The challenge of validating methods for nanoparticle analysis

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Definition of "Validation"

- *"Process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires"* *(EURACHEM Guide 1998)*
- Or: Demonstration of fitness for purpose

Analysis of nanoparticles

Measurand definition

Dynamic processes

Lack of routine methods

Particle Size Distributions

Challenges

Method suitability

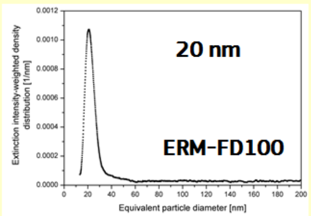
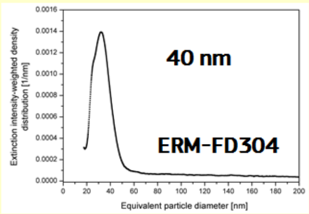
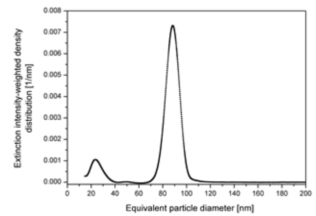
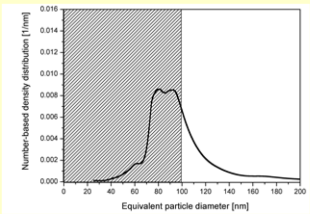
Working ranges

Sample preparation

Constituent particles/ Agglomerates/Aggregates

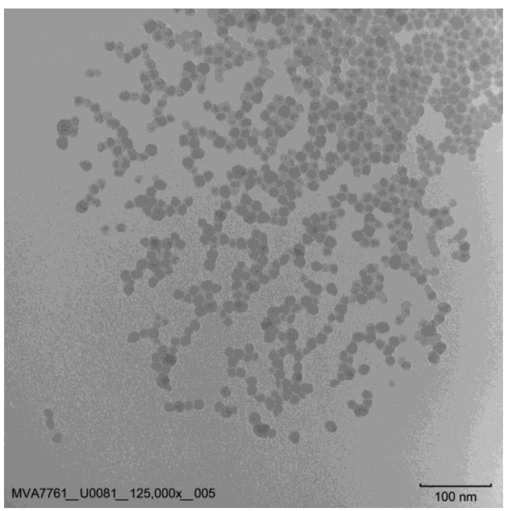
Test materials: ideal vs. real world

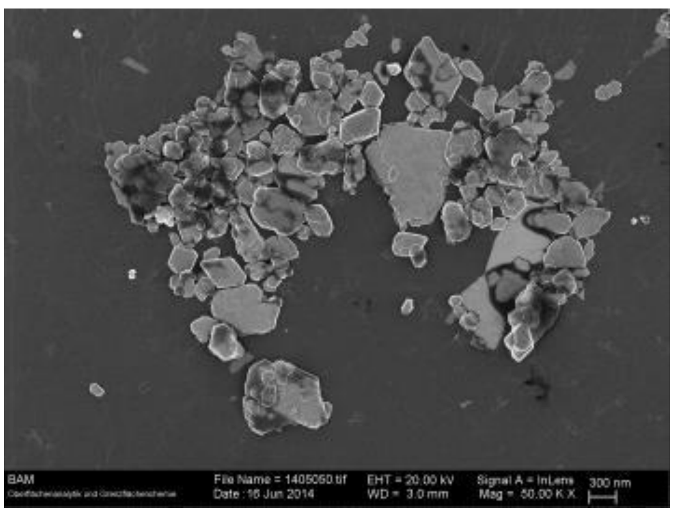
Certified reference materials for implementation of definition

Silica nanoparticles with monomodal particle size distributions	Silica nanoparticles with bimodal particle size distribution	Polydisperse particle size distribution *(most industrial materials)*
 	 20 nm and 80 nm ERM-FD102	

