Proficiency test ACEnano for gold nanoparticles in water

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Distribution list:
- 26 participating laboratories of which 21 European participants, 3 Asian participants and 2 North-American participants

Contents

Summary

In May 2018 a proficiency test (PT) for gold nanoparticles in water was organized by RIKILT Wageningen University & Research. This PT enabled laboratories to evaluate their competence for the analysis of gold nanoparticles in water.

For this proficiency test, one material was prepared by diluting a NanoComposix citrate stabilised gold nanoparticle suspension. During homogeneity testing, the material proved to be sufficiently homogenous for proficiency testing. Stability tests demonstrated that a consequential decrease of the particle size occurred during the timescale of the proficiency test. This decrease was accounted for in the calculations of the z-scores.
Twenty-six laboratories subscribed for participation in this test and all reported results and 18 showed optimal performance by reporting satisfactory z-scores for both particle diameter and particle number concentration. An overview of each participant's performance is shown in Annex 7. Ten questionable/unsatisfactory z-scores were reported, three for the diameter, seven for the particle number.

1 Introduction

Proficiency testing is conducted to provide laboratories with a powerful tool to evaluate and demonstrate the reliability of the data that are produced. Next to validation and accreditation, proficiency testing is an important requirement of the EU Additional Measures Directive 93/99/EEC [1] and is required by ISO 17025:2005 [2].

The aim of this proficiency test was to give laboratories the possibility to evaluate or demonstrate their competence for the analysis of nanoparticles in an aqueous suspension using single particle inductively coupled plasma mass spectrometry (spICP-MS).

2 Material and methods

2.1 Sample preparation

Samples were produced from a NanoComposix citrate stabilised gold nanoparticle suspension. The original material was diluted in the same citrate matrix and bottled in vials.

2.2 Sample identification

The vials for the participants were randomly selected and coded through a website application designed for proficiency tests. The codes of the sample for each participant are presented in Annex 1. The remaining vials were used for homogeneity and stability testing and were stored in the refrigerator.

2.3 Participants

Twenty-six laboratories subscribed for participation in the proficiency test of which 21 are situated within Europe, three in Asia and two in North-America. Each participant was asked to report the results through a web application designed for proficiency tests.

2.4 Homogeneity study

The homogeneity of the materials was tested according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [3] and ISO 13528 [4]. For homogeneity a target standard deviation for proficiency assessment (σ_P) of 20% was used as a fit-for-purpose standard deviation for the particle concentration. For the particle diameter a fit-for-purpose σ_P of 10% was used. With this procedure the between-sample standard deviation (s_s) and the within-sample standard deviation (s_w) are compared with the fit-for-purpose standard deviations. The method applied for homogeneity testing is considered suitable if $s_w < 0.5*\sigma_P$ and a material is considered adequately homogeneous if $s_s < 0.3*\sigma_P$.

Ten containers of the aqueous suspension were analysed in duplicate for the concentration of Au in ng/l and the particle size in nm to determine the homogeneity of the material. The results of the homogeneity study and their statistical evaluation are presented in Annex 2. The material was demonstrated to be sufficiently homogeneous for use in the proficiency test.

2.5 Sample distribution and instructions

Each of the participating laboratories received a randomly assigned laboratory code, generated by the website application. The sample with the corresponding number (Annex 1) was sent to the participating laboratories on May 28, 2018. The sample, together with 1 g of citrate buffer, was packed in carton box and dispatched to the participants immediately by courier. The sample was accompanied by a protocol (Annex 3) describing the requested analyses and an acknowledgement of the receipt form. By e-mail the laboratories received instructions on how to use the web application to report results.

The laboratories were asked to store the samples in the refrigerator until analysis. A single analysis of each sample was requested. The deadline for submitting the quantitative results was July 13, 2018, allowing at least six weeks for the quantitative analysis.

2.6 Stability

Six vials were analysed at the beginning of the PT, at June 6, 2018. Six other vials, which were stored in the refrigerator during 36 days, were analysed at the end of the PT, at July 12, 2018.

It was determined whether a 'consequential instability' occurred [3,4]. A consequential instability occurs when the average value of the samples analysed at the end of the PT is more than $0.3\sigma_P$ below the average value of the samples analysed at the beginning of the PT. If so, the instability has a significant influence on the calculated z-scores.

