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Department of Industry, Innovation and Science
National Measurement Institute

Proficiency Test Report
AQA 19-13
Heroin

January 2020

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.

Lesley Johnston

Luminita Antin

Jenny Xu

Raluca Iavetz

A/g Manager, Chemical Reference Values
105 Delhi Rd, Riverside Corporate Park,
North Ryde NSW 2113

Phone: 61-2-9449 0178
raluca.iavetz@measurement.gov.au

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SUMMARY

AQA 19-13 was conducted in August 2019. Three test samples of heroin hydrochloride were sent to thirty-eight 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 **1**, **3**, **7**, **8**, **9**, **10**, **11**, **12**, **15**, **16**, **17**, **18**, **19**, **21**, **22**, **23**, **24**, **26**, **27**, **29**, **32**, **33**, **34**, **35**, **36**, **38**, **39**, and **40** returned satisfactory z and E_n-scores for all results.

Of the 120 results for which z-scores were calculated, 107 (89%) returned $|z| \leq 2$, indicating a satisfactory performance.

Of the 120 results for which E_n-scores were calculated, 102 (85%) 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*

Of the 120 results, 114 (95%) were reported with an associated expanded uncertainty. Laboratories **4** and **25** did not report uncertainty. Laboratory **4** is not accredited. Laboratory **25** reported that they are accredited to ISO 17025 and ANAB.

The magnitude of reported uncertainties was within the range 0.1% to 58% 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 75% base (m/m) supplied by the Australian Federal Police. The study coordinator added baking soda in Sample S1, caffeine in Sample S2, and glucodin and niacinamide in Sample S3.

Thirty-nine participants (98%) reported on the identity of the cutting agents. Laboratories **12** and **13** correctly identified all cutting agents used. The majority of laboratories did not report baking soda as a cutting agent in Sample S1.

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;
- inorganic analytes 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 the Proficiency Testing of Analytical Chemistry Laboratories.[4]

2 STUDY INFORMATION

2.1 Study Timetable

The timetable of the study was:

Invitation issued:	24 May 2019
Samples dispatched:	30 August 2019
Results due:	15 November 2019
Interim report issued:	10 December 2019

2.2 Participation

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

Thirty-eight laboratories agreed to participate and thirty-eight submitted results. 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 2019. The starting material was heroin hydrochloride, approximately 75% base (m/m) supplied by the Australian Federal Police. Caffeine and niacinamide purchased from Sigma Aldrich, baking soda purchased from a local supermarket, and glucodin purchased from a local pharmacy were used as cutting agents. Baking soda was used to prepare Sample S1, caffeine was used for Sample S2, and glucodin and niacinamide 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 ~54% heroin base (m/m).

Sample S2 was prepared to contain ~54% heroin base (m/m).

Sample S3 was prepared to contain ~15% 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 30 August 2019.

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 18 October 2019, as late results may not be included in the report; and
- any other comment.

The due date for the results was extended to 15 November 2019 for all participants, as some participants received the test samples late.

2.8 Interim Report

An interim report was emailed to all participants on 10 December 2019.

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	Chloroform/methanol	2,2,2-Triphenylacetophenone	1	GC	FID	HP5
2	Chloroform	Benzopinacolone	1	GC	FID	HP5
3	acetonitrile/water (86/14)	none	4	HPLC	UV	NH2
4	Ethanol	Propylparaben	8	UPLC	DAD	BEH Shield RP18
5	Acetonitrile	Strychnine	6	GC	FID	Capillary Column Model HP 1909Z-431 Stationary Phase HP-1 15mx250umx0.25um
6	Chloroform and Methanol (9:1)	Beta- benzapinacolone	1	GC	FID	HP5
7	Chloroform-d	Dimethyl terephthalate		1H QNMR	Bruker AVIII 600 with BBFO probe	N/A
8	Dichloromethane	Tetracosane	7	GC	FID	Equity 5
9	Acetonitrile/water	None	5	HPLC	UV	Kinetex 5u C18
10	acetonitrile / water	none	1	HPLC	UV	Kromasil
11	Ethanol	2,2,2-triphenylacetophenone (TPAP)	3	GC	FID	HP-1MS
12	chloroform	c28 n-alkane	4	GC	FID	HP1
13	Acetonitrile	N/A	6	UPLC	DAD	Acquity UPLC ® BEH C18 1.7um 2.1x100mm Column
14	Chloroform	B-benzopinacolone	1	GC	FID	HP5
15	Methanol	None	5	HPLC	DAD	Kinetex C-18-XB
16	Chloroform	Benzopinacolone	4	GC	FID	HP1
17	Phosphate buffer with 75% acetonitrile		5	HPLC	UV-DAD	XTERRA TM C18 reversed phase
18	Methanol	Alprazolam	1	LC	DAD	Hypersil-5-ODS
19	water/acetonitrile/n10 sulphuric acid 90:10:0.1	None	3	HPLC	Diode array	Shimpack XR-ODS
20	ethanol : dimethylformamide (9:1)	tribenzylamine	6	GC	FID	HP1

Lab. Code	Extraction solvent	Internal standard	Calib. points	Technique	Detector	Column
21	acetonitrile Acetic acid	No IS	4	HPLC	Uv DAD	Poroshell 120 EC-C18
22	Methanol	No (External Standard)	7	HPLC	DAD	Poroshell 120 EC-C18(4.6x150;2.7microns pore size)
23	ACN:H2O; 75:25	Benzocaine	3	UPLC	DAD	Acquity BEH C18 1.7um
24	Chloroform	2,2,2-Triphenylacetophenone	1	GC	FID	HP5
25	Chloroform, Methanol	2,2,2-Triphenylacetophenone	1	GC	FID	HP5
26	Methanol	None	6	HPLC	UV, 225nm	Phenomenex Luna C18
27	Chloroform	Beta-benzopinacolone	1	GC	FID	HP5
28	CH3CN/H20 (80/20)	External standard	2	HPLC	DAD	Column NH2
29	Chloroform and Methanol	Beta-benzapinacolone	1	GC	FID	HP5
30	Methanol	Mepivacaine	4	HPLC	DAD	C18
31	Acetonitrile	N/A	6	LC	PDA	Acquity BEH C18 1.7um 2.1x100mm
32	ACN/MeOH/H2O	Analog of heroin	7	UPLC	MSMS	C-18 column
33	chloroform	Nortriptyline	1	GC	FID	HP5
34	Methanol	Methadone	4	GC	FID	RXI-5MS
35	Methanol	None	3	HPLC	Diode array	Silica 1500mm x 4.6mm
36	Methanol	Diazepam	6	GC	FID	J&W 128-5512
37	Acetonitrile/Methanol (95:5)	Pholcodine 1mg/ml	3	UPLC	PDA	ACQUITY C-18
38	Methanol	Alprazolam	1	LC	DAD	Hypersil-5-ODS
39	Ammonium Formate, pH 3	No	4	LC	MS	Ascentis Express Phenyl-Hexyl (2.7 µm)
40	Chloroform	Octacosane	5	GC	FID	HP5

