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# 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]

This standard is issued under the fixed designation E2909; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

## 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

[1] This guide is under the jurisdiction of ASTM Committee E56 on Nanotechnology and is the direct responsibility of Subcommittee E56.01 on Informatics and Terminology.

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[2] Available from ISAtools, http://isa-tools.org.

[3] Available from Nanotechnology Characterization Laboratory (NCL), Frederick National Laboratory for Cancer Research, SAIC-Frederick, Inc., P.O. Box B, Frederick, MD 21702-1201, http://ncl.cancer.gov.

[4] Available from Nanotechnology Characterization Laboratory (NCL), Frederick National Laboratory for Cancer Research, SAIC-Frederick, Inc., P.O. Box B, Frederick, MD 21702-1201, http://ncl.cancer.gov. Download available from http://ncl.cancer.gov/120406.pdf.

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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.

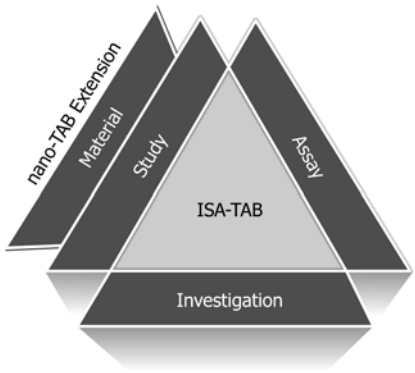


**FIG. 1 ISA-TAB-Nano File Structure**

[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.

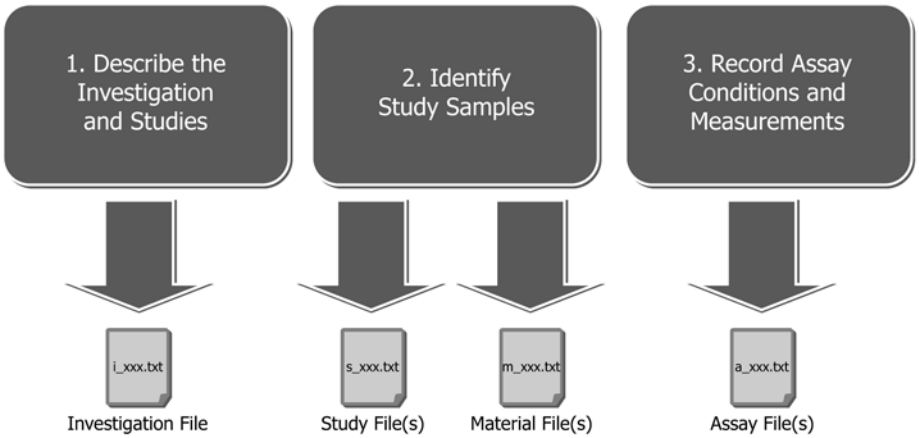


**FIG. 2 ISA-TAB-Nano File Development Process**

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 |

line, animal, and so forth). The nanomaterial applied to a biospecimen in an *in-vitro* or *in-vivo* assay is considered as a study factor along with other factors that vary in the experiment (for example, dose). The study file is required as it functions as the primary mapping file between the samples and the assay. For studies involving nanomaterials (also applies to small molecules), the material file allows for a detailed description of the composition and characteristics of the nanomaterial, and the ISA-TAB-Nano material file name is referenced as a qualifier for the source sample (nanomaterial) in the study file.

4.4.2.2 *File Format*—The ISA-TAB-Nano study file is a horizontal-based spreadsheet format with column headers in the first row. An example subset of this format is provided in Table 3. Please note, the concept specified within the bracket "[ ]" is a variable depending on the type of sample.

4.4.3 *Material File:*

4.4.3.1 *Description:*

*(1)* The material file describes the composition of the material formulation. The material file is an important file that is designed to allow for nanomaterial comparison across nanotechnology resources.

*(2)* An investigation typically involves the preparation of multiple materials that are tested. These materials could be of the same chemical composition (same type and amount of chemical components in the sample) or different chemical compositions. Materials having the same chemical composition should be described using one material file. Materials of different chemical composition should be described separately using multiple materials files.

4.4.3.2 *File Format*—The material file format leverages a horizontal-based spreadsheet format with rows representing multiple values. Table 4 shows an example subset of the material file.

4.4.4 *Assay File:*

**TABLE 3 Example Subset of the Study File Format**

| A | B | C |
|---|---|---|
| Source Name | Material Type | Characteristics [cell type] |
| LLC-PK1 | biospecimen | porcine proximal tubule cells |
| LLC-PK1 | biospecimen | porcine proximal tubule cells |

**TABLE 4 Example Subset of the Material File Format**

| A | B | C | D | E |
|---|---|---|---|---|
| Material Source Identifier | Material Name | Manufacturer Lot ID | Material Description | Material Synthesis |
| NCL-22-1 | g45_coona_ dendrimer | | G4.5 COONa terminated PAMAM dendrimer | |
| NCL-23-1 | g45_coona_ dendrimer_ magnevist_ complex | | G4.5 COONa terminated PAMAM dendrimer-Magnevist[A] complex | |
| NCL-24-1 | magnevist | | gadolinium based image contrast agent | |

[A] Magnevist is a registered trademark by Berlex Laboratories, Inc., http://www.berlex.com.

4.4.4.1 *Description*—The assay file provides references to assay results including measurements, raw data files, derived data files, image files, and other file types. There can be multiple assay files per study.

4.4.4.2 *File Format*—The ISA-TAB-Nano assay file is a horizontal-based spreadsheet format with column headers and row values. An example subset of this format is provided in Table 5. Concepts specified within the bracket "[ ]" are variables depending on the type of assay. Please note, ISA-TAB-Nano allows the entry of summary data in the assay files through an optional field called Measurement Value, as described in Table 6.

## 5. Keywords

5.1 data sharing; ISA-TAB-Nano; nanomedicine; nanotechnology; ontologies

**TABLE 5 Example Subset of the Assay File Format**

| A | B | C | D | E |
|---|---|---|---|---|
| Sample Name | Assay Name | Measurement Value [Hydrodynamic Diameter] | Statistic | Unit |
| NCL-20-1 | size by DLS assay | 5.2 | z-average | Nm |
| NCL-20-2 | size by DLS assay | 8.6 | z-average | Nm |

**TABLE 6 Extensions and Constraints Applied to the ISA-TAB Assay File in Support of ISA-TAB-Nano**

| Field | Change | Description | Field Status (if applicable) |
|---|---|---|---|
| Measurement Value [ ] | Addition | The measurement value is leveraged to capture derived data values associated with each assay. In ISA-TAB, this is typically represented in a raw or derived data file; however, in assays in which there are only a few measurements (for example, Z-average size), it is useful to display the factors along with the measurements, and the measurement value field is leveraged. | Optional |
| Statistic | Addition | The ISA-TAB format allows for the specification of units (for example, mg/mL) but not a statistic (for example, mean, SD, and so forth). | Optional |

# APPENDIXES

**(Nonmandatory Information)**

## X1. ISA-TAB-NANO DETAILED STRUCTURE

X1.1 The following sections provide a detailed overview of the structure of each ISA-TAB-Nano file along with example data and recommendations for use of concepts in the field of nanotechnology and nanomedicine.

X1.2 Appendix X1 of this guide provides a set of ISA-TAB-Nano files leveraging data from NCL as an example (NCL200612A).

### X1.3 Investigation File

X1.3.1 The field names in an investigation file are organized vertically in the first column. These fields are divided into the following eleven sections as defined by ISA-TAB. These sections are:

Ontology Source Reference (X1.3.2)
Investigation (X1.3.3)
Investigation Contacts (X1.3.4)
Investigation Publication (X1.3.5)
Study (X1.3.6)
  Study Design Descriptors (X1.3.7)
  Study Contacts (X1.3.8)
  Study Publications (X1.3.9)
  Study Assays (X1.3.10)
  Study Factors (X1.3.11)
  Study Protocols (X1.3.12)

X1.3.2 *Ontology Source Reference:*

X1.3.2.1 This section is used to define the vocabulary source from which a term is selected and referenced in the ISA-TAB-Nano files. Table X1.1 shows an example of the Ontology Source Reference Section along with example data.