For the particle size, a consequential difference was observed between the samples analysed at the beginning and at the end of the PT. There was a significant decrease of 6%. This decrease is taken into account in the calculation of the z_{ai}-scores.

For the particles per litre (diluted 1.10^6 times), a consequential difference was observed among the samples analysed at the beginning and at the end of the PT. There was a significant increase of 11%. This increase is not incorporated in the calculation of the z_a-scores.

3 Statistical evaluation

The statistical evaluation of the quantitative part of the study was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [3], elaborated by ISO, IUPAC and AOAC and ISO 13528 [4] in combination with the insights published by the Analytical Methods Committee [5,6] regarding robust statistics.

For the evaluation of the quantitative results, the consensus value, the uncertainty of the consensus value, a standard deviation for proficiency assessment and z-scores were calculated.

3.1 Calculation of the consensus value (X)

The consensus value (X) was determined using robust statistics [8,11,12]. The advantage of robust statistics is that all values are taken into account: outlying observations are retained, but given less weight. Furthermore, it is not expected to receive normally distributed data in a proficiency test. When using robust statistics, the data do not have to be normally distributed in contrast to conventional outlier elimination methods.

The robust mean of the reported results of all participants, calculated from an iterative process that starts at the median of the reported results, using a cut-off value depending on the number of results, was used as the consensus value [4,5].

3.2 Calculation of the uncertainty of the consensus value (u)

The uncertainty of the consensus value is calculated to determine the influence of this uncertainty on the evaluation of the laboratories. A high uncertainty of the consensus value will lead to a high uncertainty of the calculated participants z_a-scores. If the uncertainty of the consensus value and thus the uncertainty of the z_a-score is high, the evaluation could indicate unsatisfactory method performance without any cause within the laboratory. In other words, illegitimate conclusions could be drawn regarding the performance of the participating laboratories from the calculated z_a-scores if the uncertainty of the consensus value is not taken into account.

The uncertainty of the consensus value (the robust mean) is calculated from the estimation of the standard deviation of the consensus value and the number of values used for the calculation of the consensus value [4]:

$$u = 1.25 * \frac{\hat{\sigma}}{\sqrt{n}}$$

where:

u = uncertainty of the consensus value;
n = number of values used to calculate the consensus value;
$\hat{\sigma}$ = estimate of the standard deviation of the consensus value resulting from robust statistics.

According to ISO 13528 [4] the uncertainty of the consensus value (u) is negligible and therefore does not have to be included in the statistical evaluation if:

$u \leq 0.3\sigma_P$

where:
u = the uncertainty of the consensus value;
σ_P = standard deviation for proficiency assessment (§3.3).

In case the uncertainty of the consensus value does not comply with this criterion, the uncertainty of the consensus value should be taken into account when evaluating the performance of the participants regarding the accuracy (§3.4). In case the uncertainty is > $0.7\sigma_P$ the calculated z-scores should not be used for evaluation of laboratories' performance and are presented for information only.

3.3 Calculation of the standard deviation for proficiency assessment (σ_P)

A target standard deviation for proficiency test assessment (σ_P) for the particle number concentration was set at 20%. For the particle diameter this was set at 10%.

3.4 Performance characteristics with regard to the accuracy

For illustrating the performance of the participating laboratories with regard to the accuracy, a z_a-score is calculated. For the evaluation of the performance of the laboratories, ISO 13528 [4] is applied. According to these guidelines z_a-scores are classified as presented in Table 1.

Table 1 *Classification of z_a-scores.*

$\|z_a\| \leq 2$	Satisfactory
$2 < \|z_a\| < 3$	Questionable
$\|z_a\| \geq 3$	Unsatisfactory

If the calculated uncertainty of the consensus value complies with the criterion mentioned in §3.2, the uncertainty is negligible. In this case the accuracy z-score is calculated from:

$$z_a = \frac{\overline{x} - X}{\sigma_P} \qquad \text{Equation I}$$

where:
z_a = accuracy z-score;
$\overline{x}$ = the result of the laboratory;
X = consensus value;
σ_P = standard deviation for proficiency assessment.

