LABQUALITY

External Quality Assessment Scheme

Pipette control Round 1, 2024

Specimens

Please find enclosed 2 liquid samples S001A 5mL and S001B 35mL.

Storage and use

Once samples are received, they should be stored at $+2...8^{\circ}\text{C}$ or RT. Samples are ready for use. Allow the samples to reach room temperature before pipetting. The samples are pipetted according to laboratory's own instructions using pipette tips suitable for the pipette. The sample volumes to be pipetted and weighed are from sample S001A 100 μL and sample S001B 1000 μL . Five parallels are to be pipetted from both samples. Rinse the pipette tip before pipetting the parallels. Weigh each pipetted sample volume on a calibrated scale. Tare the scale before weighing and between parallels. If you need a new sample, please inform the EQA Coordinator.

Result reporting

Please enter the results and methods via LabScala (www.labscala.com). If you cannot find your pipette manufacturer from the registry in LabScala, please contact the EQA Coordinator. Preanalytical questions are found on the LabScala results sheet in the "Results" section and on the subsection "Preanalytical part". Please respond to these when submitting your results.

Report the average value of parallel weighings in <u>milligrams</u>. Results of five different pipettes can be reported from one sample. A new result form opens by clicking the "Add result +" button on the right side of the blue bar of each sample.

- Add the average value for sample S001A and S001B for a single pipette. If you only want to report the result of one specific pipette for one of the samples, then leave the result space empty for the other sample. Report the average value of parallel weighings with an accuracy of 0.01 mg and your pipetting technique.
- 2. Add the requested information about the used pipette (pipette manufacturer and model, max volume of the pipette, and room temperature at the time the samples were pipetted). <u>Save</u>.
- Move forward with the green Next button. The preanalytical question form opens. Information on calibration frequency and calibration site for both pipettes and the scale used are needed, please have these available when reporting. <u>Save</u>.
- 4. Accept and submit the results.

2024-02-27

INSTRUCTIONS

Product no. 8205 LQ754524011-012/FI

Subcontracting: Sample pretesting

If the kit is incomplete or contains damaged specimens, please report immediately to info@labquality.fi.

The results should be reported no later than **March 14, 2024**.

Inquiries

EQA Coordinator Riitta Viertola riitta.viertola@labquality.fi

Labquality Oy

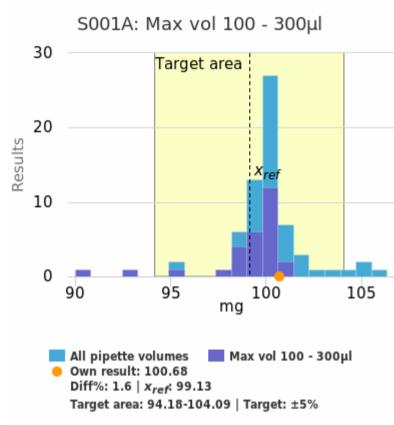
Kumpulantie 15 FI-00520 HELSINKI Finland

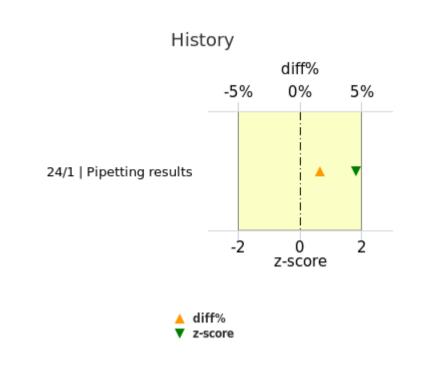
Tel. + 358 9 8566 8200

info@labquality.fi www.labquality.com



Form 1 | xxxxxxxx

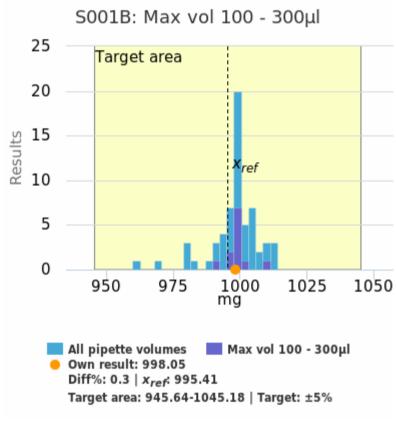




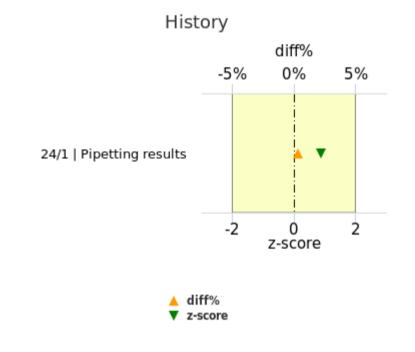
		24/1
/ %	n	

Round	Sample	x _{ref} /x pt	Result	diff%	z-score
24/1	Pipetting results	99.13	100.68	1.6%	1.81

		^X pt	sd	SEM	CV%	n
Max vol 1	L00 - 300μl	99.53 mg	0.86	0.16	0.9	27
All pipet	te volumes	99.97 mg	1.02	0.12	1.0	67
25	S001B: M		.00 -	300μ	I	
23	Target a	area				



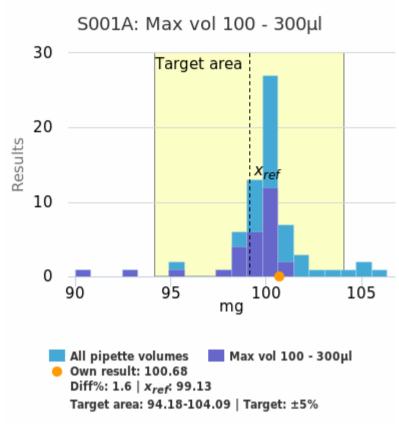
All pipette volumes Max vol 100 - 300μl Own result: 998.05 Diff%: 0.3 <i>x_{ref}</i> : 995.41 Target area: 945.64-1045.18 Target: ±5%						
	x _{pt}	sd	SEM	CV%	n	
Max vol 100 - 300µl	999.02 mg	2.96	0.85	0.3	10	
All pipette volumes	999.15 mg	6.64	0.85	0.7	61	



Round	Sample	x _{ref} /x pt	Result	diff%	z-score
24/1	Pipetting results	995.41	998.05	0.3%	0.89

XXXXX

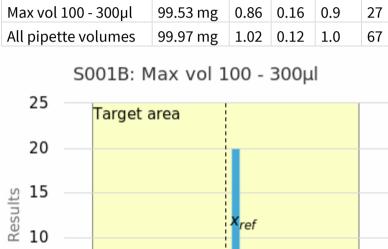
Form 2 | xxxxxxxx



History				
	-5%	diff% 0%	5%	
24/1 Pipetting results		A	•	
	-2	0 z-score	2	
△ diff% ▼ z-score				

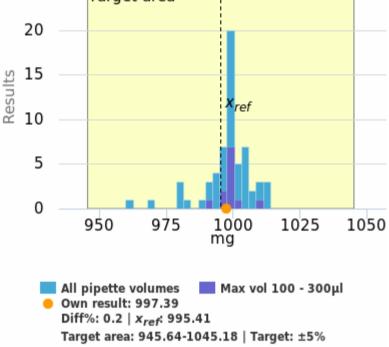
			24/1
SEM	CV%	n	

Round	Sample	x _{ref} /x pt	Result	diff%	z-score
24/1	Pipetting results	99.13	100.68	1.6%	1.81