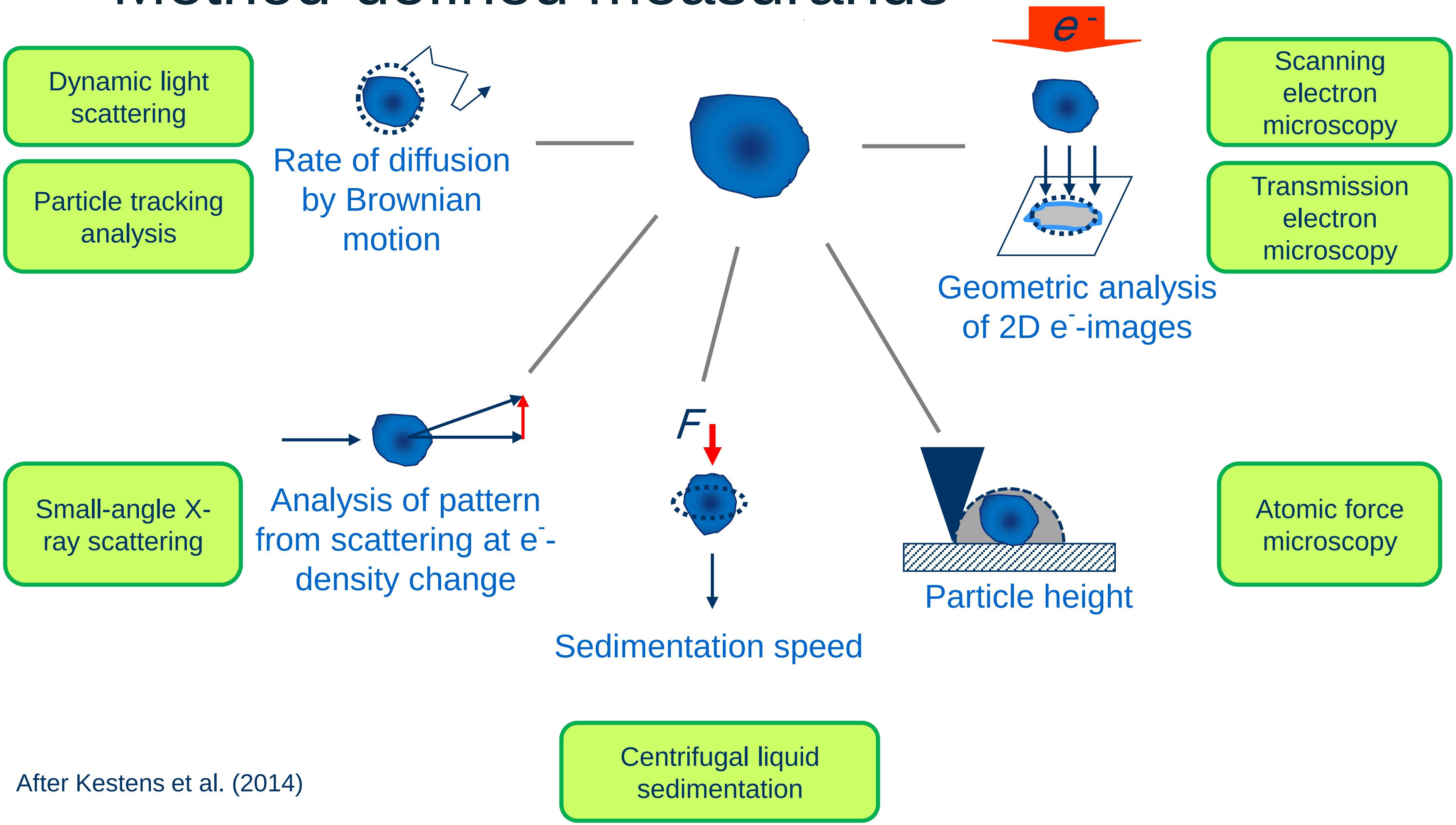
2011 | 2012 | 2014 | Future needs

Increasing complexity





Method-defined measurands

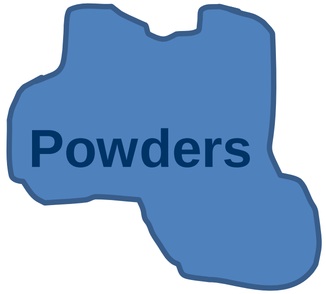


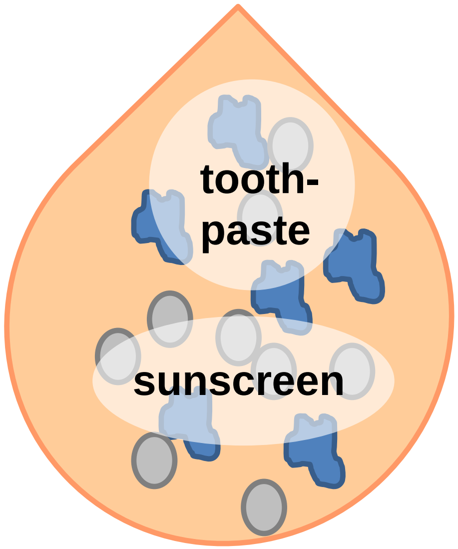
After Kestens et al. (2014)

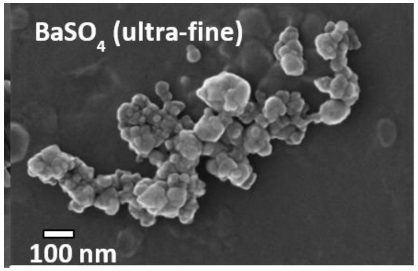
The NanoDefine validation concept

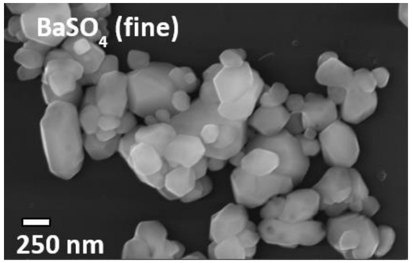
- Strongly harmonised
- Common test samples (reference materials)
- Common protocols (SOPs)
- Common in-house validation approach
- Co-ordination and support
- Collaboration with standardisation organisations
- International interlaboratory validation studies

