FP7-NANOSAFETY: TOWARDS FULL INTEGRATION OF STANDARDIZATION IN THE PROJECTS

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Introduction

Standardization :

- capitalize and disseminate knowledge
- have it implemented in the industry ("reference documents")

➔ Particularly relevant for nanosafety: progress, public debate.

Barriers to researchers

➔ **Solution : Preparation <u>in</u> projects**

- Organizational: NSBs & nanoSTAIR
- Technical: Pre-normative work

Motivations for researchers

Share your knowledge / research

- Make your work be widely used
- Moral / contractual obligation (public funding!)
- Get your work recognized

Have your say in next standards

- Adequate in your view
- compatible with your protocols

  ➔ Continuity in your practices and data

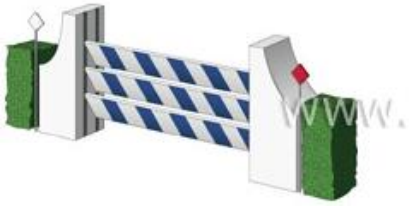
Improve your work

- Gain full discussion on your protocol(s)
- Networking effect

Barriers to researchers

Lack of awareness
Process : - complicated
- **not flexible and fast enough**

Low own impact on standard
Ignorance of each other's activities



No resources (MM) in project / organisation
No funding after project!
No funding out of project!

Low recognition (no author list) # ISI

Nanosafety-FP7's contribution to Standardization

Initiation of ISO/CEN standards, specifications, reports, CWA

- Published: 0 / ~ 22
- Under development: 0 ? / ~17
- Submitted for start: 3-4 / ~ 5-6
  + ~ 5 coming from NanoValid!
- **Others? PLEASE INFORM US!**

Participation:

- 16 /31 NSC projects involved (Compendium 2014)
- 8 Liaisons with CEN TC 352 + 2 coming
- Large participation of researchers in committees ISO, CEN, OECD

Potential:

- Almost all FP7 projects working on SOPs
- Small extra effort ➔ **Big impact**…

… if integrated … **from the proposal** … **in project objectives & plan**

I) Organisational preparation

Support from National Standardization Bodies (NSBs)

- Explain and guide
- Vote for new items
- Appoint experts

NSBs

nanoSTAIR's services to **researchers**

1. Platform : web & team
   - Shares information & contacts
2. Seminar: standardization & nanosafety
3. Check of results from research
   - Identify potential for and way to standardization:
     Semantic analysis + Expert review
   - Pool resources, consortia, experts ➔ Critical mass

Story 1: Determination of Hydrochemical reactivity of nano-objects for tox. studies

0) **13 candidates**

Doc. #	Topic of research candidate	Type of candidate
ACOSTA	Atomic Force Microscopy Based Micro/Nanomanipulation	PhD Thesis[10]
BLEEKER	Considerations on the EU definition of a nanomaterial: Science to support policy making.	Paper of Scientific journal[11]
MOTZKUS	Size characterization of airborne SiO2 nanoparticles with on-line and off-line measurement techniques: an interlaboratory comparison study	Paper in JNR - Springer (source: VAMAS : J.M. Aublant)
DENG	Study on RAFT Polymerization and Nano-structured Hybrid System of POSS macromers	PhD Thesis[13]
UKNSPG	Working Safely with Nanomaterials in Research & Development	NanoSafety Cluster document[14]
SCOPE OF GUIDANCE	Facilitating the safety evaluation of manufactured nanomaterials by characterising their potential genotoxic hazard	NANOGENOTOX Scientific Report[15].
RIEDEKER	Experimental protocol validation to design a standard using 2'7-dichlorodihydrofluorescin (DCFH) for nanoparticle oxidative reactivity detection	Paper in grey literature (source: M. Riedeker) [16].
KOIVISTO	SOURCE SPECIFIC RISK ASSESSMENT OF INDOOR AEROSOL PARTICLES	PhD Thesis - KOIVISTO
LYNCH	Labelling nanoparticles with non-radioactive isotopes	Paper of scientific journal - Lynch
NANODEVICE	Hydrochemical reactivity and biodurability of nanoparticles used for toxicity studies in NANODEVICE	Project report-NANODEVICE
NANOGENOTOX	Hydrochemical reactivity, solubility, and biodurability of NANOGENOTOX nanomaterials	Project Report-NANOGENOTOX
HINAMOX	Data on metal leaching and dissolution rates of NPs under different physiological conditions	Project Report-HINAMOX
ENPRA	Physical-chemical Data for QSAR-like modelling -In vitro sedimentation rates, dissolution and hydrochemical reactivity of the ENPRA nanomaterials	Project report- ENPRA

