Australian Government
Department of Industry, Innovation and Science
National Measurement Institute

Proficiency Test Report
AQA 18-11
Heroin

November 2018

ACKNOWLEDGMENTS

This study was conducted by the National Measurement Institute (NMI). Support funding was provided by the Australian Government Department of Industry, Innovation and Science.

I would like to thank the management and staff of the participating laboratories for supporting the study. It is only through widespread participation that we can provide an effective service to laboratories.

The assistance of the following NMI staff members in the planning, conduct and reporting of the study is acknowledged.

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SUMMARY

AQA 18-11 was conducted in July 2018. Three test samples of heroin hydrochloride were sent to twenty-nine laboratories. Two laboratories submitted extra sets of results analysed independently by different analysts.

The assigned values were the robust average of participants' results.

Traceability: The consensus of participants' results is not traceable to any external reference, so although expressed in SI units, metrological traceability has not been established.

The outcomes of the study were assessed against the aims as follows:

- *assess the proficiency of laboratories measuring heroin in samples typical of a routine seizure;*

Laboratory performance was assessed by z-score and E_n-score.

Laboratories **5**, **6**, **11**, **15**, **17**, **18**, **21**, **22**, **24**, **29** and **30** returned satisfactory z and E_n-scores for all results.

Of the 90 results for which z-scores were calculated, 61 (68%) returned $|z| \leq 2$ indicating a satisfactory performance.

Of the 90 results for which $|E_n|$-scores were calculated, 62 (69%) returned $|E_n| \leq 1$ indicating agreement of the participants' results with the assigned value within their respective expanded uncertainties.

- *develop a practical application of traceability and measurement uncertainty and provide participants with information that will assist uncertainty estimates; and*

Eighty-four of the ninety numeric results (93%) were reported with an associated expanded uncertainty. Laboratories **2** and **4** did not report uncertainty. These laboratories were not accredited.

Laboratory **16** reported significantly different estimates of uncertainty for heroin in the duplicate pair samples S1 and S2.

The magnitude of reported uncertainties was within the range 1% to 50% relative.

- *test the ability of participants to identify a cutting agent commonly found in controlled drug preparation*

Samples were prepared using an illicit seizure of heroin hydrochloride, approximately 74.4% base (m/m) supplied by the Australian Federal Police. The study coordinator added paracetamol in duplicate pair Samples S1 and S2, and paracetamol and caffeine in Sample S3.

Twenty-eight participants (93%) reported on the identity of the cutting agents and twenty-seven participants correctly identified paracetamol in Samples S1 and S2 and both paracetamol and caffeine in Sample S3.

1 INTRODUCTION

1.1 NMI Proficiency Testing Program

The National Measurement Institute (NMI) is responsible for Australia's national measurement infrastructure, providing a range of services including a chemical proficiency testing program.

Proficiency testing (PT) is: 'evaluation of participant performance against pre-established criteria by means of interlaboratory comparison.'[1] NMI PT studies target chemical testing in areas of high public significance such as trade, environment, law enforcement and food safety. NMI offers studies in:

- pesticide residues in fruit and vegetables, soil and water;
- petroleum hydrocarbons in soil and water;
- PFAS in water, soil and biota;
- metals in soil, water, food and pharmaceuticals;
- controlled drug assay and clandestine laboratory;
- allergens in food; and
- folic acid in flour.

1.2 Study Aims

The aims of the study were to:

- assess the proficiency of laboratories measuring heroin in samples typical of a routine seizure;
- develop a practical application of traceability and measurement uncertainty and provide participants with information that will assist uncertainty estimates; and
- test the ability of participants to identify a cutting agent commonly found in controlled drug preparation.

The choice of the test method was left to the participating laboratories.

1.3 Study Conduct

NMI is accredited by the National Association of Testing Authorities, Australia (NATA) to ISO 17043[1] as a provider of proficiency testing schemes. This controlled drug proficiency test is within the scope of NMI's accreditation.

The conduct of NMI proficiency tests is described in the NMI Chemical Proficiency Testing Study Protocol.[2] The statistical methods used are described in the NMI Chemical Proficiency Testing Statistical Manual.[3] These documents have been prepared with reference to ISO 17043 and The International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories.[4]

2 STUDY INFORMATION

2.1 Study Timetable

The timetable of the study was:

Invitation issued:	23 May 2018
Samples dispatched:	23 July 2018
Results due:	2 October 2018
Interim report issued:	5 October 2018

2.2 Participation

A total of ninety-five international, national, state government and private laboratories were invited to participate.

Twenty-nine laboratories agreed to participate and twenty-eight submitted results. These laboratories are listed in Appendix 1. Two laboratories requested two sets of test samples in order to be analysed by different analysts and reported two sets of results.

2.3 Test Material Specification

Three test samples were prepared in May 2018. The starting material was heroin hydrochloride approximately 74.4% base (m/m) supplied by the Australian Federal Police. Paracetamol and caffeine purchased from Sigma Aldrich were used as cutting agents. Paracetamol was used to prepare Samples S1 and S2, while paracetamol and caffeine were used for Sample S3.

The heroin was ground and sieved through a 180 μm sieve. The cutting agents were processed similarly to the heroin powder.

Test samples were prepared by mixing a known mass of sieved drug material with a known mass of sieved cutting agent in a tumbler overnight.

Portions of 150 mg of each of the test samples were weighed into labelled glass vials.

Sample S1 was prepared to contain ~38% heroin base (m/m).

Sample S2 was prepared to contain ~38% heroin base (m/m) (duplicate of S1).

Sample S3 was prepared to contain ~18% heroin base (m/m).

2.4 Laboratory Code

Each participant was randomly assigned a confidential laboratory code.

2.5 Test Sample Homogeneity

The preparation of homogeneous test samples is an important part of a proficiency testing study. Given the small (<150 mg) test portions normally used for controlled substances analysis the particle size must be sufficiently small and uniformly distributed to ensure minimal influence on analytical precision.

The procedure for the preparation of the study samples has been validated in previous studies. No homogeneity testing was conducted in this proficiency study. Results returned by the participants gave no reason to question the homogeneity of the test samples.

2.6 Sample Dispatch and Receipt

A set of three samples, each containing approximately 150 mg of test material, were dispatched on 23 July 2018.

The following items were packaged with the samples:

- a covering letter with instructions for participants; and
- a form for participants to confirm the receipt of the test samples.

An Excel spreadsheet for the electronic reporting of results was e-mailed to participants.

2.7 Instructions to Participants

Participants were asked to analyse the samples using their routine quantitative method and return the following information:

- one result for each sample as % (m/m) heroin base;
- an estimate of the expanded uncertainty associated with the result as % (m/m) heroin base at the 95% confidence level;
- brief detail on how the uncertainty was calculated e.g. uncertainty budget method;
- the identity of the cutting agents in all three samples, if part of routine analysis;
- origin and stated purity of the analytical reference standard used;
- brief summary of the quantitative method used;
- the completed results sheet by 21 September 2018, as late results cannot be included in the report; and
- any other comment.

2.8 Interim Report

An interim report was emailed to all participants on 5 October 2018.

Laboratory 16 results were omitted from the interim report. This laboratory reported results before the study's closing date, however NMI received the results after the interim report was issued.

3 PARTICIPANT LABORATORY INFORMATION

3.1 Test Method Summaries

Participants' method summaries are presented for information in Table 1.

Table 1 Summary of Participants' Test Methods

Lab. Code	Extraction solvent	Internal standard	Calib. points	Technique	Detector	Column
1	ACN/MeOH/H2O	Analog off heroin	7	UPLC	MSMS	C-18 coloumn
2	Ethanol	Propylparaben	5	UPLC	DAD	BEH shield RP18
3	20:80 acetonitrile/water		5	HPsLC	UV	Kinetex 5u C18
4	Methanol	Loxapine	5	HPLC	DAD	Xterra C18
5	Acetonitrile/Water (75:25)	Benzocaine	3	UPLC	PDA	Acquity UPLC BEH C18 1.7µm (2.1x100mmm)
6	Acetonitrile/Methanol(95:5)	Pholcodine 1mg/Ml	3	UPLC	PDA	TY C-18
7	MeOH	Mepivacaine	4	HPLC	DAD	C18
8	Ethanol	Triphenylacetophenone (TPAP) Dichloromethane (30ml per L) was used to dissolve the TPAP	3	GC	FID	HP-1MS
Pa9	Acetonitrile	Strychnine	6	GC	FID	HP-1
10	water/acetonitrile/ n10 sulphuric acid 90:10:1		3	HPLC	Diode array	Shimpack XR-ODS
11	Phosphate buffer with 75% aceto nitrile		5	HPLC	UV-DAD	XTERRA TM C18 reversed phase
12	Acetonitrile		6	UPLC	UV	Acquity UPLC C18 1.7um 2.1 x 100mm
13	ethanol/DMF (9/1)	Tribenzylamine	5	GC	FID	HP1
14	Methanol	Methadone	4	GC	FID	RXI-5MS
15	chloroform	benzopinacolone	5	GC	FID	HP5
16	Pyridine	Tropine	5	GC	MS	DB-5MS
17	Methanol	Alprazolam	1	LC	DAD	Hypersil-5-ODS
18	dichloromethane	5a-cholestane	5	GC	FID	HP5

Lab. Code	Extraction solvent	Internal standard	Calib. points	Technique	Detector	Column
19	Acetonitrile		6	UPLC	DAD	Acquity UPLC® BEH C18 1.7µm 2.1 x 100 mm Column
20	Acetic acid Acetonitrile Water		4	HPLC	UV DAD	POROSHELL 120 EC-C18
21	Eluent: Acetonitrile, ammonium acetate, diethylamine and water		3	HPLC	Diode Array	LiChrosphere RP-18 (5 um)
22	acetonitrile/water (86/14)		4	HPLC	UV	NH2
23	Methanol	Diazepam	6	GC	FID	J&W 128-5512
24	Methanol	Alprazolam	1	LC	DAD	Hypersil-5-ODS
25	Methanol	No (External Standard)	7	HPLC	DAD	Poroshell 120 EC-C18 (4.6x150; 2.7 microns pore size)
27	Ammonium Formate, pH 3		4	LC	MS	Ascentis Express Phenyl-Hexyl (2,7 µm)
28	Deuterium oxide	maleic acid		1H QNMR	Bruker AV III 600 with BBFO probe	N/A
29	Acetonitrile / water		1	HPLC	UV	Kromasil
30	Methanol		5	HPLC	DAD	Kinetex C-18-XB
31	Methanol		3	HPLC	DAD	Luna 5µm silica (2) 100A 150 x 4.6mm

3.2 Reported Basis of Participants' Measurement Uncertainty Estimates

Participants' responses as received are listed in Table 2.