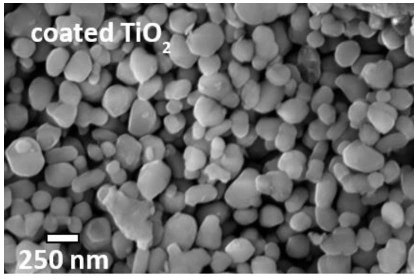
Test samples for validations

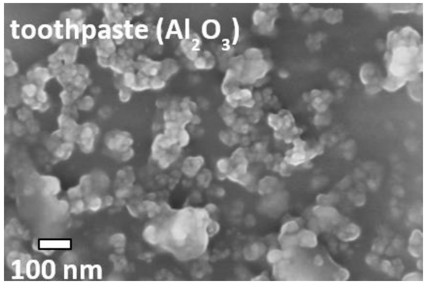


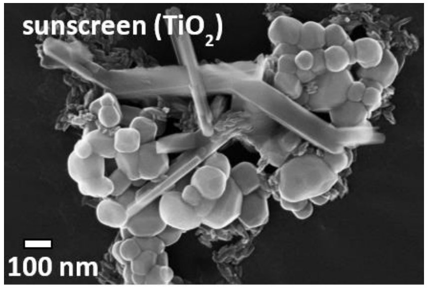












Challenges for method validation

- Often little experience available (for NMs)
- Well developed, optimized methods (SOPs)
- Only "first attempt" possible
- Very limited time
- External laboratories for ILCs needed
- Sufficient data sets for ILCs

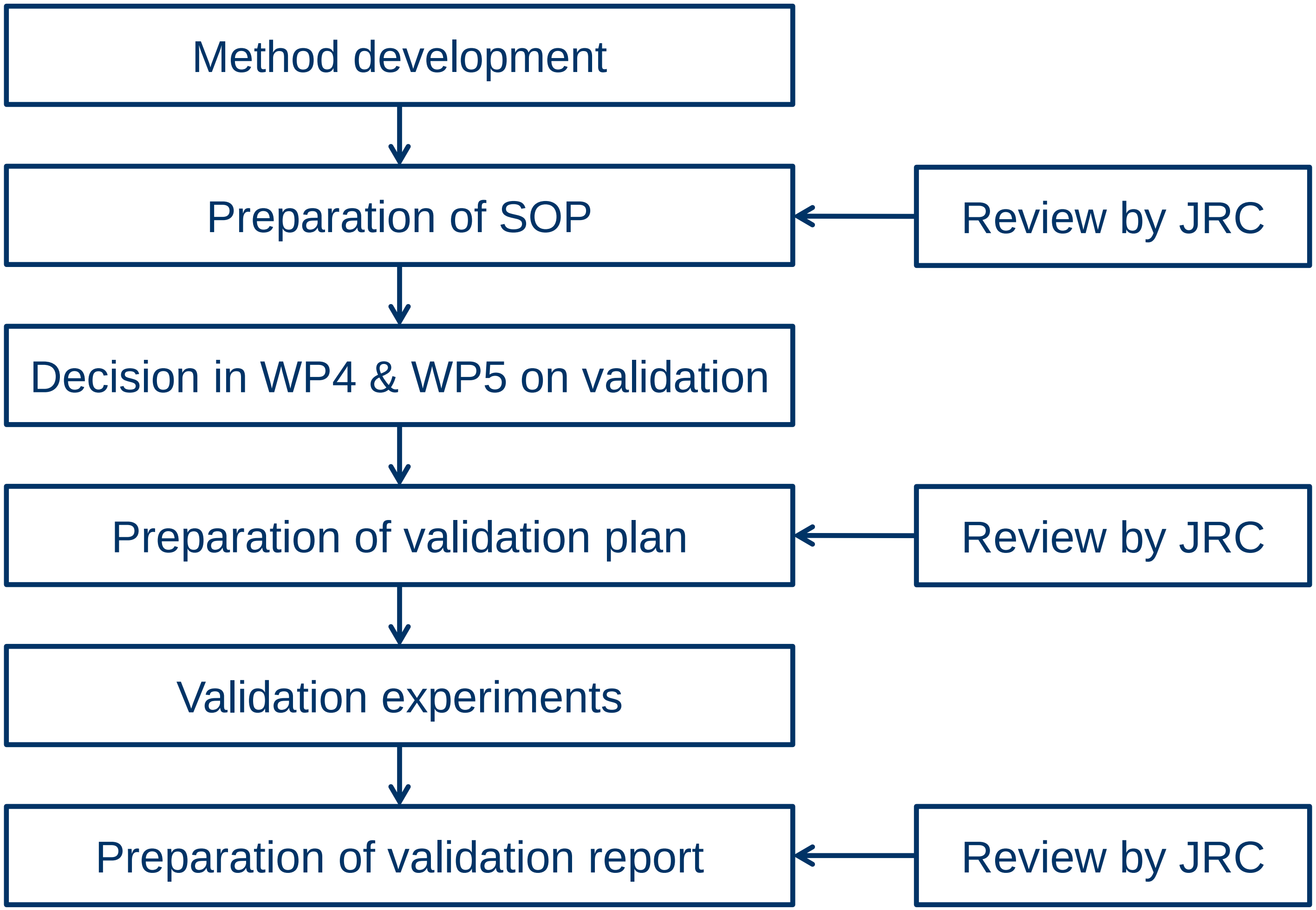
In-house validation approach

- Definition of a "method"
  - Based on International Vocabulary of Metrology (VIM) definition of a "measurement procedure" ("test method", "SOP")
  - a combination of
    - a sample preparation protocol
    - the measurement technique (e.g. TEM)
    - a specific type of sample/nanomaterial(s).
  - Clear definition of the measurand (in the SOP)
  - Having the *EC Definition of a NM* in mind

In-house validation approach

- Harmonized approach
  - Common validation guideline
  - Based on international guidelines
  - Co-ordinated approach
    - Validation parameters
    - Common templates
    - Based on detailed SOPs
    - Using reference materials