1) Semantic distance Texts vs. TCs or WGs

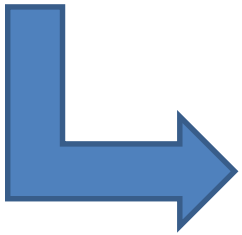
	TC24-SC4	TC201	TC202	TC209	TC229-WG1	TC229-WG2	TC229-WG3	TC229-WG4	
Acosta	0,76	0,73	0,85	0,94	0,88	0,91	0,82	0,82	New Topic
Bleeker	0,56	0,75	0,75	0,81	0,63	0,69	0,31	0,75	Close
Motzkus	0,52	0,65	0,70	0,78	0,96	0,65	0,74	0,78	Quite new
Deng	0,82	0,76	0,88	0,91	0,85	0,82	0,76	0,85	New topic
UKNSPG	0,83	0,78	0,91	0,83	0,87	0,87	0,22	0,87	Close
NanoGenotox	0,65	0,70	0,70	0,75	0,95	0,80	0,60	0,75	Quite new
Riedeker	0,59	0,53	0,71	0,71	0,88	0,71	0,53	0,71	Quite new
Lynch	0,80	0,60	0,85	0,95	0,95	0,75	0,75	0,85	Quite new
Koivisto	0,68	0,74	0,84	0,79	0,95	0,89	0,42	0,89	Close
NANODEVICE	0,67	0,57	0,71	0,81	0,81	0,62	0,62	0,76	Quite new
Nanogenotox	0,76	0,76	0,94	0,82	0,94	0,82	0,76	0,82	New topic
HINAMOX	0,47	0,53	0,71	0,65	0,82	0,71	0,53	0,65	Quite new
ENPRA	0,58	0,63	0,74	0,68	0,84	0,79	0,58	0,68	Quite new

2) Expert review

Fits best to

- CEN/TC 352
- ISO/TC 229

„Nanotechnologies“



CEN/TC 352 contacted

3) Process with CEN/TC 352

July 2013 — First **working draft** (DIN + proposer)
Form N „**New Work Item Proposal**“

Oct. 2013 — Presentation to CEN/TC 352 Strategy Group

End 2013-Aug. 2014 — Ballot on the topic (NSBs) ➔ **Yes** ➔ Pre-Work Item

nanoSTAIR‘s call for expert:
- 9 volunteering experts
- 6 countries > 5 requested

Oct. 2014 - Begin 2015 — Ballot (NSBs) for creation of a **Project Group**

2016 (?) — **TS** or **EN** (?)

1) Method

1) Introduction – Info on standardization
Opening and presentation of NanoValid (Project Coordinator)
Standardization process (CEN)
Expectations from Standardization committees (CEN/TC 352)

2) Physico-chemical characterization

3) Hazard identification, risk assessment and LCA

Existing Standards (DIN)
Methods developed in project (WP leader)
Selection of methods (WP leader)

4) Conclusion (Project Coordinator)

2) Outcome

SOP	Partner	Standardization work	Committee	NSB	Remaining Technical Preparation	Remaining Organizational Preparation
SOP1	XXX	…	…	…	~ 10 items !	…
SOP2	YYY	…	…	…		…
SOP3	ZZZ	…	…	…	…	…

Standardization work: New Work Item Proposal (NWIP), revision, participate in ongoing project

NSB: National Standardization Body

NanoValid

Addition: action plan

nanoSTAIR

DIN

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II) Technical: **Pre-normative Work within projects**

- **Harmonization** of protocols
- **Inter-Lab training**
- **Inter Laboratory Comparison** (ILC) or **Round Robin** (RR)
  Also with external labs (→ subcontracting ?)

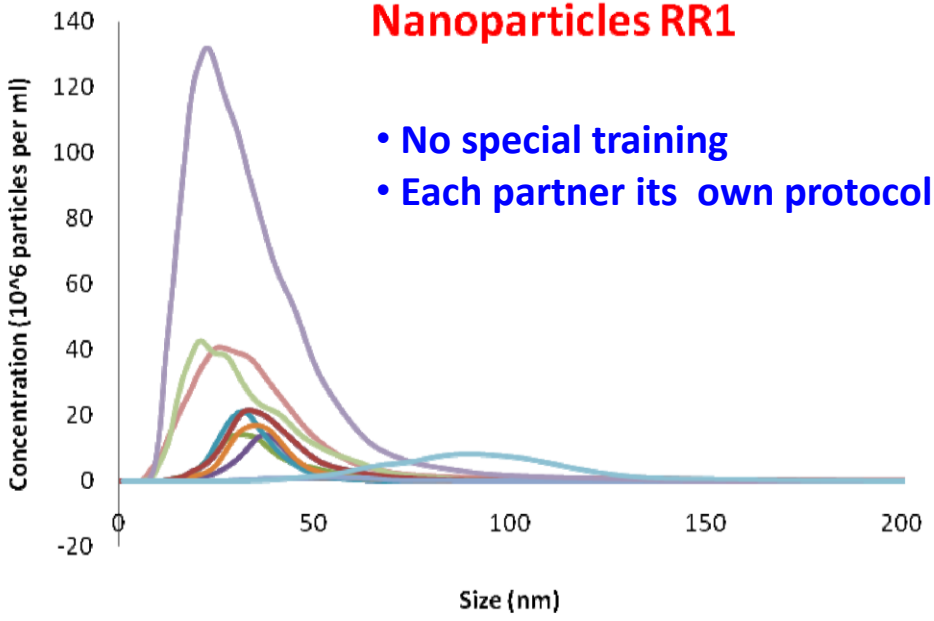
- **Working draft** - also with external experts

+ **Publish** : scientific publication, white paper (with authors!)

1) First 3 rounds

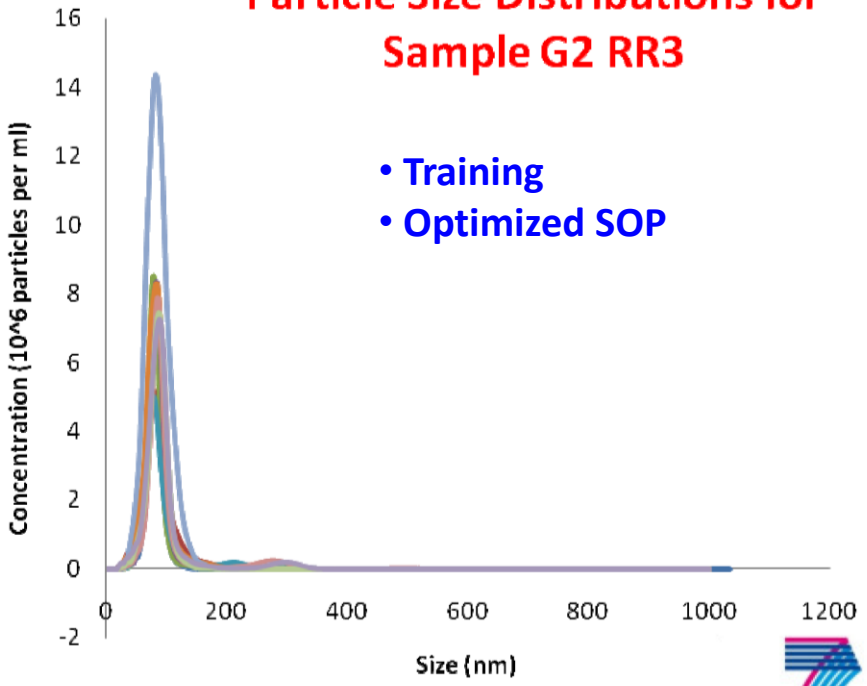
10 partners

Partical Size Distributions for Gold Nanoparticles RR1



- No special training
- Each partner its own protocol

Particle Size Distributions for Sample G2 RR3



- Training
- Optimized SOP

Source: NanoSight (Dr. P. Hole)