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	Duplicate analysis	Recoveries of SS Standard purity Instrument calibration	Eurachem/CITAC Guide
2	Top Down - precision and estimates of the method and laboratory bias	Control samples Duplicate analysis	Standard purity Instrument calibration Masses and volumes	Eurachem/CITAC Guide
3	Top Down - precision and estimates of the method and laboratory bias	Control samples – RM	Laboratory bias from PT studies Standard purity	ISO 11352
4	No MU reported			
5	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples Duplicate analysis	Laboratory bias from PT studies Standard Purity Instrument calibration Masses and volumes Homogeneity of sample	NATA - Estimating and reporting MU of Chemical Test Results
6	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Standard purity Matrix effects	Eurachem/CITAC Guide
7	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples – CRM Duplicate analysis	Standard purity Instrument calibration Masses and Volumes	
8	Top Down - precision and estimates of the method and laboratory bias	Control samples – CRM Duplicate analysis	Laboratory bias from PT studies Standard purity Matrix effects Instrument calibration Masses and volumes Homogeneity of sample	
9	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Standard purity Matrix effects Instrument calibration Masses and volumes Homogeneity of sample	Eurachem/CITAC Guide
10	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	
11	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples – CRM Duplicate analysis	Standard purity Recoveries of SS Matrix effects Instrument calibration Masses and volumes Homogeneity of sample	Eurachem/CITAC Guide
12	Top Down - precision and estimates of the method and laboratory bias	Control samples – RM Duplicate analysis		Internal document based on Eurachem/CITAC, ISO/GUM
13	Top Down - precision and estimates of the method and laboratory bias	Control samples Duplicate analysis	Laboratory bias from PT studies Standard purity Instrument calibration Masses and volumes Homogeneity of sample	NATA - Estimating and reporting MU of Chemical Test Results
14	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis		
15		Control samples – RM Duplicate analysis	Standard Purity Recoveries of SS Instrument calibration Masses and volumes	Eurachem/CITAC Guide
16	validation data (k=2)			
17	Top Down - precision and estimates of the method and laboratory bias	Control samples – RM	Laboratory bias from PT studies	Nordtest Report TR537
18	Top Down - precision and estimates of the method and laboratory bias	Control samples – SS Duplicate analysis	Laboratory bias from PT studies Recoveries of SS	Eurachem/CITAC Guide
19	Professional judgement	Control samples – CRM Duplicate analysis	Standard purity Instrument calibration	ISO/GUM
20	Top Down - precision and estimates of the method and laboratory bias	Control samples – RM	Standard purity	
21	Accuracy profile - based on intermediate precision and repeatability	Control samples – RM	Standard purity	ISO 5725-2 and ISO/TS 21748
22	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
23	Top Down - precision and estimates of the method and laboratory bias	Control samples – RM Duplicate analysis	Standard purity Masses and volumes	Eurachem/CITAC Guide

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU**
		Precision	Method Bias	
24	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Standard purity Matrix effects	Eurachem/CITAC Guide
25	No MU reported			
26	Top Down - reproducibility (standard deviation) from PT studies used directly	Control samples – RM Duplicate analysis	Laboratory bias from PT studies	Eurachem/CITAC Guide
27	Bottom up	Control samples – CRM	Recoveries of SS Instrument calibration Masses and volumes Homogeneity of sample	EURACHEM, ANAB
28		Control samples	Standard purity Instrument calibration	ISO/GUM
29	Top Down Approach	Duplicate analysis	Standard purity	
30	Top Down - precision and estimates of the method and laboratory bias	Control samples	Laboratory bias from PT studies Standard purity Recoveries of SS Matrix effects Instrument calibration Masses and volumes Homogeneity of sample	EA-4/16: 2003 and ILAG G-17:2002
31	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples – RM Duplicate analysis	Standard purity Instrument calibration Masses and volumes Homogeneity of sample	NATA - Estimating and reporting MU of Chemical Test Results
32	Top Down - precision and estimates of the method and laboratory bias	Control samples – RM		
33	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Recoveries of SS Masses and volumes	Eurachem/CITAC Guide
34	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis	Masses and volumes	ISO/GUM
35	Professional judgement	Control samples – CRM		ISO/GUM
36	Estimating Measurement Uncertainty by black box by pairs of values	Standard deviation from PT studies only		ISO/GUM
37	Top Down - precision and estimates of the method and laboratory bias	Control samples – CRM Duplicate analysis	Standard purity Instrument calibration Masses and volumes Homogeneity of sample	NATA - Estimating and reporting MU of Chemical Test Results

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU**
		Precision	Method Bias	
38	Top Down - precision and estimates of the method and laboratory bias	Control samples – SS Duplicate analysis	Laboratory bias from PT studies Recoveries of SS	Eurachem/CITAC Guide
39	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples – real samples from Police case Duplicate analysis	Laboratory bias from PT studies	Nordtest Report TR537
40	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

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

**Some entries have been modified so that the participant cannot be identified.

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	In house	94.31
2	Toronto Research Chemicals	98
3	Lipomed	99.827
4	NMI	99.4
5	NMI	99.4
6	Toronto Research Chemical	98
7	Sigma Aldrich	99.95 ± 0.21
8	Lipomed	99.827 +/-0.006 - free base content 87.7
9	Johnson/Matthey	99.5
10	Lipomed	99.6
11	NMI	99.4
12	Siegfried	99.3
13	NMI	99.4
14	In house	98
15	LGC	99.7
16		
17	Johnson Matthey	100
18	Lipomed	99.827 ± 0.006
19	LGC	99.7

Lab. Code	Reference Standard*	Purity (%)
20	Lipomed	99.6
21	Lipomed	99.6
22	Lipomed	99.6
23	NMI	99.4
24	In house	98
25	In house	98
26	NMI	99.4
27	In house	94
28	NMI	99.4
29	Toronto Research Chemical Inc	98
30	Lipomed	99.6 +/- 0.020
31	NMI	99.4
32	Lipomed	100
33	In house	94.31
34	Sigma Aldrich (Cerilliant)	99.63
35	Sigma Aldrich	
36	LIPOMED	99.1
37	NMI	99.4
38	Lipomed	99.827 ± 0.006
39	In house	ca 100
40	NMI	99.4

*Some entries have been modified so that the participant cannot be identified.

3.4 Participants' Comments

The study manager welcomes comments or suggestions from participants as it provides information which will improve future studies. All responses 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
3	Send samples of very different grades between 3 and 50%	Samples were prepared to be of various concentrations to cater for the need of different laboratories. Samples S1 and S2 was slightly greater than 50% base (m/m) and Sample S3 was approximately 15% base (m/m).
7	Method: Simultaneous observation of analyte and I.S. 1H peaks under quantitative NMR conditions	

Lab. Code	Participant comments	Study Manager's response
11	The cation of the cutting agent in sample S1 was not determined, but the cutting agent is likely to be sodium bicarbonate (baking soda). Method: A small amount of dichloromethane was used to dissolve the TPAP prior to the addition of ethanol.	
13	Acetylcodeine detected in all samples.	
16	Acetylcodeine present in all samples. S3 was analysed using a purity estimate only due to failure of initial full quantitative analysis and having insufficient sample for a full repeat	
19	MuM determined from 3 x std deviation of multiple injections expanded by professional judgement. No analysis carried out for inert bulking agents	
21	Method: 0; 5; 20; 100 mg/L	
22	Qualitative analysis was carried out by GC-MS	
25	No Expanded Uncertainty was established (not a common drug here). This test is only for study purpose.	
36	Estimating Measurement Uncertainty by black box by pairs of values: Guide ENAC G 09 or ISO 21748	

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.