Table 2 Reported Basis of Uncertainty Estimate

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation		Guide Document for Estimating MU
		Precision*	Method Bias	
1	Top Down - precision and estimates of the method and laboratory bias	Control Samples – RM		
3	Top Down - precision and estimates of the method and laboratory bias	Duplicate Analysis	Recoveries of Spiked Samples	Eurachem/CITAC Guide
5	Top Down - precision and estimates of the method and laboratory bias	Control Samples – RM Duplicate Analysis	Standard Purity Homogeneity of Sample	Eurachem/CITAC Guide
6	Top Down - precision and estimates of the method and laboratory bias	Control Samples – CRM Duplicate Analysis	Standard Purity	Nata Technical Note 33
7	Top Down - precision and estimates of the method and laboratory bias	Control Samples	Laboratory bias from PT studies Recoveries of Spiked Samples Standard Purity Matrix Effects Instrument Calibration Masses and Volumes Homogeneity of Sample	EA-4/16: 2003 and ILAG G-17:2002
8	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram)	Control Samples – CRM Duplicate Analysis	Recoveries of Spiked Samples Standard Purity Matrix Effects Instrument Calibration Masses and Volumes Homogeneity of Sample	Eurachem/CITAC Guide
9	Top Down - reproducibility (standard deviation) from PT studies used directly	Control Samples – RM Duplicate Analysis	Standard Purity Matrix Effects Instrument Calibration Masses and Volumes	
10	Professional judgment	Control Samples – CRM Duplicate Analysis	Standard Purity Instrument Calibration	ISO/GUM
11	Top Down - precision and estimates of the method and laboratory bias	Control Samples – RM	Laboratory bias from PT studies	Nordtest Report TR537
12	Standard deviation of replicate analyses multiplied by 2 or 3	Control Samples – RM Duplicate Analysis	Standard Purity Instrument Calibration Homogeneity of Sample	Nata Technical Note 33
13	Top Down - precision and estimates of the method and laboratory bias	Control Samples – RM	Standard Purity	
14	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate Analysis	Masses and Volumes	ISO/GUM

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation		Guide Document for Estimating MU
		Precision*	Method Bias	
15	Top Down - precision and estimates of the method and laboratory bias	Control Samples – previously analysed police seizures Duplicate Analysis	Standard Purity Matrix Effects Instrument Calibration Masses and Volumes Homogeneity of Sample	Eurachem/CITAC Guide
16	Top Down - precision and estimates of the method and laboratory bias	Control Samples - CRM		
17	Top Down - precision and estimates of the method and laboratory bias	Control Samples – SS Duplicate Analysis	Laboratory bias from PT studies Recoveries of Spiked Samples	Eurachem/CITAC Guide
18	repeatability, sample heterogeneity (ENFSI quantitative sampling guideline)	Control Samples – RM Duplicate Analysis	Homogeneity of Sample	Eurachem/CITAC Guide
19	Top Down - precision and estimates of the method and laboratory bias	Control Samples – RM Duplicate Analysis	Standard Purity Instrument Calibration Masses and Volumes Homogeneity of Sample	Nata Technical Note 33
20		Control Samples – RM	Standard Purity	ISO 5725-2 & ISO/TS 21748
21	Uncertainty Budget Method	Control Samples – RM Duplicate Analysis	Standard Purity Instrument Calibration Masses and Volumes	In-house document "Uncertainty of Measurement"
22	Top Down - precision and estimates of the method and laboratory bias	Control Samples - RM	Laboratory bias from PT studies Standard Purity	Norme NF V03-110
23	Estimating Measurement Uncertainty by black box by pairs of values			Guide ENAC G 09 or ISO 21748
24	Top Down - precision and estimates of the method and laboratory bias	Control Samples - SS Duplicate Analysis	Laboratory bias from PT studies Recoveries of Spiked Samples	Eurachem/CITAC Guide
25	Top Down - precision and estimates of the method and laboratory bias	Control Samples - CRM Duplicate Analysis	Laboratory bias from PT studies Standard Purity Instrument Calibration Masses and Volumes Homogeneity of Sample	Eurachem/CITAC Guide
27	Standard deviation of replicate analyses multiplied by 2	Control Samples – Real samples from a police case	Laboratory bias from PT studies	Nordtest Report TR537
28	The larger of (standard deviation of duplicate measurements multiplied by 3) and (bottom-up propagation of errors)	Duplicate Analysis	Standard Purity Instrument Calibration Masses and Volumes	Nata Technical Note 33
29	Standard deviation of replicate analyses multiplied by 2 or 3	Control Samples - RM		ISO/GUM

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation		Guide Document for Estimating MU
		Precision*	Method Bias	
30		Control Samples - CRM Duplicate Analysis	Standard Purity Recoveries of Spiked Samples Instrument Calibration	Eurachem/CITAC Guide
31	Professional judgment	Control Samples - CRM Duplicate Analysis	Standard Purity Instrument Calibration	ISO/GUM

*SS = Spiked Samples, RM = Reference Material, CRM = Certified Reference Material

3.3 Details of Participant Calibration Standards

Participants' responses as received are listed in Table 3.

Table 3 Participant Calibration Standard

Lab. Code	Reference Standard*	Purity (%)
1	Lipomed	100
2	LGC standard	99.4
3	Johnson Matthey	99.5
4	Lipomed 1 mg/ml	>98
5	NMI	99.4 ± 2.0
6	NMI	99.4
7	Lipomed	99.6
8	NMI	99.4
9	NMI	99.4
10	LGC	99.7
11	Johnson Matthey	100
12	NMI	99.4
13	LGC	99.4
14	Sigma Aldrich (Cerilliant)	99.4
15	NMI	99.4
16	NMI	99.4
17	Lipomed	99.827 ± 0.006
18	Macfarlan Smith	99.9
19	NMI	99.4
20	Lipomed	99.95
21	NMI	99.4
22	LIPOMED	99.827
23	LIPOMED	99.1
24	Lipomed	99.827 ± 0.006
25	LIPOMED	99.6
27	Norsk medisinal depot	ca 100
28	Sigma Aldrich Prod. no. 92816	99.98±0.13
29	Lipomed	99.6
30	NMI	99.4 +/- 2.0
31	MacFarlan Smith	99.1

3.4 Participants' Comments

The study manager welcomes comments or suggestions from participants as it provides information which will improve future studies. All returns are listed as received in Table 4 along with the study manager's response, where appropriate.

Table 4 Participant Comments

Lab. Code	Participant comments	Study Manager's response
10	MuM determined from 3 x std deviation of multiple injections expanded by professional judgement. No analysis carried out for inert bulking agents	
21	Insufficient sample to repeat analysis if needed. S3 failed to meet internal quality control requirements. Due to the limited quantity of sample received, insufficent sample was available to repeat analysis to determine purity.	Most participants use less than 50 mg for each analysis. For reasons of security and accountability, NMI conducts these PTs using the minimum practical amount of drug.
22	send samples of different grades	Samples S1 and S2 were prepared as duplicates, and sample S3 was prepared to be of lower concentration than S1 and S2.
25	Qualitative analysis was carried out by GC-MS	

4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

4.1 Results Summary

Participant results are listed in Tables 5 to 7 with resultant summary statistics: mean, median, maximum, minimum, robust average, robust standard deviation (Robust SD) and robust coefficient of variation (Robust CV).

Bar charts of results and performance scores are presented in Figures 2 to 4.

An example chart with interpretation guide is shown in Figure 1.

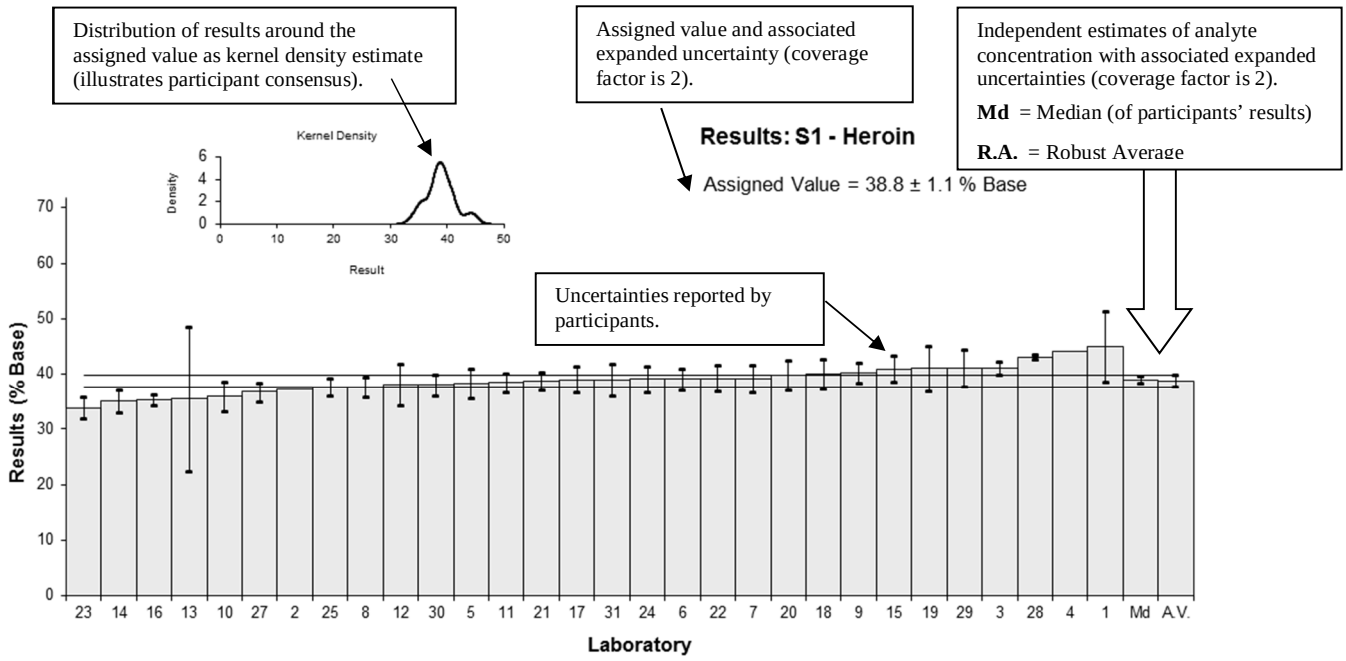


Figure 1 Guide to Presentation of Results

4.2 Assigned Value

The assigned value is defined as: 'value attributed to a particular quantity and accepted, sometimes by convention, as having an uncertainty appropriate for a given purpose'. [1]

For a proficiency test, the assigned value is the best available measurement of the true concentration of an analyte in the test sample.