^Xpt

sd

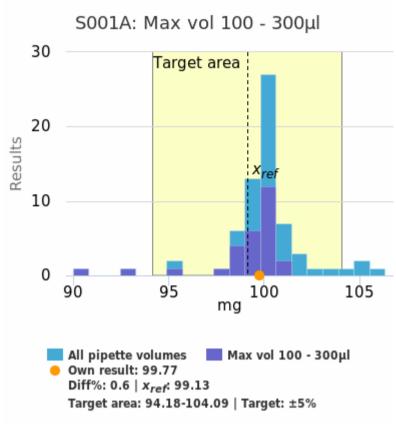


History				
	-5%	diff% 0%	5%	
24/1 Pipetting results		•		
	-2	0 z-score	2	
diff% ▼ z-score				

	^x pt	sd	SEM	CV%	n
Max vol 100 - 300µl	999.02 mg	2.96	0.85	0.3	10
All pipette volumes	999.15 mg	6.64	0.85	0.7	61

Round	Sample	x _{ref} /x pt	Result	diff%	z-score
24/1	Pipetting results	995.41	997.39	0.2%	0.67

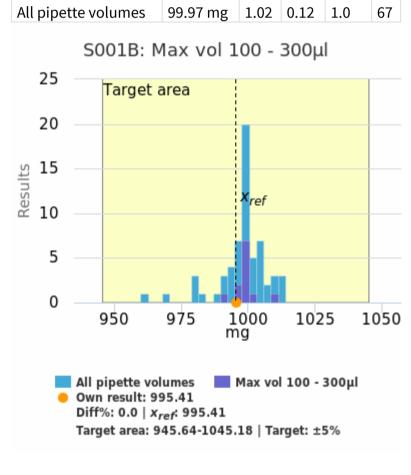
Form 3 | xxxxxxxx

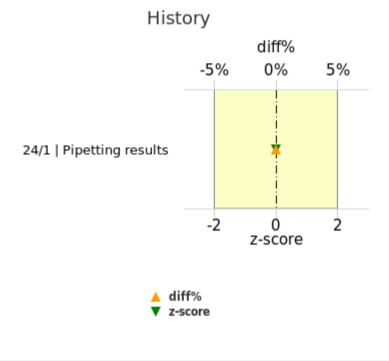


History					
	-5%	diff% 0%	5%		
	-5 /0	0 70	J /0		
		į			
24/1 Pipetting results		▲ ▼			
	-2	0 z-score	2		
diff% ▼ z-score					

	^X pt	sd	SEM	CV%	n
ax vol 100 - 300µl	99.53 mg	0.86	0.16	0.9	27

Round	Sample	x _{ref} /x pt	Result	diff%	z-score
24/1	Pipetting results	99.13	99.77	0.6%	0.74

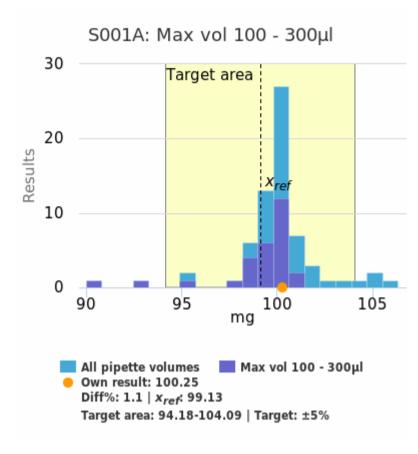




	^X pt	sd	SEM	CV%	n
Max vol 100 - 300µl	999.02 mg	2.96	0.85	0.3	10
All pipette volumes	999.15 mg	6.64	0.85	0.7	61

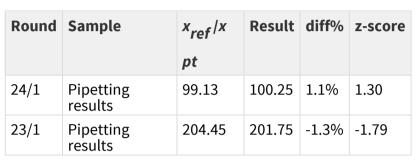
Round	Sample	x _{ref} /x pt	Result	diff%	z-score
24/1	Pipetting results	995.41	995.41	0.0%	0.00