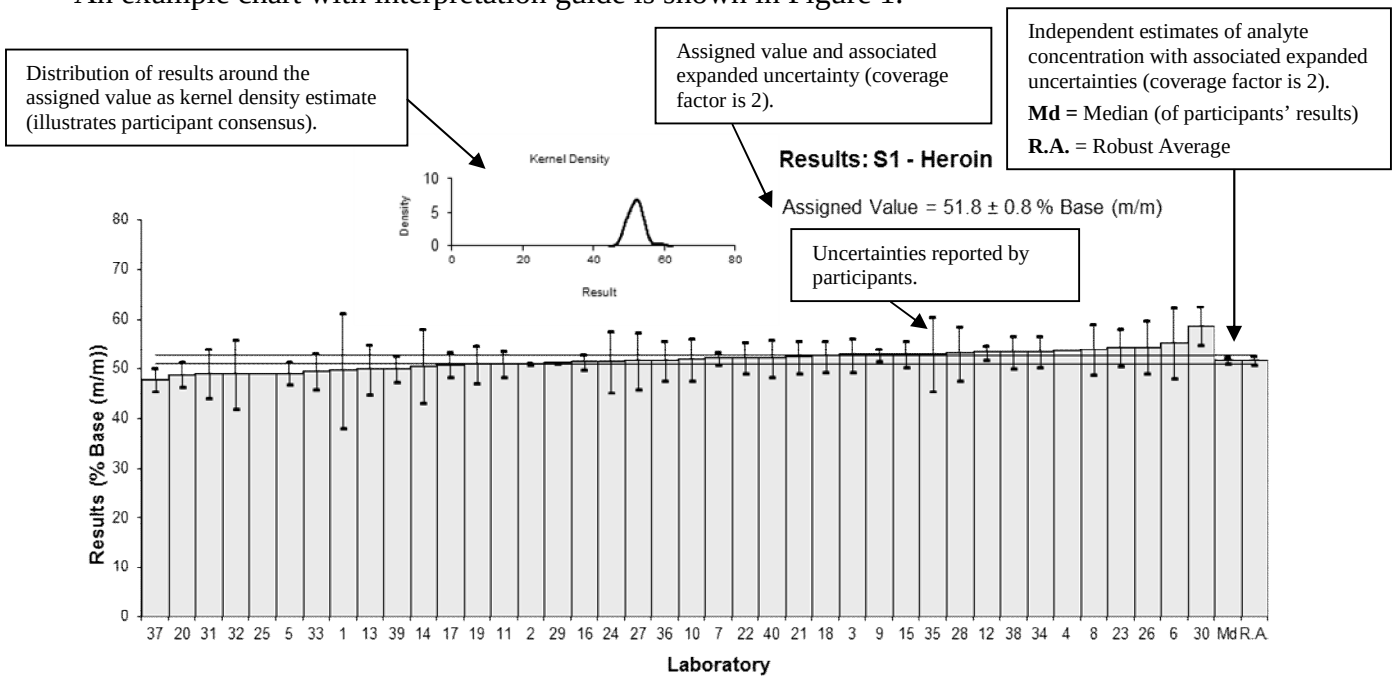


Figure 1 Guide to Presentation of Results

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 'ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons'.[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.[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. It is a measure of the between laboratory variation that in the judgement of the study coordinator would be expected from participants given the analyte concentration. 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 the 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 the 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

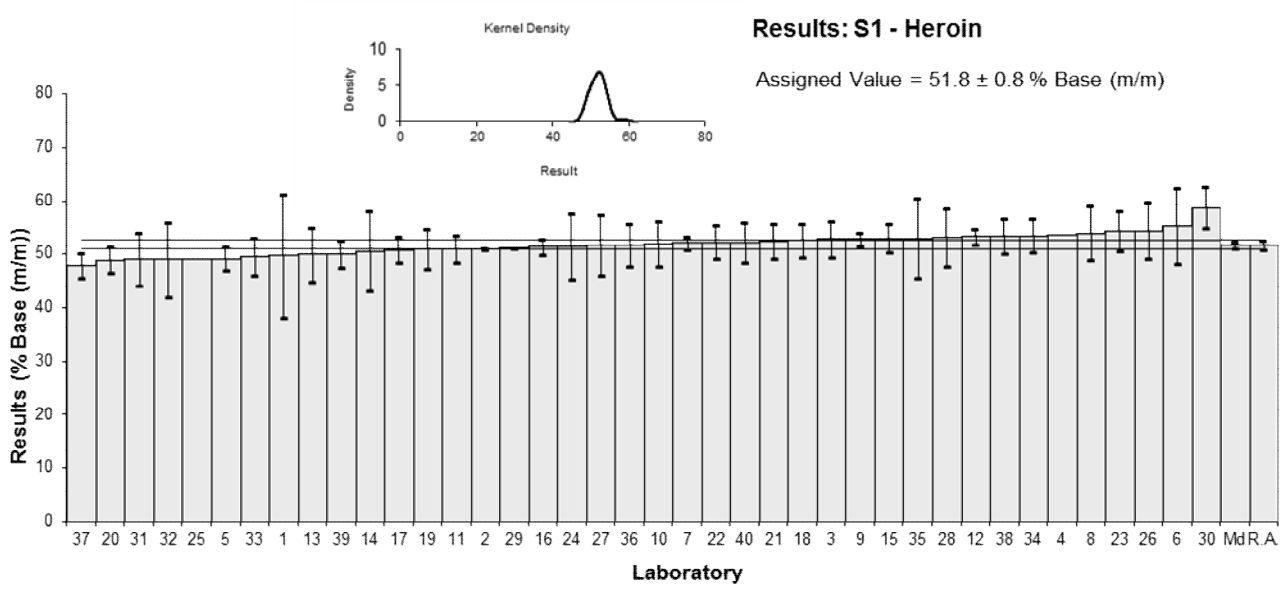
Sample No.	S1
Matrix.	Powder
Analyte.	Heroin
Units	% Base (m/m)

