Standard Guide for
Investigation/Study/Assay Tab-Delimited Format for Nanotechnologies (ISA-TAB-Nano): Standard File Format for the Submission and Exchange of Data on Nanomaterials and Characterizations[1]

1. Scope

1.1 This guide (ISA-TAB-Nano) specifies the format for representing and sharing information about nanomaterials, small molecules and biological specimens along with their assay characterization data (including metadata, and summary data) using spreadsheet or TAB-delimited files.

1.2 The Appendices Sections contain a detailed listing of ISA-TAB-Nano fields (Appendix X1), a practical example (Appendix X2), a discussion of optional files (Appendix X3), and summary of background (Appendix X4).

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ISA-TAB Format Specification:*[2]
ISA-TAB Release Candidate 1.0 ISA-TAB 1.0

2.2 *Assay Protocol Documents from NCL:*[3]
NCL Method GTA-1 (Version 1.1) LLC-PK1 Kidney Cytotoxicity Assay

2.3 *Reports from NCL:*[4]
NCL200612A Dendrimer-Based MRI Contrast Agents, Prepared for Dendritic Nanotechnologies, Inc. December 2006

3. Significance and Use

3.1 This guide is intended to facilitate the meaningful submission and exchange of nanomaterial descriptions and characterization data (metadata and summary data) along with the other files (raw/derived data files, image files, protocol documents, etc.) among individual researchers and to or from nanotechnology resources. This guide also serves to empower organizations to adopt standard methods for representing data in nanotechnology publications; and to provide researchers with guidelines for representing nanomaterials and characterizations to achieve cross-material comparison. This guide encourages standardization in the field of informatics, where adherence ISA-TAB-Nano concepts nanotechnology to a broader and established bioinformatics community.

3.2 The format of this guide is of a file structure that contains four primary files—investigation, study, assay (ISA), and material files.

3.2.1 In addition, raw or derived data files and any other files (for example, image files, protocol documents) specific to each assay can be shared along with the four primary ISA-TAB-Nano files if the data files are referenced in the primary ISA-TAB-Nano files. The specification does not provide format specification for files other than the four primary files: investigation, study, assay, and material files. The ISA-TAB-Nano investigation file is used for three purposes: (*1*) to record all declarative information referenced in other files; (*2*) to

relate assay files to study files; and (*3*) to group multiple study files that are part of the same investigation. The ISA-TAB-Nano study file is used to record information about the source, sampling methodology, treatment, preparation, and characteristics of the subjects (biospecimens) studied using one or more assays under an investigation. The ISA-TAB-Nano assay file records the assay protocol names, endpoint measurements and references to image/data files; and the material file is used to represent the composition and characteristics of nanoparticle formulations and small molecules.

3.2.2 *Use of Ontologies and Standard Terminologies in ISA-TAB-Nano:*

3.2.2.1 ISA-TAB-Nano provides fields for entering and referencing terms selected from ontologies and standard terminologies. The ontologies are available at BioPortal,[5] which is maintained by the National Center for Biomedical Ontologies. Though the investigator may use alternative ontology and vocabulary sources, the ability to evaluate and share data require that all parties have access to those being used (they should be available to the investigators). All terms and fields used in this guide utilize the NCI EVS and Nanoparticle Ontology elements. The NanoParticle Ontology (NPO)[6] provides a subset of the terms and relationships for the description and characterization of nanomaterials in the ISA-TAB-Nano file format.

3.2.2.2 *Distinction Between Biological and Non-Biological Samples*—In nanotechnology, samples from biological and non-biological sources can be the primary subjects of a study. Therefore, in ISA-TAB-Nano, samples derived from biological sources are called *biological specimens* or *biospecimens* (for example, cell line, body fluids, organs, etc.). Whereas, samples derived from non-biological sources are simply called *material samples* (for example, nanomaterials, nanoparticle formulations, small molecules). For physico-chemical characterizations of nanomaterials, the sample is the nanomaterial. For *in-vitro* and *in-vivo* characterizations, the sample is the biological specimen (cell line, animal, and so forth). Hence, in

ISA-TAB-Nano, the concept of a sample is defined to include both biological specimens and material samples. The ISA-TAB-Nano study file can only be used to record the source and characteristics of biospecimens studied in an assay and cannot support the representation of materials. Therefore, in ISA-TAB-Nano, the material file is used to describe *material samples*, while the study file is used to describe *biospecimens*.