Hole et al, (2013), Interlaboratory comparison of size measurements on nanoparticles using Nanoparticle Tracking Analysis (NTA), Journal of Nanoparticle Research"

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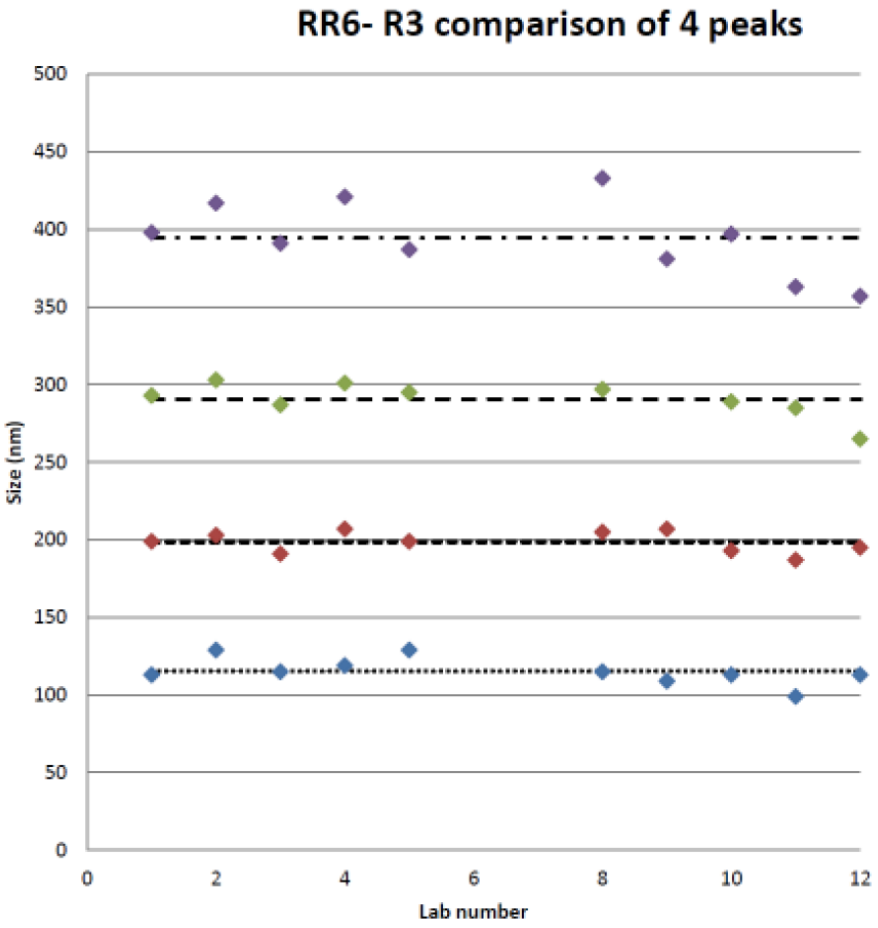
2) Further rounds

NTA RR 4-6: increasing complexity



RR6	R3			
Lab	Peak 1 (nm)	Peak 2 (nm)	Peak 3 (nm)	Peak 4 (nm)
1	113	199	293	398
2	129	203	303	417
3	115	191	287	391
4	119	207	301	421
5	129	199	295	387
8	115	205	297	433
9	109	207		381
10	113	193	289	397
11	99	187	285	363
12	113	195	265	357
Mean	115.4	198.6	290.6	394.5
Stdev	8.88	6.98	11.35	24.40
%cv	7.70	3.51	3.91	6.19

Source: NanoSight (Dr. P. Hole)

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Summary: Actions for Researchers

0) Always: Be prepared in your team / your project

- Standardization **seminar** : NSB / nanoSTAIR

1) Planning/ proposal stage of a research

- Check potential and way: NSB / nanoSTAIR check
- Plan **harmonization** / **Interlab. comparison**
- Plan standard **drafting group**

} Internal + External partners

+ Include your "Standardization records" in proposal

2) Running phase of a research

- Project-specific **seminar** : NSB / nanoSTAIR
- Check potential and way: nanoSTAIR check

} Action plan

- Prepare **working draft**, **submit topic** & prepare **NSB vote**,...