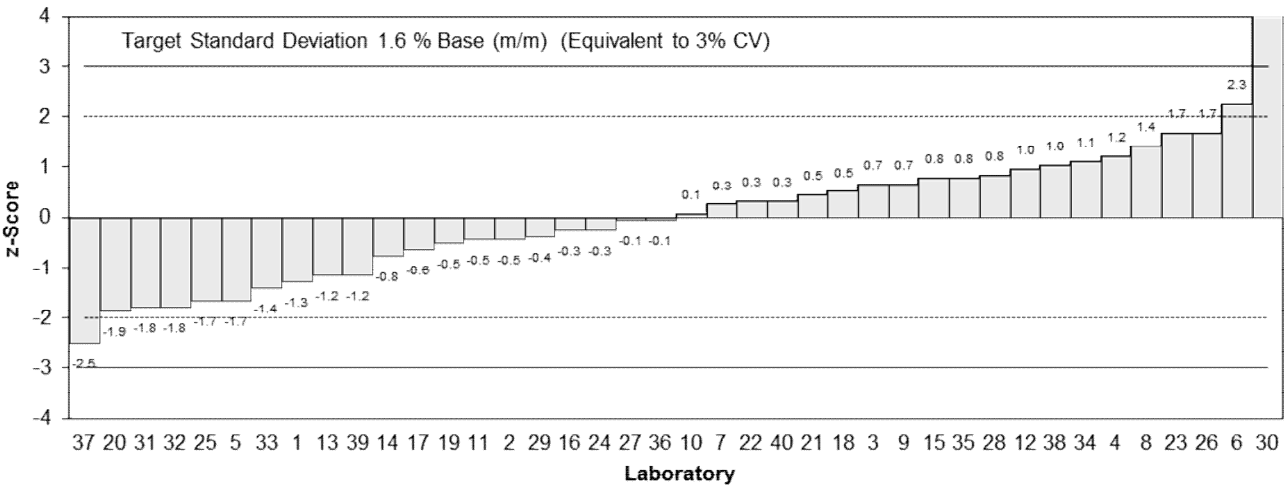
Participant Results

Lab Code	Result	Uncertainty	z-Score	E_n-Score
1	49.8	11.5	-1.29	-0.17
2	51.1	0.1132	-0.45	-0.87
3	52.82	3.33	0.66	0.30
4	53.7	NR	1.22	2.38
5	49.2	2.2	-1.67	-1.11
6	55.3	7.0	2.25	0.50
7	52.2	1.2	0.26	0.28
8	54	5	1.42	0.43
9	52.82	1.20	0.66	0.71
10	51.9	4.2	0.06	0.02
11	51.1	2.5	-0.45	-0.27
12	53.3	1.4	0.97	0.93
13	50	5.0	-1.16	-0.36
14	50.6	7.4	-0.77	-0.16
15	53	2.7	0.77	0.43
16	51.4	1.5	-0.26	-0.24
17	50.8	2.4	-0.64	-0.40
18	52.6	3.2	0.51	0.24
19	51	3.8	-0.51	-0.21
20	48.9	2.4	-1.87	-1.15
21	52.5	3.2	0.45	0.21
22	52.3	3.1	0.32	0.16
23	54.4	3.8	1.67	0.67
24	51.4	6.1	-0.26	-0.07
25	49.2	NR	-1.67	-3.25
26	54.4	5.3	1.67	0.49
27	51.7	5.6	-0.06	-0.02
28	53.1	5.4	0.84	0.24
29	51.2	0.04	-0.39	-0.75
30	58.8	3.8	4.50	1.80
31	49	4.9	-1.80	-0.56
32	49	6.9	-1.80	-0.40
33	49.6	3.4	-1.42	-0.63
34	53.53	3.13	1.11	0.54
35	53	7.5	0.77	0.16
36	51.7	3.9	-0.06	-0.03
37	47.9	2.3	-2.51	-1.60
38	53.4	3.3	1.03	0.47
39	50	2.5	-1.16	-0.69
40	52.3	3.7	0.32	0.13

Statistics

Assigned Value	51.8	0.8
Robust Average	51.8	0.8
Median	51.8	0.6
Mean	51.8	
N	40	
Max.	58.8	
Min.	47.9	
Robust SD	2.0	
Robust CV	3.9%	


Kernel Density
Density
Result
Results: S1 - Heroin
Assigned Value = 51.8 ± 0.8 % Base (m/m)
Results (% Base (m/m))
Laboratory
37 20 31 32 25 5 33 1 13 39 14 17 19 11 2 29 16 24 27 36 10 7 22 40 21 18 3 9 15 35 28 12 38 34 4 8 23 26 6 30 Md R A


z-Scores: S1 - Heroin
Target Standard Deviation 1.6 % Base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
37 20 31 32 25 5 33 1 13 39 14 17 19 11 2 29 16 24 27 36 10 7 22 40 21 18 3 9 15 35 28 12 38 34 4 8 23 26 6 30

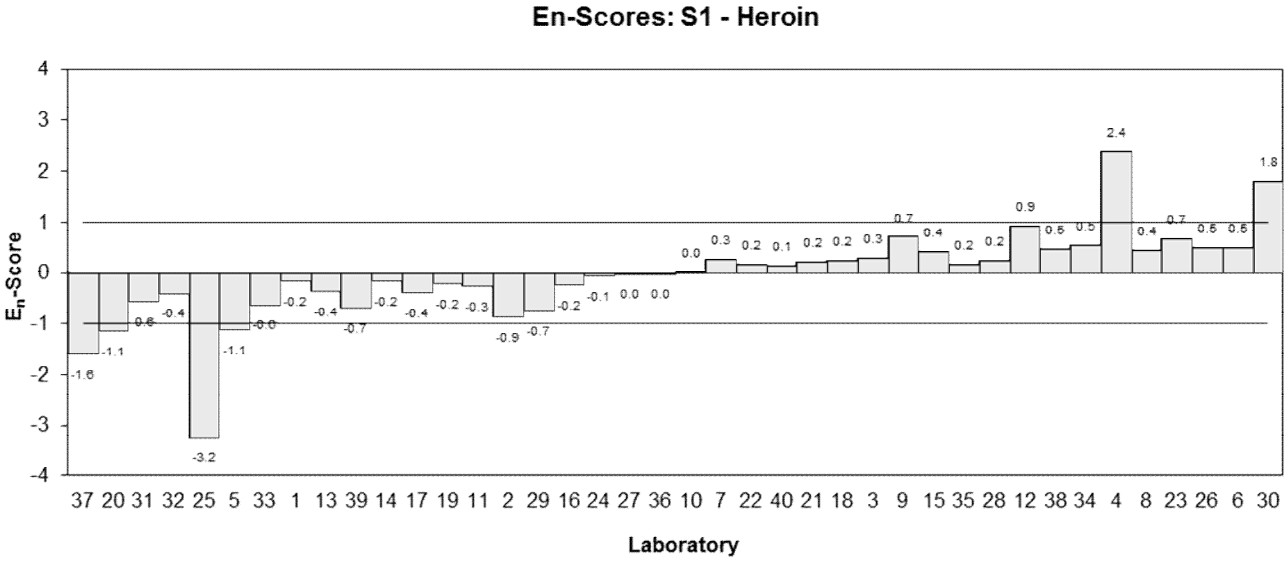

En-Scores: S1 - Heroin
En-Score
Laboratory
37 20 31 32 25 5 33 1 13 39 14 17 19 11 2 29 16 24 27 36 10 7 22 40 21 18 3 9 15 35 28 12 38 34 4 8 23 26 6 30