However, if the uncertainty of the consensus value does not comply with the criterion mentioned in §3.2, it could influence the evaluation of the laboratories. Although, according to ISO 13528 in this case no z-scores can be calculated, we feel that evaluation of the participating laboratories is of main importance justifying the participating laboratories' effort. Therefore in this case, the uncertainty is taken into account by calculating the accuracy z-score [4]:

$$z'_a = \frac{\overline{x} - X}{\sqrt{\sigma_P^2 + u^2}}$$ Equation II

where:
z'_a = accuracy z-score taking into account the uncertainty of the consensus value;
$\overline{x}$ = the result of the laboratory;
X = consensus value;
σ_P = standard deviation for proficiency assessment;
u = uncertainty of the consensus value.

If a consequential instability of the proficiency test materials is observed, this can influence the evaluation of the laboratory performance. Therefore, in that case the consequential instability is taken into account when calculating z-scores. Because instability only regards one side of the confidence interval (a decrease of the concentration) this correction only applies to the lower 2s limit and results in an asymmetrical confidence interval.

In the case of a consequential instability the accuracy z-score for the laboratories that reported an amount below the consensus value is corrected for this instability by:

$$z_{ai} = \frac{\overline{x} - X}{\sqrt{\sigma_P^2 + \Delta^2}}$$ Equation III

where:
z_{ai} = accuracy z-score taking into account the instability of the consensus value;
$\overline{x}$ = the result of the laboratory;
X = consensus value;
σ_P = standard deviation for proficiency assessment;
Δ = difference between parameter of Au particle at the beginning and at the end of the PT.

In some cases the uncertainty of the consensus value does not comply with the criterion in §3.2 and a consequential instability is observed. In this case the z'_a score for the laboratories that reported an amount below the consensus value is corrected for this instability by:

$$z'_{ai} = \frac{\overline{x} - X}{\sqrt{\sigma_P^2 + \Delta^2 + u^2}}$$ Equation IV

where:
z'_{ai} = accuracy z-score taking into account the uncertainty and instability of the consensus value;
$\overline{x}$ = the result of the laboratory;
X = consensus value;
σ_P = standard deviation for proficiency assessment;
Δ = difference between parameter of Au particle at the beginning and at the end of the PT;
u = uncertainty of the consensus value.

For the particle size Equation III was used. For the particle per litre Equation II was used.

4 Results and discussion

Twenty-six laboratories registered for participation and all reported results for the proficiency test for gold nano particles in water. An overview of the applied methods is presented in Annex 5. The z-scores are summarized in Annex 6.

4.1 Particle diameter

All laboratories reported results for the particle diameter (Annex 6).
The lowest value reported is 49.9 nm and the highest value is 80 nm. The consensus value is 61 nm with a robust standard deviation of 4.9 nm. This is lower than the fit-for-purpose value of 10% of the consensus value (=6.1 nm). The uncertainty of the consensus value is 1.2 nm which does not exceed $0.3\sigma_P$ (§3.2). With regard to the accuracy one result was questionable (PT023) and one was unsatisfactory (PT025). If no decrease in particle diameter was observed (§2.6), the results would remain the same: one questionable and one unsatisfactory results.

4.2 Particle number concentration

After reporting the results, seven participants were asked to check their reported concentration one more time, since it appeared that the dilution factor of 1.10^6 was not taken into account or the result was reported per millilitre and not per litre. Two participants forgot the dilution factor, two reported per millilitre and three results remained deviating.

All laboratories reported results for the particle number concentration (Annex 6), but lab PT9993 reported a qualitative result ('detected').
The lowest value reported is $1.73.10^7$ parts/l and the highest value is $4.1.10^{13}$ parts/l. The consensus value is $1.44.10^{13}$ parts/l with a robust standard deviation of $0.6.10^{13}$ parts/l. This is more than 2 times higher than the fit-for-purpose value of 20% of the consensus value (=$0.29.10^{13}$ parts/l). The uncertainty of the consensus value is $0.15.10^{13}$ parts/l which exceeds $0.3\sigma_P$ (§3.2), so the uncertainty is taken into account when calculating z-scores. With regard to the accuracy four results were questionable (PT025, PT9994, PT9995 and PT9996) and three were unsatisfactory (PT023, PT030 and PT9955).

5 Conclusions

Twenty-six laboratories registered for participation and all reported results for the proficiency test for gold nanoparticles in an aqueous suspension.