X1.3.2.2 The Ontology Source Reference Section uses four concepts. These concepts are all taken from ISA-TAB and are described below:

*(1) Term Source Name*—The name of the source from where a term is selected and referenced in the ISA-TAB-Nano files. The source could be an ontology or a controlled vocabulary. The source name is the full name or the acronym of the ontology/controlled vocabulary. This is a required field if the term source name is referenced in any of the ISA-TAB-Nano files.

*(2) Term Source File*—A file name or a URI of the source named in the *term source name* field.

*(3) Term Source Version*—Version number of the vocabulary source file. This is a required field if the field for *term source file* has a value.

*(4) Term Source Description*—Text description to disambiguate resources when homologous acronyms are used.

**TABLE X1.2 Example Ontology Source Reference Section**

| A | B | C |
|---|---|---|
| **Ontology Source Reference** | | |
| Term Source Name | MO | NPO |
| Term Source File | http://purl.bioontology.org/ontology/MO | http://purl.bioontology.org/ontology/npo |
| Term Source Version | v. 1.3.1.1 | v. 2011-02-12 |
| Term Source Description | MGED Ontology | NanoParticle Ontology |

X1.3.3 *Investigation:*

X1.3.3.1 This section is used to describe an investigation. An example of the Investigation Section is in Table X1.2 along with example data.

X1.3.3.2 The Investigation Section uses nine concepts. These concepts are taken from ISA-TAB unless otherwise noted and are described below:

*(1) Investigation Identifier*—A locally unique identifier or an accession number provided by a repository.

**TABLE X1.3 Example Investigation Section**

| A | B |
|---|---|
| **Investigation** | |
| Investigation Identifier | NCL200612A |
| Investigation Title | Dendrimer-Based MRI Contrast Agents |
| Investigation Description | The goal of this investigation is to characterize a PAMAM dendrimer with an associated gadolinium chelate MRI contrast agent. |
| Investigation Disease | |
| Term Accession Number | |
| Term Source REF | |
| Investigation Outcome | |
| Investigation Submission Date | 2002-11-30 |
| Investigation Public Release Date | 2002-11-30 |

**TABLE X1.4 Example Investigation Contacts Section**

| A | B |
|---|---|
| **Investigation Contacts** | |
| Investigation Person Last Name | McNeil |
| Investigation Person First Name | Scott |
| Investigation Person Middle Initials | E |
| Investigation Person Email | mcneils@mail.nih.gov |
| Investigation Person Telephone | +1 301-846-6939 |
| Investigation Person Fax | +1 610-832-9599 |
| Investigation Person Address | MSC 1050 Boyles Street, Frederick, MD 21702 |
| Investigation Person Affiliation | Nanotechnology Characterization Laboratory |
| Investigation Person Role | investigator |
| Term Accession Number | |
| Term Source REF | MO |

*(2) Investigation Title*—A concise phrase used as a title for the investigation.

*(3) Investigation Description*—A textual description of the investigation.

*(4) Investigation Disease*—Disease(s) that are the subject of an investigation, if applicable. This concept is introduced in ISA-TAB-Nano to identify the disease(s) related to the subject of the investigation.

*(5) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *investigation disease*.

*(6) Term Source REF*—The name which identifies the source from where the term for *investigation disease* is selected. This name should match one of the names entered in the *term source name* field.

*(7) Investigation Outcome*—A textual description of the outcome(s) of an investigation. This concept is introduced in ISA-TAB-Nano to provide a brief summary or conclusion of an investigation.

*(8) Investigation Submission Date*—The date on which the investigation was reported to a repository (format: YYYY-MM-DD).

*(9) Investigation Public Release Date*—The date on which the investigation is publicly released or published (format: YYYY-MM-DD).

X1.3.4 *Investigation Contacts:*

X1.3.4.1 The Investigation Contacts Section allows for the identification of the point(s) of contact for an investigation. An example of the Investigation Contacts Section of the spreadsheet is in Table X1.3 along with example data.

X1.3.4.2 There are eleven concepts used as field names in the Investigation Contacts Section. These concepts are all taken from ISA-TAB and are described below:

*(1) Investigation Person Last Name*—The last name of a person who is the point of contact for the investigation.

*(2) Investigation Person First Name*—The first name of a person who is the point of contact for the investigation.

*(3) Investigation Person Middle Initials*—The middle initial(s) of a person who is the point of contact for the investigation.

*(4) Investigation Person Email*—The email address of a person who is the point of contact for the investigation.

*(5) Investigation Person Telephone*—The telephone number of a person who is the point of contact for the investigation.

*(6) Investigation Person Fax*—The fax number of a person who is the point of contact for the investigation.

*(7) Investigation Person Address*—The mailing address of a person who is the point of contact for the investigation.

*(8) Investigation Person Affiliation*—The name of the organization to which the point of contact belongs.

*(9) Investigation Person Role*—The term which classifies the role(s) performed by person who is the point of contact for the investigation.

*(10) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *investigation person role*.

*(11) Term Source REF*—Name of the ontology or controlled vocabulary from which a term is selected and entered as a value for *investigation person role*.

X1.3.5 *Investigation Publication:*

X1.3.5.1 The Investigation Publication Section allows for the identification of articles (published) associated with the investigation. Table X1.4 shows an example of the Investigation Publication Section along with example data.

X1.3.5.2 There are seven concepts used in the Investigation Publication Section. These concepts are taken from ISA-TAB and are described below:

*(1) Investigation PubMed ID*—A Digital Object Identifier (DOI) of the publication associated with the investigation.

*(2) Investigation Publication DOI*—A semicolon-delimited (";") list of authors of a publication associated with the investigation.

**TABLE X1.5 Example Investigation Publications Section**

| A | B |
|---|---|
| **Investigation Publication** | |
| Investigation PubMed ID | 18095846 |
| Investigation Publication DOI | 10.2217/17435889.2.6.789 |
| Investigation Publication Author List | Hall JB; Dobrovolskaia MA; Patri AK; McNeil SE |
| Investigation Publication Title | Characterization of nanoparticles for therapeutics |
| Investigation Publication Status | published |
| Term Accession Number | |
| Term Source REF | |

*(3) Investigation Publication Author List*—A semicolon-delimited (";") list of authors of a publication associated with the investigation.

*(4) Investigation Publication Title*—A concise phrase used as a title for the publication associated with the investigation.

*(5) Investigation Publication Status*—A term describing the status of a publication (that is, submitted, in preparation, published).

*(6) Term Accession Number*—The identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *investigation publication status*.

*(7) Term Source REF*—The name which identifies the source from where the term for *investigation publication status* is selected. This name should match one of the names entered in the *term source name* field.

X1.3.6 *Study:*

X1.3.6.1 The Study Section allows for the description of one or more studies conducted as part of an investigation. Table X1.5 shows an example of the Study Section along with example data.

X1.3.6.2 There are eleven concepts used in the STUDY section. These concepts are taken from ISA-TAB unless otherwise noted and are described below:

*(1) Study Identifier*—A unique identifier used for the study. It is either a temporary identifier supplied by users or one generated by a repository or other database.

*(2) Study Title*—A concise phrase used to encapsulate the purpose and goal of the study.

*(3) Study Submission Date*—The date on which the study is submitted to an archive (format: YYYY-MM-DD).

**TABLE X1.6 Example Study Section**

| A | B |
|---|---|
| **Study** | |
| Study Identifier | NCL200612A-CytoxicityLLC-PK1 |
| Study Title | Cytotoxicity characterization in LLC-PK1 cells |
| Study Submission Date | 2002-11-30 |
| Study Public Release Date | 2002-11-30 |
| Study Description | Nanoparticle biocompatibility was evaluated in the porcine renal proximal tubule cell line, LLC-PK1. Cytotoxicity was determined as described in the NCL protocol for LLC-PK1 Kidney Cytotoxicity Assay (NCL Method GTA-1). Briefly, test materials were diluted to the desired assay concentrations in cell culture media. Cells were preincubated for 24 h prior to adding test material, reaching an approximate confluence of 80%. Cells were exposed to test material for 6, 24 and 48 h, and cytotoxicity was determined using the MTT cell viability and LDH membrane integrity assays. |
| Study Disease | |
| Term Accession Number | |
| Term Source REF | |
| Study Outcome | NCL22, NCL23 and NCL24 were found to be minimally cytotoxic, under the testing conditions utilized. |
| Study File Name | s_cytotoxicity-LLC-PK1.xls |
| Study File Description | |

*(4) Study Public Release Date*—The date on which the study is publicly released or published (format: YYYY-MM-DD).

