBI project** (Minimum Information for Biological and Biomedical Investigations; [http://mibbi.org](http://mibbi.org))
Biomarkers Guidelines


STROBE-The STRengthening the reporting of Observational Studies in Epidemiology


Data reporting/sharing


Biomarkers Guidelines


All five biomarker category studies should at least report:

- Study design and sampling methods (indicate if possible setting and recruitment periods)
- Study size (number of groups and individuals/group) indicating how was assessed
- Diagnosis criteria and severity grade, indicating how was assessed
- Demographic and clinical data of participants
- Variables studied, statistical methods
- Limitations and potential bias

BIOMARKER CATEGORY SPECIFIC CDE RECOMMENDATIONS

- Since all five biomarker categories selected for study by this Working Group require analysis of human derived samples (stools, body fluids or tissues), a selection of guidelines to record and report pre-analytical variables that could impact downstream applications as well as some quality control recommendations are shown:

  Preanalytical variables: Biospecimen reporting for improved study quality (BRISQ) and Sample PREanalytical Code (SPREC) expected to facilitate and consolidate international multicenter biomarker identification research and biospecimen research in the clinical Biobank environment


Biomarkers Guidelines


Sample quality control (QC):

ISBER (International Society for Biological and Environmental Repositories; http://www.isber.org):
Table 9. QC tools for samples used in proteomics, metabolomics, transcriptomics, or targeted analytical applications
Table 10. QC tools for molecular and cellular derivatives


Study design, study size assessment and statistical analysis:


1. Microbiome/Microorganisms

International Human Microbiome Standards (IHMS) project: http://www.microbiome-standards.org/#SOPS
Biomarkers Guidelines

The Microbiome Quality Control project: http://www.mbqc.org/
NIH Human Microbiome Project: https://www.hmpdacc.org/hmp/publications.php
NIH Human Microbiome Project Tools and Technology: https://www.hmpdacc.org/resources/tools_protocols.php


Microbiome/Microorganisms should at least report:
- Sample collection, transport and storage
- Nucleic acid extraction methods
- Any amplification (details)
- Next-Generation Sequencing (NGS) protocol [Whole Genome Shotgun Sequencing(WGSS), rRNA]
- NGS platforms
- Sequence analysis & pipelines

2. Proteome/Proteins

MIAPE (Minimal Information about a Proteomics Experiment; http://psidev.info/miape)


Proteome/Proteins studies should at least report:
Biomarkers Guidelines

- Biological material that was extracted, how it was collected and stored
- Extraction procedure—reagents, pH, detergent concentration, resultant protein concentration and how protein concentration was determined
- How digestion and cleanup was carried out
- What quality control is performed on the mass spectroscopy (MS) system
- Brand and model of mass spec system
- Separation column used
- How long a gradient was used
- Pooling strategy of samples

3. Metabolome/Metabolism


Data reporting/sharing:


Metabolome studies should at least report:

- Biological material that was extracted, how it was collected, stored and prepared for analysis
- Extraction procedure – solvent, instrumentation (e.g. magnetic/mechanical stirrer, sonication, temperature, duration)
- Whether and what type of cleanup was carried out, e.g., filtration, centrifugation, concentration or complete evaporation and re-uptake in a different solvent
- How samples are stored – temperature, duration
- What quality control is performed on the MS system, e.g., standards used
Biomarkers Guidelines

- Brand and model of mass spec system, ionization mode, brand and model of high performance liquid chromatography (HPLC) system
- Separation column used
- Solvent gradient parameters

Data evaluation, quantitation and validation:
- Targeted or non-targeted analysis
- Relative quantitation or absolute quantitation (using stable isotope labeled internal standards)
- MS/MS validation using multiple fragment ions for quantitation plus qualifier fragment ions when needed

Together with MS I think it is fair to include nuclear magnetic resonance (NMR) as a complementary technique, including all parameters for this method.

As complement to liquid chromatography (LC) we should mention gas chromatography (GC), capillary electrophoresis (CE), capillary electrochromatography (CEC) and super critical fluid chromatography (SFC) as other separation techniques commonly used in metabolomics.

Maybe also something about the statistics used for data evaluation together with bioinformatics tools applied.

4. Genome/Epigenome

Minimum Information about a Genotyping Experiment (MIGEN)


STrengthening the REporting of Genetic Association Studies (STREGA)

Biomarkers Guidelines


Table 1 STREGA Reporting Recommendations, Extended from STROBE Statement

Genome/Epigenome studies should at least report:

- Source of DNA (cells, tissue, etc. and how was prepared)
- How DNA was prepared and stored
- DNA quality parameters
- Method and platform (type and vendor) used to obtain expression levels
- How bioinformatic analysis was performed

5. Gene expression/Transcriptome

The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments


Minimum Information About a Microarray Experiment (MIAME)


Gene expression/Transcriptome studies should at least report:
Biomarkers Guidelines

- Source of RNA (cells, tissue, etc. and how was prepared)
- How RNA was prepared and stored
- RNA quality parameters
- Method and platform (type and vendor) used to obtain expression levels
- How bioinformatic analysis was performed

Technology for gene expression analysis is developing rapidly. Cost considerations are significant and some investigators will be limited to certain platforms. Most popular among currents are:

Biased (targeted): Quantitative Polymerase Chain Reaction following Retrotranscription (qRT-PCR), microarrays, differential display, etc.

Unbiased (non-targeted): Next Generation sequencing (NGS) based strategies (various vendors)

Data quality control and Data reporting/sharing:

