

# ISA-TAB-Nano Overview

June 2015

# ISA-TAB-Nano Goal

**To develop a specification that facilitates the import/export of data on nanomaterials and their characterizations to/from nanotechnology resources**

- **Challenges and Opportunities:**
  - Development of a standard flexible enough to support the numerous (100+) diverse characterization assays performed by the nanotechnology community
  - Development of a standard that can represent the complex structures of nanomaterials and their components
  - Identification of the minimal information required to achieve cross-material comparison

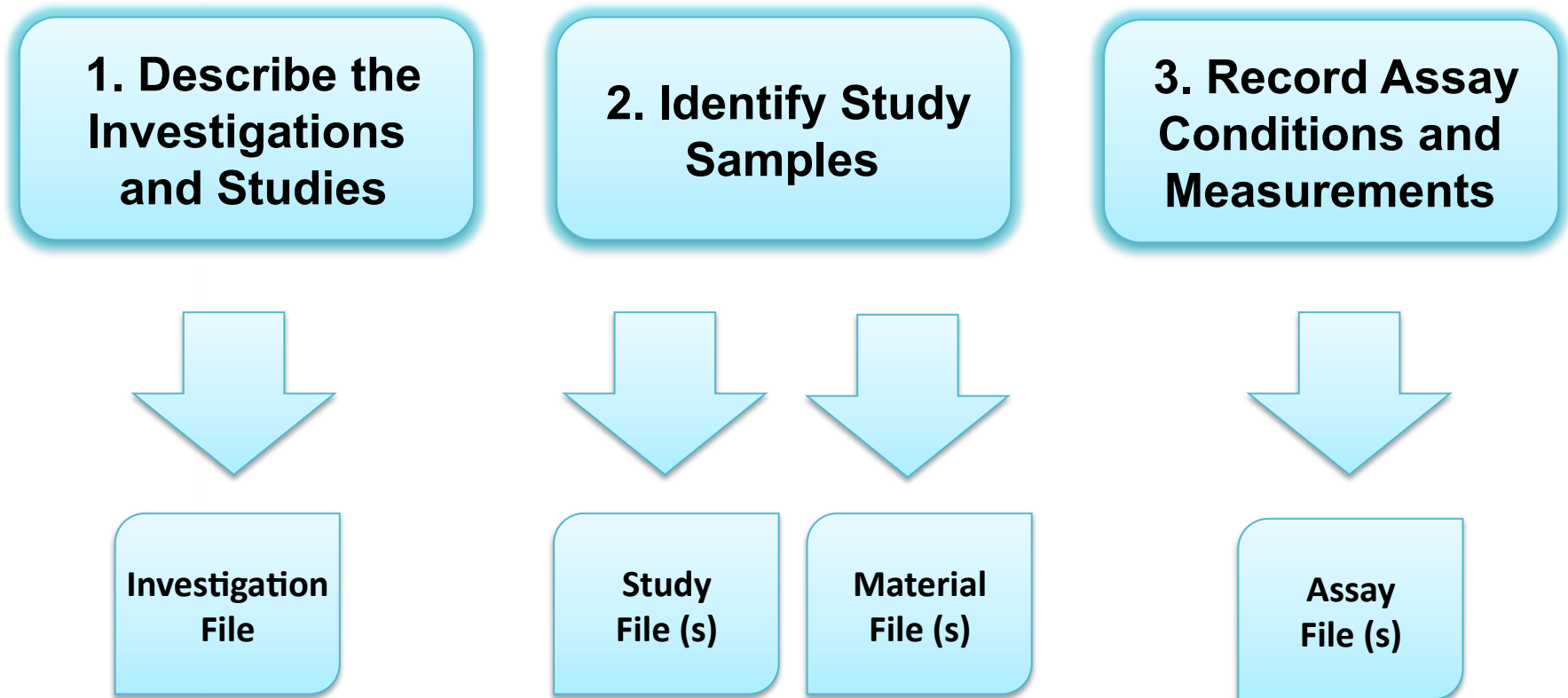
# What is ISA-TAB-Nano?