In-house validation approach



Method selection for validations

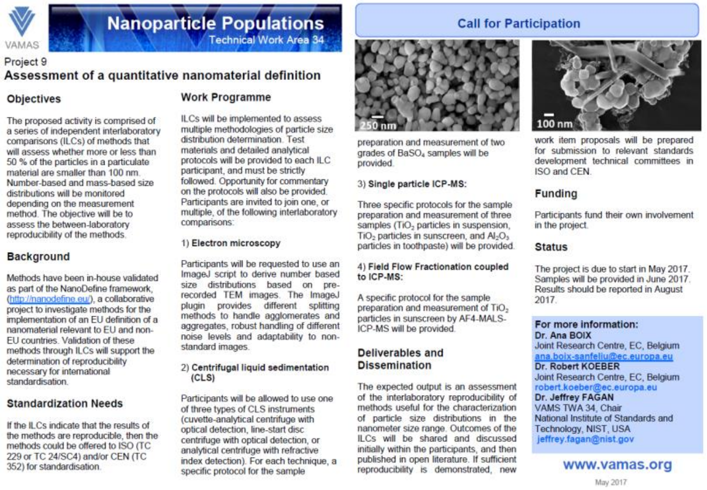
- Out of the in-house validated methods, 4 needed to be selected for interlaboratory validation
- Selection of
  - spICP-MS (TiO_2, TiO_2 in sunscreen, Al_2O_3 in toothpaste)
  - ParticleSizer (EM image analysis; SiO_2, TiO_2, gold, CeO)
  - Centrifugational methods (Disc-CLS, Cuvette CLS, AUC; $BaSO_4$)
  - AF4-MALS-ICP-MS (TiO_2 in sunscreen)

In-house validation results

Measurement technique	Measurand	Test samples	Working range	LOD/LOQ	Repeatability	day–to-day precision	Trueness (u_t or R)	Standard measurement uncertainty(u_x)
spICP-MS	Particle size [nm]	TiO_2 in suspension	25 nm – 500 nm	25 nm/30 nm	3.2 %	7.9 %	n.a.	**9.0 %**
spICP-MS	Number concentration [1/mg]	TiO_2 in suspension	> 250 pg L^{-1}	250/750 pg L^{-1}	23 %	24 %	n.a.	**36 %**
spICP-MS	Mass concentration [%]	TiO_2 in suspension	> 250 pg L^{-1}	250/750 pg L^{-1}	20 %	21 %	83 %	**32 %**
spICP-MS	Particle size [nm]	TiO_2 in sunscreen	20 nm – 500 nm	20 nm/23 nm	2.4 %	4.0 %	n.a.	**5.0 %**
spICP-MS	Number concentration [1/mg]	TiO_2 in sunscreen	> 12 mg/kg	12/30 mg kg^{-1}	8.0 %	16.8 %	n.a.	**20 %**
spICP-MS	Mass concentration [%]	TiO_2 in sunscreen	> 12 mg/kg	12/30 mg kg^{-1}	19.9 %	21.3 %	83 %	**34 %**
spICP-MS	Particle size [nm]	Al_2O_3 in toothpaste	48 nm – 500 nm	48/70 nm	5.3 %	15 %	n.a.	**17 %**
spICP-MS	Number concentration [1/mg]	Al_2O_3 in toothpaste	> 61 mg/kg	61/180 mg kg^{-1}	5.8 %	24 %	n.a.	**25 %**
spICP-MS	Mass concentration [%]	Al_2O_3 in toothpaste	> 61 mg/kg	61/180 mg kg^{-1}	2.3 %	29 %	64 - 81 %	**33 %**
ParticleSizer	Median $Feret_{min}$ diameter	SiO_2 (default mode)	0.16 – 477.7 nm pixel size	0.16/1.7 – 1.6/11.7 nm ps	1.2 – 2.0 %	1.3 – 3.0 %	3.0 – 4.1 %	**4.3 – 5.1 %**
ParticleSizer	Median $Feret_{min}$ diameter	Gold nanorods (irr. watershed mode)	0.16 – 477.7 nm pixel size	0.16/1.7 – 1.6/11.7 nm ps	3.1 – 6.9 %	3.4 – 7.4 %	4.5 – 8.0 %	**5.7 – 10.9 %**
ParticleSizer	Median $Feret_{min}$ diameter	TiO_2 (ellipse fitting mode)	0.16 – 477.7 nm pixel size	0.16/1.7 – 1.6/11.7 nm ps	1.9 – 2.6 %	2.1 – 2.6 %	3.6 – 4.0 %	**4.3 – 4.8 %**
ParticleSizer	Median $Feret_{min}$ diameter	TiO_2, CeO (single particle mode)	0.16 – 477.7 nm pixel size	0.16/1.7 – 1.6/11.7 nm ps	2.0 – 8.5 %	2.5 – 9.0	3.7 – 9.5 %	**8.5 – 26.2 %**
CLS (Disc)	Number-based median diameter	BaSO4 (nano & non-nano)	0.2 – 2.6 mg/mL	30 nm	9.6 – 10.7 %	3.4 %	n.a.	**10.3 – 10.9 %**
CLS (Disc)	Mass-based median diameter	BaSO4 (nano & non-nano)	0.2 – 2.6 mg/mL	30 nm	3.2 – 4.3 %	1.0 %	n.a.	**3.3 – 4.6 %**
CLS (Cuvette)	Number-based median diameter	BaSO4 (nano & non-nano)	0.6 g/kg – 10 g/kg	0.6 g/kg	6.3 – 7.3 %	2.8 – 5.9 %	n.a.	**9.4 – 9.7 %**
CLS (Cuvette)	Mass-based median diameter	BaSO4 (nano & non-nano)	0.6 g/kg – 10 g/kg	0.6 g/kg	3.3 – 7.2 %	2.8 – 2.9 %	n.a.	**4.7 – 7.7 %**
CLS (AUC)	Number-based median diameter	BaSO4 (nano & non-nano)	2 – 2173 nm	0.05 mg/mL	4.1 – 6.0 %	2.1 – 4.1 %	n.a.	**4.6 – 7.2 %**
CLS (AUC)	Mass-based median diameter	BaSO4 (nano & non-nano)	2 – 2173 nm	0.01 mg/mL	3.6 – 4.3 %	0.2 – 1.9 %	n.a.	**4.1 – 4.6 %**
AF4-MALS-ICP-MS	Hydrodynamic radius (MALS)	TiO_2 in sunscreen	20 – 269 nm	9 nm/ 27 nm 2.1 µg/6.4 µg L^{-1}	2.7 %	2.8 %	12.0 %	**12.6 %**
AF4-MALS-ICP-MS	Hydrodynamic radius (ICP-MS)	TiO_2 in sunscreen	20 – 269 nm	9 nm/ 27 nm 2.1 µg/6.4 µg L^{-1}	3.2 – 3.3 %	3.1 – 4.4 %	8.0– 20.0%	**9.1 – 20.8 %**
AF4-MALS-ICP-MS	Mass-based PSD	TiO_2 in sunscreen	20 – 269 nm	9 nm/ 27 nm 2.1 µg/6.4 µg L^{-1}	3.8 – 5.5 %	3.4 – 7.1 %	5.0 – 52 %	**10.1 – 52.2 %**