*(5) Study Description*—A textual description of the study, with components such as objectives or goals.

*(6) Study Disease*—Disease(s) that are the subject of the study, if applicable. This concept is introduced in ISA-TAB-Nano to identify the disease(s) related to the study.

*(7) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study disease*.

*(8) Term Source REF*—The name which identifies the source from where the term for *study disease* is selected. This name should match one of the names entered in the *term source name* field.

*(9) Study Outcome*—A textual description about the outcome(s) of the study. This concept is introduced in ISA-TAB-Nano to provide a summary or conclusion of a study.

*(10) Study File Name*—The name of the ISA-TAB-Nano study file, which lists information about the biological specimens (cells, tissues, organs, animal model, body fluids), nanoparticles, small organic molecules, and other types of samples assayed in a study. There can be only one file name per cell.

*(11) Study File Description*—A textual description which provides additional information on the ISA-TAB-Nano study file.

X1.3.7 *Study Design Descriptors:*

X1.3.7.1 The Study Design Descriptors Section allows for the identification of design type of the study. Table X1.6 shows an example of the Study Design Descriptors Section along with example data.

X1.3.7.2 There are three concepts used in the Study Design Descriptors Section. These concepts are taken from ISA-TAB and are described below:

*(1) Study Design Type*—A term describing the classification of the study based on the overall study (experimental) design (for example, comparison study). The term can be free-text or taken from a controlled vocabulary/ontology.

*(2) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study design type*.

*(3) Term Source REF*—The name which identifies the source from where the term for *study design type* is selected. This name should match one of the names entered in the *term source name* field.

X1.3.8 *Study Contacts:*

X1.3.8.1 The Study Contacts Section allows the identification of the point of contact for a study. An example of the Study Contacts Section is shown in Table X1.7 along with example data.

**TABLE X1.7 Example Study Design Descriptors Section**

| A | B |
|---|---|
| **Study Design Descriptors** | |
| Study Design Type | comparison |
| Term Accession Number | |
| Term Source REF | |

**TABLE X1.8 Example Study Contacts Section**

| A | B |
|---|---|
| **Study Contacts** | |
| Study Person Last Name | Dobrovolskaia |
| Study Person First Name | Marina |
| Study Person Middle Initials | A |
| Study Person Email | marina@mail.nih.gov |
| Study Person Telephone | +1 301-846-6352 |
| Study Person Fax | +1 610-832-9599 |
| Study Person Address | MSC 1050 Boyles Street, Frederick, MD 21702 |
| Study Person Affiliation | Nanotechnology Characterization Laboratory |
| Study Person Role | investigator |
| Term Accession Number | |
| Term Source REF | MO |

X1.3.8.2 The Study Contacts Section has eleven concepts. These concepts are taken from ISA-TAB and are described below:

*(1) Study Person Last Name*—The last name of a person who is the point of contact for the study.

*(2) Study Person First Name*—The first name of a person who is the point of contact for the study.

*(3) Study Person Middle Initials*—The middle initial(s) of a person who is the point of contact for the study.

*(4) Study Person Email*—The email address of a person who is the point of contact for the study.

*(5) Study Person Telephone*—The telephone number of a person who is the point of contact for the study.

*(6) Study Person Fax*—The fax number of a person who is the point of contact for the study.

*(7) Study Person Address*—The mailing address of a person who is the point of contact for the study.

*(8) Study Person Affiliation*—The name of the organization to which the point of contact belongs.

*(9) Study Person Role*—The term which classifies the role(s) performed by person who is the point of contact for the study.

*(10) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study person role*.

*(11) Term Source REF*—The name which identifies the source from where the term for *study person role* is selected. This name should match one of the names entered in the *term source name* field.

X1.3.9 *Study Publications:*

X1.3.9.1 The Study Publications Section allows for the identification of articles (published) associated with the study. Table X1.8 shows an example of the Study Publications Section along with example data.

X1.3.9.2 The Study Publications Section has seven concepts. These concepts are taken from ISA-TAB and are described below:

*(1) Study PubMed ID*—PubMed identifier of the publication associated with the study.

*(2) Study Publication DOI*—A Digital Object Identifier (DOI) of the publication associated with the study.

*(3) Study Publication Author List*—A semicolon-delimited (";") list of authors of a publication associated with the study.

**TABLE X1.9 Example Study Publications Section**

| A | B |
|---|---|
| **Study Publications** | |
| Study PubMed ID | 18095846 |
| Study Publication DOI | 10.2217/17435889.2.6.789 |
| Study Publication Author list | Hall JB; Dobrovolskaia MA; Patri AK; McNeil SE |
| Study Publication Title | Characterization of nanoparticles for therapeutics |
| Study Publication Status | published |
| Term Accession Number | |
| Term Source REF | |

*(4) Study Publication Title*—A concise phrase used as a title for the publication associated with the study.

*(5) Study Publication Status*—A term describing the status of a publication (that is, submitted, in preparation, published), associated with the study.

*(6) Term Accession Number*—The identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study publication status*.

*(7) Term Source REF*—The name which identifies the source from where the term for *study publication status* is selected. This name should match one of the names entered in the *term source name*.

X1.3.10 *Study Assays:*

X1.3.10.1 The Study Assays Section allows for the identification of type of measurement and the type of technology used for the measurement in an assay that is part of a study. Table X1.9 shows an example of the Study Assays Section along with example data.

X1.3.10.2 The Study Assays Section has eleven concepts. These concepts are taken from ISA-TAB unless otherwise noted and are described below:

*(1) Study Assay Measurement Type*—A term to qualify the endpoint, or what is being measured.

*(2) Term Accession Number*—Identification number of the term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study assay measurement type*.

**TABLE X1.10 Example Study Assays Section**

| A | B | C |
|---|---|---|
| **Study Assays** | | |
| Study Assay Measurement Type | MTT Assay | LDH Release Assay |
| Term Accession Number | | NPO_1709 |
| Term Source REF | | NPO |
| Study Assay Technology Type | | |
| Term Accession Number | | |
| Term Source REF | | |
| Study Assay Technology Platform | | |
| Study Assay Measurement Name | cell viability | LDH release |
| Term Accession Number | NPO_1343 | |
| Term Source REF | NPO | |
| Study Assay File Name | a_MTT-LLCPK1.xls | a_LDH-LLCPK1.xls |

*(3) Term Source REF*—The name which identifies the source from where the term for *study assay measurement type* is selected. This name should match one of the names entered in the *term source name* field.

*(4) Study Assay Technology Type*—The type of technology (technique or method) used for the assay measurement (for example, Dynamic light scattering).

*(5) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study assay technology type*.

*(6) Term Source REF*—The name which identifies the source from where the term for *study assay technology type* is selected. This name should match one of the names entered in the *term source name* field.

*(7) Study Assay Technology Platform*—The manufacturer and platform name of the instruments used in the study assay.

*(8) Study Assay Measurement Name*—A semicolon-delimited list of names of quantities whose values are the outputs of an assay measurement. This concept is introduced in ISA-TAB-Nano to define the variables measured as output of an assay.

*(9) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study assay measurement name*.

*(10) Term Source REF*—The name which identifies the source from where the term for *study assay measurement name* is selected.

*(11) Study Assay File Name*—Name of the ISA-TAB-Nano Assay file corresponding to the study assay. This is a required field. There can be only one assay file name per cell.

X1.3.11 *Study Factors:*

X1.3.11.1 The Study Factors Section allows for the identification of factors associated with the study. Table X1.10 shows an example of the Study Factors Section along with example data.

X1.3.11.2 The Study Factors Section has six concepts. These concepts are taken from ISA-TAB unless otherwise noted and are described below:

*(1) Study Factor Name*—The name of one independent variable (factor) manipulated by the experimentalist with the intention to affect the subject of study (that is, Stressor). This is a required field, if there are factors in an assay study. Only one factor name is allowed per cell. The value of a factor is given either in the study or in the assay file.