4.3 Robust Average

The robust averages and associated expanded measurement uncertainties were calculated using the procedure described in 'Statistical methods for use in proficiency testing by inter-laboratory comparisons, ISO 13528:2015(E)'.[5]

4.4 Robust Between-Laboratory Coefficient of Variation

The robust between-laboratory coefficient of variation (robust CV) is a measure of the variability of participants' results and was calculated using the procedure described in ISO 13528:2015(E).[5]

4.5 Target Standard Deviation

The target standard deviation (σ) is the product of the assigned value (*X)* and the performance coefficient of variation (PCV) as presented in Equation 1. This value is used for calculation of participant z-score.

$\sigma = X * PCV$ *Equation 1*

It is important to note that the PCV is a fixed value established by the study coordinator and is not the standard deviation of participants' results. By setting a fixed value for the PCV, the participants' performance can be compared from study to study.

4.6 z-Score

For each participant result a z-score is calculated according to Equation 2 below:

$$z = \frac{(\chi - X)}{\sigma} \qquad \textit{Equation 2}$$

where:

z is z-score

χ is participants' result

X is the assigned value

σ is the target standard deviation from equation 1

A z-score with absolute value (|z|):

- $|z| \leq 2$ is satisfactory;
- $2 < |z| < 3$ is questionable;
- $|z| \geq 3$ is unsatisfactory.

4.7 E_n-Score

The E_n-score is complementary to the z-score in assessment of laboratory performance. E_n-score includes measurement uncertainty and is calculated according to Equation 3 below:

$$E_n = \frac{(\chi - X)}{\sqrt{U_\chi^2 + U_X^2}} \qquad \textit{Equation 3}$$

where:

E_n is E_n-score

χ is a participants' result

X is the assigned value

U_χ is the expanded uncertainty of the participants' result

U_X is the expanded uncertainty of the assigned value

An E_n-score with absolute value ($|E_n|$):

- $|E_n| \leq 1$ is satisfactory;
- $|E_n| > 1$ is unsatisfactory.

4.8 Traceability and Measurement Uncertainty

Laboratories accredited to ISO/IEC Standard 17025:2017[6] must establish and demonstrate the traceability and measurement uncertainty associated with their test results. Guidelines for quantifying uncertainty in analytical measurement are described in the Eurachem /CITAC Guide. [7]

5 TABLES AND FIGURES

Table 5

Sample Details

Sample No.	S1
Matrix.	Powder
Analyte.	Heroin
Units	% Base (m/m)

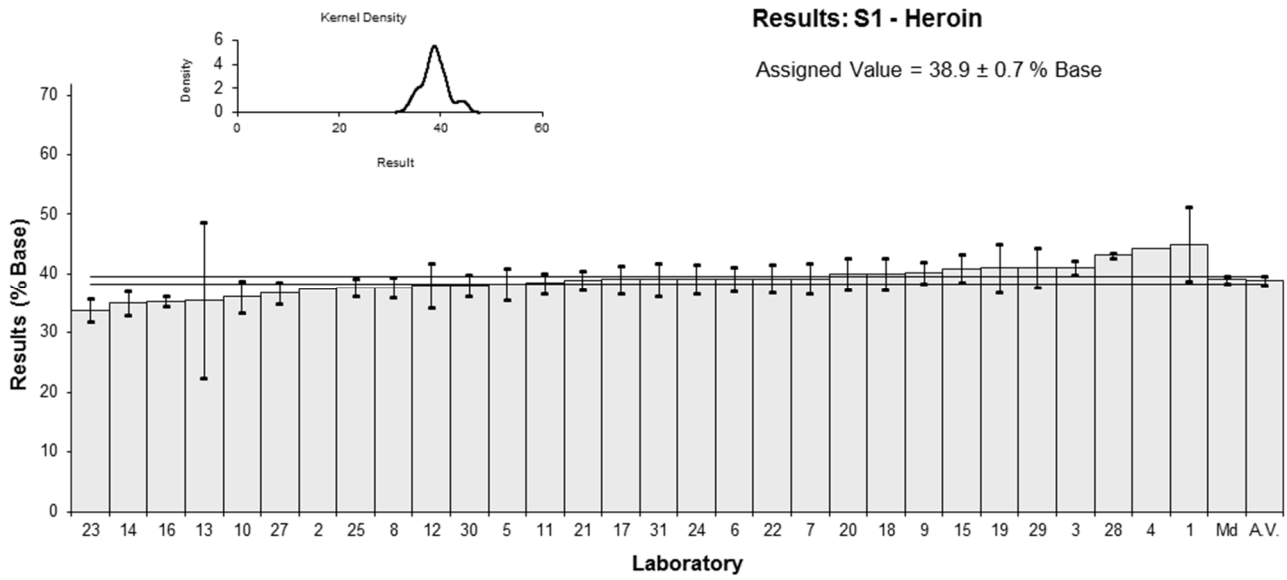
Participant Results

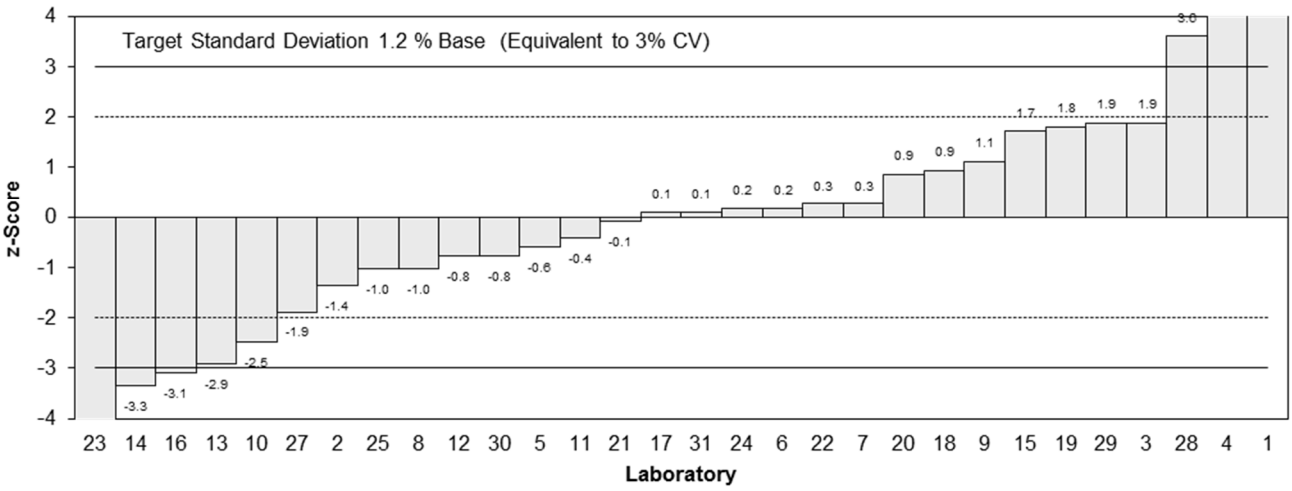
Lab Code	Result	Uncertainty	z-Score	E_n-Score
1	45	6.3	5.23	0.96
2	37.3	NR	-1.37	-2.29
3	41.1	1.2	1.89	1.58
4	44.2	NR	4.54	7.57
5	38.2	2.7	-0.60	-0.25
6	39.1	2	0.17	0.09
7	39.2	2.5	0.26	0.12
8	37.7	1.80	-1.03	-0.62
9	40.2	1.8	1.11	0.67
10	36	2.7	-2.49	-1.04
11	38.4	1.7	-0.43	-0.27
12	38	3.8	-0.77	-0.23
13	35.5	13.1	-2.91	-0.26
14	35	2	-3.34	-1.84
15	40.9	2.38	1.71	0.81
16	35.3	0.9	-3.08	-3.16
17	39.0	2.4	0.09	0.04
18	40.0	2.7	0.94	0.39
19	41	4.1	1.80	0.50
20	39.9	2.7	0.86	0.36
21	38.8	1.6	-0.09	-0.06
22	39.2	2.4	0.26	0.12
23	33.8	1.9	-4.37	-2.52
24	39.1	2.4	0.17	0.08
25	37.7	1.6	-1.03	-0.69
27	36.7	1.8	-1.89	-1.14
28	43.1	0.4	3.60	5.21
29	41.1	3.3	1.89	0.65
30	38	1.9	-0.77	-0.44
31	39	2.9	0.09	0.03

Statistics

Assigned Value*	38.9	0.7
Robust Average	38.8	1.1
Median	39.0	0.7
Mean	38.9	
N	30	
Max.	45	
Min.	33.8	
Robust SD	2.4	
Robust CV	6.2%	

[a]The assigned value was calculated as the robust average of the combined results of duplicate pair Samples S1 and S2.