Interlaboratory validation: preparation

- Involvement of the *Versailles Project on Advanced Materials and Standards* (VAMAS)
- Establishment of a project in TWA 34
- Publishing international call
- Managing registrations
- Sending test samples
- Clarify questions from labs
- Collect data sets
- Evaluate data
- Reporting



VAMAS

Nanoparticle Populations
Technical Work Area 34

Project 9
Assessment of a quantitative nanomaterial definition

Objectives

The proposed activity is comprised of a series of independent interlaboratory comparisons (ILCs) of methods that will assess whether more or less than 50 % of the particles in a particulate material are smaller than 100 nm. Number-based and mass-based size distributions will be monitored depending on the measurement method. The objective will be to assess the between-laboratory reproducibility of the methods.

Background

Methods have been in-house validated as part of the NanoDefine framework, (http://nanodefine.eu/), a collaborative project to investigate methods for the implementation of an EU definition of a nanomaterial relevant to EU and non-EU countries. Validation of these methods through ILCs will support the determination of reproducibility necessary for international standardisation.

Standardization Needs

If the ILCs indicate that the results of the methods are reproducible, then the methods could be offered to ISO (TC 229 or TC 24/SC4) and/or CEN (TC 352) for standardisation.

Work Programme

ILCs will be implemented to assess multiple methodologies of particle size distribution determination. Test materials and detailed analytical protocols will be provided to each ILC participant, and must be strictly followed. Opportunity for commentary on the protocols will also be provided. Participants are invited to join one, or multiple, of the following interlaboratory comparisons:

1) **Electron microscopy**

Participants will be requested to use an ImageJ script to derive number based size distributions based on pre-recorded TEM images. The ImageJ plugin provides different splitting methods to handle agglomerates and aggregates, robust handling of different noise levels and adaptability to non-standard images.

2) **Centrifugal liquid sedimentation (CLS)**

Participants will be allowed to use one of three types of CLS instruments (cuvette-analytical centrifuge with optical detection, line-start disc centrifuge with optical detection, or analytical centrifuge with refractive index detection). For each technique, a specific protocol for the sample preparation and measurement of two grades of $BaSO_4$ samples will be provided.

3) **Single particle ICP-MS:**

Three specific protocols for the sample preparation and measurement of three samples (TiO_2 particles in suspension, TiO_2 particles in sunscreen, and Al_2O_3 particles in toothpaste) will be provided.

4) **Field Flow Fractionation coupled to ICP-MS:**

A specific protocol for the sample preparation and measurement of TiO_2 particles in sunscreen by AF4-MALS-ICP-MS will be provided.

Deliverables and Dissemination

The expected output is an assessment of the interlaboratory reproducibility of methods useful for the characterization of particle size distributions in the nanometer size range. Outcomes of the ILCs will be shared and discussed initially within the participants, and then published in open literature. If sufficient reproducibility is demonstrated, new work item proposals will be prepared for submission to relevant standards development technical committees in ISO and CEN.

Call for Participation

250 nm

100 nm

Funding

Participants fund their own involvement in the project.

Status

The project is due to start in May 2017. Samples will be provided in June 2017. Results should be reported in August 2017.