**TABLE X1.11 Example Study Factors Section**

| A | B | C | D |
|---|---|---|---|
| **Study Factors** | | | |
| Study Factor Name | nanoparticle sample | particle concentration | time of exposure |
| Term Accession Number | NPO_1404 | NPO_1830 | NPO_1819 |
| Term Source REF | NPO | NPO | NPO |
| Study Factor Type | | | |
| Term Accession Number | | | |
| Term Source REF | | | |

*(2) Term Accession Number*—Identification number of the term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study factor name*.

*(3) Term Source REF*—The name which identifies the source from where the term for *study factor name* is selected. This name should match one of the names entered in the *term source name* field.

*(4) Study Factor Type*—A term used for the classification of factors associated with the study (for example, condition). This is a required field if there are factors in an assay study. More than one term is allowed per cell; multiple terms are separated by semicolons.

*(5) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study factor type*.

*(6) Term Source REF*—The name which identifies the source from where the term for *study factor type* is selected. This name should match one of the names entered in the *term source name* field.

X1.3.12 *Study Protocols:*

X1.3.12.1 The Study Protocols Section allows for the identification of the type of protocols, and the parameters and components of a protocol used in a study. A protocol describes the formal plan of an experiment or research activity, including the objective, rationale, design, materials and methods for the conduct of the study; intervention description, and method of data analysis. Table X1.11 shows an example of the Study Protocols Section along with example data.

X1.3.12.2 The Study Protocols Section has fourteen concepts. These concepts are taken from ISA-TAB unless otherwise noted and are described below:

*(1) Study Protocol Name*—The name of the protocols used within a study and will be referenced in the ISA-TAB-Nano Study or Assay files.

*(2) Study Protocol Type*—The term used to classify the protocol (for example, synthesis, assay, etc.).

*(3) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *study protocol type*.

*(4) Term Source REF*—Name of the ontology or controlled vocabulary from which a term is selected and entered as a value for *study protocol type*.

*(5) Study Protocol Description*—A textual description of the protocol.

*(6) Study Protocol URI*—Pointer to protocol documents or resources external to ISA-TAB-Nano, which can be accessed by their Uniform Resource Identifier (URI). If URI is not available, enter the protocol document file name and make the protocol document available along with ISA-TAB-Nano files.

*(7) Study Protocol Version*—A semicolon-delimited (";") list of parameter names used as an identifier in the ISA-TAB-Nano Study or Assay files. A protocol parameter is a constant associated with a protocol, which is not varied as part of an experiment.

**TABLE X1.12 Example Study Protocols Section**

| A | B | C |
|---|---|---|
| **Study Protocols** | | |
| Study Protocol Name | Time-6-24-48 plate MTT assay | Test plate LDH assay |
| Study Protocol Type | | |
| Term Accession Number | | |
| Term Source REF | | |
| Study Protocol Description | Test Plates: 6, 24, and 48 hour exposures (MTT Assay)<br>5.4.1 Remove appropriate test plate from incubator and replace media from Triton-X positive control wells (see plate format in Appendix) with 200 µL 1% Triton-X (made in step 4.1.2). Let the plate set for 10 minutes at room temperature. Spin plate at 700 × g for 3 minutes.<br>5.4.2 Remove 100 µL of media from each well and transfer it to another plate, maintaining plate format. Use this plate immediately for the LDH assay (see section 5.5).<br>5.4.3 Remove remaining media from original plate and discard.<br>5.4.4 Add 200 µL of fresh media to all wells.<br>5.4.5 Add 50 µL of MTT to all wells.<br>5.4.6 Cover in aluminum foil and incubate for 37°C for 4 hours.<br>5.4.7 Remove plate from incubator and spin at 700 × g for 3 minutes.<br>5.4.8 Remove media and MTT.<br>5.4.9 Add 200 µL of DMSO to each well.<br>5.4.10 Add 25 µL of glycine buffer to each well. Place on shaker to mix.<br>5.4.11 Read absorbance at 570 nm on plate reader using a reference wavelength of 680 nm. | Test Plates: 0, 6, 24 and 48 hour exposures (LDH Assay) (Adapted from Biovision LDH Cytotoxicity Assay Kit, K311-400)<br>5.5.1 Add 100 µL of the Reaction Mixture (step 4.3.2) to each well of transfer plate. Shake plate on an orbital shaker briefly to mix samples.<br>5.5.2 Incubate at room temperature for up to 20 minutes in the dark.<br>5.5.3 Read the plate on plate reader at 490 nm using a reference wavelength of 680 nm. |
| Study Protocol URI | NCL_Method_GTA-1.pdf | NCL_Method_GTA-1.pdf |
| Study Protocol Version | 1.1 | 1.1 |
| Study Protocol Parameter Name | | |
| Term Accession Number | | |
| Term Source REF | | |
| Study Protocol Component Name | MTT; acetaminophen; dimethyl sulfoxide; glycine; sodium chloride; triton-X-100; M199 cell culture media; fetal bovine serum; nanoparticle; costar 96 well flat bottom cell culture plates; plate reader; centrifuge set at 700-800 × g (Allegra X-15R, Beckman Coulter) with 96 well plate adapter; orbital plate shaker | acetaminophen; dimethyl sulfoxide; glycine; sodium chloride; triton-X-100; M199 cell culture media; fetal bovine serum; biovision LDH-cytoxicity assay kit; nanoparticle; costar 96 well flat bottom cell culture plates; plate reader; centrifuge set at 700-800 × g (Allegra X-15R, Beckman Coulter) with 96 well plate adapter; orbital plate shaker |
| Study Protocol Component Type | reagent; reagent; reagent; reagent; reagent; reagent; reagent; reagent; material; instrument; instrument; instrument | reagent; reagent; reagent; reagent; reagent; reagent; reagent; reagent; material; instrument; instrument; instrument |
| Term Accession Number | NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290;NPO_290; ; NPO_1436; NPO_1436; NPO_1436 | NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290; NPO_290;NPO_290; ; NPO_1436; NPO_1436; NPO_1436 |
| Term Source REF | NPO; NPO; NPO; NPO; NPO; NPO; NPO; NPO; NPO; ; NPO; NPO; NPO | NPO; NPO; NPO; NPO; NPO; NPO; NPO; NPO; NPO; ; NPO; NPO; NPO |

*(8) Study Protocol Parameter Name*—A semicolon-delimited (";") list of parameter names used as an identifier in the ISA-TAB-Nano Study or Assay files. A protocol parameter is a constant associated with a protocol, which is not varied as part of an experiment.

*(9) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for the *study protocol parameter name*.

*(10) Term Source REF*—The name which identifies the source from where the term for *study protocol parameter name*. This name should match one of the names entered in the *term source name* field.

*(11) Study Protocol Component Name*—A semicolon-delimited (";") list of names identifying the components of a protocol. Component names include instrument names, software names, and reagent names.

*(12) Study Protocol Component Type*—The term to classify the protocol component, for example, instrument, software, and reagent.

*(13) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for the *study protocol component type*.

*(14) Term Source REF*—The name which identifies the source from where the term for *study protocol component type* is selected. This name should match one of the names entered in the *term source name* field.

## X1.4 Study File

X1.4.1 The study file is a horizontally oriented spreadsheet, where the field names are organized as column headers in the first row. Table X1.12 and Table X1.13 show an example of a study file. According to the ISA-TAB specification, the Study

**TABLE X1.13 Example Study File (Columns A-F)**

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| Source Name | Protocol REF | Performer | Parameter Value [pH] | Parameter Value [particle concentration] | Unit |
| NCL-20-1 | Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering | Anil Patri | 7.4 | 1 | mg/mL |

**TABLE X1.14 Example Study File (Columns G-L)**

| G | H | I | J | K | L |
|---|---|---|---|---|---|
| Sample Name | Material Type | Term Accession Number | Term Source Ref | Material File | Provider |
| NCL-20-1 | nanoparticle sample | NPO_1404 | NPO | m_NCL-20.xls | Dendritic Nanotechnologies, Inc. |

file has different types of fields called nodes, attributes of nodes, qualifiers of nodes' attributes, and other valid fields. The concepts used in a study file are taken from ISA-TAB and described below:

X1.4.2 *Study Nodes:*

X1.4.2.1 *Source Name*—The unique identification name of the source from where the sample is derived. Source names can be qualified using the following column attributes: *characteristic [ ], material type, material file, provider*, and *comment*.