3.2.2.3 *ISA-TAB-Nano File Names*—ISA-TAB-Nano file names may end in either .txt or .xls extensions. The ISA-TAB-Nano files used as examples in this document were prepared in excel spreadsheets, and so their filenames have the .xls extension.

4. ISA-TAB-Nano Release Candidate 1.0 Files and Structure

4.1 *ISA-TAB-Nano File Structure:*

4.1.1 ISA-TAB-Nano uses four primary files– investigation file, study file, assay file and material file (Fig. 1). Other files such as raw/derived data files, image files, protocol documents, etc., referenced in the ISA-TAB-Nano files have to be shared along with the ISA-TAB-Nano files.

4.1.2 When sharing primary ISA-TAB-Nano files, other files referenced in these files have to be shared along with the primary files.

4.2 *ISA-TAB-Nano File Development Process:*

4.2.1 In Fig. 2, the ISA-TAB-Nano file development process is described. Typically, the investigation file is developed first and describes the overall investigation, associated studies and assays. The investigation file is a text file with a naming convention of "i_xxx.txt" or "i_xxx.xls," in which xxx can be any name provided by the investigator. Once the investigation file has been completed, one or more study files (following the convention "s_xxx.txt" or "s_xxx.xls") can be created. Similarly, one or more material files can be created. The material file describes the nanomaterial (or small molecule) and its components including structural information and follows the naming convention "m_xxx.txt" or "m_xxx.xls". Assay files (following the convention "a_xxx.txt" or "a_xxx.xls") are created for all assays performed. Each assay is defined by the endpoint measured and the technique used to measure that endpoint. Data files (raw or derived) specific to each type of assay can be associated to the respective assay files by referencing the names of the data files in the assay files.
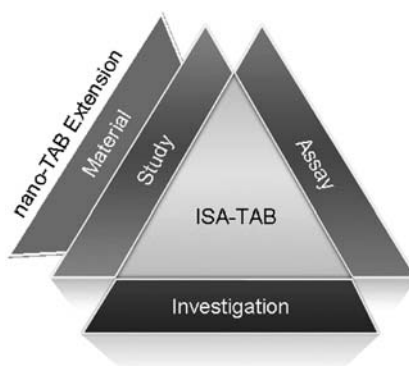
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[5] Available from BioPortal, The National Center for Biomedical Ontology, Stanford Biomedical Informatics Research, Medical School Office Building X-215, 1265 Welch Road, Stanford, CA 94305-5479, http://www.bioontology.org.

[6] Thomas, D.G., Pappu, R.V., and Baker, N.A., "Nanoparticle Ontology for Cancer Nanotechnology Research," *Journal of Biomedical Informatics*, Vol 44, No. 1, 2011, pp. 59–74, http://www.nano-ontology.org.