Eighteen labs showed optimal performance by reporting a correct diameter and a correct particle number concentration. An overview of each participant's performance is shown in Annex 7. A total number of nine questionable/unsatisfactory z-scores was reported, two for the diameter part and seven for the particle number concentration. For the latter, some of the questionable/unsatisfactory z-scores are possibly reported because the dilution factor of 1,000,000 was not taken into account.

References

1 Council directive 93/99/EEC of 29 October 1993 on the subject of additional measures concerning the official control of foodstuffs. Off. J. Eur. Commun. L290, 24/11/1993, 0014 – 0017.

2 ISO/IEC 17025:2005(E). 2005. General Requirements for the Competence of Calibration and Testing Laboratories.

3 Thompson M, Ellison SL, Wood R. 2006. The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. Pure Appl. Chem. 78(1):145-196.

4 ISO 13528:2015(E). 2015. Statistical methods for use in proficiency testing by inter-laboratory comparison.

5 Analytical Methods Committee. 1989. Robust statistics – How not to reject outliers Part 1. Basic concepts. Analyst 114:1693-1697.

6 Analytical Methods Committee. 1989. Robust statistics – How not to reject outliers Part 2. Inter-laboratory trials. Analyst. 114:1699-1702.

Annex 1 Codification of the samples

Lab number	Codes
PT018	802
PT019	456
PT020	721
PT021	448
PT022	569
PT023	855
PT024	803
PT025	715
PT026	921
PT027	871
PT028	520
PT029	689
PT030	427
PT9892	455
PT9954	930
PT9955	190
PT9956	118
PT9957	320
PT9991	385
PT9992	306
PT9993	924
PT9994	816
PT9995	541
PT9996	394
PT9997	629
PT9998	820

* All sample codes start with NANO/2018/.

Annex 2 Statistical evaluation of homogeneity data

Concentration Au in ng/l		
Sample number	Replicate 1	Replicate 2
Hom/001	49	53
Hom/002	49	52
Hom/003	48	46
Hom/004	50	46
Hom/005	46	47
Hom/006	47	48
Hom/007	45	46
Hom/008	49	43
Hom/009	46	45
Hom/010	42	49
Grand mean	47	
Cochran's test		
C	0.366	
C_{crit}	0.602	
$C<C_{crit}$?	NO OUTLIERS	
Target s = σ_P	9.5	
s_x	2.02	
s_w	2.59	
s_s	0.85	
Critial = $0.3\sigma_P$	2.84	
s_s<critical?	ACCEPTED	
$s_w<0.5\sigma_P$?	ACCEPTED	

Particle size Au in nm		
Sample number	Replicate 1	Replicate 2
Hom/001	59	58
Hom/002	58	58
Hom/003	59	58
Hom/004	58	58
Hom/005	59	58
Hom/006	58	58
Hom/007	58	59
Hom/008	58	58
Hom/009	58	58
Hom/010	58	58
Grand mean	58	
Cochran's test		
C	0.250	
C_{crit}	0.602	
$C<C_{crit}$?	NO OUTLIERS	
Target s = σ_P	5.8	
s_x	0.26	
s_w	0.45	
s_s	0.0	
Critial = $0.3\sigma_P$	1.75	
s_s<critical?	ACCEPTED	
$s_w<0.5\sigma_P$?	ACCEPTED	

Annex 3 Instruction letter

Inter-laboratory study ACEnano (2018-02) of the measurement of particle number concentration and particle size of gold nanoparticles: Protocol for sample handling, preparation, measurement and reporting

May 2018

1 Introduction

Nanoparticles are increasingly used and a vast number of nano-technological products including consumer products, are entering the market. To determine whether a product contains a nanomaterial, particle size and number concentration need to be measured. The EU project ACEnano innovates and optimises analytical techniques to detect and characterise nanoparticles. Task 5.3 in this project foresees the development of a proficiency testing scheme for nanomaterial analysis to assure comparable performance of laboratories. Participation in proficiency tests are essential to improve or maintain the quality of a laboratory.

RIKILT Wageningen University & Research, a partner in ACEnano, is experienced in the organisation of proficiency tests and was already involved in the organisation of three interlaboratory studies (ILS) on nanomaterials[1,2,3]. The samples for this proficiency test will be gold nanoparticles in an aqueous suspension and will be provided by ACEnano. You are asked to determine particle size and particle number concentration using the technique of single particle inductively coupled plasma mass spectrometry (spICP-MS).