Figure 2

Table 6

Sample Details

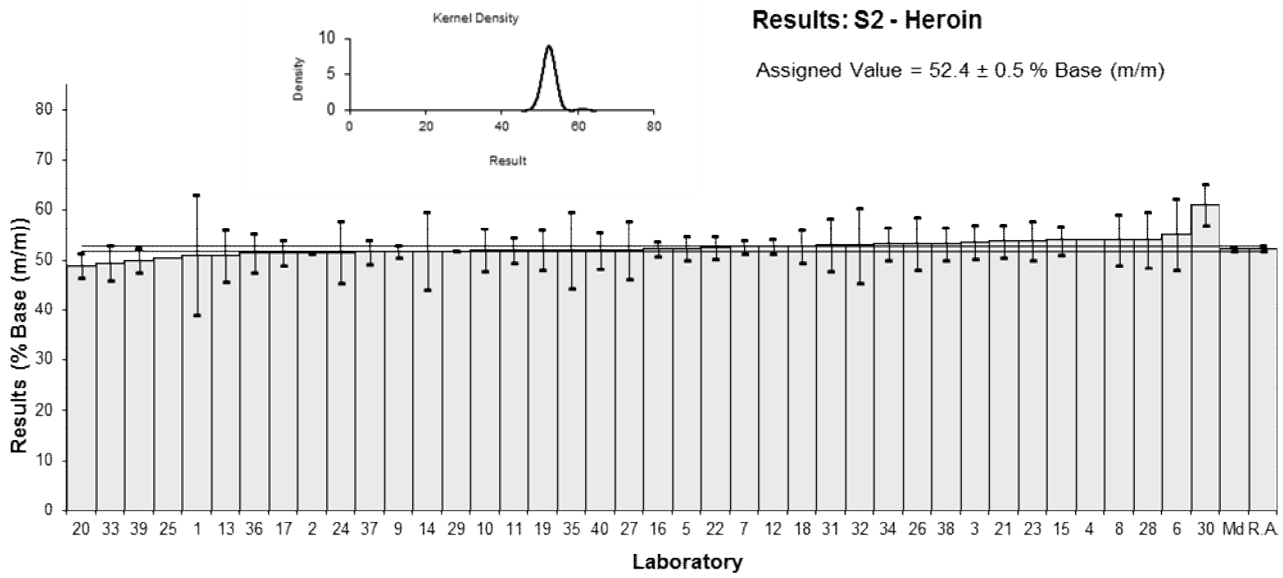
Sample No.	S2
Matrix.	Powder
Analyte.	Heroin
Units	% Base (m/m)

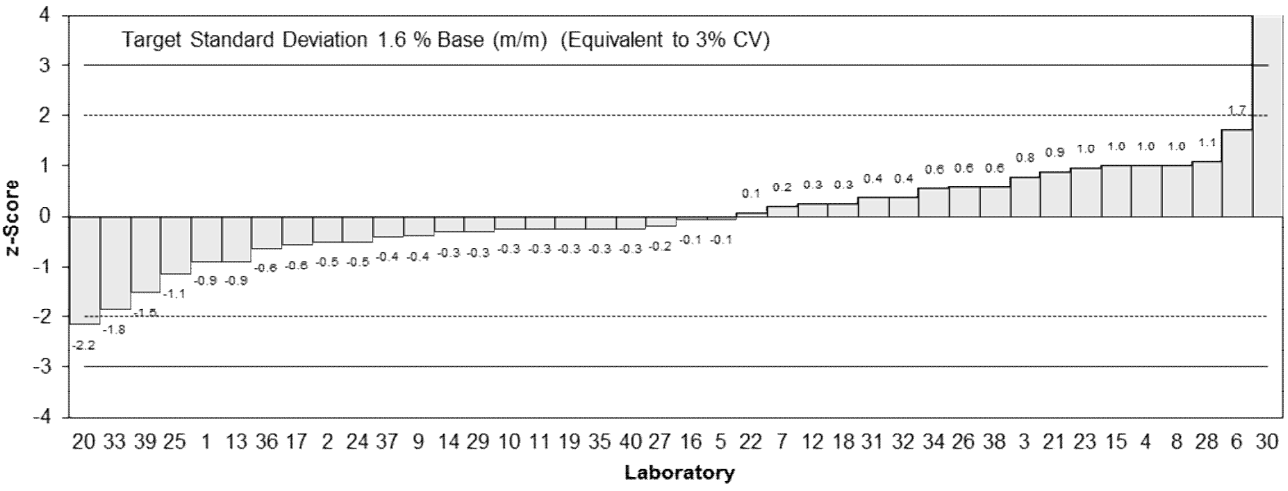
Participant Results

Lab Code	Result	Uncertainty	z-Score	E_n-Score
1	51.0	11.8	-0.89	-0.12
2	51.6	0.1132	-0.51	-1.56
3	53.64	3.38	0.79	0.36
4	54.0	NR	1.02	3.20
5	52.3	2.3	-0.06	-0.04
6	55.1	6.9	1.72	0.39
7	52.7	1.2	0.19	0.23
8	54	5	1.02	0.32
9	51.78	1.20	-0.39	-0.48
10	52.0	4.2	-0.25	-0.09
11	52.0	2.5	-0.25	-0.16
12	52.8	1.4	0.25	0.27
13	51	5.1	-0.89	-0.27
14	51.9	7.6	-0.32	-0.07
15	54	2.8	1.02	0.56
16	52.3	1.5	-0.06	-0.06
17	51.5	2.4	-0.57	-0.37
18	52.8	3.2	0.25	0.12
19	52	3.9	-0.25	-0.10
20	49.0	2.4	-2.16	-1.39
21	53.8	3.2	0.89	0.43
22	52.5	2.1	0.06	0.05
23	53.9	3.8	0.95	0.39
24	51.6	6.1	-0.51	-0.13
25	50.6	NR	-1.15	-3.60
26	53.3	5.2	0.57	0.17
27	52.1	5.7	-0.19	-0.05
28	54.1	5.5	1.08	0.31
29	51.9	0.04	-0.32	-1.00
30	61.1	4.0	5.53	2.16
31	53	5.3	0.38	0.11
32	53	7.4	0.38	0.08
33	49.5	3.4	-1.84	-0.84
34	53.28	3.11	0.56	0.28
35	52	7.5	-0.25	-0.05
36	51.4	3.9	-0.64	-0.25
37	51.76	2.3	-0.41	-0.27
38	53.3	3.2	0.57	0.28
39	50	2.5	-1.53	-0.94
40	52.0	3.6	-0.25	-0.11

Statistics

Assigned Value	52.4	0.5
Robust Average	52.4	0.5
Median	52.2	0.4
Mean	52.5	
N	40	
Max.	61.1	
Min.	49	
Robust SD	1.3	
Robust CV	2.5%	


Kernel Density
Density
Result
Results: S2 - Heroin
Assigned Value = 52.4 ± 0.5 % Base (m/m)
Results (% Base (m/m))
Laboratory
20 33 39 25 1 13 36 17 2 24 37 9 14 29 10 11 19 35 40 27 16 5 22 7 12 18 31 32 34 26 38 3 21 23 15 4 8 28 6 30 Md R.A


z-Scores: S2 - Heroin
Target Standard Deviation 1.6 % Base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
20 33 39 25 1 13 36 17 2 24 37 9 14 29 10 11 19 35 40 27 16 5 22 7 12 18 31 32 34 26 38 3 21 23 15 4 8 28 6 30