+ Publish!

3) After project: Finalization only !

Summary: Actions for Funding agencies

- **Post-project funding** for standardization
- **Support the support** to standardization (like ESS)
- Integrate standardization in the **calls** and in the **evaluation**

  ➔ Criteria:

  – Candidates' references in standardization activities (require a list!)
  – Standardization workshop early in project
  – Harmonization of protocols and inter-lab training
  – ILC or RR (→ budget for external labs)
  – NWIPs
  – Draft standard (submitted) = deliverables
- Require **open access** to main protocols

Conclusion

Preparation in projects

- Organizational:
  NSBs & nanoSTAIR
- Technical:
  Pre-normative work

Required and supported
by funding agencies

(Process complicated
not flexible /fast enough)

~~No resources in project / organisation~~

(**No funding after /out of project**!)

~~Lack of awareness~~
~~Low own impact on standard~~
~~Ignorance of each other's activities~~

~~**Low recognition** (no author list) #ISI~~

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DLO/RIKILT | WU | DGUV | TAU | VITO | SMU | TCD | FIOH | UOE | HW | CNRS | INERIS | UBRM | RIVM

EU-VRi - European Virtual Institute for Integrated Risk Management

European Economic Interest Grouping (EEIG)

5 Founding Members

- Bay Zoltán Foundation
- INERIS
- Steinbeis GmbH & Co. KG für Technologietransfer
- Technologica Group c.v.
- Univ. of Stuttgart

~ 50 associated members:

Industry, R&D & Education, GO/NGOs.

Industrial safety & Emerging risks

- Creation of consortia, project management
- Dissemination, exploitation, standardisation

Connection / Knowledge Transfer :

- Industry
- R&D
- Authorities
- Society

Round-robin *versus* interlaboratory comparison studies

Round-robin (RR):

- Small group (3-4) of expert labs
- Optimize and verify existing SOPs

Aim: develop best-practice SOPs and make available for ILCs and nanosafety research community

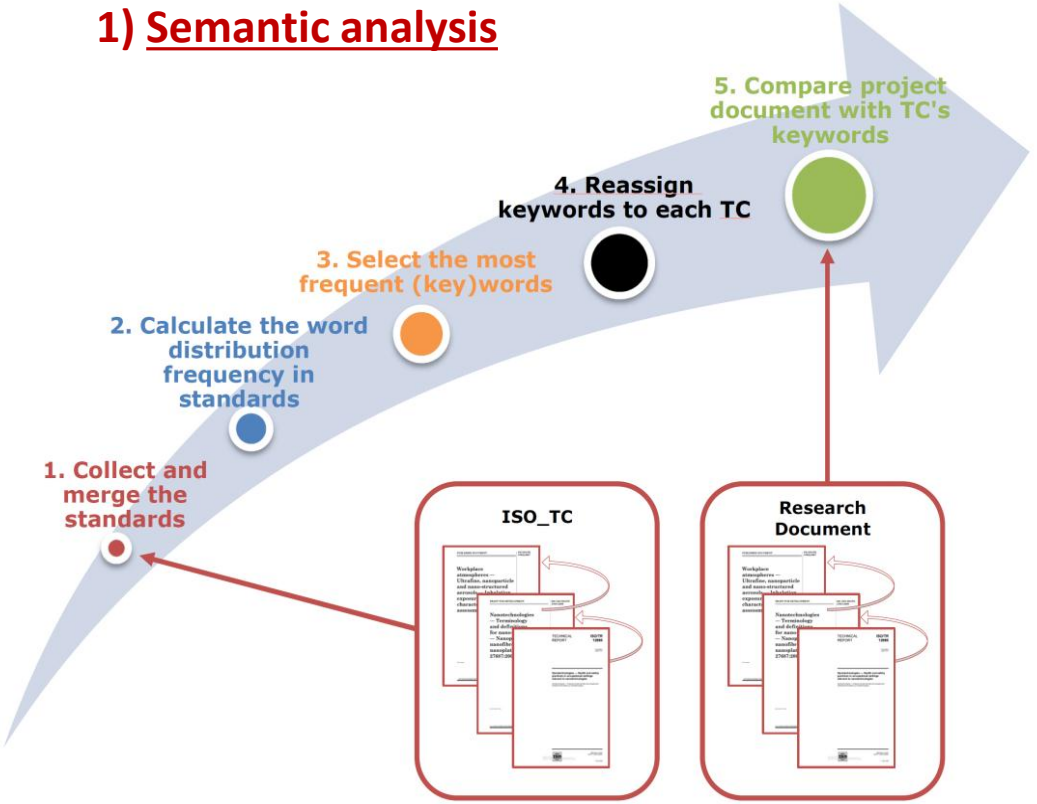
Interlaboratory comparison study (ILC):