Form 4 | xxxxxxxx

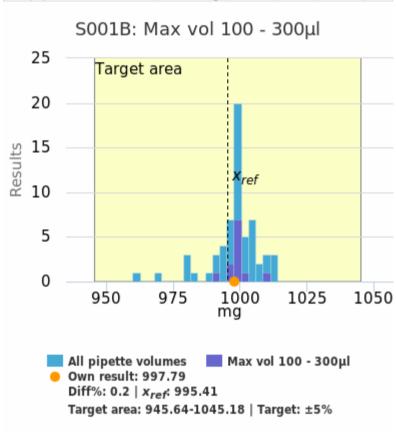


Histo	ory	115507	
	-5%	diff% 0%	5%
24/1 Pipetting results		A '	v
23/1 Pipetting results	-2	^	2
		z-score	2
▲ dif	f%		

	^X pt	sd	SEM	CV%	n
Max vol 100 - 300µl	99.53 mg	0.86	0.16	0.9	27
All pipette volumes	99.97 mg	1.02	0.12	1.0	67



z-score

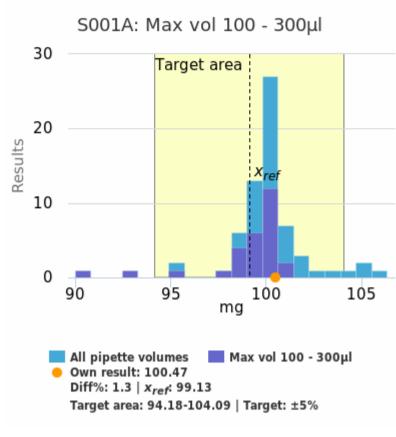


History					
	diff%				
	-5% 0% 5%				
24/1 Pipetting results	•				
23/1 Pipetting results	▲ ▼				
	-2 0 2 z-score				
△ diff% ▼ z-score					

	x _{pt}	sd	SEM	CV%	n
Max vol 100 - 300μl	999.02 mg	2.96	0.85	0.3	10
All pipette volumes	999.15 mg	6.64	0.85	0.7	61

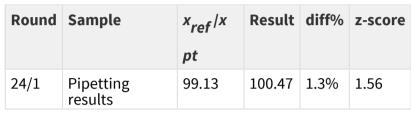
Round	Sample	x _{ref} /x pt	Result	diff%	z-score
24/1	Pipetting results	995.41	997.79	0.2%	0.80
23/1	Pipetting results	125.63	121.10	-3.6%	-0.60

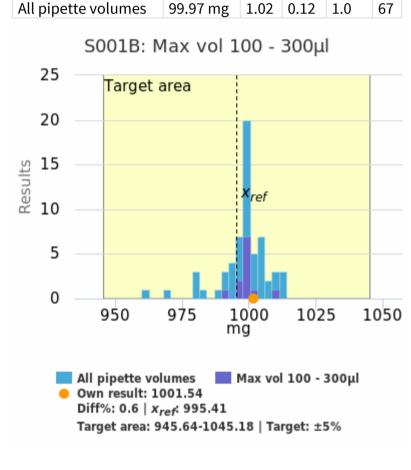
Form 5 | xxxxxxxx

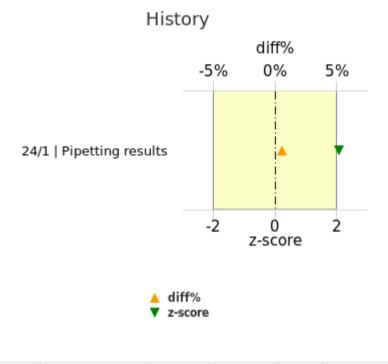


History					
	-5%	diff% 0%	5%		
24/1 Pipetting results		A	•		
	-2	0 z-score	2		
△ diff% ▼ z-score					

	x _{pt}	sd	SEM	CV%	n
Max vol 100 - 300μl	99.53 mg	0.86	0.16	0.9	27







	^X pt	sd	SEM	CV%	n
Max vol 100 - 300µl	999.02 mg	2.96	0.85	0.3	10
All pipette volumes	999.15 mg	6.64	0.85	0.7	61

Round	Sample	x _{ref} /x pt	Result	diff%	z-score
24/1	Pipetting results	995.41	1001.54	0.6%	2.07

LABQUALITY Pipette control (an analytical Scale is also needed), February, 1-2024

XXXXX

Report info

Participants

27 participants from 12 countries.