Kernel Density
Density
Result
Results: S1 - Heroin
Assigned Value = 38.9 ± 0.7 % Base
Results (% Base)
Laboratory
23 14 16 13 10 27 2 25 8 12 30 5 11 21 17 31 24 6 22 7 20 18 9 15 19 29 3 28 4 1 Md A.V.


z-Scores: S1 - Heroin
Target Standard Deviation 1.2 % Base (Equivalent to 3% CV)
z-Score
Laboratory
23 14 16 13 10 27 2 25 8 12 30 5 11 21 17 31 24 6 22 7 20 18 9 15 19 29 3 28 4 1

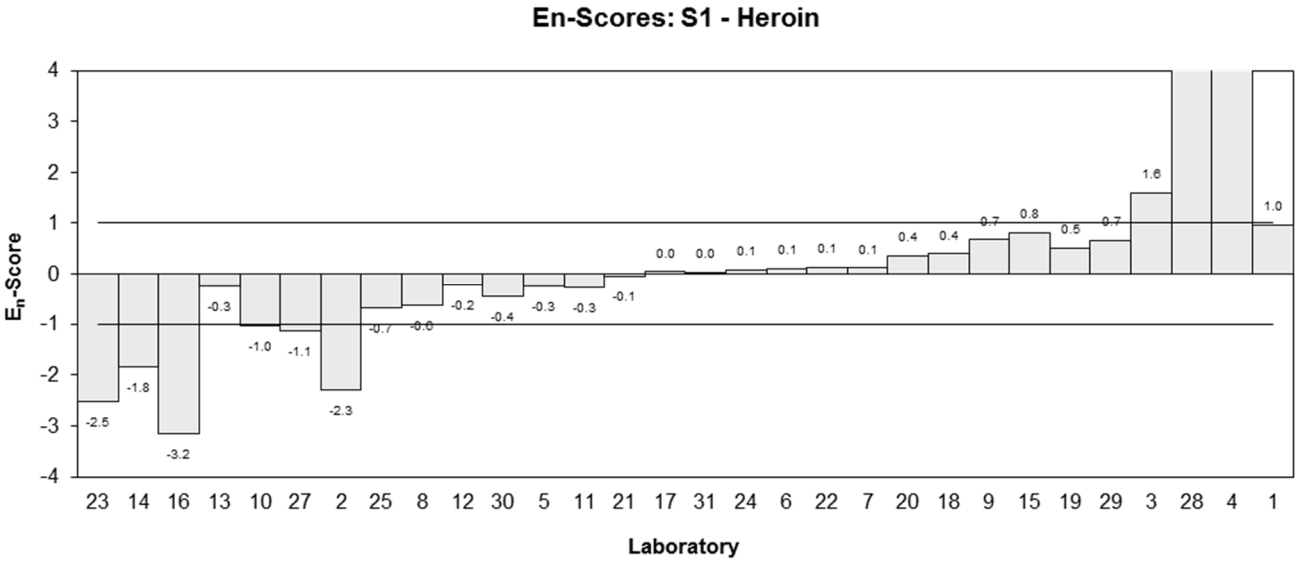

En-Scores: S1 - Heroin
En-Score
Laboratory
23 14 16 13 10 27 2 25 8 12 30 5 11 21 17 31 24 6 22 7 20 18 9 15 19 29 3 28 4 1

Figure 2

Table 6

Sample Details

Sample No.	S2
Matrix.	Powder
Analyte.	Heroin
Units	% Base (m/m)

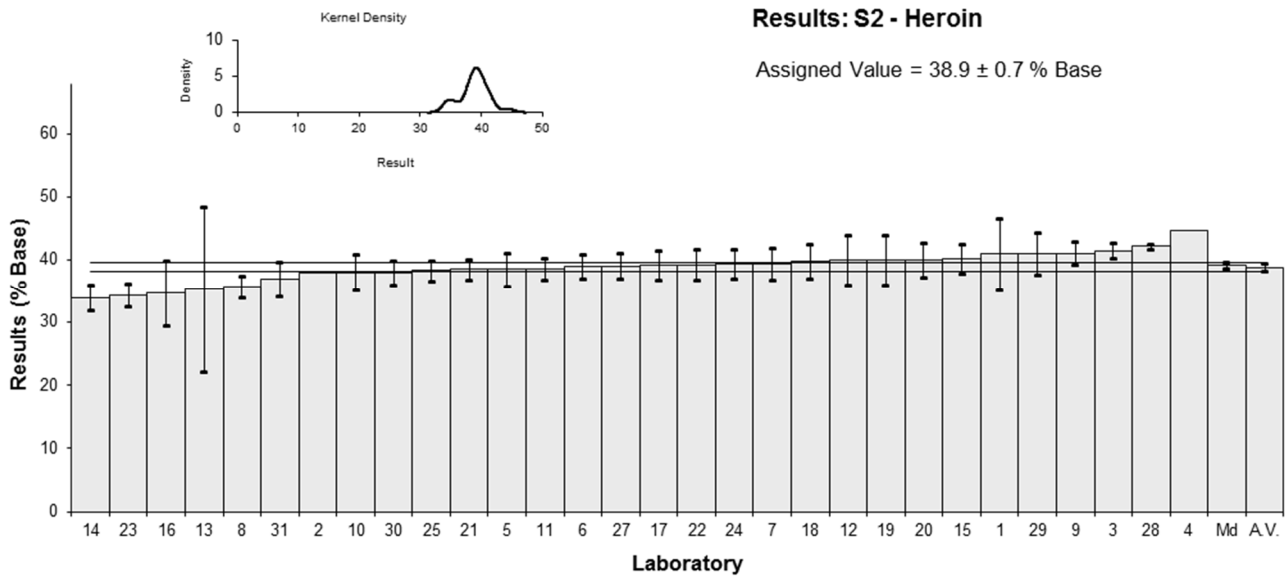
Participant Results

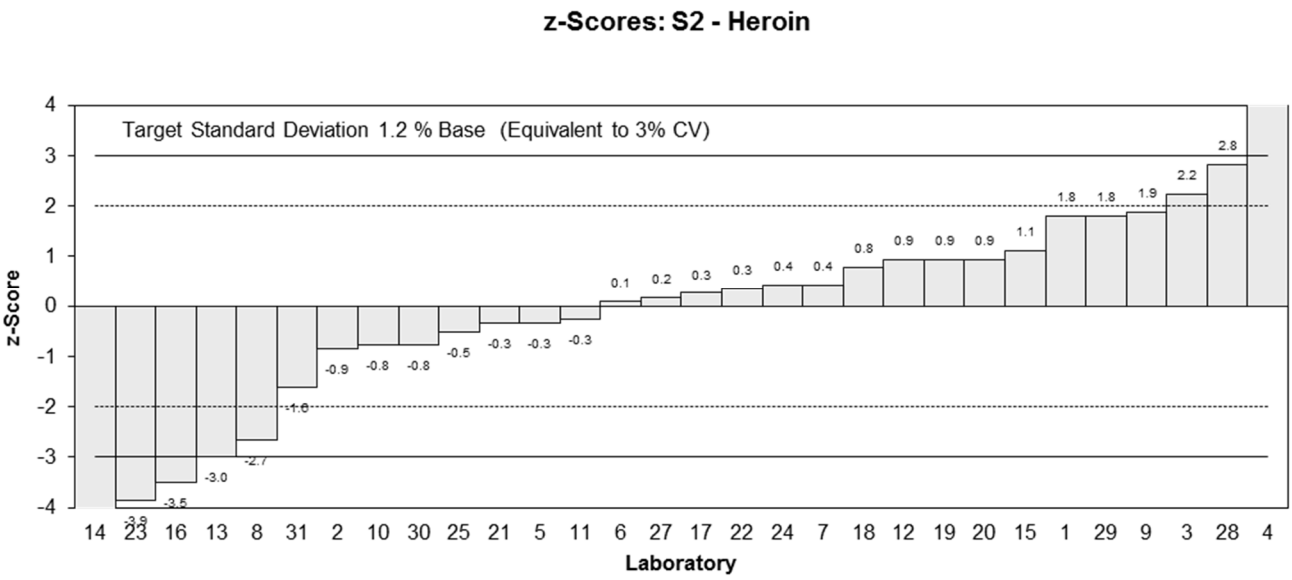
Lab Code	Result	Uncertainty	z-Score	E_n-Score
1	41	5.7	1.80	0.37
2	37.9	NR	-0.86	-1.43
3	41.5	1.2	2.23	1.87
4	44.7	NR	4.97	8.29
5	38.5	2.7	-0.34	-0.14
6	39.0	2	0.09	0.05
7	39.4	2.6	0.43	0.19
8	35.8	1.71	-2.66	-1.68
9	41.1	1.8	1.89	1.14
10	38	2.85	-0.77	-0.31
11	38.6	1.7	-0.26	-0.16
12	40	4.0	0.94	0.27
13	35.4	13.1	-3.00	-0.27
14	34	2	-4.20	-2.31
15	40.2	2.34	1.11	0.53
16	34.8	5.2	-3.51	-0.78
17	39.2	2.4	0.26	0.12
18	39.8	2.7	0.77	0.32
19	40	4.0	0.94	0.27
20	40.0	2.7	0.94	0.39
21	38.5	1.6	-0.34	-0.23
22	39.3	2.4	0.34	0.16
23	34.4	1.9	-3.86	-2.22
24	39.4	2.4	0.43	0.20
25	38.3	1.7	-0.51	-0.33
27	39.1	2.0	0.17	0.09
28	42.2	0.4	2.83	4.09
29	41.0	3.3	1.80	0.62
30	38	1.9	-0.77	-0.44
31	37	2.7	-1.63	-0.68

Statistics

Assigned Value*	38.9	0.7
Robust Average	38.9	1.0
Median	39.2	0.6
Mean	38.9	
N	30	
Max.	44.7	
Min.	34	
Robust SD	2.2	
Robust CV	5.7%	

[a]The assigned value was calculated as the robust average of the combined results of duplicate pair Samples S1 and S2.


Kernel Density
Density
Result
Results: S2 - Heroin
Assigned Value = 38.9 ± 0.7 % Base
Results (% Base)
Laboratory
14 23 16 13 8 31 2 10 30 25 21 5 11 6 27 17 22 24 7 18 12 19 20 15 1 29 9 3 28 4 Md A.V.


z-Scores: S2 - Heroin
Target Standard Deviation 1.2 % Base (Equivalent to 3% CV)
z-Score
Laboratory
14 23 16 13 8 31 2 10 30 25 21 5 11 6 27 17 22 24 7 18 12 19 20 15 1 29 9 3 28 4