*(1)* If the sample assayed is a biological specimen, its source is the starting biological material from which the sample was derived after the application of a protocol. The name of the source typically refers to the cell line or animal number for biological specimens.

*(2)* If the sample assayed is a material that is not derived from a biological source, then the corresponding source name should refer to the starting sample that was modified by a protocol for the assay. In this case, the source name should match the value recorded for *material source identifier* in the corresponding ISA-TAB-Nano material file.

X1.4.2.2 *Sample Name*—The unique identification name of the sample. The sample is obtained after the application of a protocol. Sample names can be qualified using the following column attributes: *characteristic [ ], material type, material file, provider*, and *comment*.

X1.4.3 *Attributes of study nodes*—One or more attributes are used to provide more information about a study node. The different node attributes are described below:

X1.4.3.1 *Material type*—An attribute for the *source name* or *sample name* (for example, biospecimen, nanoparticle sample, small molecule etc.). The term can be a free-text description or taken from an ontology or a controlled vocabulary. If it is the latter, then the following qualifiers are used: *term accession number* and *term source REF*.

X1.4.3.2 *Characteristics[ ]*—An attribute for *source name* and *sample name*. The term for each characteristics[ ] attribute is written within brackets (for example, Characteristics [organism], Characteristics [cell type], etc.).

X1.4.3.3 *Material File*—The name of the ISA-TAB-Nano material file that contains detailed descriptions of the source sample. This column is not applicable for biospecimens.

X1.4.3.4 *Provider*—An attribute for *source name*, which refers to the name of the person or the vendor providing the source sample.

X1.4.4 *Attributes of Processing Events for Study Nodes*—One or more attributes are used to describe a step in the preparation of a sample.

X1.4.4.1 *Protocol REF*—The name of the protocol used to prepare the sample. This name should match a value for the *field study protocol name* in the investigation file (within the study protocols section).

X1.4.4.2 *Performer*—The name of the person who carried out the protocol.

X1.4.4.3 *Date*—The calendar day on which the protocol was carried out. The date format should be in YYYY-MM-DD. If there are other dates to be recorded (for example, the date when a sample was received from a vendor), one should create a *comment [ ]* column and specify the type of date within the square brackets (for example, Comment [date received]).

X1.4.4.4 *Parameter Value [<parameter term>]*—Value of a parameter, which is kept constant, when applying a protocol. The parameter term is written within brackets and must match the term used as value for the *study protocol parameter name* in the ISA-TAB-Nano investigation file.

X1.4.5 *Qualifiers for Study Nodes' Attributes*—Each node attribute may be qualified using the following concepts, if applicable.

X1.4.5.1 *Unit*—The standard of measurement used if the values for *characteristics [ ], parameter value [ ]* or *factor value [ ]* columns are quantitative and dimensional. If the term for unit is taken from an ontology/controlled vocabulary, then its *term accession number* and the *term source REF* should be defined in the ISA-TAB-Nano investigation file.

X1.4.5.2 *Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value in *source name, material type, characteristics [ ], parameter value [ ], unit*, or *factor value [ ]* columns.

X1.4.5.3 *Term Source REF*—The name which identifies the source from where a term is selected and entered in ISA-TAB-Nano study files. This name should match one of the names entered in the *term source name* field in the ISA-TAB-Nano investigation file.

X1.4.6 *Other Study Fields*

X1.4.6.1 *Factor Value [<factor name>]*—The value of an independent variable manipulated by the experimentalist with the intention to affect the subject of study (that is, stressor). Factor terms are given in brackets, and must be defined in the ISA-TAB-Nano investigation file in the STUDY FACTORS section.

X1.4.6.2 *Comment [ ]*—Any comment that provides additional information, which is added only when no other appropriate field exists.

X1.5 *Material File*—The material file is a horizontally oriented spreadsheet, where the field names are organized as column headers in the first row. The file describes the material sample and its components, the material relationships such as constituents and linkages, and the material file:

X1.5.1 *Material Sample*

X1.5.1.1 Material sample concepts allow for the description of the nanomaterial formulation and any materials (including material constituents) associated with the nanomaterial formulation. A formulation is the nanomaterial and any other components or medium. A formulation can also be any non-biological material sample (for example, small molecule) analyzed in experiment. Material sample concepts include the following types of information:

*(1)* Material source identifier, material name, material type, manufacturer lot identifier, and material description;

*(2)* Description of the synthesis and rationale for design;

*(3)* Chemical name and characteristics of the material; and

*(4)* Intended application of the material.

X1.5.1.2 Table X1.14 shows an example of how a material sample is described. The concepts are described below.

**TABLE X1.15 MATERIAL Sample Concepts Example**

| A | B | C | D | E |
|---|---|---|---|---|
| Material Source Identifier | Material Name | Manufacturer Lot ID | Material Description | Material Synthesis |
| NCL-22-1 | g45_coona_ dendrimer | | G4.5 COONa terminated PAMAM dendrimer | |
| NCL-23-1 | g45_coona_ dendrimer_ magnevist_ complex | | G4.5 COONa terminated PAMAM dendrimer-Magnevist[A] complex | |
| NCL-24-1 | magnevist | | gadolinium based image contrast agent | |

| F | G | H | I | J | K |
|---|---|---|---|---|---|
| Material Design Rationale | Material Intended Application | Term Accession Number | Term Source REF | Material Type | Term Accession Number |
| | delivery of image contrast agent | | | dendrimer; conjugated component | NPO_735; NPO_1826 |
| | | | | nanoparticle sample | NPO_1404 |
| | MRI contrast agent | NPO_ 581 | NPO | small molecule; imaging payload agent; conjugated component | NCIt_C48809; NPO_1534; NPO_1826 |

| M | N | O | P | Q |
|---|---|---|---|---|
| Material Chemical Name | Term Accession Number | Term Source REF | Characteristics [dendrimer branch {NPO:NPO_776}] | Characteristics [molecular weight {NPO:NPO_1171}] |
| | | | 1-4 | 26.28 |
| magnevist | 31797 | ChEBI | | nanoparticle sample small molecule; imaging payload agent; conjugated component |

[A] Magnevist is a registered trademark by Berlex Laboratories, Inc., http://www.berlex.com.

*(1) Material Source Identifier*—The unique identification name of the source from which the material sample is derived. Its value is used as the value for *source name* in ISA-TAB-Nano study files, thereby linking the ISA-TAB-Nano material file and the study file. For example, NCL-23 (see Table X1.14) is the name that identifies a material characterized by the NCL. This example indicates that the sample NCL-23 is a complex formed out of NCL-22 (a G4.5 PAMAM dendrimer terminated with carboxyl groups) and NCL-24 (Magnevist[7]).

*(2) Material Name*—The unique identification name for the sample and its different components. This name is used to identify or reference the different components across the ISA-TAB-Nano files.

*(3) Manufacturer Lot Identifier*—A distinctive numeric, alpha, or alpha-numeric identification code assigned by the manufacturer or distributor. It is assigned to a specific quantity of manufactured material or product that is produced in a manner that is expected to render it homogeneous.

*(4) Material Description*—A textual description of the material sample.

*(5) Material Synthesis*—A text or a single term description of how the material was made.

*(6) Material Design Rationale*—A text description for the underlying design rationale is the property, process or phenomenon taken into consideration when formulating a nanoparticle or other substance in order to achieve the intended use of the formulation.

*(7) Material Type*—One or more terms used to classify the type of material sample. Multiple terms are entered as a semicolon-delimited list.

*(8) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *material type*.

*(9) Term Source REF*—The name which identifies the source from where the term for *material type* is selected. This name should match one of the names entered in the *term source name* field in the investigation file.

*(10) Material Chemical Name*—The chemical name of the material or its constituent material.

*(11) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *material chemical name*.

*(12) Term Source REF*—The name which identifies the source from where the term for *material chemical name* is selected. This name should match one of the names entered in the *term source name* field in the investigation file.

*(13) Characteristics [ ]*—An attribute for *material name*. The term for each characteristics [ ] attribute is written within brackets (for example, Characteristics [dendrimer branch], Characteristics [molecular weight], etc.).