Report info

Assigned value (target value) calculation and its uncertainty.

Your own result should be compared to others using the same method. The assigned values (Xrob) are calculated according to the robust procedure described in the standard ISO 13528 (Statistical methods for use in proficiency testing by interlaboratory comparisons, Annex C, Algorithm A). The standard uncertainty of the assigned value is expressed as 1.25 x the standard error of mean (SEM) and marked as "u" in numerical summary. Due to its iterative mode algorithm A adds the uncertainty of the assigned value and with this factor we want to adjust uncertainty accordingly. In case there are 2-11 results in a method group, the robust calculation is not used but assigned values (Xpt) are means of the results where results deviating more than +/- 3*standard deviation from the median are removed. The standard uncertainty (u) of the assigned value is reported as standard error of the mean (SEM). Additionally, if the measurement uncertainty of the target value is too large (u > 0.1 * maximum allowable error) an automatic text is printed on the report: "The uncertainty of the assigned value is not negligible, and evaluations could be affected." Please notice also that for groups that have only 1 result only the client's own result is shown. No target value (except for reference method values) is calculated, no target areas are shown.

Z score In case there are 2-4 results in a method group, no z-score is calculated, and a text is printed on the report: "Due to the small number of results, the z score is not calculated." In case there are 5-11 results, the report has a text: "Z score is uncertain due to the small number of observations.". Results reported with < or > -signs cannot be included in the statistics. For information on report interpretation and performance evaluation, please see the "EQAS Interpretation guidelines" in LabScala User instructions (top right corner? Help link).

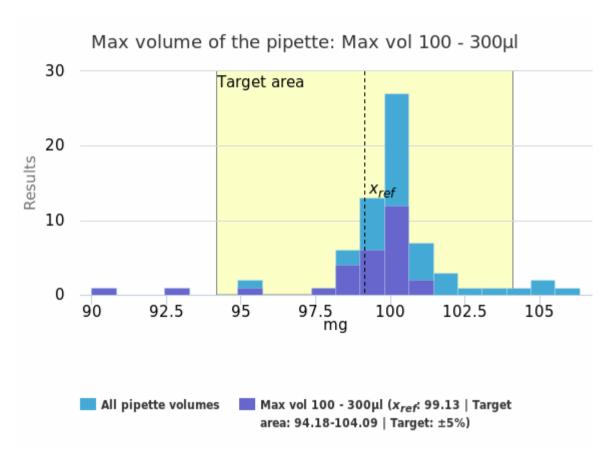
For information on report interpretation and performance evaluation, please see the "EQAS Interpretation guidelines" in LabScala User instructions (top right corner? Help link).

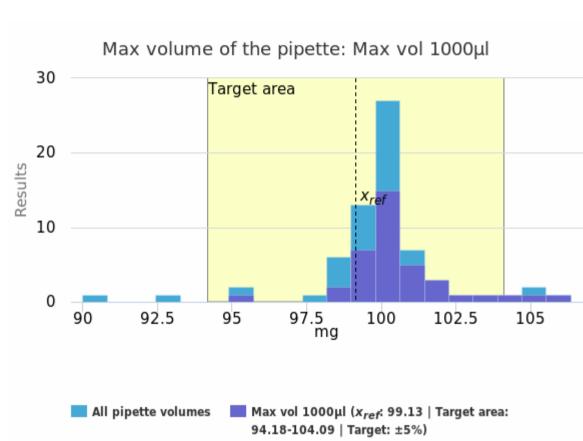
LΔBQUΔLITY Pipette control (an analytical Scale is also needed), February, 1-2024

