Particle number concentration measurement for cellular analysis (CCQM-P222) :Protocol update review

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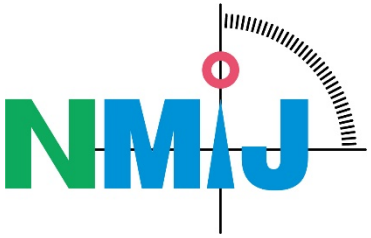


Project overview

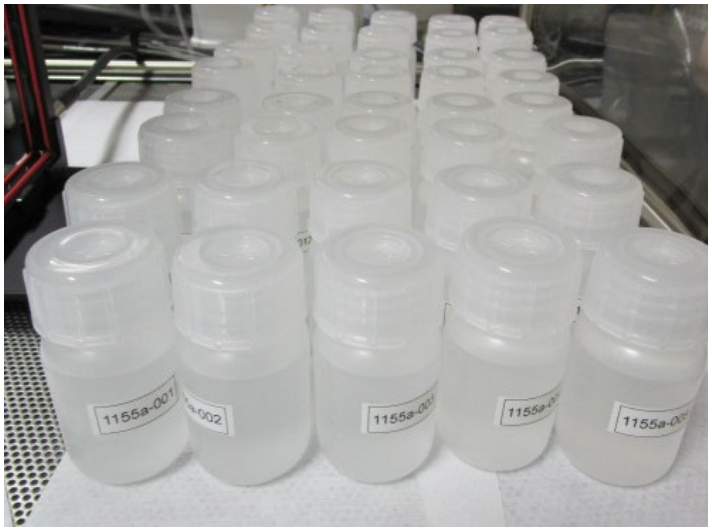
- The pilot study is intended for the first step to establish validated methods to understand the NMIs' capability of absolute particle number concentration (PNC) measurement for cellular analysis.

  1. To confirm the NMI's measurement capability of PNC.
  2. To investigate difference between the measurement principles/instruments.
  3. To elucidate the applicability of the artificial particles to the evaluation of measurement capability of cell analyzers.
  4. Establishment of a solid protocol for future key comparisons and a basis for CMC registrations for measurement of number concentration of cells/particles with artificial particles.

- In this pilot study, we use non-fluorescent 5 μm polystyrene latex (PSL) particles as surrogate of RBC.
  →Monodisperse, spherical, and stable artificial particles.

Study material: specification

- Material
  →Aqueous suspension of polystyrene latex (PSL) microspheres
- Nominal mean diameter
  →5 μm (65 fL)
- Coefficient of variation in diameter
  →1 %
- Dispersion medium
  →Water
- Nominal particle number concentration:
  →0.5×10^{6} –1.5×10^{6} particles g^{-1}
- Volume per bottle
  →49 mL
- Packaging bottle
  →Polypropylene bottle with screw cap
- Number of samples to be sent
  →2 bottles



Totally, 40 bottles were prepared.
(Distribution: 30 bottles)
(Control: 10 bottles)