*(14) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *characteristics*.

*(15) Term Source REF*—The name which identifies the source from where the value for *characteristics* is selected.

[7] Magnevist is a registered trademark by Berlex Laboratories, Inc., http://www.berlex.com.

This name should match one of the names entered in the *term source name* field in the investigation file.

*(16) Unit*—The standard of measurement used if the value for *characteristics* is quantitative and dimensional.

*(17) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *unit*.

*(18) Term Source REF*—The name which identifies the source from where the term for *unit* is selected.

*(19) Statistic*—The type of statistical measure attributed to a numerical value (for example, mean, standard deviation, z-average, etc.). This is a required field, if the characteristics value is a statistical measure.

*(20) Term accession number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *statistic*.

*(21) Term Source REF*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *statistic*. This name should match one of the names entered in the *term source name* field in the investigation file.

*(22) Material Intended Application*—The application for which a drug, nanoparticle or other substance is formulated and tested (for example, MRI).

*(23) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *material intended application*.

*(24) Term Source REF*—The name which identifies the source from where the term for *material intended application* is selected. This name should match one of the names entered in the *term source name* field in the investigation file.

X1.5.2 *Material Relationship:*

X1.5.2.1 Material relationship concepts are used to record the name and types of relationships between materials including material constiutents and material linkages. Material constiutents represent the components within a material. Material linkages represent association between materials (for example, encapsulation, attachment, entrapment). Table X1.15 illustrates an example of material relationship concepts. The concepts used for describing the relationship are given below.

*(1) Material Constituent*—The material name for each of the components of the material sample. The names should be obtained from the entries for the *material name* field.

*(2) Material Linkage*—A unique identification name for material components (whole or part) that are linked to each other in the nanoparticle sample. The names should be obtained from the entries for the *material name*. This is a required field, if the *material linkage type* field is not empty. A cell must have the names of the two linked components, separated by a semicolon.

*(3) Material Linkage Type*—The type of linkage present in a nanoparticle sample (for example, attachment, encapsulation, entrapment etc.).

*(4) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *material linkage type*.

*(5) Term Source REF*—The name which identifies the source from where the term for *material linkage type* is selected. This name should match one of the names entered in the *term source name* field in the investigation file.

X1.5.3 *Material File:*

X1.5.3.1 Material file concepts allow for the association of any files (for example, image files) to the ISA-TAB-Nano material file. Table X1.16 illustrates an example of material file concepts. The concepts are described below.

*(1) Material File Name*—The name of files (for example, Image, Structures file) containing information about the material sample. There can be only one file name per cell.

*(2) Material File Type*—The name that defines the type of the material file (for example, image, graph).

*(3) Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value for *material file type*.

*(4) Term Source REF*—The name which identifies the source from where the term for *material file type* is selected.

*(5) Material File Version*—The version number of the material file.

*(6) Material File Description*—A textual description providing additional information on the material file.

## X1.6 Assay File

X1.6.1 The assay file is a horizontally oriented spreadsheet, where the field names are organized as column headers in the first row. Table X1.17, Table X1.18, and Table X1.19, show examples of an assay file. According to the ISA-TAB specification, the assay file has different types of fields called nodes, attributes of nodes, qualifiers of nodes' attributes, and other valid fields. The assay file supports the following types of information:

X1.6.1.1 Source or sample name;

X1.6.1.2 Assay protocol reference and protocol parameter values;

X1.6.1.3 Assay name, performer, and date the assay was performed;

X1.6.1.4 Assay factor values including units and statistics, if applicable;

**TABLE X1.16 Material Relationship Concepts Example**

| A | B | C | D | E |
|---|---|---|---|---|
| Material Constituent | Material Linkage | Material Linkage Type | Term Accession Number | Term Source REF |
| g45_coona_dendrimer; magnevist | magnevist | covalent linkage | NPO_563 | NPO |

**TABLE X1.17 Material File Concepts Example**

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| Material File Name | Material File Type | Term Accession Number | Term Source REF | Material File Version | Material File Description |
| Magnevist.jpg | image | | | | |

**TABLE X1.18 Assay File Example for Size by DLS Assay (Columns A-F)**

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| Source | Protocol REF | Performer | Assay Name | Factor Value [temperature] | Unit |
| NCL-20-1 | Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering | Anil Patri | size by DLS assay | 25 | celsius |
| NCL-20-2 | Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering | Anil Patri | size by DLS assay | 25 | celsius |
| NCL-22-1 | Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering | Anil Patri | size by DLS assay | 25 | celsius |
| NCL-22-2 | Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering | Anil Patri | size by DLS assay | 25 | celsius |
| NCL-22-1 | Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering | Anil Patri | size by DLS assay | 37 | celsius |
| NCL-23-1 | Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering | Anil Patri | size by DLS assay | 25 | celsius |
| NCL-23-2 | Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering | Anil Patri | size by DLS assay | 25 | celsius |
| NCL-23-1 | Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering | Anil Patri | size by DLS assay | 37 | celsius |

**TABLE X1.19 Assay File Example for Size by DLS Assay (Columns G-L)**

| G | H | I | J | K | L |
|---|---|---|---|---|---|
| Factor Value [solvent medium] | Term Accession Number | Term Source Ref | Measurement Value [hydrodynamic diameter] | Statistic | Unit |
| Saline | NPO_1842 | NPO | 5.2 | z-average | nm |
| PBS | NPO_1846 | NPO | 8.6 | z-average | nm |
| Saline | NPO_1842 | NPO | 8.5 | z-average | nm |
| PBS | NPO_1846 | NPO | 6.6 | z-average | nm |
| PBS | NPO_1846 | NPO | 7.9 | z-average | nm |
| Saline | NPO_1842 | NPO | 7.4 | z-average | nm |
| PBS | NPO_1846 | NPO | 8.4 | z-average | nm |
| PBS | NPO_1846 | NPO | 9.8 | z-average | nm |

X1.6.1.5 Assay measurement values including units and statistics, if applicable; and

X1.6.1.6 Raw data files, derived data files or image files, if applicable.

X1.6.2 Specific concepts leveraged in the assay file are:

X1.6.2.1 *Source Name*—The unique identification name of the source referred to from within the study file. This column is used only if the source is same as the sample in an assay. Source names can be qualified only using *comment*.

X1.6.2.2 *Sample Name*—The unique identification name of the sample, which is referred to from within the study file. The sample is obtained after the application of a protocol. Sample names can be qualified only using *comment*.

X1.6.2.3 *Image File*—The name or URI of an image file generated from an assay.

X1.6.2.4 *Raw Data File*—The name or URI of the raw data files.

X1.6.2.5 *Derived Data File*—The name or URI of the file resulting from data transformation or processing.

X1.6.3 *Attributes of Assay nodes*—One or more attributes are used to provide more information about a assay node. The different assay node attributes are described below:

X1.6.3.1 *Material Type*—An attribute for the sample name (for example, biospecimen, nanoparticle sample, small molecule etc.) if the same attribute is not given in the study file. The term can be a free-text description or taken from an ontology or a controlled vocabulary. If it is the latter, then the following qualifiers are used: *term accession number* and *term source REF*.

X1.6.3.2 *Characteristics [ ]*—An attribute for *sample name* if the same attribute is not given in the study file.

X1.6.3.3 *Assay Name*—The name of the assay performed. This name is used as an identifier within the assay file.

X1.6.4 *Attributes of Processing Events for Assay Nodes*—One or more attributes are used to describe a step in the assay of a sample.

X1.6.4.1 *Protocol REF*—The name of the protocol used to perform the experiment. This name should be obtained from a value for the field *study protocol name* in the investigation file.

X1.6.4.2 *Performer*—The name of the person who carried out the protocol.

X1.6.4.3 *Date*—The calendar day on which the protocol was carried out. The date format should be in YYYY-MM-DD.

X1.6.4.4 *Parameter Value [<parameter term>]*—Value of a parameter, which is kept constant, when applying a protocol. The parameter term is written within brackets and must match the term used as value for the *study protocol parameter name* in the investigation file.

X1.6.4.5 *Measurement Value [<measurement term>]*—The endpoint of the assay. This field is used to capture measurement outputs recorded in summary data.