- Provides a standard tab-delimited format for describing data related to:
  - Investigations
  - Materials (Nanomaterials)
  - Studies (Specimens)
  - Assays
- Leverages and extends the Investigation/Study/Assay (ISA-TAB) format
  - Standard tab-delimited file format developed by the European Bioinformatics Institute (EBI) for representing a variety of assays (e.g. MAGE-TAB) and technology types
- Supports ontology-based curation
  - Nanomaterials and concepts from the NanoParticle Ontology (NPO) as well as other ontologies

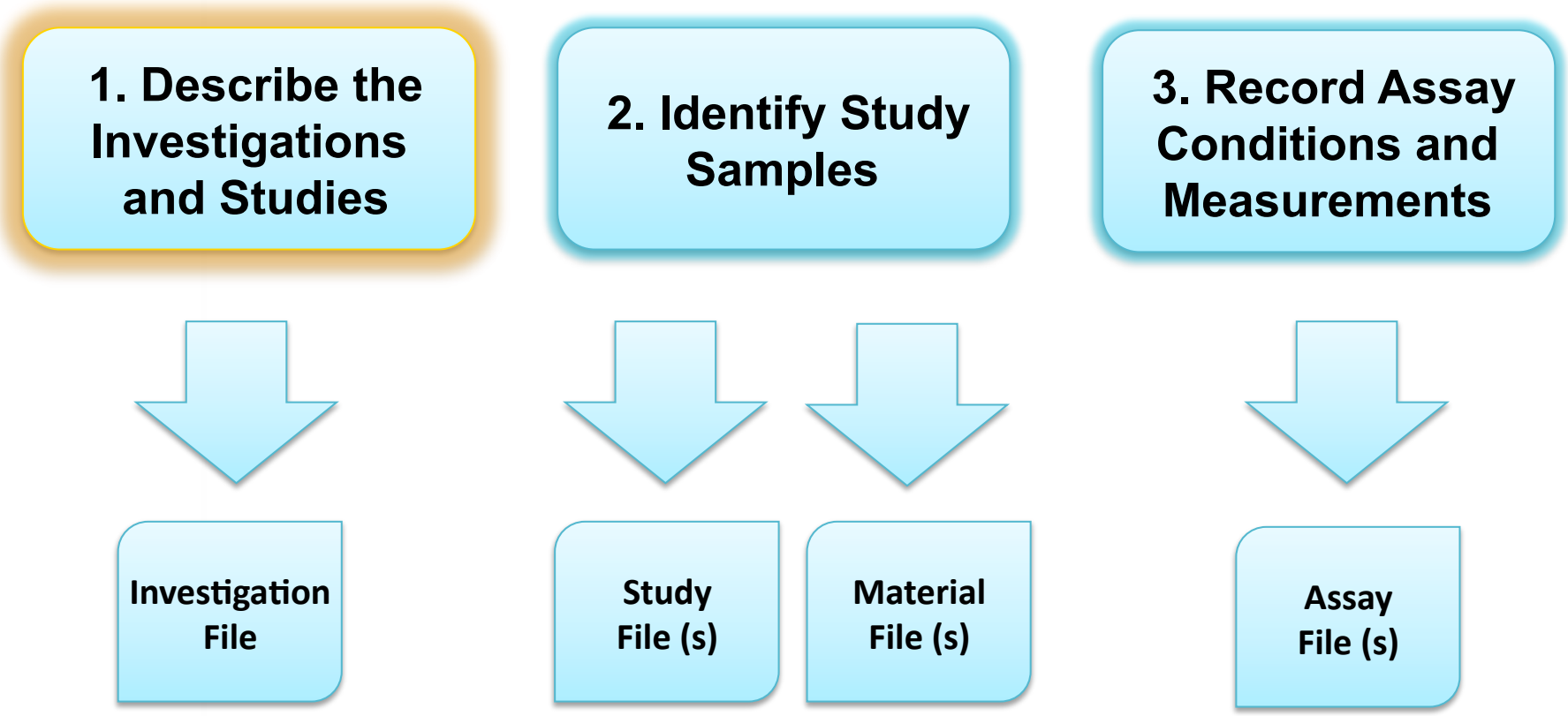
# ISA-TAB Extensions for Nanotechnology

Extension Field Name	File	Purpose
Investigation Disease, Investigation Disease Term Accession Number, Investigation Disease Term Source REF	Investigation	To capture and retrieve investigations associated with specific disease modalities such as cancer
Investigation Outcome	Investigation	To enable researchers to review the outcomes of investigations for assessing the utility of the investigation in achieving scientific endpoints
MATERIAL, Material File Name, Material Source Name	Investigation	To allow for the identification of materials used in the investigation and associated studies
Study Disease, Study Disease Term Accession Number, Study Disease Term Source REF	Investigation	To capture and retrieve studies associated with specific disease modalities such as cancer
Study Outcome	Investigation	To enable researchers to review the outcomes of studies for assessing the utility of the investigation in achieving scientific endpoints
Study Assay Measurement Name, Study Assay Measurement Name Term Accession Number, Study Assay Measurement Name Term Source REF	Investigation	To capture the variables measured in an assay to support cross study analysis
Measurement Value	Assay	To record the endpoint value of an assay measurement within the assay file
All Fields	Material	To describe nanomaterials and small molecules

# ISA-TAB-Nano Structure



# ISA-TAB-Nano Structure



# ISA-TAB-Nano Investigation File

- Describes:
  - Primary investigation
  - Associated materials, studies, assays, and protocols
- Descriptive information about the study includes:
  - Design descriptors and factors
  - Publications
  - Assays and protocols
  - Contacts
- Vertical-based spreadsheet format with columns representing multiple values

# Investigation File (1 of 3)

<b>Ontology References</b>	ONTOLOGY SOURCE REFERENCE		
	Term Source Name	MO	NPO
	Term Source File	<a href="http://purl.bioontology.org/ontology/MO">http://purl.bioontology.org/ontology/MO</a>	<a href="http://purl.bioontology.org/ontology/npo">http://purl.bioontology.org/ontology/npo</a>
	Term Source Version	v. 1.3.1.1	v. 2011-02-12
<b>Investigation Description</b>	Term Source Description	MGED Ontology	NanoParticle Ontology
	INVESTIGATION		
	Investigation Identifier	NCL200612A	
	Investigation Title	Dendrimer-Based MRI Contrast Agents The goal of this investigation is to characterize a PAMAM dendrimer with an associated gadolinium chelate MRI contrast agent.	
	Investigation Description		
	Investigation Submission Date	2002-11-30	
	Investigation Public Release Date	2002-11-30	
	Investigation Disease		
	Investigation Disease Term Accession Number		
	Investigation Disease Term Source REF		
<b>Investigation Publications</b>	Investigation Outcome		
	INVESTIGATION PUBLICATIONS		
	Investigation PubMed ID	18095846	
	Investigation Publication DOI	10.2217/17435889.2.6.789	
	Investigation Publication Author List	10.2217/17435889.2.6.789	
	Investigation Publication Title	Characterization of nanoparticles for therapeutics	
	Investigation Publication Status	published	
	Investigation Publication Status Term Accession Number		
	Investigation Publication Status Term Source REF		
	INVESTIGATION CONTACTS		
<b>Investigation Contacts</b>	Investigation Person Last Name	Doe	
	Investigation Person First Name	John	
	Investigation Person Mid Initials	E	
	Investigation Person Email	<a href="mailto:doej@mail.nih.gov">doej@mail.nih.gov</a>	
	Investigation Person Phone	1231231234	
	Investigation Person Fax		
	Investigation Person Address	Laboratory Street, City, State 111111	
	Investigation Person Affiliation	Doe Laboratories	
	Investigation Person Roles	investigator	
	Investigation Person Roles Term Accession Number		
Investigation Person Roles Term Source REF	MO		