[1] TPJ Linsinger, RJB Peters, S Weigel. *International interlaboratory study for sizing and quantification of Ag nanoparticles in food simulants by single-particle ICPMS.* Anal. Bioanal. Chem., 2014, 406:3835-3843.
[2] RJB Peters, Z Herrera Rivera, H Bouwmeester, S Weigel, HJP Marvin. *Advanced Analytical Techniques for the Measurement of Nanomaterials in Complex Samples: A Comparison.* Qual. Ass. Safe. Crop. Food., 2014, 6:281-290.
[3] S Weigel, R Peters, K Loeschner, R Grombe, T Linsinger. *Results of an interlaboratory method performance study for the size determination and quantification of silver nanoparticles in chicken meat by single-particle-inductively coupled plasma-mass spectrometry (sp-ICP-MS).* Anal. Bioanal. Chem., 2017, 409:4839–4848.

2 Timetable

You should complete this work and send the results via the electronic reporting system (https://crlwebshop.wur.nl/apex/f?p=307:login) by 13th July 2018. If you cannot do so or need extra time please inform Ingrid Elbers (pt.rikilt@wur.nl).

3 This package

This package contains:

- this protocol,
- one vial containing spherical gold colloids labelled as ACEnano (2018-02) (is ILC number); NANO/2018/xxx (is your sample code); PTxxx (is your lab code).
- 1 g of citrate buffer (Sodium Citrate Tribasic Dihydrate) labelled "citrate buffer".
- a letter containing your username, password and labcode
- an acknowledgement of receipt: upon receipt of the samples, please send this document by e-mail (pt.rikilt@wur.nl) that everything is received in good order.

4 Sample handling and storage

Samples were produced from a NanoComposix citrate stabilised gold nanoparticle suspension. The original material was diluted and bottled in vials.

Upon receipt, the samples should immediately be placed into a refrigerator (2 - 8 °C) for storage. Under no circumstances should the samples be exposed to temperatures above 40 °C or below freezing point. The samples are stable for at least 8 weeks under such conditions, but should be analysed as soon as it is possible within this timeframe.

When the samples are removed from the refrigerator for sample preparation and characterisation, allow at least 30 min for them to return to room temperature before opening the container. Use clean pipettes, pipette tips or syringes for handling the sample liquid.

5 spICP-MS measurement

The sample will require dilution before analysis. For dilution it is recommended to use tri-sodium citrate buffer which was provided in the package. The recommended buffer concentration is 1 mM in ultrapure water. If an ionic gold standard and Reference Material (RM) is used for size calibration and the determination of the transport efficiency, these should also be diluted in 1mM citrate buffer.

You should use your own *in house* method and practice experience to select the most appropriate dwell time for the spICP-MS analysis. Guidance for choosing dwell time and analysis time can be found in e.g. ISO/TS 19590:2017. Information regarding the dwell time used should be recorded in the electronic reporting form. Please carry out a single analysis.

Transport efficiency should be determined following your own *in house* method and practice experience. Guidance on transport efficiency determination can be found in e.g. ISO/TS 19590:2017.

Information regarding the transport efficiency determination approach followed should be recorded in the electronic reporting form.

You should use your own *in house* method and practice experience to calculate the particle size and number-based concentration of the sample. Guidance on calculation of these parameters can be found in e.g. ISO/TS 19590:2017, and a practical spreadsheet can be downloaded from the RIKILT website: http://www.wageningenur.nl/en/Expertise-Services/Research-Institutes/rikilt/Software-and-downloads.htm.

6 Reporting

An electronic reporting form is available at https://crlwebshop.wur.nl/apex/f?p=307:login were you can report your results after entering your lab code. Please report the following:

- Particle number concentration in particles per litre in the original (non-diluted) sample
- Measurement uncertainty particle number concentration in particles per litre
- Particle size (diameter) in nm
- Measurement uncertainty particle size in nm

The reporting form also allows you to report the method you used and the analytical conditions. Please do so. In addition there is a box for specific remarks per result. Use if required.