**TABLE X1.20 Assay File Example for Size by DLS Assay (Columns M-R)**

| M | N | O | P | Q | R |
|---|---|---|---|---|---|
| Measurement Value [Peak Size] | Unit | Measurement Value [PDI] | Derived Data File | Derived Data File | Image File |
| 4.4 | nm | 0.122 | NCL-DNT-Report.pdf | SizeSummaryTable.jpg | SizeNCL20_Saline_25.jpg |
| 6.2 | nm | 0.211 | NCL-DNT-Report.pdf | SizeSummaryTable.jpg | SizeNCL20_PBS_25.jpg |
| 6 | nm | 0.2 | NCL-DNT-Report.pdf | SizeSummaryTable.jpg | SizeNCL22_Saline_25.jpg |
| 5.2 | nm | 0.214 | NCL-DNT-Report.pdf | SizeSummaryTable.jpg | SizeNCL22_PBS_25.jpg |
| 5.1 | nm | 0.282 | NCL-DNT-Report.pdf | SizeSummaryTable.jpg | SizeNCL22_PBS_37.jpg |
| 5.3 | nm | 0.235 | NCL-DNT-Report.pdf | SizeSummaryTable.jpg | SizeNCL23_Saline_25.jpg |
| 6.1 | nm | 0.265 | NCL-DNT-Report.pdf | SizeSummaryTable.jpg | SizeNCL23_PBS_25.jpg |
| 5.6 | nm | 0.358 | NCL-DNT-Report.pdf | SizeSummaryTable.jpg | SizeNCL23_PBS_37.jpg |

X1.6.5 *Qualifiers for Aassay Nodes' Attributes*—Each node attribute may be qualified using the following concepts, if applicable.

X1.6.5.1 *Unit*—The standard of measurement used if the values for *characteristics [ ], parameter value [ ]* or *factor value [ ]* columns are quantitative and dimensional. If the term for unit is taken from an ontology/controlled vocabulary, then its *term accession number* and the *term source REF* should be defined in the ISA-TAB-Nano investigation file.

X1.6.5.2 *Term Accession Number*—Identification number of a term selected from an ontology or a controlled vocabulary, if the term is entered as a value in *source name, material type, characteristic [ ], parameter value [ ], unit* or *factor value [ ]* columns.

X1.6.5.3 *Term Source REF*—The name which identifies the source from where a term is selected and entered in ISA-TAB-Nano study files. This name should match one of the names entered in the *term source name* field in the ISA-TAB-Nano investigation file.

X1.6.5.4 *Statistic*—The type of statistical measure attributed to a numerical value (for example, mean, standard deviation, z-average, etc.). This is a required field, if the parameter value, factor value, or measurement value is a statistical measure.

X1.6.6 *Other Assay Fields:*

X1.6.6.1 *Factor Value [<factor name>]*—The value of an independent variable manipulated by the experimentalist with the intention to affect the subject of study (that is, stressor). Factor terms are given in brackets, and must be defined in the ISA-TAB-Nano investigation file in the Study Factors Section (X1.3.11). Factor Value [ ] in assay file should reference technical variations (such as software, instrument, or protocol variations).

X1.6.6.2 *Comment [ ]*—Any comment that provides additional information, which is added only when no other appropriate field exists.

X1.6.7 The assay file will vary depending on the type of assay performed and protocol and technology type leveraged. Provided below are examples of assay files for two common types of assays identified across nanotechnology resources.

X1.6.8 *Example Assay File: Size by Dynamic Light Scattering (DLS)*—DLS is a type of spectroscopy that uses a laser beam to irradiate a sample containing particles in suspension resulting in light scattering. Rapid fluctuations in scattering intensity around a mean value at a certain angle occur because of particle diffusion and are dependent upon on particle size. The calculated correlation function yields a diffusion coefficient, for a given temperature and viscosity, that can be used to calculate particle size. Table X1.17, Table X1.18, and Table X1.19 illustrate an example assay file for size by DLS.

X1.6.9 *Example Assay File: Assay File for MTT Cytotoxicity*—The MTT assay is a colorimetric assay that can assess the viability of cells by quantitation of the reduction of the yellow substrate MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to a product that has a purple color. This assay can measure the cytotoxicity of a chemical or drug by determining the affect of treatment on cell viability. Table X1.20 illustrates an example for a MTT cytotoxicity assay (MTT) performed on three nanoparticle samples, where nine dilutions of each nanoparticle sample are exposed to porcine proximal tubule cells for three different times of exposure (6 h, 2 h, and 48 h).

**TABLE X1.21 Assay File Example for MTT Cytotoxicity Assay in Porcine Proximal Tubule Cells**

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| Sample Name | Protocol REF | Performer | Assay Name | Derived Data File | Image File |
| LLC-PK1-6h-NCL22-1 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL22-2 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL22-3 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL22-4 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL22-5 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL22-6 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL22-7 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL22-8 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL22-9 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL23-1 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL23-2 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL23-3 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL23-4 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL23-5 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL23-6 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL23-7 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL23-8 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL23-9 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL24-1 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL24-2 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL24-3 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL24-4 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL24-5 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL24-6 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-6h-NCL24-7 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
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| LLC-PK1-6h-NCL24-9 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL22-1 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL22-2 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL22-3 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL22-4 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL22-5 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL22-6 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL22-7 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL22-8 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL22-9 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL23-1 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |

**TABLE X1.20** *Continued*

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| Sample Name | Protocol REF | Performer | Assay Name | Derived Data File | Image File |
| LLC-PK1-24h-NCL23-2 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL23-3 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL23-4 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL23-5 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL23-6 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL23-7 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL23-8 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL23-9 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
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| LLC-PK1-24h-NCL24-3 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL24-4 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL24-5 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL24-6 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL24-7 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL24-8 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-24h-NCL24-9 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL22-1 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL22-2 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL22-3 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL22-4 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL22-5 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL22-6 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL22-7 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL22-8 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL22-9 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL23-1 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL23-2 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL23-3 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL23-4 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL23-5 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL23-6 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL23-7 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL23-8 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL23-9 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL24-1 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL24-2 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |

**TABLE X1.20** *Continued*

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| Sample Name | Protocol REF | Performer | Assay Name | Derived Data File | Image File |
| LLC-PK1-48h-NCL24-3 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL24-4 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL24-5 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL24-6 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL24-7 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL24-8 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |
| LLC-PK1-48h-NCL24-9 | Time-6-24-48 plate MTT assay | Marina Dobrovolskaia | MTT assay | NCL-DNT-Report.pdf | MTT_LLC-PK1.jpg |

## X2. EXAMPLE ISA-TAB-NANO FILES FROM THE NCL DENDRIMER MRI INVESTIGATION

X2.1 Sample Description—As described in NCL200612A, the objective of the Dendritic Nanotechnologies, Inc.-NCL collaboration is to characterize a PAMAM dendrimer with an associated gadolinium chelate MRI contrast agent. The nanomaterials submitted for testing at the NCL were (NCL20) G4 tris (hydroxyl) terminated PAMAM dendrimer, (NCL21) G4 pyrrolidinone terminated PAMAM dendrimer, (NCL22) G4.5 COONa terminated PAMAM dendrimer, (NCL23) G4.5 COONa terminated PAMAM dendrimer-Magnevist complex, (NCL25) G4 tris (hydroxyl) terminated PAMAM dendrimer-Magnevist complex, and (NCL26) G4 pyrrolidinone terminated PAMAM dendrimer-Magnevist complex. Commercially available Magnevist (NCL24) was used as a control. NCL studies addressed in this report can be divided into three main categories: physicochemical characterization, immunotoxicology, and *in-vitro* toxicology.

## X3. OPTIONAL FILES

### X3.1 Structure File

X3.1.1 The structure file describes the 3D composition of the material and is not included in the ISA-TAB-Nano Release Candidate 1.0. The structure file is an optional file and typically developed for modeling and simulation activities in support of structure-activity-relationships (SAR). The ability to develop this file requires a detailed understanding of the 3D composition of the particles and the ability to represent each atom of the nanomaterial and relationships to other atoms. The structure file is referenced from the material file.

X3.1.2 Since there are no standard structure files for nanotechnology, investigators can include structure files of any format.