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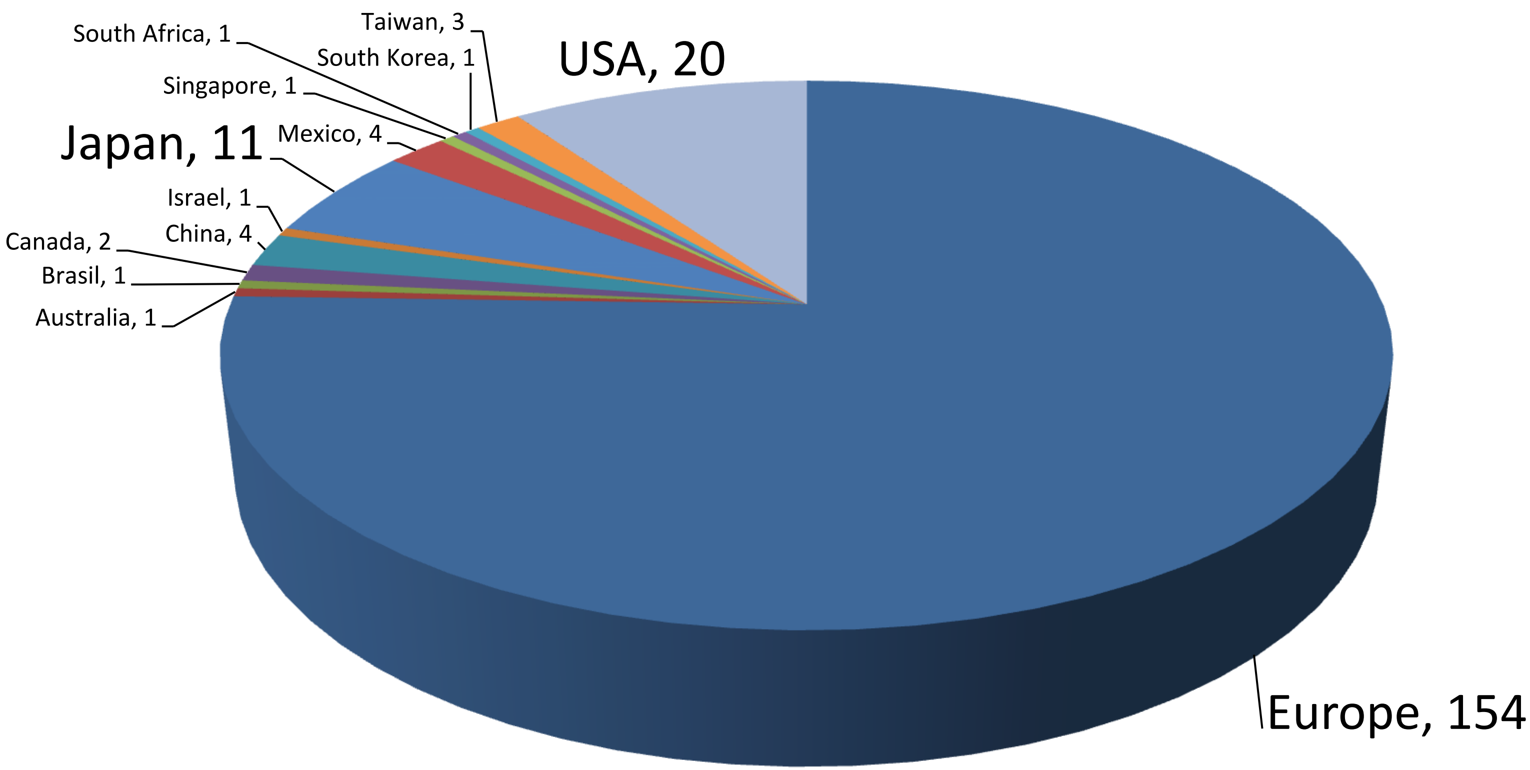
May 2017