# Investigation File (2 of 3)

<b>Material Identifiers</b>	MATERIAL		
	Material File Name	m_NCL-20.xls	m_NCL-21.xls
	Material Source Name	NCL-20	NCL-21
<b>Study Description</b>	STUDY		
	Study Identifier	NCL200612A-Size	
	Study Title	Hydrodynamic Size/Size Distribution via Dynamic Light Scattering (DLS) Dynamic light scattering(DLS) technique was used to measure the hydrodynamic size of dendritic nanomaterials. The effects of sample concentration, buffer and temperature on the hydrodynamic size (stability) also were measured.	
	Study Description		
	Study Submission Date	2002-11-30	
	Study Public Release Date	2002-11-30	
	Study Disease		
	Study Disease Term Accession Number		
	Study Disease Term Source REF		
		Hydrodynamic size (diameter) of the dendrimer samples NCL22, NCL23 and NCL20 were measured in aqueous solutions using DLS at 25 °C and 37 °C. An instrument with a backscattering detector was used for these measurements in batch mode (no fractionation). This technique does not have the resolving power of differentiating monomers and dimers without fractionation. Samples were weighed, dissolved in deionized (DI) water, aliquoted, lyophilized and resuspended in desired buffer solutions to a final concentration of 1 mg/mL, and filtered through a 0.2-µm filter, unless otherwise indicated. The measurements were taken in saline (154 mM NaCl) and phosphate-buffered saline (PBS) at pH 7.4. Three measurements were taken for each sample.	
	Study Outcome		
<b>Study Design</b>	Study File Name	s_size-DLS.xls	
	STUDY DESIGN DESCRIPTORS		
	Study Design Type	comparison	
	Study Design Type Term Accession Number		
	Study Design Type Term Source REF		
<b>Study Publications</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	
<b>Study Factors</b>	Study Publication Status Term Accession Number		
	Study Publication Status Term Source REF		
	STUDY FACTORS		
	Study Factor Name	temperature	solvent medium
	Study Factor Type	temperature	solvent medium
	Study Factor Type Term Accession Number	PATO_0000146	NPO_1855
	Study Factor Type Term Source REF	PATO	NPO



# Investigation File (3 of 3)

## Study Assays

STUDY ASSAYS	
Study Assay Measurement Type	hydrodynamic size
Study Assay Measurement Type Term Accession Number	
Study Assay Measurement Type Term Source REF	
Study Assay Technology Type	dynamic light scattering(DLS)
Study Assay Technology Type Term Accession Number	NPO_1469
Study Assay Technology Type Term Source REF	NPO
Study Assay Technology Platform	
Study Assay Measurement Name	hydrodynamic diameter; peak size; PDI
Study Assay Measurement Name Term Accession Number	; ; NPO_1155
Study Assay Measurement Name Term Source REF	; ; NPO
Study Assay File Name	a_size-DLS.xls