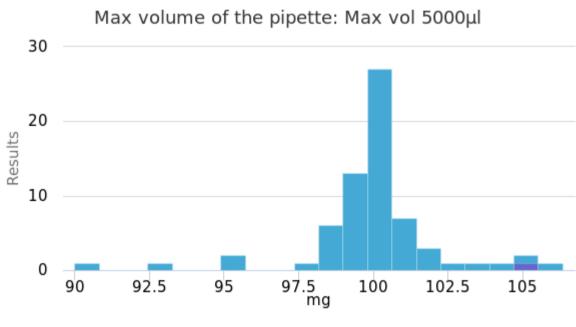
S001A

Max volume of the pipette	x _{pt}	Median	sd	CV%	SEM	min	max	Outliers	n
Max vol 100 - 300µl	99.53	99.80	0.86	0.9	0.16	90.00	100.68	3	27
Max vol 1000µl	100.38	100.00	1.28	1.3	0.21	95.23	106.34	3	33
Max vol 5000µl	-	_	-	-	-	105.05	105.05	-	1
All	99.97	100.00	1.02	1.0	0.12	90.00	106.34	15	67

S001A histogram summaries in LabScala





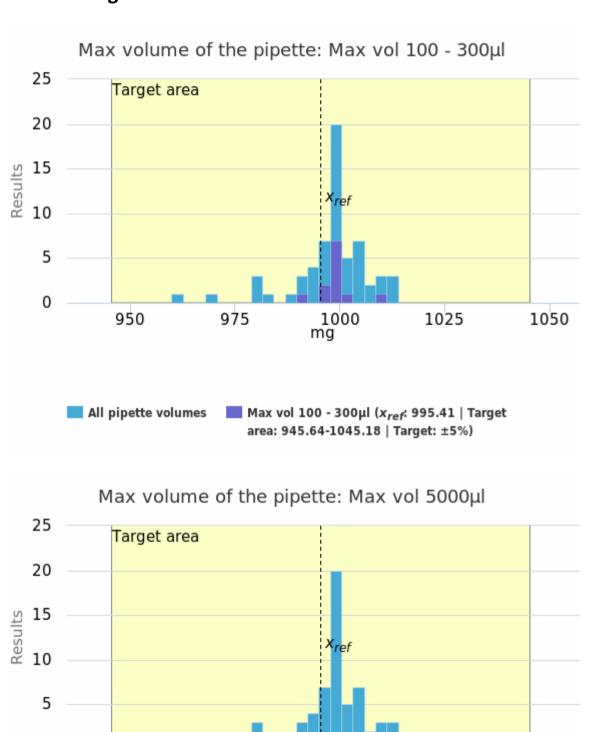


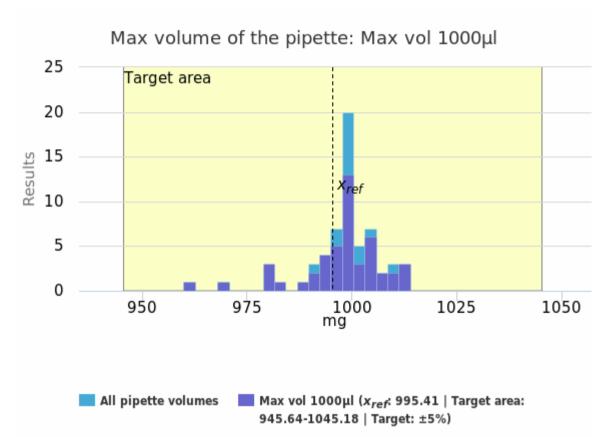
LΔBQUΔLITY Pipette control (an analytical Scale is also needed), February, 1-2024