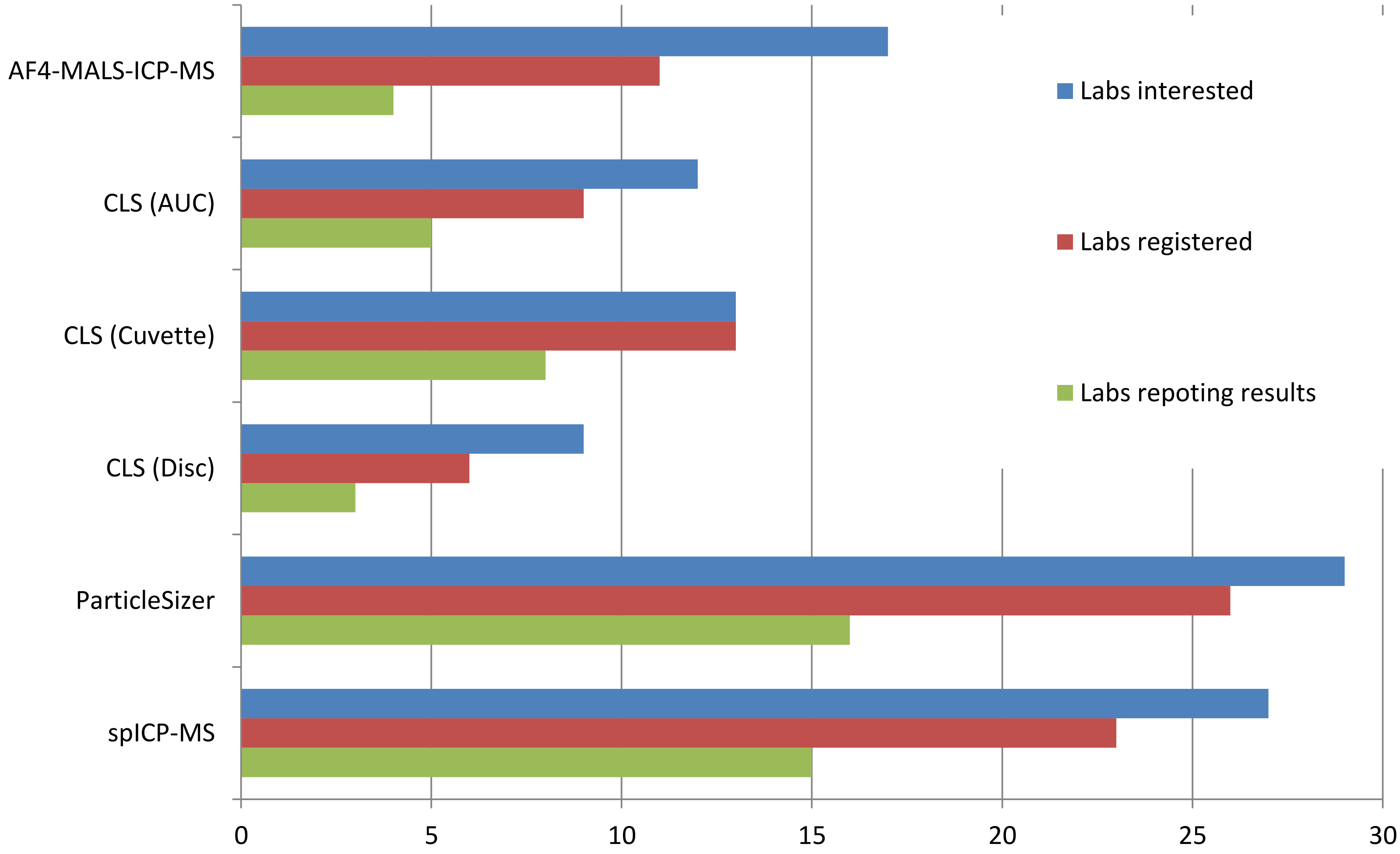
Interlaboratory validation: participation