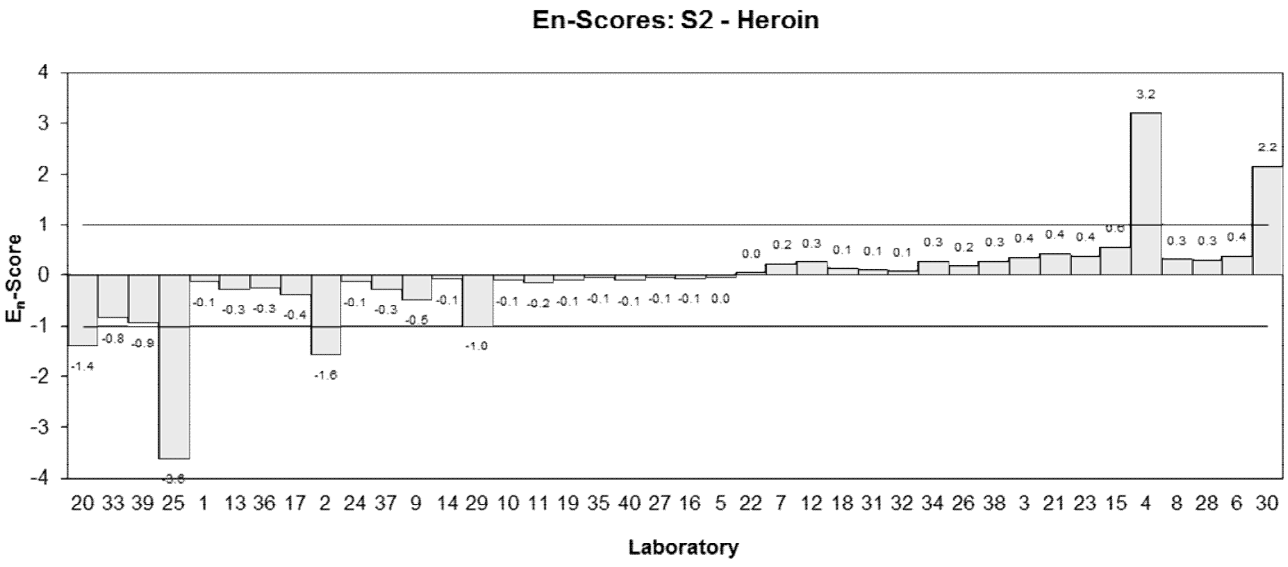

En-Scores: S2 - Heroin
En-Score
Laboratory
20 33 39 25 1 13 36 17 2 24 37 9 14 29 10 11 19 35 40 27 16 5 22 7 12 18 31 32 34 26 38 3 21 23 15 4 8 28 6 30

Figure 3

Table 7

Sample Details

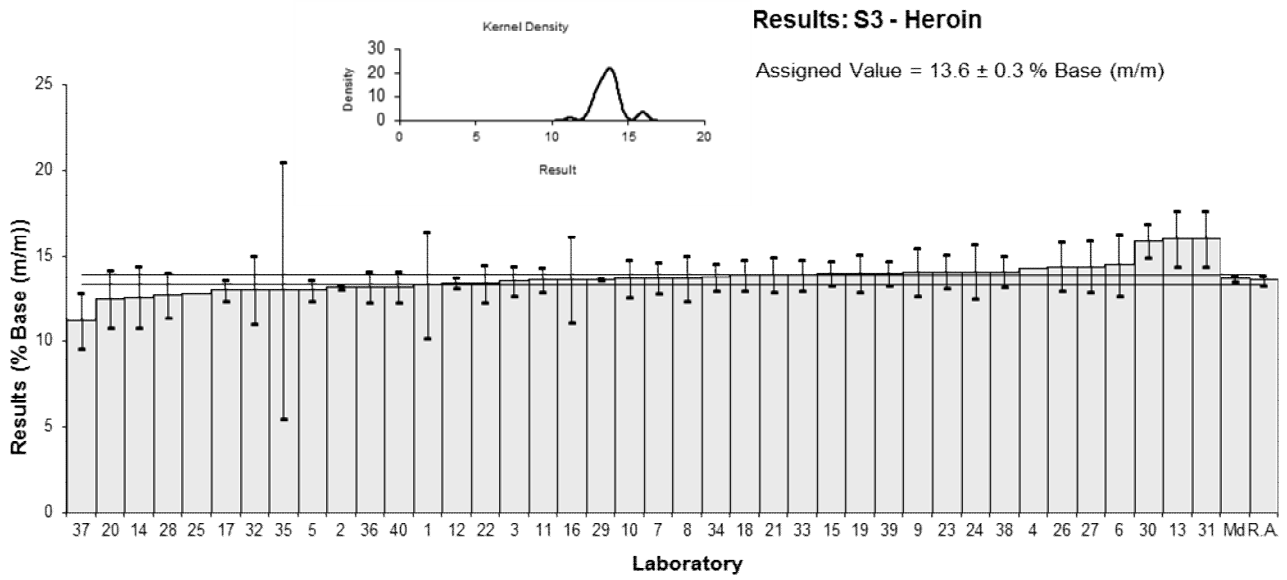
Sample No.	S3
Matrix.	Powder
Analyte.	Heroin
Units	% Base (m/m)

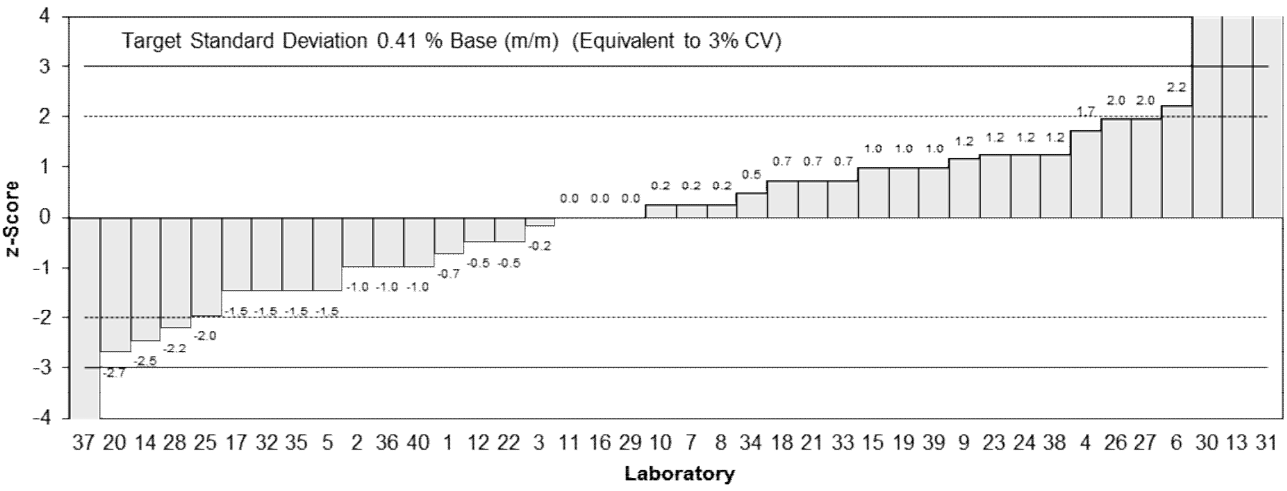
Participant Results

Lab Code	Result	Uncertainty	z-Score	E_n-Score
1	13.3	3.1	-0.74	-0.10
2	13.2	0.1132	-0.98	-1.25
3	13.53	0.85	-0.17	-0.08
4	14.3	NR	1.72	2.33
5	13	0.6	-1.47	-0.89
6	14.5	1.8	2.21	0.49
7	13.7	0.9	0.25	0.11
8	13.7	1.3	0.25	0.07
9	14.07	1.40	1.15	0.33
10	13.7	1.1	0.25	0.09
11	13.6	0.7	0.00	0.00
12	13.4	0.3	-0.49	-0.47
13	16	1.6	5.88	1.47
14	12.6	1.8	-2.45	-0.55
15	14	0.7	0.98	0.53
16	13.6	2.5	0.00	0.00
17	13.0	0.6	-1.47	-0.89
18	13.9	0.9	0.74	0.32
19	14	1.1	0.98	0.35
20	12.5	1.7	-2.70	-0.64
21	13.9	1	0.74	0.29
22	13.4	1.1	-0.49	-0.18
23	14.1	1.0	1.23	0.48
24	14.1	1.6	1.23	0.31
25	12.8	NR	-1.96	-2.67
26	14.4	1.4	1.96	0.56
27	14.4	1.5	1.96	0.52
28	12.7	1.3	-2.21	-0.67
29	13.6	0.04	0.00	0.00
30	15.9	1.0	5.64	2.20
31	16	1.6	5.88	1.47
32	13	2.0	-1.47	-0.30
33	13.9	0.9	0.74	0.32
34	13.79	0.80	0.47	0.22
35	13	7.5	-1.47	-0.08
36	13.2	0.9	-0.98	-0.42
37	11.2	1.6	-5.88	-1.47
38	14.1	0.9	1.23	0.53
39	14	0.7	0.98	0.53
40	13.2	0.9	-0.98	-0.42