Annex 4 Statistical evaluation of stability data

Statistical evaluation for particle diameter in nm		
Time in refrigerator (days)	0	36
Calculated amounts (nm)	60.8	57.2
	61.1	57.3
	60.3	56.6
	60.4	56.5
	60.7	57.8
	60.5	56.8
Average amount (nm)	60.6	57.1
n	6	6
Standard deviation (nm)	0.29	0.48
Difference		3.6
$0.3\sigma_P$		1.8
Consequential difference? Diff < 0.3 σ_P		YES

Statistical evaluation for particle concentration/l after dilution		
Time in freezer (days)	0	36
Calculated particles/l	18789615	20566241
	18767854	20876226
	18229774	18924222
	19033613	20342810
	17313825	20922359
	17620785	20564983
Average amount (parts/l)	18292578	20366140
n	6	6
Standard deviation (parts/l)	697893	738758
Difference		-2073563
$0.3\sigma_P$		3658516
Consequential difference? Diff < 0.3 σ_P		NO

Annex 5 Overview of the applied methods

CYP

Lab	Sample preparation	Internal standard	Detection method
PT019	Sonication with ultrasonic bath (180W) then dilution in utrapure water by weight and analysis	- Au ionic calibration 0-1-5-10 µg/L in ultrapure water - AuNP standard BBI 60 nm - AuNP control BBI 40 nm	spICP-MS Perkin Elmer NexION 300 - Micromist 0.4 mL/min nebulizer - Cyclonic Spray Chamber - Ni cones - Sample flowrate 0.164 mL/min - 197Au (19.3 g/cm3) - 100 µs dwell time - 100 seconds acquisition time
PT021	Dilution in 1 mM citrate buffer, sonication	NIST SRM 8013	sp-ICP-MS iCAP Q Thermo Scientific, sample flow 0.323 ml/min, dwell time 3 ms
PT022	Dilution with sodium citrate (tribasic dihydrate)	NIST SRM 8013	Single-Particle ICP-MS
PT025	Only dilution, no sonication		
PT026		no internal standard;	single Particle ICP-MS (7900 ICP-MS from Agilent Technologies; P=1550W, Ar gas flow rate = 15 L/min, sample introduction flow rate = 0.346 mL/min; MicroMist Nebulizer, quartz glass Scott spray chamber)
PT028	NA	NA	Single Particle ICP-MS. NexION 2000 Dwell time 50 us, sampling time 60 seconds TE 7.46% Flow rate 0.118 ml/min Calibration Standards. ionic and NP standards were used to calibrate. Ionic Blank, 1, 2 and 3 ppb, 50 nm and 100 nm PKI Au standards.
PT029	All samples and standards have been diluted by using 1mM trisodium citrate buffer in ultrapure water and, they were prepared by weight. Different dilutions of the samples has been measured: 1E6, 2E5 and 1E5.	Transport efficiency has been calculated by the use of a 50nm Au NPs standard from NanoComposix after approximately 2.5E5 times dilution giving a value of 8.5%.	The SP-ICP-MS measurements have been done by using the NexIon ICP-MS from Perkin Elmer. This instrument is a quadrupole based ICP-MS equipped with a collision/reaction cell and its detector allows the use of dwell times as low as 10 Âµs
PT030	sonication dilution with citrate buffer and sonication again		

Lab	Sample preparation	Internal standard	Detection method
PT9954	Dilution: The sample, Au particle standard and ionic Au standards were diluted with tri-sodium citrate buffer (1 mM)	Calibration with ionic Au standards (prepared in tri-sodium citrate 1 mM): 0 µg/L 0.25 µg/L 0.50 µg/L 1.0 µg/L 2.0 µg/L ICP-MS response to ion standard: 207629 cps/(µg/L)	SP-ICP-MS: ICP-MS Agilent 8800 dwell time 3 ms nebulization efficiency 4.53%
PT9956	none	none	Sector-field ICPMS Thermo Element XR MicroMist pneumatic Nebulizer Cyclonoc Spray chamber Mass window 5% Samples/peak 1000 Sample time 1 ms Settling time 1 ms Runs 5000 Passes 1 Repeats 3 Time 4 min, 16 s
PT9992			spICPMS Forward Power: 1600 Nebulizer: Standard type Glas flow (plasma): 18 L/min, gas flow (Nebulizer): 1.02 L/min, Gas flow (Auxiliary): 1.2 L/min Sample flow rate: 0.323 mL/min Dwell time: 50 µs (microsecs) Total acqu. time: 60s
PT9993	-	-	ICP-MS
PT9995	-10 seconds vortex -2 times 1:1000 dilution with vortexing in between	Au NIST Standard 60nm ionic Au Standard	sp-ICP-MS
PT9996	Dilution: 100x100x10 in 1mM citratbuffer	No internal standard	sp-ICPMS
PT9997	Dilution and sonication		sp-ICP-MS
PT9998	Stock nanoparticle suspensions were prepared by gravimetric dilution using the method outlined in NPL report AS98. The dilution matrix was 1 mM trisodium citrate. Measurements were made immediately upon dilution.	Calibration standards for particle size: nanocomposix, nanoxact gold nanosheres (citrate) 20, 40, 60, 80 and 100 nm. Size calibration was checked using NIST RM 8013 60 nm gold nanoparticles.	spICPMS (FAST) using Perkin Elmer Nexion 300D ICPMS and Syngistix nano application module. Read time: 60 s; dwell time: 50 Âµs; flow rate ca. 0.25 ml/minute.