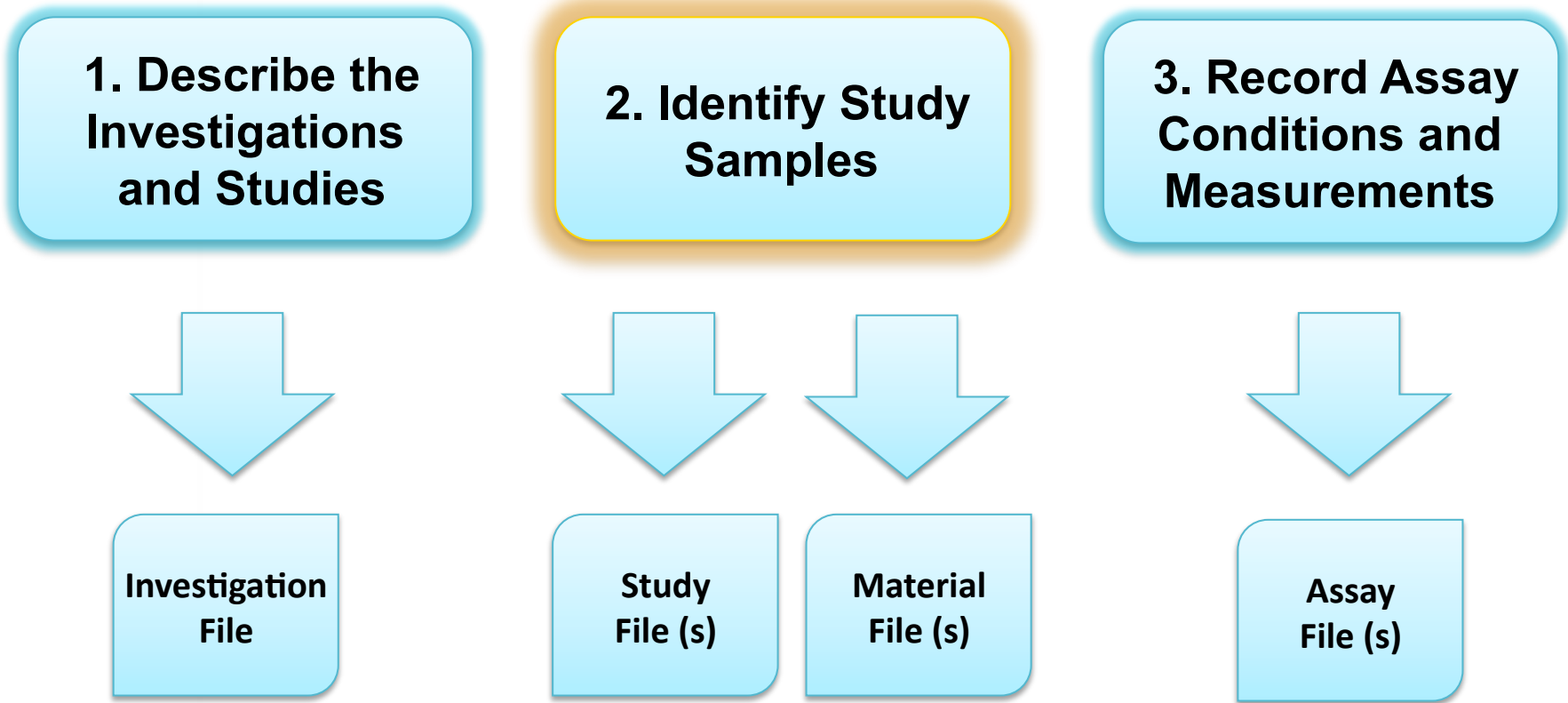
## Study Protocols

STUDY PROTOCOLS	
Study Protocol Name	Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering
Study Protocol Type	sample preparation procedure; particle size measurement procedure
Study Protocol Type Term Accession Number	
Study Protocol Type Term Source REF	
Study Protocol Description	This assay protocol outlines procedures for sample preparation and the determination of mean nanoparticle size (hydrodynamic diameter) using batch-mode dynamic light scattering (DLS) in dilute aqueous suspensions. Although particle size is the primary determinant of the measured diffusion coefficient, other parameters can impact these measurements and influence the measured size. Therefore, guidelines for making successfully size measurements in the nanosize range are provided, as well as a discussion of relevant standards and data analysis.
Study Protocol URI	NCL_Method_PCC-1.pdf
Study Protocol Version	1.1
Study Protocol Parameters Name	pH; NaCl concentration; particle concentration
Study Protocol Parameters Name Term Accession Number	UO_0000196; ; NPO_1830
Study Protocol Parameters Name Term Source REF	UO; ;NPO
Study Protocol Components Name	Mastersizer 2000 (Malvern DLS)
Study Protocol Components Type	DLS instrument
Study Protocol Components Type Term Accession Number	NPO_1766
Study Protocol Components Type Term Source REF	NPO

## Study Contacts

STUDY CONTACTS	
Study Person Last Name	Smith
Study Person First Name	Jane
Study Person Mid Initials	K
Study Person Email	<a href="mailto:smithj@mail.nih.gov">smithj@mail.nih.gov</a>
Study Person Phone	1231231235
Study Person Fax	
Study Person Address	Laboratory Street, City, State 111111
Study Person Affiliation	Doe Laboratories
Study Person Roles	investigator
Study Person Roles Term Accession Number	
Study Person Roles Term Source REF	MO

# ISA-TAB-Nano Structure



## ISA-TAB-Nano Study File

- Provides mapping between the study samples, materials, and processing events
- Samples can be:
  - Biological materials
  - Nanomaterials
  - Small molecules
- For physical-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, etc.)
- Horizontal spreadsheet describing the biological materials and association with the nanomaterials described in the Material file

# Study File

## Biological Sample Identifier

## Biological Sample Characteristics

## Protocol

Source Name	Material Type	Characteristics[cell type{NCIt:C12508}]	Characteristics[ATCC#{NCIt:C15661}]	Provider	Protocol REF	Performer
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe
LLC-PK1	biospecimen	porcine proximal tubule cells	CL-101	Doe Technologies	cell preparation in four 96-well plates	Jane Doe