Statistics

Assigned Value	13.6	0.3
Robust Average	13.6	0.3
Median	13.7	0.2
Mean	13.7	
N	40	
Max.	16	
Min.	11.2	
Robust SD	0.68	
Robust CV	5.0%	


Results: S3 - Heroin
Assigned Value = 13.6 ± 0.3 % Base (m/m)
Kernel Density
Density
Result
Results (% Base (m/m))
Laboratory
37 20 14 28 25 17 32 35 5 2 36 40 1 12 22 3 11 16 29 10 7 8 34 18 21 33 15 19 39 9 23 24 38 4 26 27 6 30 13 31 Md R.A.


z-Scores: S3 - Heroin
Target Standard Deviation 0.41 % Base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
37 20 14 28 25 17 32 35 5 2 36 40 1 12 22 3 11 16 29 10 7 8 34 18 21 33 15 19 39 9 23 24 38 4 26 27 6 30 13 31

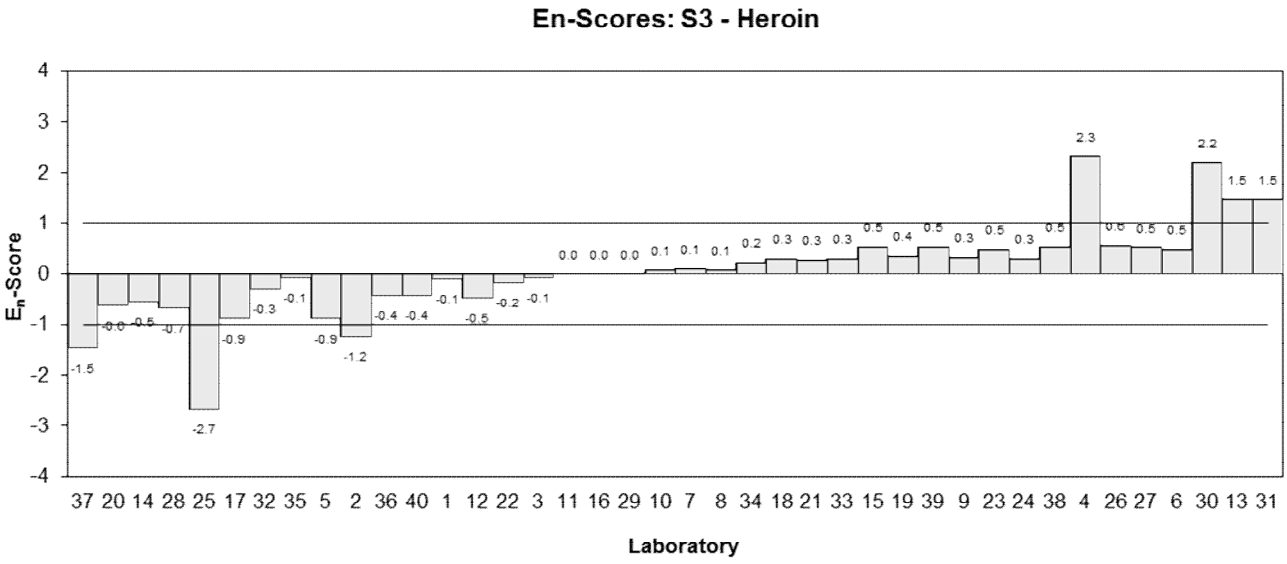

En-Scores: S3 - Heroin
En-Score
Laboratory
37 20 14 28 25 17 32 35 5 2 36 40 1 12 22 3 11 16 29 10 7 8 34 18 21 33 15 19 39 9 23 24 38 4 26 27 6 30 13 31

Figure 4

Table 8 Participants' identification of cutting agents

Lab. Code	Cutting agents		
	S1	S2	S3
1		Caffeine	Niacinamide
2			Acetylcodeine
3	None	caffeine	glucose
4		caffeine: 28.1%	glucose: 37.3%
5		Caffeine	Glucose, Nicotinamide
6	None	Caffeine	Niacinamide
7	unidentified inorganic sodium salt	Caffeine (~28.5%)	Nicotinamide (~33.4%) and an unidentified disaccharide
8	Acetylcodeine	Caffeine, acetylcodeine	Nicotinamide, acetylcodeine
9	None detected	Caffeine	Niacinamide
10	-	caffeine	nicotinamide
11	A bicarbonate salt The cation of the cutting agent in sample S1 was not determined, but the cutting agent is likely to be sodium bicarbonate (baking soda).	Caffeine	Nicotinamide & glucose
12	sodium bicarbonate	caffeine	nicotinamide and glucose
13	Sodium bicarbonate	Caffeine	Nicotinamide, Dextrose
14	NO CUTTING AGENT	NO CUTTING AGENT	NICOTINAMIDE
15	MAM, acetylcodeine, morphine	MAM, acetylcodeine, morphine, caffeine	acetylcodeine, nicotinamide, morphine
16	none detected	caffeine	nicotinamide
17	None	Caffeine	Nicotinamide
18	-	Caffeine	Nicotinamide
19	none detected	Caffeine	Niacinamide/Vitamin B3
20		29.2% caffeine	nicotinamide and glucose (not quantified)
21	-	Caffeine	Glucose
22		Caffeine	Nicotinamide
23	Not determined	Caffeine indicated	Nicotinamide indicated
24	Lactose	Lactose, caffeine	Niacinamide
25		Caffeine	Nicotinamide
26	not determined	not determined	not determined
27			
28		CAFFEINE	NICOTINAMIDE
29	None	Caffeine	Niacinamide
30		Caffeine	Nicotinamide
31	Not detected	Caffeine	Nicotinamide

Lab. Code	Cutting agents		
	S1	S2	S3
32	None	Caffeine	none
33	No	caffeine	niacinamide
34		Caffeine	Nicotinamide and Glucose
35	None	Caffeine	Niacinamide
36		Caffeine	Niacinamide
37		Caffeine	nicotinamide
38	-	Caffeine	Nicotinamide
39		caffeine	nicotinamide
40		caffeine	nicotinamide

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 'ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons'.[5] The calculation procedure for the expanded uncertainty in Sample S1 is presented in Appendix 1.

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 accreditation program.