- > 200 laboratories invited

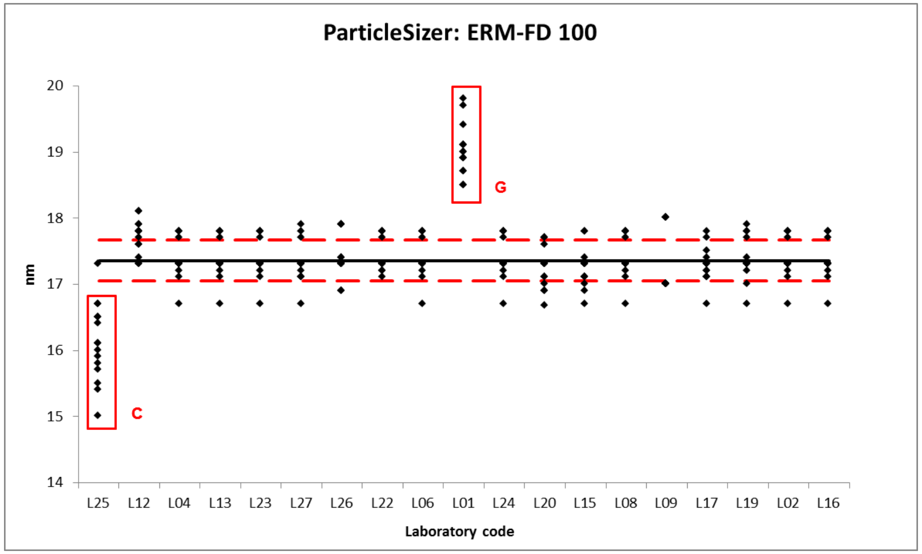


Interlaboratory validation: participation

NanoDefine



Interlaboratory validation: examples



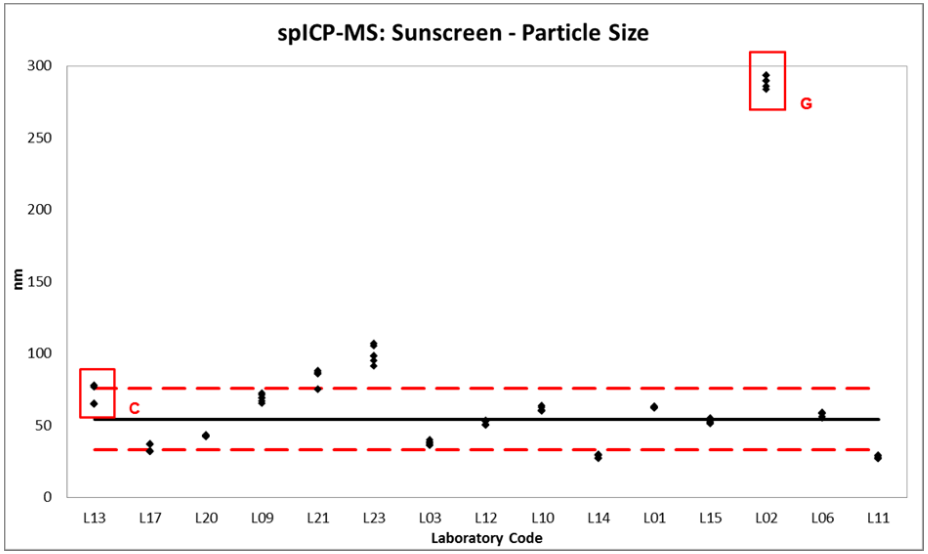
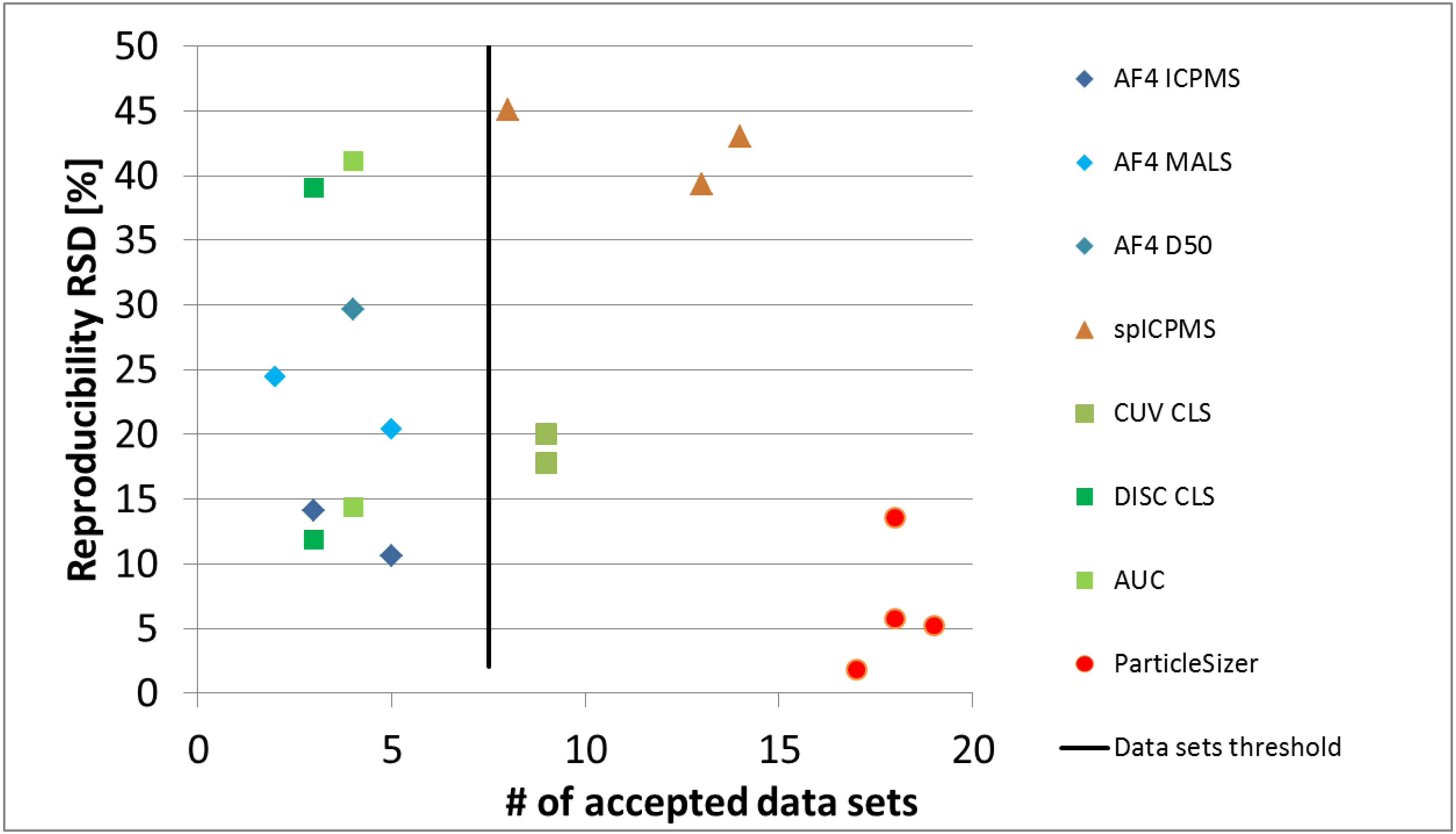


Figure 1: Performance of ParticleSizer for ERM-FD 100. All replicates per laboratory are presented. The solid line refers to overall mean after outlier exclusion (C: Cochran, G: Grubbs outlier tests). Dashed lines refer to the observed acceptance range for the overall mean (Xobs ± reproducibility standard deviation, SR).

Figure 1: Performance spICP-MS for BAM-13 (TiO_2 in sunscreen). All replicates for particle size per laboratory are presented. The solid line refers to overall mean after outlier exclusion (C: Cochran, G: Grubbs outlier tests). Dashed lines refer to the observed acceptance range for the overall mean (X_{obs} ± reproducibility standard deviation, S_R).

Interlaboratory validation: overview



Conclusions

- Test methods (for NMs) must be specific
- Sample preparation is crucial
- Harmonised validation conditions are essential
- In-house validations were successful
- With international call via VAMAS: study launched and interlaboratory validation carried out
- Reproducibility data obtained for 8 methods
- Cuvette CLS and ParticleSizer performed well
- Follow-up of FFF and spICPMS methods desirable