## Biological Sample

## Study Factors

Sample Name	Factor Value[nanoparticle sample]	Factor Value[particle concentration]	Unit	Term Accession Number	Term Source Ref	Factor Value[time of exposure]	Unit
LLC-PK1-6h-NCL22-1	NCL-22	0.004	mg/mL	UO_0000176	UO	6	hour
LLC-PK1-6h-NCL22-2	NCL-22	0.008	mg/mL	UO_0000176	UO	6	hour
LLC-PK1-6h-NCL22-3	NCL-22	0.016	mg/mL	UO_0000176	UO	6	hour
LLC-PK1-6h-NCL22-4	NCL-22	0.032	mg/mL	UO_0000176	UO	6	hour
LLC-PK1-6h-NCL22-5	NCL-22	0.064	mg/mL	UO_0000176	UO	6	hour
LLC-PK1-6h-NCL22-6	NCL-22	0.128	mg/mL	UO_0000176	UO	6	hour
LLC-PK1-6h-NCL22-7	NCL-22	0.256	mg/mL	UO_0000176	UO	6	hour
LLC-PK1-6h-NCL22-8	NCL-22	0.512	mg/mL	UO_0000176	UO	6	hour
LLC-PK1-6h-NCL22-9	NCL-23	1	mg/mL	UO_0000176	UO	6	hour

# ISA-TAB-Nano Material File

- Primary file for describing:
  - Nanomaterial composition and formulation
  - Physical properties
  - Structure
- Allows for:
  - Comparison of nanomaterials across nanotechnology resources
  - Association with optional files; e.g., a Structure file for representing the 3D structure of the nanomaterial
- Horizontal spreadsheet describing the nanomaterial sample, associated components, material characteristics, and material linkages

# Material File (1 of 2)

Material Identifiers		Material Description			Intended Application
Material Source Name	Material Name	Material Description	Material Synthesis	Material Design Rationale	Material Intended Application
NCL-22	g45_coona_dendrimer	G4.5 COONa terminated PAMAM dendrimer			delivery of image contrast agent
NCL-23	g45_coona_dendrimer_magnevist_complex	G4.5 COONa terminated PAMAM dendrimer-Magnevist® complex			MRI
NCL-24	magnevist	gadolinium based image contrast agent			MRI contrast agent

Material Type			Chemical Name			Characteristics
Material Type	Term Accession Number	Term Source REF	Material Chemical Name	Term Accession Number	Term Source REF	Characteristics[dendrimer branch]
dendrimer; conjugated component	NPO_735; NPO_1826	NPO;NPO				1-4
nanoparticle sample	NPO_1404	NPO				
small molecule; imaging payload agent; conjugated component	NCIt_C48809; NPO_1534; NPO_1826	NCIt;NPO;NPO	gadopentetate dimeglumine	31797	ChEBI	

# Material File (2 of 2)

## Characteristics

Characteristics[dendrimer generation]	Characteristics[molecular weight]	Unit	Term Accession Number	Term Source REF	Characteristics[molecular formula]
4.5	26.28	kDa	UO_0000222	UO	
					[Gd+3] .CNC[C@H] (O) [C@@H] (O) [C@H] (O) [C@H] (O) CO.CNC[C@H] (O) [C@@H] (O) [C@H] (O) [C@H] (O) CO.OC(=O) CN(CCN(CCN(CC(O) =O) CC([O- ] ) =O) CC([O- ] ) =O) CC([O- ] ) =O