▼

Up to 15 laboratories can participate…

Today's topics

Updates from last Spring CAWG meeting

1. Evaluation of the study material
   (Results of homogeneity and long-term stability test)

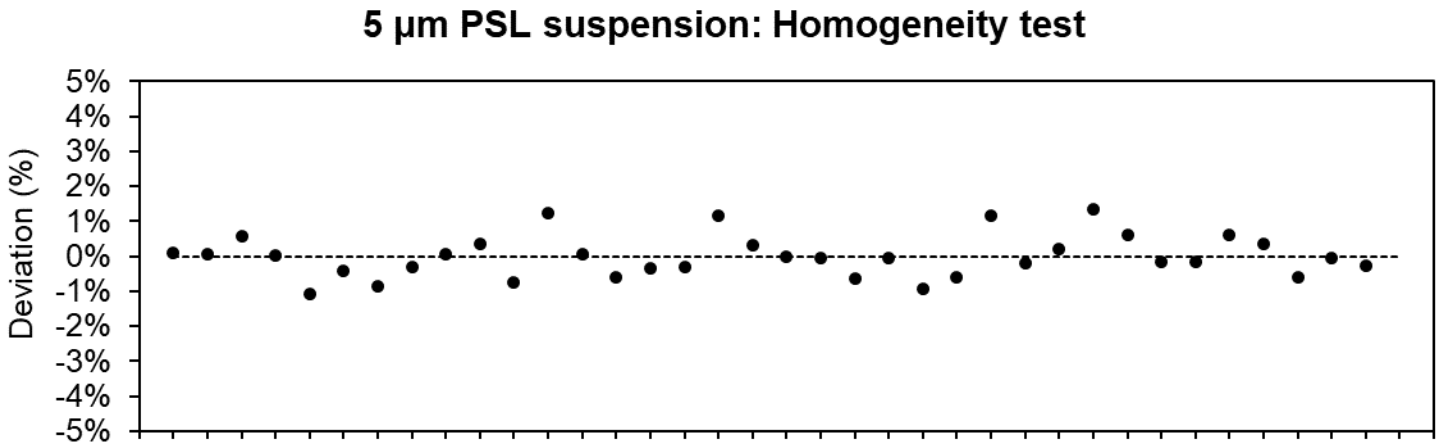
2. Reporting results

3. Instructions for receiving, storage and use

4. Reschedule for the pilot study

1. Evaluation of the study material

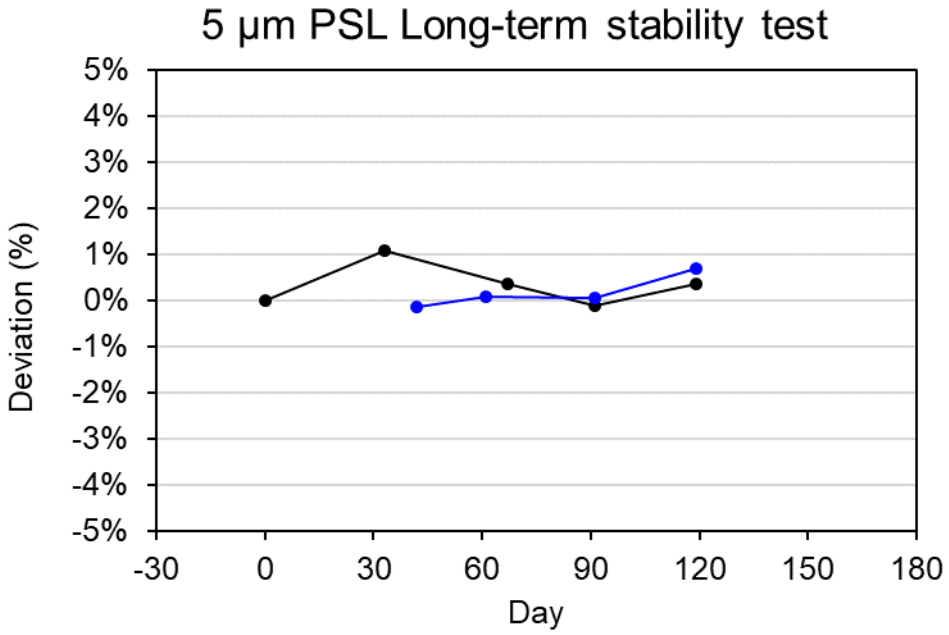
Homogeneity test (Full inspection)



- Number concentrations of each bottle were sufficiently homogeneous.
- Uncertainty analysis based on ANOVA, u_{bb} = 0.30% was obtained.

1. Evaluation of the study material

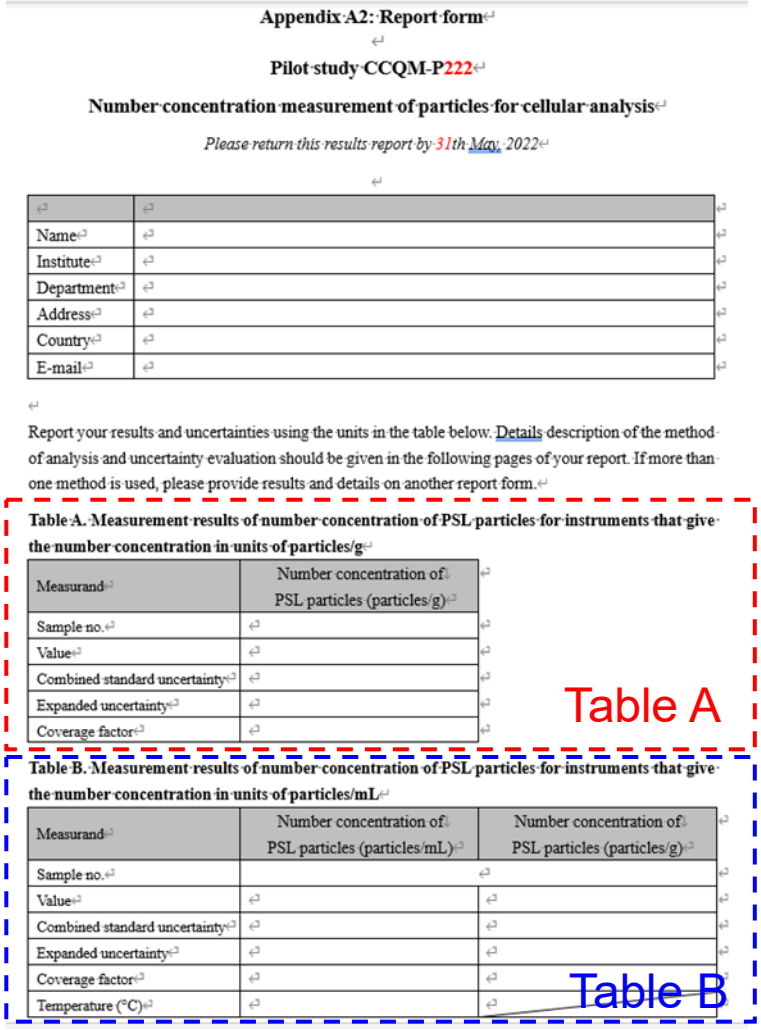
Long-term stability test



- Based on ISO Guide 35, long-term stability of the study material is sufficiently stable ($|\beta| < t_{0.95} \times u(\beta)$).