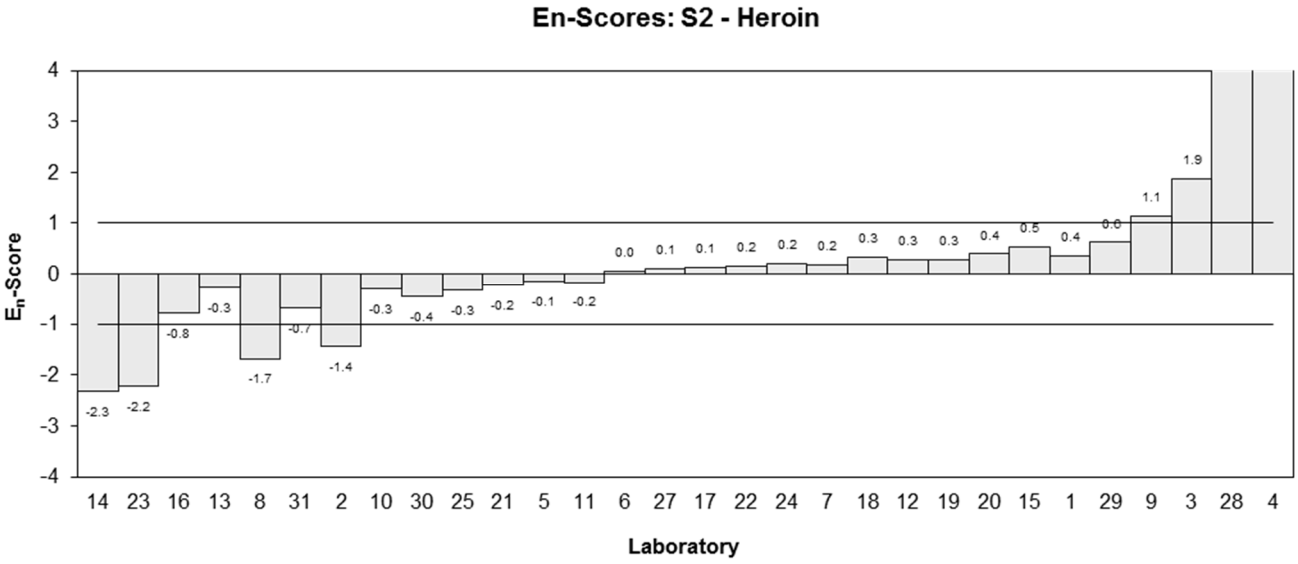

En-Scores: S2 - Heroin
En-Score
Laboratory
14 23 16 13 8 31 2 10 30 25 21 5 11 6 27 17 22 24 7 18 12 19 20 15 1 29 9 3 28 4

Figure 3

Table 7

Sample Details

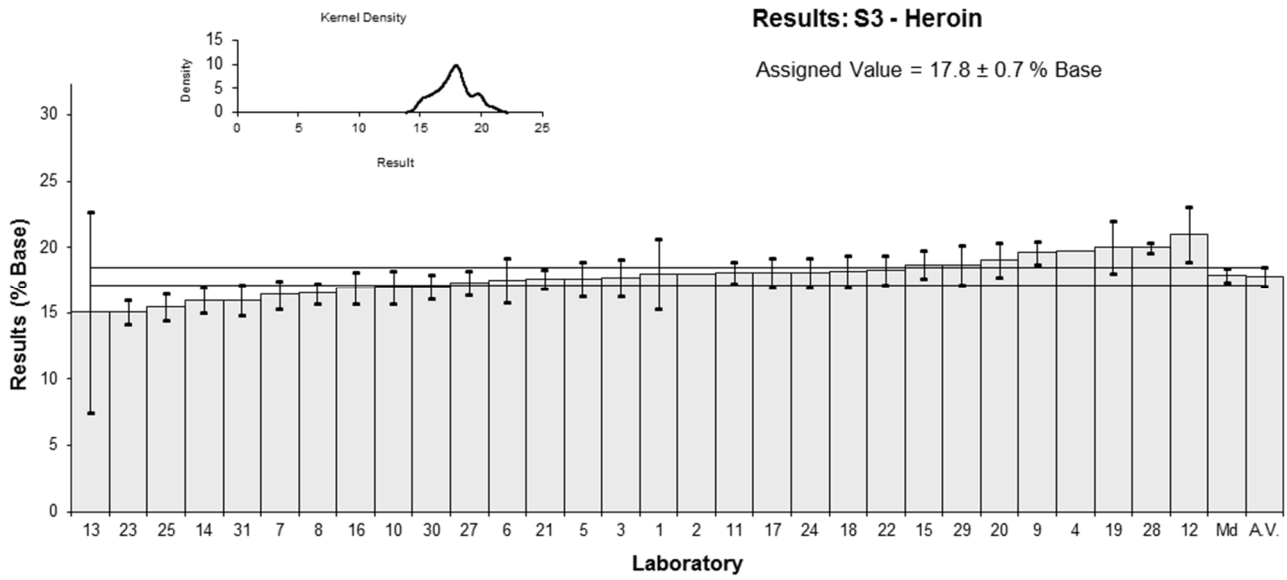
Sample No.	S3
Matrix.	Powder
Analyte.	Heroin
Units	% Base (m/m)

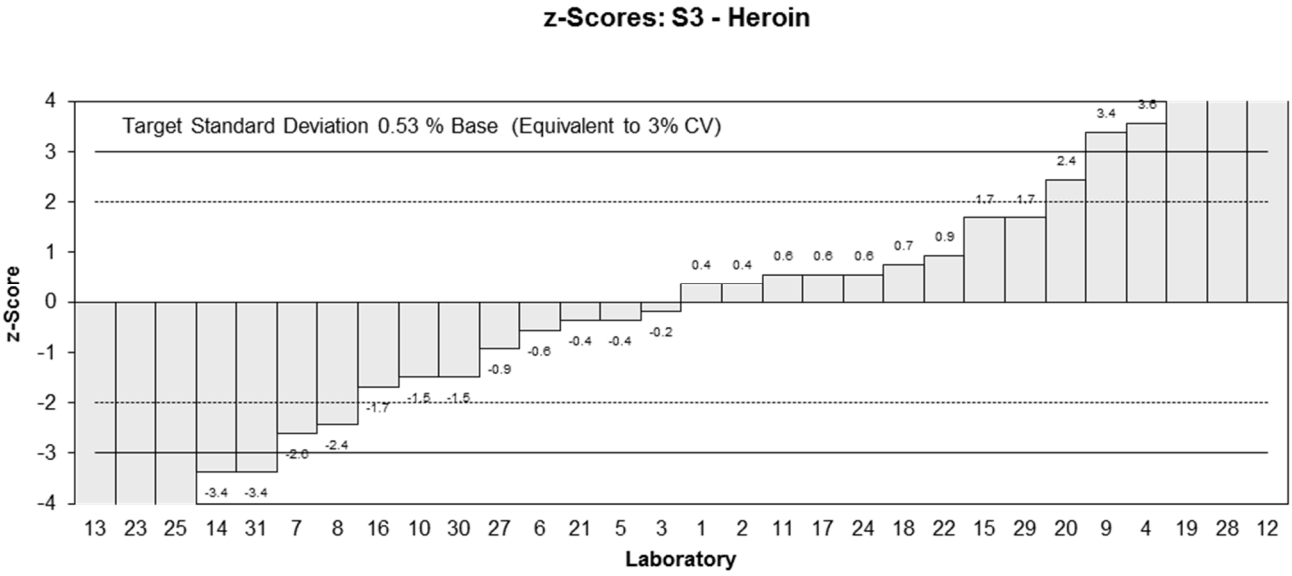
Participant Results

Lab Code	Result	Uncertainty	z-Score	E_n-Score
1	18	2.7	0.37	0.07
2	18.0	NR	0.37	0.29
3	17.7	1.4	-0.19	-0.06
4	19.7	NR	3.56	2.71
5	17.6	1.3	-0.37	-0.14
6	17.5	1.7	-0.56	-0.16
7	16.4	1.1	-2.62	-1.07
8	16.5	0.79	-2.43	-1.23
9	19.6	0.9	3.37	1.58
10	17	1.28	-1.50	-0.55
11	18.1	0.8	0.56	0.28
12	21	2.1	5.99	1.45
13	15.1	7.6	-5.06	-0.35
14	16	1	-3.37	-1.47
15	18.7	1.09	1.69	0.69
16	16.9	1.2	-1.69	-0.65
17	18.1	1.1	0.56	0.23
18	18.2	1.2	0.75	0.29
19	20	2.0	4.12	1.04
20	19.1	1.3	2.43	0.88
21	17.6	0.7	-0.37	-0.20
22	18.3	1.1	0.94	0.38
23	15.1	0.9	-5.06	-2.37
24	18.1	1.1	0.56	0.23
25	15.5	1.0	-4.31	-1.88
27	17.3	0.9	-0.94	-0.44
28	20.0	0.4	4.12	2.73
29	18.7	1.5	1.69	0.54
30	17	0.9	-1.50	-0.70
31	16	1.2	-3.37	-1.30

Statistics

Assigned Value	17.8	0.7
Robust Average	17.8	0.7
Median	17.9	0.5
Mean	17.8	
N	30	
Max.	21	
Min.	15.1	
Robust SD	1.6	
Robust CV	9 %	


Kernel Density
Density
Result
Results: S3 - Heroin
Assigned Value = 17.8 ± 0.7 % Base
Results (% Base)
13 23 25 14 31 7 8 16 10 30 27 6 21 5 3 1 2 11 17 24 18 22 15 29 20 9 4 19 28 12 Md A.V.
Laboratory


z-Scores: S3 - Heroin
Target Standard Deviation 0.53 % Base (Equivalent to 3% CV)
z-Score
13 23 25 14 31 7 8 16 10 30 27 6 21 5 3 1 2 11 17 24 18 22 15 29 20 9 4 19 28 12
Laboratory

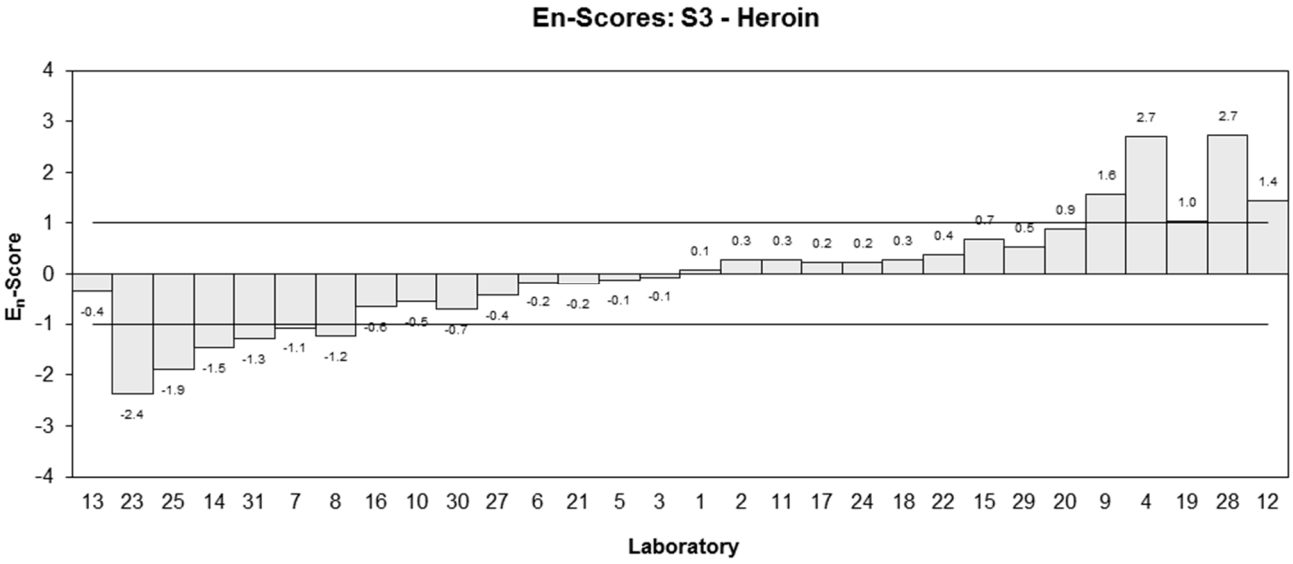

En-Scores: S3 - Heroin
En-Score
13 23 25 14 31 7 8 16 10 30 27 6 21 5 3 1 2 11 17 24 18 22 15 29 20 9 4 19 28 12
Laboratory