S001B

Max volume of the pipette	^X pt	Median	sd	CV%	SEM	min	max	Outliers	n
Max vol 100 - 300µl	999.02	1000.00	2.96	0.3	0.85	990.00	1010.00	2	10
Max vol 1000µl	998.73	999.80	7.99	0.8	1.17	960.00	1013.95	2	40
Max vol 5000μl	1003.80	1003.80	2.97	0.3	2.10	1001.94	1005.65	-	2
All	999.15	1000.00	6.64	0.7	0.85	960.00	1013.95	11	61

S001B histogram summaries in LabScala





0

950

975

1000 mg

945.64-1045.18 | Target: ±5%)

All pipette volumes Max vol 5000μl (x_{ref}: 995.41 | Target area:

1025

1050

LABQUALITY Pipette control (an analytical Scale is also needed), February, 1-2024

Report info

Participants

27 participants from 12 countries.

Report info

Your own result should be compared to others using the same method. Assigned values (x_{pt}, target values) are means of the results where results deviating more than +/- 3*standard deviation from the median are removed. The standard uncertainty (u) of

the assigned value is reported as standard error of the mean (SEM). Additionally, if the measurement uncertainty of the target value is large an automatic text is printed on the report: "The uncertainty of the assigned value is not negligible, and evaluations could be affected."

In case the client's result is the only one in the method group, no assigned value will be calculated, no target area shown, and no statistics calculated. In case there are only a few results in the client's own method group, the result can be compared to all method mean or to a group that is similar to the own method.

If a reference value (x_{ref}), is used as an assigned value, uncertainty of the assigned value and metrological traceability will be reported in the Final report letter.

Results reported with < or > -signs cannot be included in the statistics.

For information on report interpretation and performance evaluation, please see the "EQAS Interpretation guidelines" LabScala User instructions (top right corner? Help link).

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22.04.2024 3/3

LABQUALITY

External Quality Assessment Scheme

Pipette control Round 1, 2024

Specimens

Sample S001A (LQ754524011) and sample S001B (LQ754524012) were purified water. The samples were tested in an accredited Finnish reference laboratory. Based on the pre-testing and the results of this round, the samples were homogeneous, stable, and suitable for the external quality assessment scheme. The materials were sent without temperature control packaging.

Pre-test methods: direct and reverse pipetting. The target values were measured in a specialized calibration room with air humidifier and weighed with a scale equipped with evaporation trap.

The reference laboratory's measurement conditions before and after:

Air humidity: 25.2% Air temperature: 21.7°C Air pressure 1013 hPa

The target values for the samples were as follows:

S001A: 99.13 mg (+/-5%: 94.18 - 104.9) / direct pipetting / 100 μ L S001B: 995.41 mg (+/-5%: 945.64 - 1045.18) / direct pipetting / 1000 μ L

Report info

Description of the data analysis is provided on the last page of the laboratoryspecific reports and global reports. It is important to read the Final report first, as it contains important information about the samples and results in each round.

Comments - Expert

S001A and S001B where both aqueous materials. Direct pipetting is the recommended technique for aqueous solutions. The participants were instructed to pipette 100 μL from S001 with five parallel measurements. The participants were instructed to do the same for S001B, but with a volume of 1000 μL . The average of the five measurements was compared to the target value.

For S001A and S001B the pipette groups with maximum volumes $100-300~\mu L$ are shown in the global report in the same group and histogram. For S001B the participants were instructed to pipet a volume of 1000 μL . It is not recommended to use a pipette that cannot withdraw the whole volume in one operation. By using a pipette with lower volume and doing several pipetting steps to achieve the full volume gives great uncertainty to the pipette results and is not recommended.

Discussion of the results

For both samples and all pipette groups in this round, many reported results (around 80% for S001A and 70% for S001B) were above the target values. The distribution around the target value is different from the pilot round, where most reported results (around 90%) for the sample containing water were below the reference value.