2. Reporting results

- Participants should report the number concentration of 5 μm PSL particles in units of "**particles/g**".
- Fill in the provided report form (Appendix A2). A full description of the method of analysis and uncertainty evaluation is also needed.
- If your instruments give the gravimetric number concentration (**particles/g**), use **Table A**.
- If your instruments give the volumetric number concentration (**particles/mL**), use **Table B**.



Appendix A2: Report form

Pilot study CCQM-P222

Number concentration measurement of particles for cellular analysis

Please return this results report by 31th May, 2022

Name	
Institute	
Department	
Address	
Country	
E-mail	

Report your results and uncertainties using the units in the table below. Details description of the method of analysis and uncertainty evaluation should be given in the following pages of your report. If more than one method is used, please provide results and details on another report form.

Table A. Measurement results of number concentration of PSL particles for instruments that give the number concentration in units of particles/g

Measurand	Number concentration of PSL particles (particles/g)
Sample no.	
Value	
Combined standard uncertainty	
Expanded uncertainty	
Coverage factor	

Table A

Table B. Measurement results of number concentration of PSL particles for instruments that give the number concentration in units of particles/mL

Measurand	Number concentration of PSL particles (particles/mL)	Number concentration of PSL particles (particles/g)
Sample no.		
Value		
Combined standard uncertainty		
Expanded uncertainty		
Coverage factor		
Temperature (°C)		

Table B

Pattern A: Gravimetric concentration

- If your instruments give the gravimetric number concentration (**particles/g**), fill in **Table A** and make a full description of your measurement.

Table A. Measurement results of number concentration of PSL particles for instruments that give the number concentration in units of particles/g

Measurand	Number concentration of PSL particles (particles/g)
Sample no.	
Value	
Combined standard uncertainty	
Expanded uncertainty	
Coverage factor	

Pattern B: Volumetric concentration

- Fill in **Table B** and report both the units (particles/mL and particles/g) by converting the “particles/mL” with the density of the study material at 23℃.
- A density of 0.998 g/mL can be assumed for the particle suspension for conversion of volumetric concentrations measured at 23℃ to concentration per mass.
- If the measurement temperature cannot be set at **23℃±1℃**, the unit conversion with the density at 23℃ should not be applied.
  →In that case, report only the concentration in particles/mL.

Report the measurement temperature in pattern B.

Table B. Measurement results of number concentration of PSL particles for instruments that give the number concentration in units of particles/mL

Measurand	Number concentration of PSL particles (particles/mL)	Number concentration of PSL particles (particles/g)
Sample no.		
Value		
Combined standard uncertainty		
Expanded uncertainty		
Coverage factor		
Temperature (°C)		

Others

- If multiple bottles are evaluated by a single method, the concentration and its uncertainty for each bottle may be reported.
  →Please identify which value should be used in the calculation of the reference value.

- When you report the results, please e-mail the report form to the pilot-Lab (NMIJ). and the co-pilot-Lab (PTB) by the deadline.

3. Instructions for receiving, storage, and use

- Study material will be shipped under the room temperature condition. (15℃–25 ℃)

- Once receiving the study material, participants should weigh the study material and check any leakage.
  →Please report us by e-mail **before starting the measurement**.

- The study material should be stored in a clean environment, at a temperature between 4℃–30℃. **DO NOT freeze**!

- When volumetric dilution is needed, the temperature at which the **dilution is performed should be 23℃±3℃**.
  →If the dilution is done at out of the range, unit conversion from particles/mL to particles/g using a density (0.998 g/mL) can't be applied.

4. Reschedule for the pilot study

5. → Schedule (*Updated on 8th November, 2021*)

Date	Timeline
25 November 2020	First proposal
February 2021-	Questionnaire distributed
Late April 2021	Draft protocol distributed
6 May 2021	Proposal in CCQM meeting
June 2021 CCQM	Pilot number distributed
June 2021 CCQM	Specification of study material fixed
June 2021 -	Preparation of study material
June - November 2021	homogeneity and stability test
November 2021 CCQM	Call for participation
November - December 2021	Final protocol circulated
November - 20 December 2021	Registration for participation (End of registration)
January 2022	Study material distribution
31 May 2022	Submission of results
…	…

- Sample shipping will be started from January.
- Participants are expected to submit the result by the end of May.

Call for participation

- We are planning to start sample shipping from January 2022.
- If you have any comments about the draft protocol, email us by the end of November.
- Final protocol will be circulated after this CAWG meeting by early December at the latest.
- If you plan to participate in the pilot study, please e-mail us **by 20 December**.

Please inform us of your information…
Name, Institute, Department, Full address(City, Province/State, Postal/Zip code, Country), E-mail address, Phone number

If you have any questions about this pilot study, please e-mail us
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Shin-ichiro Fujii (s-fujii@aist.go.jp) (NMIJ)
Andreas Kummrow (Andreas.Kummrow@ptb.de) (PTB)