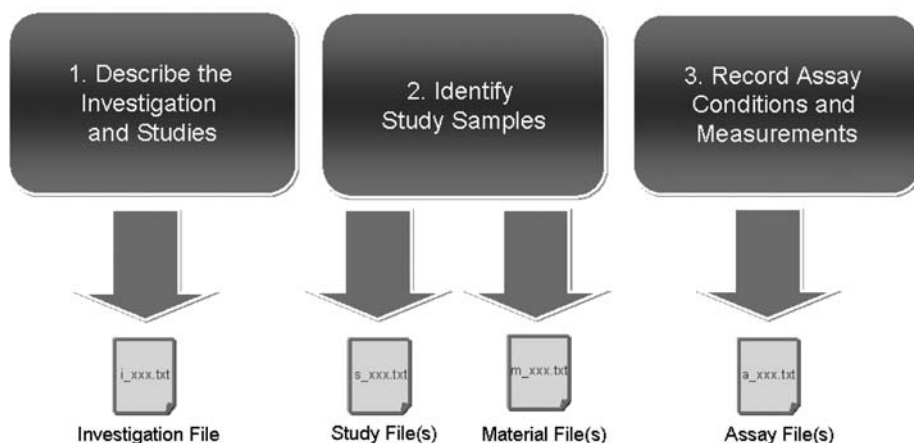**FIG. 1 ISA-TAB-Nano File Structure**

FIG. 2 ISA-TAB-Nano File Development Process

4.3 Once the ISA-TAB-Nano files have been created, the files can be validated and submitted into nanotechnology resources that support the ISA-TAB-Nano specification.

4.4 *ISA-TAB-Nano File Descriptions*

4.4.1 *Investigation File:*

4.4.1.1 *Description:*

(1) The ISA-TAB-Nano investigation allows for the description of the primary investigation and associated studies including assays and protocols. An investigation can have one or more studies. For example, an investigation titled "Dendrimer-Based MRI Contrast Agents" may have two studies titled as "Characterizing the Size of Dendrimer based MRI Contrast Agents" and "Determining the cytotoxicity property of Dendrimer based MRI Contrast Agents in porcine proximal tubule cells." Each study can have one or more assays depending on the endpoint measured and the technique used. For example, a cytotoxicity study may be conducted using an MTT assay and a LDH release assay. A size characterization study can include two types of assays based on the technique used – one using DLS and the other using AFM.

(2) The ISA-TAB-Nano guide provides flexibility in representing the level of granularity in information associated with a study; however, the level of granularity should factor in the effective representation of assays and protocols in conformance with the specification. For example, a study focusing on "Size Characterizations" will have multiple size measurements (for example, Z-average size, hydrodynamic size) and may involve the use of multiple techniques (for example, size by DLS, size by AFM). These can be represented effectively in the ISA-TAB-Nano file structure.

(3) The investigation file provides descriptive information about studies including design descriptors, publications, factors, assays, protocols, and contacts. This descriptive information lays the foundation for other ISA-TAB-Nano files. For example, Table 1 shows a subset of the Investigation File, which is the study factors section of the investigation file. This section provides the names of factors (for example, temperature, solvent medium) used in the study and their associated units of measurement (if the factors are quantitative). The values of these factors (for example, PBS, 25 Celsius) are specified either in the study or the assay file.

TABLE 1 Example Subset of the Investigation File Format

A	B	C
STUDY FACTORS		
Study Factor Name	temperature	solvent medium
Term Accession Number	PATO_0000146	NPO_1855
Term Source REF	PATO	NPO
Study Factor Type	condition	condition
Term Accession Number		
Term Source REF		

4.4.1.2 *File Format*—The ISA-TAB-Nano investigation file is a vertical-based spreadsheet format with row headers in the first column, as shown in Table 1. The fields are divided into sections, therefore, the field values in the investigation file are entered in column order. For instance, in Table 1, Column A indicates the field names and Columns B and C contain the field values.

4.4.2 *Study File:*

4.4.2.1 *Description*—The ISA-TAB-Nano study file provides a mapping between the samples (biospecimens and material samples) and processing events (occurs whenever a protocol is applied) associated with a study. It is also used to provide values for the *parameters* and factors associated with an assay of the sample. In ISA-TAB-Nano, factors can be either entered in the study file or in the assay file. For physico-chemical characterizations of nanomaterials, the sample is the nanomaterial. For *in-vitro* and *in-vivo* characterizations, the sample is the biological specimen (cell

TABLE 2 Extensions and Constraints Applied to the ISA-TAB Investigation File in Support of ISA-TAB-Nano

Section	Field	Change	Field Status (if applicable)
INVESTIGATION	Investigation disease	Addition	Optional
INVESTIGATION	Investigation outcome	Addition	Optional
STUDY	Study disease	Addition	Optional
STUDY	Study outcome	Addition	Optional
STUDY ASSAYS	Study assay measurement name	Addition	Optional