By studying the results from the reference laboratory, we observe that there is a difference in the target values set for 100 μL water for this round compared to the pilot round. Target value for water this round was 99,13 mg (humidity 25,5%), and in the pilot round 100,37 mg (humidity 52%). The target shift may be one of the explanations for the change of the participants results related to target.

2024-04-22

FINAL REPORT

Product no. 8205

Subcontracting: Sample pretesting

 Samples sent
 2024-02-27

 Round closed
 2024-03-21

 Final report
 2024-04-22

Request for correction

Typing errors in laboratory's result forms are the laboratory's responsibility. Labquality accepts responsibility only for result processing. Requests must be notified by writing within three weeks from the date of this letter.

Authorized by

EQA Coordinator Riitta Viertola riitta.viertola@labquality.com

Expert

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Senior Biomedical laboratory scientist/PhD, Marit Sverresdotter Sylte, Bifo - Biomedical laboratory scientist research group, Department of Medical biochemistry and Pharmacology (MBF), Haukeland University Hospital, Norway

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When planning for pipetting, it is recommended to choose a pipette with a maximum volume close to the volume to be pipetted. For S001A the participants were instructed to pipette 100 μ L from the sample. There was a small difference in CV% between the max volume 100-300 μ L group and the 1000 μ L group, with a slightly better CV% for the first group which have the maximum value closest to the volume to be pipetted.

For S001B participants were instructed to pipette 1000 μ L from the sample. Of the pipettes used, 65% belonged to the max volume 1000 μ L group. CV% within this group was 0.8%, which is similar to the result from S001A for the pipettes with a maximum volume matching the volume tested.

Summary

For both samples most of the participants performed within the target area. The variation (CV%) was acceptable for both samples and all method groups, and slightly better for groups that used a pipette with maximum value close to the volume to be pipetted. The results emphasize the recommendation to select a pipette with a maximum volume close to the intended pipetting volume.

70-80% of the reported results from the participant laboratories are higher than the target value set by the reference laboratory under optimized conditions. Determination of the target value is crucial for consistent distribution of the results in relation to the target value over time. It is useful to participate in the pipette EQA round to increase competence in pipetting, and to be aware of variables that may affect both the target value and the participant's results.

It is important to point out that by participating in this pipette EQA round does not exclude laboratory's own calibrations or internal controls for the pipettes.

Preanalytics

The preanalytical pie-charts illustrate the pipette and scale calibration intervals, and whether the calibrations are carried out locally, centrally, or externally. The response rate for the pre-analytical case was 70% for the pipette questions and 67% for the questions about the scale. The responses show that 84% are calibrating their pipettes once a year, or more frequently. 89% are calibrating their scales once a year, or more frequently. The use of an external calibration agency is the most common way to perform calibrations, both for pipettes and for scales.

Annex 1. List of possible sources of uncertainty that may affect pipetting results.

Annex 2. Target area limits

End of report

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Annex 1. List of possible sources of uncertainty that may affect pipetting results.

- Insecure attachment of the pipette tip to the pipette
- Insufficient rinsing of the pipette tip
- Incorrect depth of the pipette tip in the liquid
- Incorrect angle during pipetting
- Not wiping the pipette tip on the cylinder wall
- Not using an evaporation trap

In addition, the general quality of the pipettes, varying expertise in pipetting, use of the scale, and environmental conditions during the weighing as e.g., air pressure, temperature, and vibrations may be reflected in the weighing results.

Annex 2. Target area limits

The limits of the target area have been set to 5% since the reported results may contain many uncertainties and the participation imposed few requirements for standardized conditions. In comparison, when calibrating pipettes according to NS-EN ISO 8655-2:2022, the pipettes would be approved with a demonstrated systematic deviation less than 0,8% when calibrated at 100% of the pipettes maximum volume, and correspondingly 8% at 10% of the pipettes maximum volume.