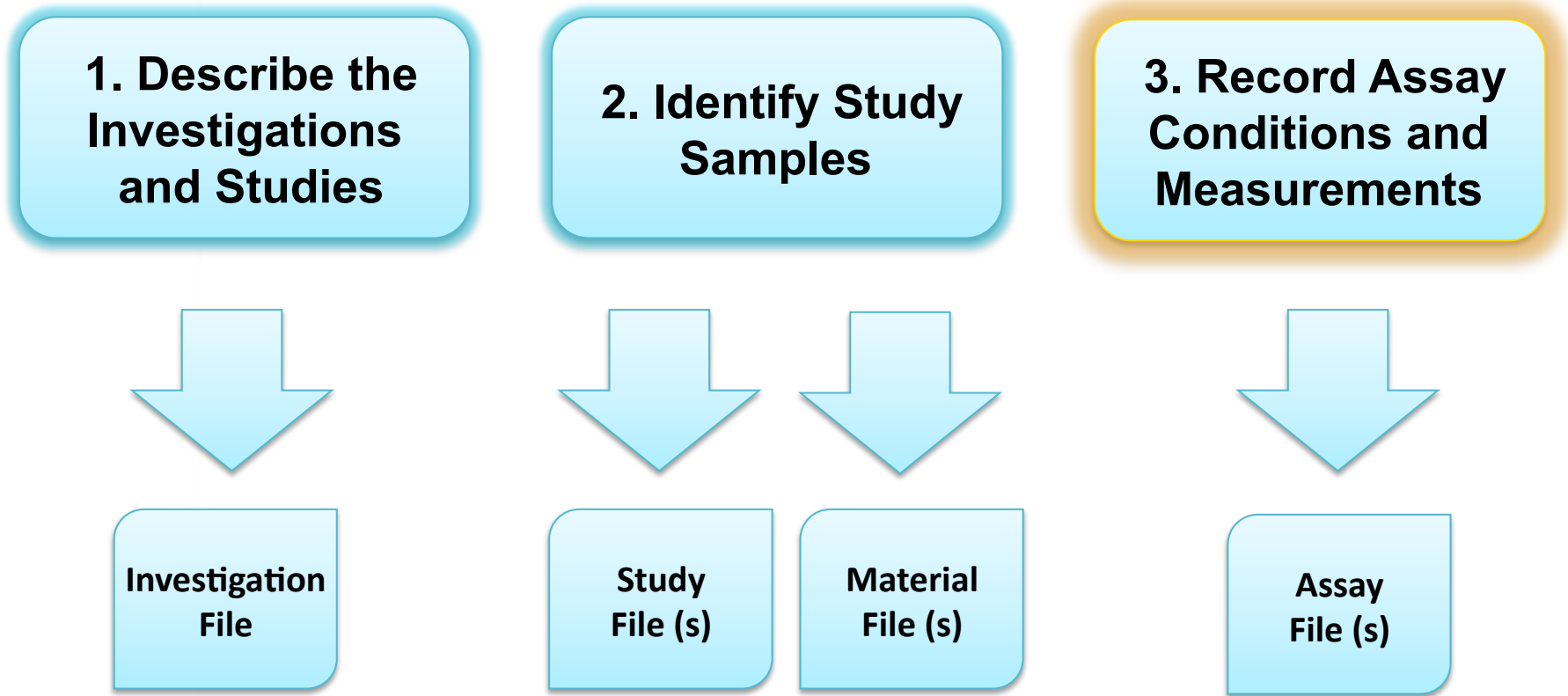
## Material Linkage

## Material File

Material Constituent	Material Linkage Type	Term Accession Number	Term Source REF	Material Data File	Material Data File Type	Term Accession Number	Term Source REF	Material Data File Version	Material Data File Description
g45_coona_dendrimer; magnevist	covalent linkage	NPO_563	NPO	magnevist.jpg	image	NCIt_C48179	NCIt		



# ISA-TAB-Nano Structure



## ISA-TAB-Nano Assay File

- Describes the protocol parameters and factors, such as:
  - Temperature
  - Media/solvent
  - Concentration
- Provides references or links to assay results, including:
  - Measurements
  - Instrumentation
  - Derived Data Files
- Templates available for the “Top Nano WG assays”
  - Size by DLS (Physico-Chemical)
  - Zeta Potential (Physico-Chemical)
  - Hemolysis (In Vitro)
  - Hepatocarcinoma Cytotoxicity (MTT and LDH) (In Vitro)
  - Caspase 3 Apoptosis (In Vitro)
  - Toxicity (Pharmacokinetics, Tissue Distribution) (In Vivo)

# Assay File

## Size by DLS

Sample Names		Protocol Parameters			Assay Factors					
Sample Name	Protocol REF	Performer	Assay Name	Factor Value[temperature]	Term Accession Number	Term Source Ref	Unit	Term Accession Number	Term Source Ref	Factor Value[solvent medium]
NCL-20-1	Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering	John Doe	size by DLS assay	25	PATO_0000146	PATO	celsius	UO_0000027	UO	saline
NCL-20-1	Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering	John Doe	size by DLS assay	25	PATO_0000146	PATO	celsius	UO_0000027	UO	PBS
NCL-22-1	Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering	John Doe	size by DLS assay	25	PATO_0000146	PATO	celsius	UO_0000027	UO	saline
NCL-22-1	Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering	John Doe	size by DLS assay	25	PATO_0000146	PATO	celsius	UO_0000027	UO	PBS
NCL-22-1	Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering	John Doe	size by DLS assay	37	PATO_0000146	PATO	celsius	UO_0000027	UO	PBS