Annex 6 Results

	Particle diameter in nm X: 61 nm Uncertainty of X: 1.2 nm Target sd: 6.1 nm Robust sd: 4.9 nm Instability: 3.6 nm		particle number concentration per litre X: $1.44.10^{13}$ p/l Uncertainty of X: $1.50.10^{12}$ p/l Target sd: $2.88.10^{12}$ p/l Robust sd: $6.01.10^{12}$ p/l	
Lab code	Result (nm)	z_{ai}-score	Result (p/l)	z'_a-score
PT018	65.24	0.62	18181479274000	1.17
PT019	62.4	0.15	14500000000000	0.03
PT020	59.52	-0.27	15030000000000	0.20
PT021	61	-0.06	10060000000000	-1.33
PT022	65	0.58	15700000000000	0.40
PT023	74	2.04	17300000	-4.43
PT024	64	0.41	11000000000000	-1.04
PT025	80	3.02	7610000000000	-2.09
PT026	55.5	-0.84	20400000000000	1.85
PT027	60	-0.20	19000000000000	1.42
PT028	61.6	0.02	12700000000000	-0.52
PT029	58.16	-0.46	16000000000000	0.50
PT030	63	0.25	44300000	-4.43
PT9892	68.8	1.20	10100000000000	-1.32
PT9954	52	-1.33	20000000000000	1.73
PT9955	57	-0.63	41000000000000	8.20
PT9956	60.2	-0.18	13400000000000	-0.30
PT9957	61.6	0.02	17000000000000	0.80
PT9991	61.25	-0.03	16100000000000	0.53
PT9992	66	0.74	11350000000000	-0.94
PT9993	49.9	-1.62	detected	
PT9994	63	0.25	6730000000000	-2.36
PT9995	58.4	-0.43	24000000000000	2.96
PT9996	54	-1.05	21000000000000	2.04
PT9997	60	-0.20	16900000000000	0.77
PT9998	66.4	0.80	7980000000000	-1.97

X consensus value.

sd standard deviation.

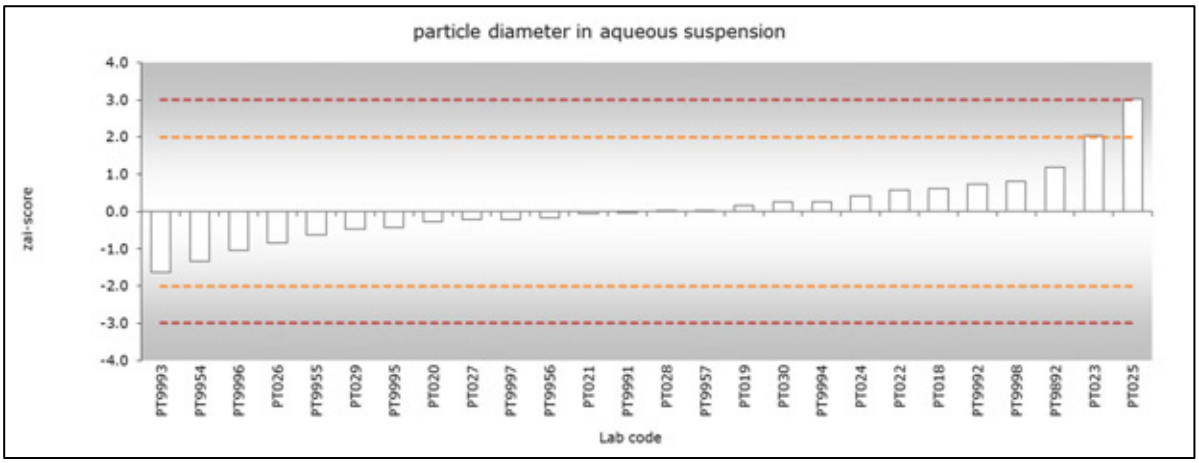

Figure a *Graphical representation of the reported results for the particle diameter. The X ± 2σ_P lines (dotted) are calculated according to equation III in §3.4.*