Figure 4

Table 8 Participants' identification of cutting agents

Lab Code	Cutting agents		
	S1	S2	S3
1	Paracetamol	Paracetamol	Caffeine, Paracetamol
2	Acetaminophen : 41.7 %	Acetaminophen : 42.6 %	Caffeine : 5.6 % Acetaminophen : 62.5 %
3	paracetamol	paracetamol	paracetamol, caffeine
4	ACETAMINOPHEN	ACETAMINOPHEN	ACETAMINOPHEN CAFFEINE
5	Paracetamol	Paracetamol	Paracetamol, Caffeine
6	paracetamol	paracetamol	paracetamol+caffeine
7	paracetamol	paracetamol	caffeine, paracetamol
8	Paracetamol	Paracetamol	Paracetamol & Caffeine
9	Paracetamol	Paracetamol	Paracetamol, Caffeine
10	Paracetamol	Paracetamol	Caffeine, paracetamol
12	Paracetamol	Paracetamol	Paracetamol, Caffeine
13	paracetamol 47.8%	paracetamol 48.4%	paracetamol 70.3%, caffeine 5.9%
14	Paracetamol	Paracetamol	Paracetamol, caffeine
15	paracetamol	paracetamol	caffeine & paracetamol
16	Paracetamol	Paracetamol	Paracetamol & Caffeine
17	Paracetamol	Paracetamol	Paracetamol, caffeine
18	not analyzed	not analyzed	not analyzed
19	Paracetamol	Paracetamol	Paracetamol, Caffeine
20	acetaminophen	acetaminophen	acetaminophen caffeine
21	indications of diacetamate, acetaminophen, 6-monoacetylmorphine, acetylcodeine	indications of diacetamate, acetaminophen, 6-monoacetylmorphine, acetylcodeine	indications of diacetamate, acetaminophen, caffeine, 6-monoacetylmorphine, acetylcodeine
22	Paracetamol, acetylcodeine	Paracetamol, acetylcodeine	Caffeine, paracetamol, acetylcodeine and 6MAM
23	Acetaminophen	Acetaminophen	Acetaminophen, caffeine
24	Paracetamol	Paracetamol	Paracetamol, caffeine
25	Paracetamol	Paracetamol	Paracetamol Caffeine
27	Paracetamol	Paracetamol	Paracetamol, caffeine
28	Paracetamol (47% m/m)	Paracetamol (48% m/m)	Paracetamol (68% m/m), caffeine (6% m/m)
29	Acetaminophen	Acetaminophen	Acetaminophen Caffeine
30	paracetamol,MAM, acetylcodeine, (noscapine)	paracetamol,MAM, acetylcodeine, (noscapine)	paracetamol,MAM,caffeine,acetylcodeine
31	Paracetamol, Caffeine and acetylcodeine	Paracetamol, Caffeine and acetylcodeine	Paracetamol and Caffeine

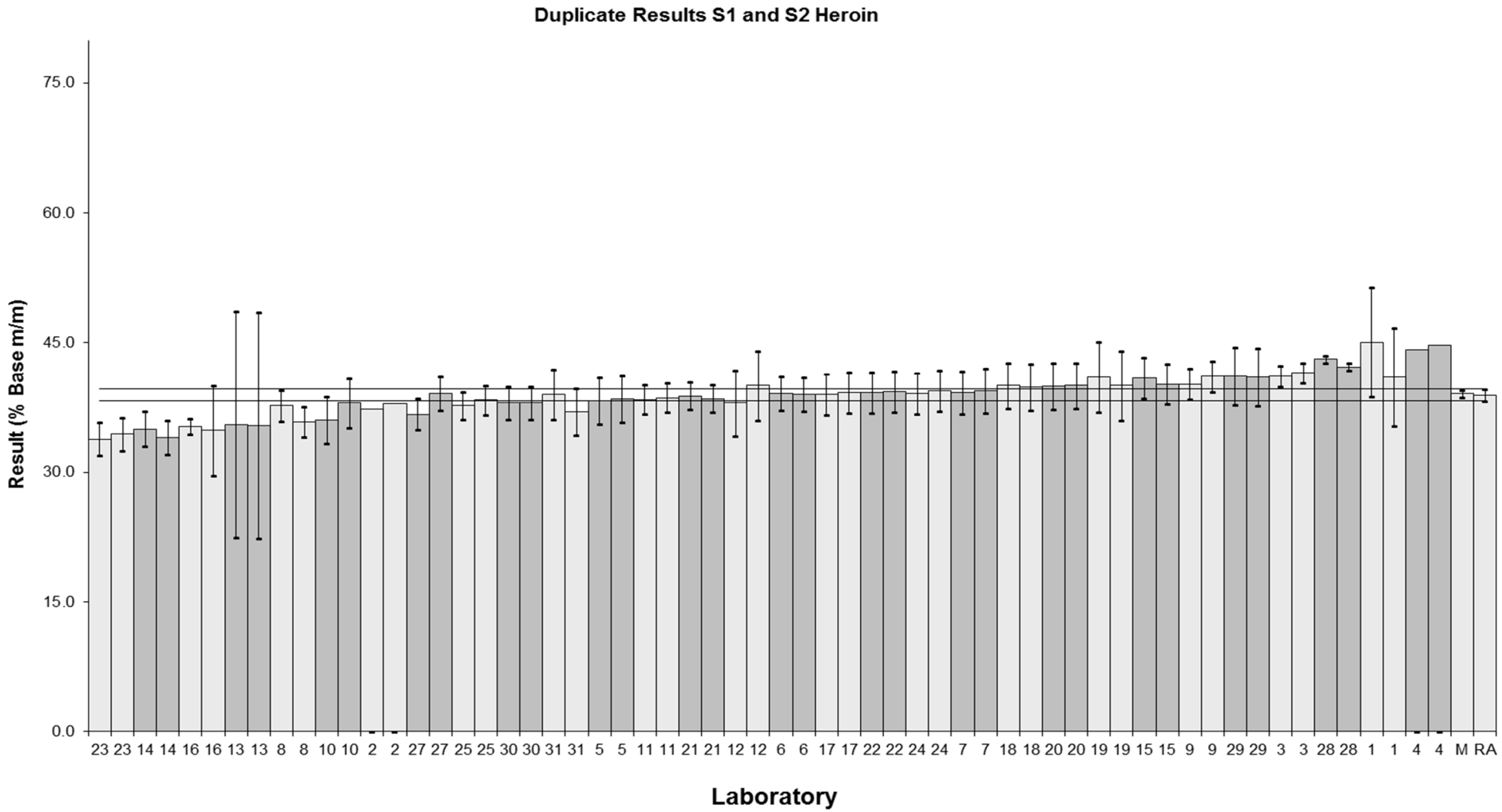


Figure 5 Results for Heroin in Duplicate Samples S1 and S2

6 DISCUSSION OF RESULTS

6.1 Assigned Value

The assigned value is the robust average of the results reported by the participants. The robust average and associated expanded uncertainties were calculated using the procedure described in 'ISO13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons'.[5] The calculation procedure for the expanded uncertainty in Sample S3 is presented in Appendix 2.

Traceability: The consensus of participants' results is not traceable to any external reference, so although expressed in SI units, metrological traceability has not been established.

6.2 Measurement Uncertainty Reported by Participants

Participants were asked to report an estimate of the expanded measurement uncertainty associated with their results and the basis of this uncertainty estimate (Table 2).

It is a requirement of the ISO Standard 17025[6] that laboratories have procedures to estimate the uncertainty of chemical measurements and to report this uncertainty in specific circumstances, including 'when the client's instruction so requires.' From 1 July 2012 this is also a requirement of ASCLD/Lab-International accreditation program.

Eighty-four results (93%) were reported with an associated expanded uncertainty. Laboratories **2** and **4** did not report uncertainty. These laboratories were not accredited.

Laboratory **16** reported significantly different estimates of uncertainty for heroin in the duplicate pair samples S1 and S2 (Figure 5).

The magnitude of reported uncertainties was within the range 1% to 50% relative.

Seventy-five of eighty-four (89%) expanded uncertainties were between 3% and 10% relative to the result. Laboratories reporting uncertainties smaller than 3% or larger than 10% relative may wish to consider whether these estimates are realistic or fit for purpose.

Laboratories having a satisfactory z-score and an unsatisfactory E_n-score are likely to have underestimated the expanded uncertainty associated with the result.

In some cases the results were reported with an inappropriate number of significant figures. The recommended format is to write the uncertainty to no more than two significant figures and then to write the result with the corresponding number of decimal places (for example instead of 40.9 ± 2.38% the recommended format is 40.9 ± 2.4%).[7]

6.3 z-Score

A target standard deviation equivalent to 3% PCV was used to calculate z-scores. Target standard deviation, the between-laboratory coefficient of variation predicted by Thomson - Horwitz equation[8] and between-laboratories coefficient of variation obtained in this study are presented in Table 9.

Table 9 Target standard deviations, coefficient of variations from predictive model and between laboratories

Sample	Analyte	Assigned value (% base m/m)	Target SD (as PCV)	Thompson Horwitz CV	Between laboratories CV
S1	Heroin	38.9	3%	2.3%	6.2%
S2	Heroin	38.9	3%	2.3%	5.7%
S3	Heroin	17.8	3%	2.6%	9%

A summary of z-scores by laboratory is presented in Figure 6.