- Multiple (> 10) labs
- Use QualityNano SOPs generated by RRs

Aim: 1) evaluate performance of partner and associated labs, and identify training needs; 2) test QualityNano positive and negative control nanomaterials

The nanoSTAIR check : how?

1) Semantic analysis



Distance of document from TCs

2) Expert review

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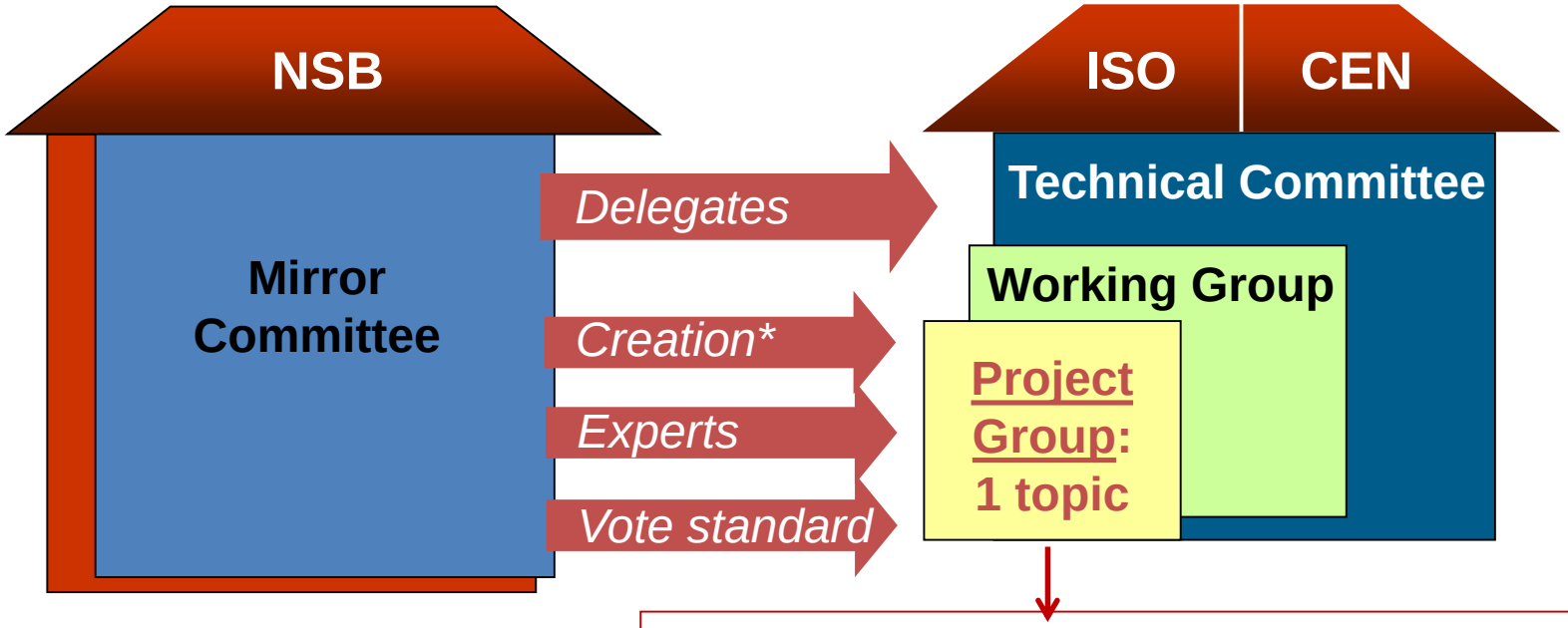
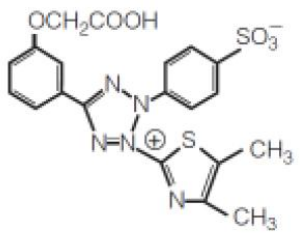
Information: how it works