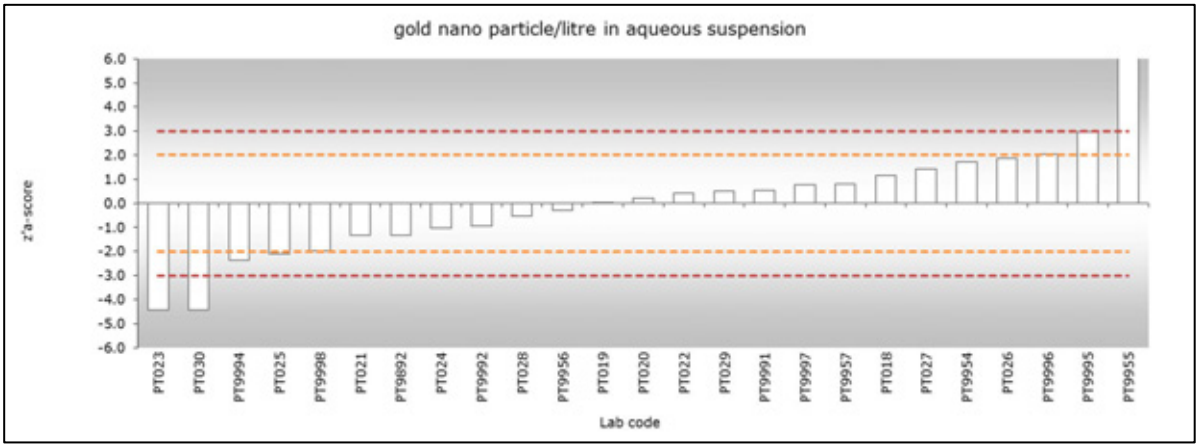

Figure b *Graphical representation of the reported results for the particle number concentration per litre. The X ± 2σ_P lines (dotted) are calculated according to equation II in §3.4.*

Annex 7 Overall performance

Lab code	Performance
PT018	**Optimal performance**, two satisfactory z-scores
PT019	**Optimal performance**, two satisfactory z-scores
PT020	**Optimal performance**, two satisfactory z-scores
PT021	**Optimal performance**, two satisfactory z-scores
PT022	**Optimal performance**, two satisfactory z-scores
PT023	Questionable z-score for diameter, unsatisfactory z-score for particle number
PT024	**Optimal performance**, two satisfactory z-scores
PT025	Unsatisfactory z-score for diameter, questionable z-score for particle number
PT026	**Optimal performance**, two satisfactory z-scores
PT027	**Optimal performance**, two satisfactory z-scores
PT028	**Optimal performance**, two satisfactory z-scores
PT029	**Optimal performance**, two satisfactory z-scores
PT030	Satisfactory z-score for diameter, unsatisfactory z-score for particle number
PT9892	**Optimal performance**, two satisfactory z-scores
PT9954	**Optimal performance**, two satisfactory z-scores
PT9955	Satisfactory z-score for diameter, unsatisfactory z-score for particle number
PT9956	**Optimal performance**, two satisfactory z-scores
PT9957	**Optimal performance**, two satisfactory z-scores
PT9991	**Optimal performance**, two satisfactory z-scores
PT9992	**Optimal performance**, two satisfactory z-scores
PT9993	Satisfactory z-score for diameter, no quantitative result for particle number
PT9994	Satisfactory z-score for diameter, questionable z-score for particle number
PT9995	Satisfactory z-score for diameter, questionable z-score for particle number
PT9996	Satisfactory z-score for diameter, questionable z-score for particle number
PT9997	**Optimal performance**, two satisfactory z-scores
PT9998	**Optimal performance**, two satisfactory z-scores

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