Assay Measurements								Assay Files	
Measurement Value[z-average(hydrodynamic diameter)]	Unit	Term Accession Number	Term Source Ref	Measurement Value[peak size]	Unit	Term Accession Number	Term Source Ref	Measurement Value[pdi]	Derived Data File
5.2	nm	UO_0000018	UO	4.4	nm	UO_0000018	UO	0.122	NCL-Dendrimer-Based_MRI_Contrast_Agent.pdf
8.6	nm	UO_0000018	UO	6.2	nm	UO_0000018	UO	0.211	NCL-Dendrimer-Based_MRI_Contrast_Agent.pdf
8.5	nm	UO_0000018	UO	6	nm	UO_0000018	UO	0.2	NCL-Dendrimer-Based_MRI_Contrast_Agent.pdf
6.6	nm	UO_0000018	UO	5.2	nm	UO_0000018	UO	0.214	NCL-Dendrimer-Based_MRI_Contrast_Agent.pdf
7.9	nm	UO_0000018	UO	5.1	nm	UO_0000018	UO	0.282	NCL-Dendrimer-Based_MRI_Contrast_Agent.pdf

# Top Nano WG Assay File Templates

The screenshot shows a web browser displaying the NCI Wiki page for Assay File Examples. The page header includes the National Cancer Institute logo and the text "NCI Wiki". The main content area features a table with four columns: Assay File, Assay Factors, Assay Measured Values, and Supporting Information. The first row of the table lists "Hemolysis" as the assay file, "Concentration" as the assay factor, and "isHemolytic" and "Percent Hemolysis" as the measured values. The supporting information includes "Analysis of Hemolytic Properties of Nanoparticles" and "Dendrimer-Based MRI Contrast Agents".

Below the table, the page is titled "In Vivo Characterization Assays" and contains a section for "Disposition and Pharmacokinetics Assay". This section includes a paragraph describing the assay: "The Disposition and Pharmacokinetics assay is performed to determine the fate of a nanoparticle formulation in an organism, which can include its absorption, tissue distribution, metabolism or excretion (ADME). This is accomplished by detecting the whole formulation or its components at various body locations and time points after dosing. A variety of methods can be used to detect and quantify nanoparticle formulations and their components in vivo."

Below the paragraph is another table with the same four columns: Assay File, Assay Factors, Assay Measured Values, and Supporting Information. The first row lists "Pharmacokinetics" as the assay file, "Time" as the assay factor, and "Clearance at Time Zero (C0)", "Area Under Curve (AUC)", "Clearance (CL)", "Half Life (T 1/2)", and "Volume of Distribution (V app)" as the measured values. The supporting information includes "Distribution and Pharmacokinetics Description" and "Ceramide Liposomes NCL200702A". The second row lists "Tissue Distribution" as the assay file, "Time" as the assay factor, and "Tissue Distribution" as the measured value. The supporting information includes "Distribution and Pharmacokinetics Description".

The page footer indicates it is powered by Atlassian Confluence 5.5.3, Team Collaboration Software, and provides a link to contact administrators. The browser's address bar shows the URL: <https://wiki.nci.nih.gov/display/ICR/Assay+File+Examples>.

<https://wiki.nci.nih.gov/display/ICR/Assay+File+Examples>

## ISA-TAB-Nano Uses and Benefits

- Addresses the data sharing challenges in nanomedicine
- Provides a standard means for identifying nanomaterials and characterizations
- Enables the submission and exchange of nanomaterial data to/from nanotechnology data resources ( e.g., NBI, caNanoLab, etc.)
- Empowers organizations to adopt standards for representing data in nanotechnology publications
- Provides researchers with guidelines for representing nanomaterials and characterizations to achieve cross-material comparison

## Current Status

- The ISA-TAB-Nano Team is working with the community and ISA-TAB Team to address feedback from ISA-TAB-Nano usage and plans to release ISA-TAB-Nano 1.3
- The ISA-TAB-Nano team participates in Nano WG discussions on identifying the minimal information required in ISA-TAB-Nano files

## References

- ISA-TAB-Nano Project Site:  
<https://wiki.nci.nih.gov/display/ICR/ISA-TAB-Nano>
- ASTM nano-TAB Work Item WK28974:  
<http://www.astm.org/DATABASE.CART/WORKITEMS/WK28974.htm>
- ISA-TAB: <http://isatab.sourceforge.net>
- Nano WG Data Standards Document:  
<https://wiki.nci.nih.gov/display/ICR/ISA-TAB-Nano>
- NanoParticle Ontology (NPO):  
<http://www.nano-ontology.org>