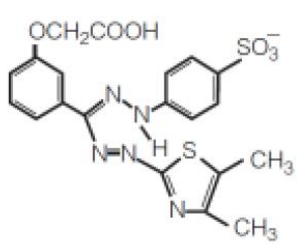
- Up to 3 years drafting, + adoption (votes, comments?)
- Meetings: very variable depending on the PG
  0 - >2 face-to-face, 0 - >2 teleconf.
- Electronic exchanges on documents
- 1 or 2 participants "hold the pen"
- Others comment, complete, amend (also essential !)
- + for PG chairman: organization, liaison with WG

* 2 x TC + NSB vote !

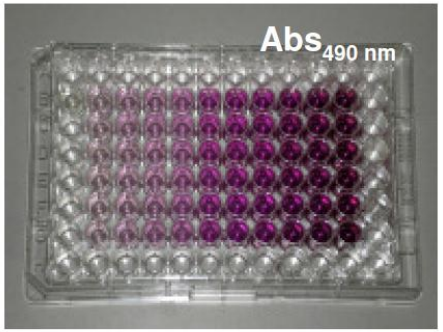
Ex: Q-Nano: Citotoxicity with MTS 1/3



MTS



Formazan



MTS bioreduction: quantity of formazan product is proportional to number of living cells

-cells (A549), serum, NP distributed from central stock
-SOPs (MTS assay) distributed

Aim:
- assessment of cytotoxicity of a chemical positive control (staurosporine)
- assessment of cytotoxicity of positive and negative control NP (amine-modified PS and carboxyl-modified PS, resp.)

Ex: Q-Nano: Citotoxicity with MTS 2/3

Compliance with acceptance criteria of test method:

# laboratories	STS	NH_2-PS NMs	COOH-PS NMs
≥ Three accepted & independent runs	8/14	5/14	6/14

Robust statistics according to ISO 13258:2005(E):

- laboratory bias >2 SD: 'warning signal'
- laboratory bias >3 SD: 'action signal'

One 'action signal' in one round, or two 'warning signals' in several rounds requires investigation.

Corrective actions:
-SOP refinement and repeat of the tests to achieve reproducibility within and across labs
-hands-on training

Source: VITO (Dr. I. Nelissen)

Ex: Q-Nano: Citotoxicity with MTS 3/3

Technical difficulties encountered in many labs => Questionnaire sent to all laboratories

→ Critical phases in SOP identified

→ SOP adjusted

- Hands-on-training planned for all participating labs

Protocol step	Critical phase
• All steps	• Verification of pipets and instruments
• Preparation and storage of staurosporine (STS) stock solution	• Pipetting • Dissolution of lyophilized product • Storage of stock and working solutions
• Preparation of STS working solution	• low pipetting volume
• Preparation of NM dilutions in culture medium	• Storage of stock solutions • Low pipetting volume • Dispersion protocol
• Preparation of dosing plate	• Different pipetting volumes • Pipetting technique
• Plating cells	• Cell counting method • Equal suspension for replicate cultures • Edge effects
• Exposure to test item	• Removal of medium from cultures • Equal suspension of test items • Application of test solutions onto cultures • Incubation time of cell cultures with NPs
• MTS assay	• Removal of medium from cultures • Interference of NMs with read-out • Air bubbles • Precipitate of MTS reagent • Spectrophotometer type