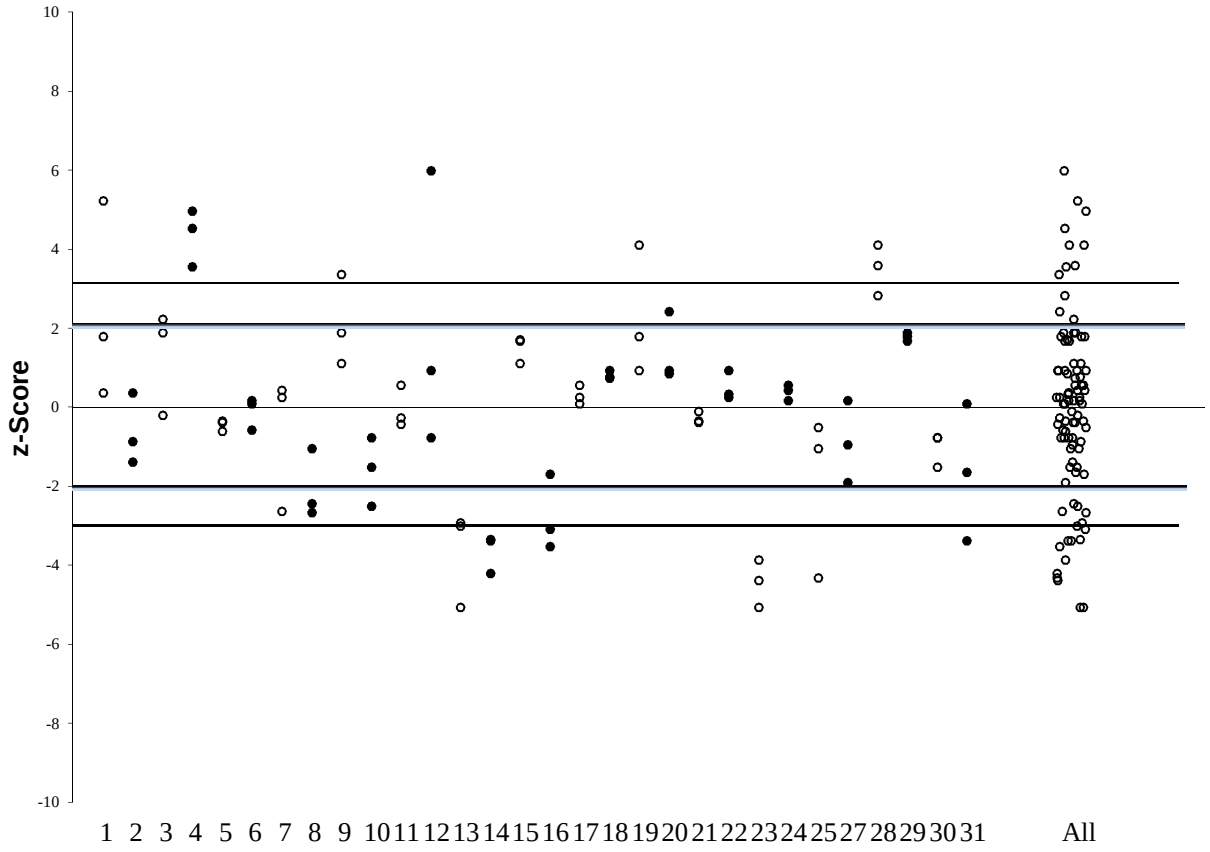


Figure 6 Summary of participants' z-score.

Sixty-one of ninety numeric results (68%) returned a satisfactory z-score with $|z| \leq 2$**.**

- Thirteen participants (43%) : **2**, **5**, **6**, **11**, **15**, **17, 18**, **21**, **22**, **24**, **27**, **29** and **30** returned satisfactory scores for all three samples;
- Seventeen participants returned at least one questionable or unsatisfactory z-score;
- Laboratories **4**, **13**, **14**, **23** and **28** returned questionable or unsatisfactory z-scores for all test samples demonstrating an unsatisfactory performance.
- Laboratories **13**, **14** and **23** reported results for all test samples lower than the assigned value (negative bias), while laboratories **4** and **28** reported all results higher than the assigned value (positive bias). These laboratories may need to investigate the source of bias.

6.4 E_n-Score

The dispersal of participants' E_n-scores is graphically presented in Figure 7. Where a laboratory did not report an expanded uncertainty with a result, an expanded uncertainty of zero (0) was used to calculate the E_n-score.

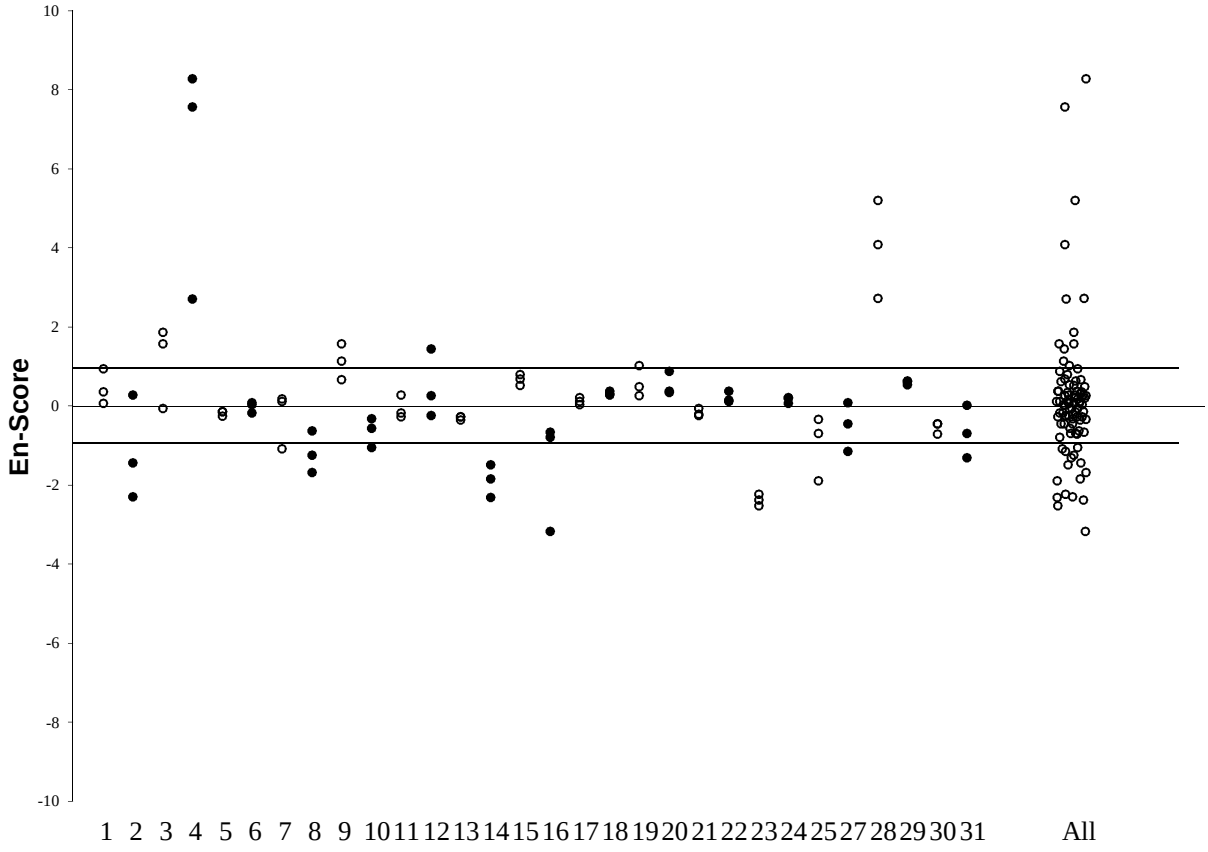


Figure 7 Summary of participants' E_n-Score

Sixty-two of ninety numeric results (69%) returned a satisfactory E_n-score with $|E_n| \leq 1$.

- Fourteen laboratories (46%) – **1**, **5**, **6**, **11**, **13**, **15**, **17**, **18**, **20**, **21**, **22**, **24**, **29** and **30** returned satisfactory scores for all three samples;
- Twelve laboratories returned at least one unsatisfactory E_n-score; and
- Laboratories **4**, **14**, **23** and **28** returned $|E_n| > 1$ for all samples.

6.5 Identification of Cutting Agent

Samples were prepared using an illicit seizure of heroin hydrochloride, approximately 74.4% base (m/m) supplied by the Australian Federal Police. The study coordinator added paracetamol in Samples S1 and S2, and both paracetamol and caffeine in Sample S3.

Twenty-eight participants (93%) reported on the identity of the cutting agents and twenty-seven identified correctly paracetamol in duplicate samples S1 and S2 and both paracetamol and caffeine in Sample S3. One participant reported incorrectly caffeine in identical Samples S1 and S2 (Table 8).

6.6 Participants' Analytical Methods

Participants were requested to analyse the samples using their normal test methods and to report a single result for each sample as they would normally report to a client. Results

reported in this way reflect the true variability of results reported to laboratory clients. The method description provided by participants are presented in Table 1.

A summary of accreditation status, participants' methods and reference standards is presented below.

Accredited	Laboratory Code
Yes to ISO 17025	1 5 6 7 8 9 10 11 12 13 14 15 18 19 21 22 23 25 29 30 31
Yes to ASCLD/Lab International	17 21 24
No	2 3 4 16 20 27 28

Sample Mass Used (mg)	Laboratory Code
4-10	4 23 27 29
11-30	3 6 8 10 11 13 14 15 16 18 19 20 22 28 30 31
31-50	1 2 5 7 9 12 17 24 25
51-100	21

Instrument Used for quantification	Laboratory Code
GC-FID or GC-MS	8 9 13 14 15 16 18 23
UPLC (w/ MSMS, PDA or UV)	1 5 6 12 19
HPLC (UPLC) (w/ DAD or UV)	2 3 4 7 10 11 17 20 21 22 24 25 29 30 31
QNMR	28
LC-MS	27

Sources of Calibration Standard	Laboratory Code
NMI Australia	5 6 8 9 12 15 16 19 21 30
Lipomed	1 4 7 17 20 22 23 24 25 29
Sigma Aldrich	14 28
MacFarlan Smith	18 31
LGC	2 10 13
Johnson Matthey	3 11
Other	27

A plot of the measurement instrument used vs z-scores is presented in Figure 8. Overall good agreement was found between the results coming from UPLC, HPLC and GC measurements. One laboratory used QNMR and reported high results for all three samples.