### X3.2 Data File

X3.2.1 Data files are optional files that can include additional spreadsheets, images, histograms, distribution graphs, and so forth. Data files are referenced within an assay file under a column header of “image file” or “derived file.”

X3.2.2 There are no standard data file formats for nanotechnology. In gene expression studies, these files typically refer to array data files such as the Affymetrix.cel file and derived array data files. Since there are no standard data files for nanotechnology, investigators can include any data files and images associated with an assay.

## X4. ISA-TAB-NANO BACKGROUND

X4.1 *Background*—The ISA-TAB-Nano specification is intended to facilitate the 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/from nanotechnology resources like the NCI's cancer Nanotechnology Laboratory (caNanoLab) portal[8] and the Nanomaterial-Biological Interactions (NBI) knowledgebase.[9] ISA-TAB-Nano 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.

X4.2 The ISA-TAB-Nano project is an effort of the National Cancer Institute (NCI) Cancer Biomedical Informatics Grid (caBIG[10]) Nanotechnology Informatics Working Group (Nano WG). Its proper use as a standard requires familiarity with other components of the caBIG complement of informatics tools that are all designed to support the meaningful exchange of data across the nanotechnology community.

X4.2.1 *ISA-TAB-Nano format's relationship to other caBIG projects:*

X4.2.1.1 *ISA-TAB*—The ISA-TAB-Nano format specification is based on an existing specification developed by the ISA Community[11], namely, the investigation/study/assay (ISA-TAB) format specification. The ISA-TAB format is used by the 'omics' (proteomics, genomics, metabolomics, and transcriptomics) communities to share data and metadata associated with different assays and technology types in their experiments. The ISA-TAB file structure relies on three primary files—investigation, study, and assay (ISA) files. Raw/derived data files and any other files (for example, image files, protocol documents) specific to each assay are shared along with the three primary ISA-TAB files if the data files are referenced in the primary ISA-TAB files. ISA-TAB does not provide format specification for files other than the investigation, study, and assay files. The ISA-TAB 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 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.

X4.2.1.2 *National Cancer Institute Enterprise Vocabulary Service (NCI EVS)*—The NCI EVS is a project of the NCI Center for Biomedical Informatics and Information Technology (CBIIT). EVS provides controlled terminologies and ontologies in support of the biomedical research and informatics activities of the NCI and its partners, including the caBIG community. The activities of the EVS include development of terminologies, development of terminology-related software, and operations support to address the broad spectrum of terminology needs in the cancer research enterprise. Among the vocabularies that EVS supports vocabularies such as the NanoParticle Ontology (NPO), by providing terminology development facilities and terminology servers, which are made available both by way of the web and programmatically through EVS server application programming interfaces (APIs). Additionally, the EVS presents NPO to the public both in a standalone format and as a component of the NCI Metathesaurus, where its concepts are mapped to the concepts of other vocabularies used by the NCI community. Also, the EVS-managed NCI Thesaurus (NCIt) includes nanotechnology concepts that have been utilized in the development of the NCI caNanoLab. Data from caNanoLab has been utilized in the ISA-TAB-Nano example files in this document.

X4.2.1.3 *Life Sciences Domain Analysis Model (LS DAM)*—The caBIG (cancer Bioinformatics Grid) LS DAM[12] provides a shared view of the semantics of the life sciences domains that are represented by the different workspaces in the caBIG infrastructure. It has a nanotechnology subdomain, which was developed based on caNanoLab object model and NPO terms. LS DAM makes a distinction between biospecimens (for example, cell line, tissue samples, body fluid samples, organ parts) and materials that are not derived from a cell, tissue, organ, or body (for example, nanoparticle formulations, drug formulations, solvent, and so forth). This motivated the use of the term "material sample" in the ISA-TAB-Nano material file. Weekly Nano WG web-conferencing was used to ensure the alignment of ISA-TAB-Nano with the LS DAM.

X4.2.1.4 *Use of Ontologies and Standard Terminologies in ISA-TAB-Nano:*

*(1)* Like ISA-TAB, ISA-TAB-Nano provides fields for entering and referencing terms selected from ontologies and standard terminologies. The ontologies are available at BioPortal, 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 standard utilize the NCI EVS and Nanoparticle Ontology elements.

*(2) NanoParticle Ontology (NPO)*—NanoParticle Ontology (NPO)[6] is an ontology that is designed and developed within the framework of the Basic Formal Ontology (BFO)[13] and implemented in the ontology web language (OWL).[14] It is being developed to represent the knowledge underlying the

[8] Access available from https://cananolab.nci.nih.gov/caNanoLab.

[9] Access available from http://nbi.oregonstate.edu.

[10] caBIG is trademarked by the U.S. Department of Health and Human Services, http://www.hhs.gov.

[11] Available from http://www.isa-tools.org.

[12] Available from https://wiki.nci.nih.gov/pages/viewpage.action?pageId=25007847.

[13] Spear, A.D., "Ontology for the Twenty First Century: An Introduction With Recommendations, Basic Formal Ontology (BFO)," available from http://www.ifomis.org/bfo/manual.

[14] Available from http://www.w3.org/TR/owl-features.

description, preparation, and characterization of nanomaterials. NPO development began with the representation of knowledge underlying the chemical composition, preparation, physiochemical, and functional/biological characterization of nanoparticles that are formulated and tested for applications in cancer diagnostics and therapeutics. The NPO provided the knowledge framework for developing the ISA-TAB-Nano material file format. The NPO provides a subset of the terms and relationships for the description and characterization of nanomaterials in the ISA-TAB-Nano file format. The NPO is being further developed for the following purposes: (*1*) to provide terms for annotating nanotechnology research data; (*2*) to provide the knowledge framework required for developing data-sharing models and standards in nanomedicine; (*3*) to enable semantic integration of data; (*4*) to enable unambiguous interpretation of the description and characterization of nanomaterials; and (*5*) to enable knowledge-based searching and comparison of nanomaterial descriptions and characterization results.

X4.2.2 *Requisite Adjustments to ISA-TAB Practice to be Found in ISA-TAB-Nano:*

X4.2.2.1 *ISA-TAB-Nano Extension to ISA-TAB*—While ISA-TAB-Nano leverages the three primary ISA-TAB files, it extends ISA-TAB by providing specification for a fourth file (called the material file) for representing the composition and characteristics of nanoparticle formulations and small molecules. Raw/ derived data files and any other files (for example, image files, protocol documents) specific to each assay have to be shared along with the four primary ISA-TAB-Nano files. ISA-TAB-Nano does not provide any specification for how to format files other than the four primary files: investigation, study, assay and material files. Although ISA-TAB-Nano adopts ISA-TAB field names and their definition in the investigation, study, and assay files, some of the definitions are modified and additional fields are introduced. These modifications and extensions are required to expand the scope of information captured from nanotechnology data sets into the ISA-TAB-Nano files.

X4.2.2.2 *ISA-TAB-Nano Extensions*—ISA-TAB-Nano extends the ISA-TAB Investigation File specification by introducing new fields, which are listed in Table 2. The field concepts are described in X1.3.2.2.

X4.2.2.3 *ISA-TAB-Nano Extensions*—ISA-TAB-Nano extends the ISA-TAB specification for the study file by redefining the concept of a sample and adding a new field called material file to reference the material files that contain description of the material samples.

X4.2.2.4 *ISA-TAB-Nano Extensions*—Since the material file does not exist in the ISA-TAB specification, the entire file is considered an extension. This file is described in detail in X1.5.

X4.2.2.5 *ISA-TAB-Nano Extensions*—In support of ISA-TAB-Nano, the extensions and constraints in Table 6 were applied to the ISA-TAB assay file and are further described in X1.3.2.2. One important extension is that allows the entry of summary data in the assay files through an optional field called Measurement Value, as described in Table 6.

X4.2.2.6 *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 Nano-TAB, 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 physicochemical 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 Nano-TAB, the concept of a sample (as used in ISA-TAB specification) is redefined to include both biological specimens and material samples. The ISA-TAB 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 Nano-TAB, the material file is used to describe *material samples*, while the study file is used to describe *biospecimens*.

X4.2.2.7 *ISA-TAB and ISA-TAB-Nano File Names*—ISA-TAB specifies that the names of the primary files end with .txt extensions. 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.

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