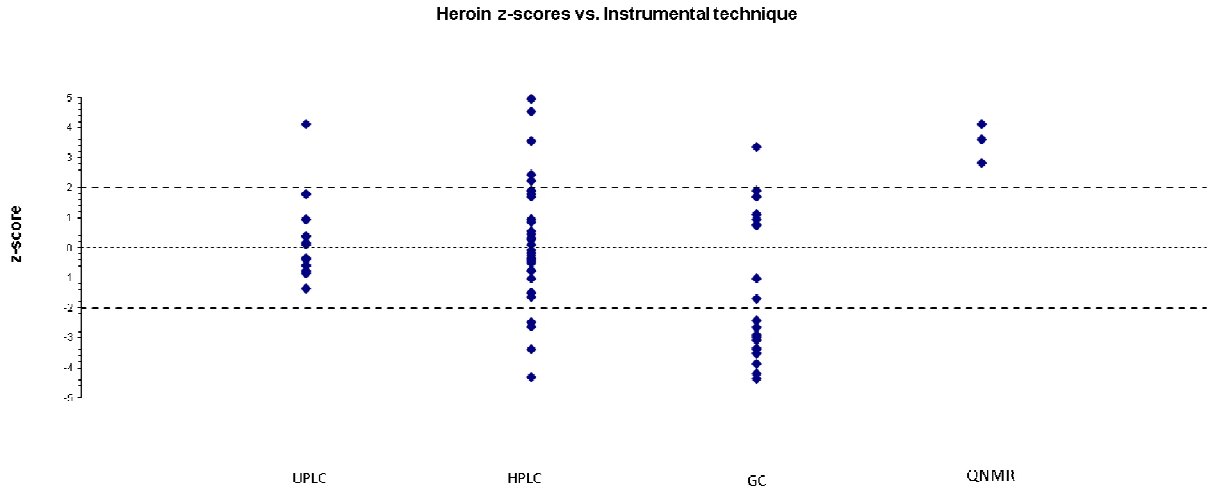


Figure 8 Measurement instrument vs z-score

6.7 Summary of participation and performance in Heroin Studies

Overall percentages of satisfactory z-scores and E_n-scores obtained by laboratories since 2009 are presented in Figure 9. The proportion of satisfactory z-scores and E_n-scores over 10 years on average is 76% and 78% respectively.

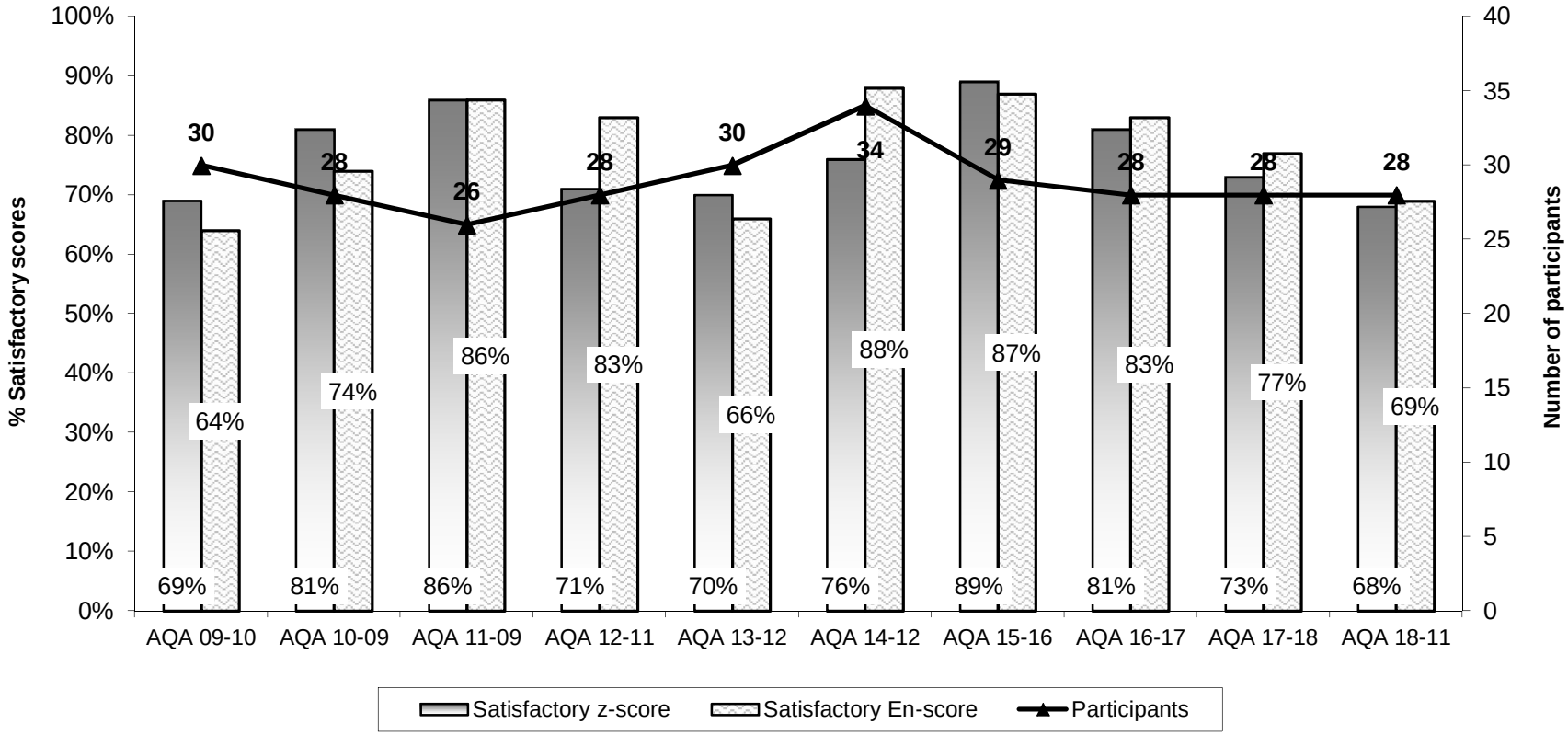


Figure 9 Summary of participants' performance since 2009

7 REFERENCES

[1] ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing, ISO Geneva

[2] NMI Chemical Proficiency Testing Study Protocol
http://www.measurement.gov.au → Products and Services → Chemical Proficiency Testing→ Details of Our Program

[3] NMI Chemical Proficiency Testing Statistical Manual
http://www.measurement.gov.au → Products and Services → Chemical Proficiency Testing→ Details of Our Program

[4] Thompson, M. E, S. L. R. and Wood, R., The international harmonized protocol for proficiency testing of (chemical) analytical laboratories, Pure Appl. Chem. 78, 145-196, 2005.

[5] ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons, ISO Geneva

[6] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories, ISO Geneva

[7] Eurachem/CITAC Guide Quantifying uncertainty in analytical measurement third edition, (2012), http://eurachem.org/images/stories/guides/pdf/quam2012_P1.pdf

[8] Thompson, M. and Lowthian, P.J., A Horwitz-like function describes precision in a proficiency test, Analyst, 120, 271-272, 1995.

APPENDIX 1 - PARTICIPANT LABORATORIES

ACT Government Analytical Laboratory ACT	Environmental Science and Research Ltd Mt. Albert Science Centre, NEW ZEALAND
CHEMCENTRE WA	Forensic & Analytical Science Services NSW
ESG Staffordshire, UK	Forensic Science SA SA
Forensic Institute, Odense Syddansk Universitet, DENMARK	Health Sciences Authority, SINGAPORE
I.N.C.C. Drogue, BELGIUM	Instituto Nacional de Toxicologia y Ciencias Forenses Departamento de Madrid, SPAIN
Instituto Nacional de Toxicologia Departamento de Barcelona, SPAIN	LABEX, FRANCE
Laboratoire Toxgen, FRANCE	Laboratoire Toxlab s.a.s., FRANCE
Lancashire Constabulary Headquarters, UK	National Criminal Investigation Service/Kripos LRA008, NORWAY
National Measurement Institute NSW	NBI - Laboratories, FINLAND
PJGN/IRCGN/ASQ, FRANCE	Queensland Health Forensic and Scientific Services QLD
Scientific Services Hampshire, UK	Scottish Police Authority Forensic Services Dundee, UK
Section of Forensic Chemistry Department of Forensic Medicine, University of Copenhagen, DENMARK	Service Commun de Laboratoires Laboratoire de Lille, FRANCE
Service Commun des Laboratoires Laboratoire de Paris, FRANCE	Swedish National Forensic Centre - NFC, SWEDEN
University of Aarhus, Institut of Forensic Medicine Department of Toxicology and Drug Analysis, DENMARK	University of New South Wales Mark Wainwright Analytical Centre, NSW
Victoria Police Forensic Services Dept. VIC	

APPENDIX 2 - MEASUREMENT UNCERTAINTY OF THE ASSIGNED VALUE

When the assigned value is calculated as the robust average using the procedure described in 'ISO13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons – Annex C'[7], the uncertainty is estimated as:

$$u_{rob\ average} = 1.25 * S_{rob\ average} / \sqrt{p} \qquad \text{Equation 4}$$

where:

$u_{rob\ average}$	robust average standard uncertainty
$S_{rob\ average}$	robust average standard deviation
p	number of results

The expanded uncertainty ($U_{rob\ average}$) is the standard uncertainty multiplied by a coverage factor of 2 at approximately 95% confidence level.

A worked example is set out below in Table 10.

Table 10 Uncertainty of assigned value for Sample S3 as % base (m/m)

No. results (p)	30
Robust Average	17.8
$S_{rob\ average}$	1.7
$u_{rob\ average}$	0.35
k	2
$U_{rob\ average}$	0.7

The robust average for Sample S3 is 17.8 ± 0.7% heroin base (m/m).

APPENDIX 3 - ACRONYMS AND ABBREVIATIONS

ASCLD	American Society of Crime Laboratory Directors
CITAC	Cooperation on International Traceability in Analytical Chemistry
CRM	Certified Reference Material
CV	Coefficient of Variation
DAD	Diode Array Detector
$\|E_n\|$	Absolute value of an E_n-score
FID	Flame Ionization Detector
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GUM	Guide to the expression of uncertainty in measurement
HPLC	High Performance Liquid Chromatography
ISO	International Standards Organisation
LC	Liquid Chromatography
Max	Maximum value in a set of results
Md	Median
Min	Minimum value in a set of results
NATA	National Association of Testing Authorities
NMI	National Measurement Institute Australia
NR	Not Reported
NT	Not Tested
PDA	Photodiode array
PT	Proficiency Test
QNMR	Quantitative Nuclear Magnetic Resonance
Robust CV	Robust Coefficient of Variation
Robust SD	Robust Standard Deviation
SI	International System of Units
Target SD (σ)	Target standard deviation
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet
$\|z\|$	Absolute value of a z-score

END OF REPORT