One hundred and fourteen results (95%) were reported with an associated expanded measurement uncertainty. Laboratories **4** and **25** did not report expanded measurement uncertainties. Laboratory **4** is not accredited. Laboratory **25** reported that they are accredited to ISO17025 and ANAB, however they stated that heroin was not a common drug tested and participation in this PT was only for study purposes.

The magnitude of reported uncertainties was within the range 0.1% to 58% relative.

Seventy-two of one hundred and fourteen (63%) 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 53.28 ± 3.11%, the recommended format is 53.3 ± 3.1%).[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 the Thompson-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	51.8	3%	1.4%	3.9%
S2	Heroin	52.4	3%	1.4%	2.5%
S3	Heroin	13.6	3%	2.7%	5.0%

A summary of z-scores by laboratory is presented in Figure 5.

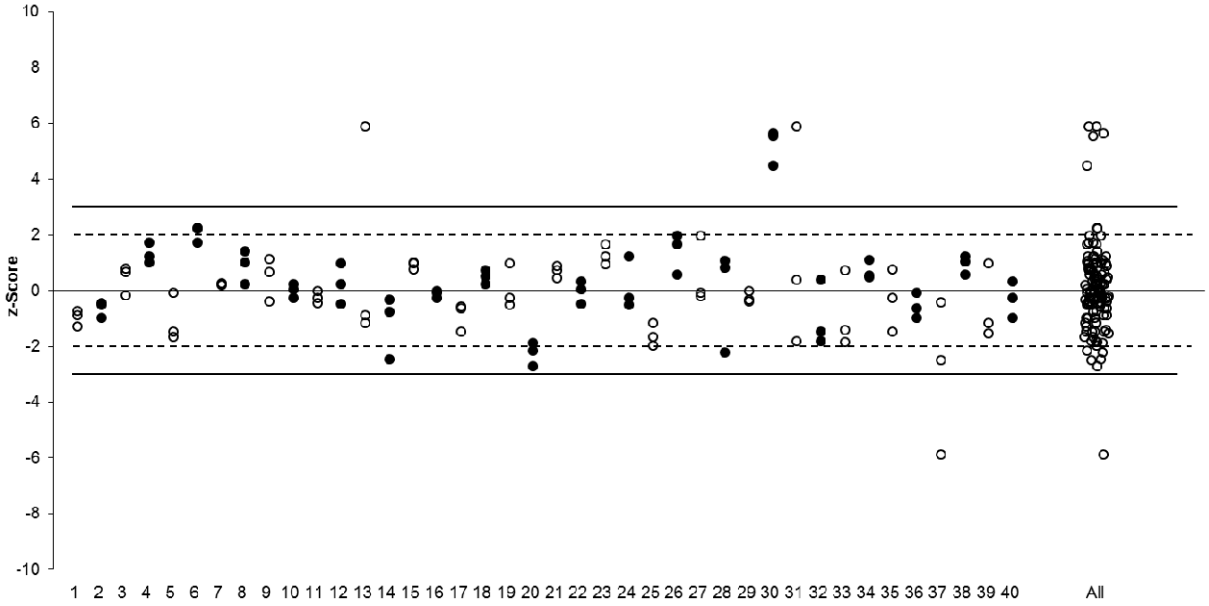


Figure 5 Summary of participants' z-scores.

One hundred and seven of one hundred and twenty results (89%) returned a satisfactory z-score with $|z| \leq 2$.

- Thirty-two participants (80%): **1**, **2**, **3**, **4**, **5**, **7**, **8**, **9**, **10**, **11**, **12**, **15**, **16**, **17**, **18**, **19**, **21**, **22**, **23**, **24**, **25**, **26**, **27**, **29**, **32**, **33**, **34**, **35**, **36**, **38**, **39** and **40** returned satisfactory scores for all three samples;
- Eight participants returned at least one questionable or unsatisfactory z-score;
- Laboratory **30** returned questionable or unsatisfactory z-scores for all test samples, demonstrating an unsatisfactory performance. Results for all test samples were higher than the assigned value (positive bias). This laboratory 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 6. 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.

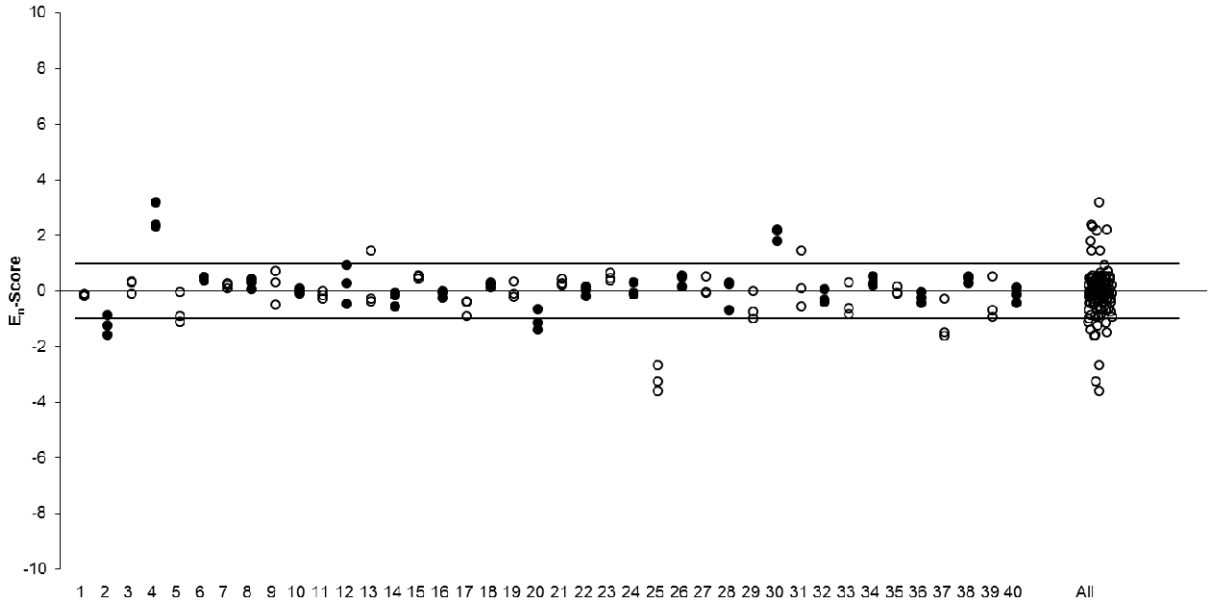


Figure 6 Summary of participants' E_n-scores.

One hundred and two of one hundred and twenty results (85%) returned a satisfactory E_n-score with $|E_n| \leq 1$.

- Thirty-one laboratories (78%): **1**, **3**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **14**, **15**, **16**, **17**, **18**, **19**, **21**, **22**, **23**, **24**, **26**, **27**, **28**, **29**, **32**, **33**, **34**, **35**, **36**, **38**, **39** and **40** returned satisfactory scores for all three samples;
- Nine laboratories returned at least one unsatisfactory E_n-score;
- Laboratories **4**, **25** and **30** returned $|E_n| > 1$ for all samples.

6.5 Identification of Cutting Agent

Samples were prepared using an illicit seizure of heroin hydrochloride, approximately 75% base (m/m) supplied by the Australian Federal Police. The study coordinator added baking soda in Sample S1, caffeine in Sample S2, and glucodin and niacinamide in Sample S3.

Thirty-nine participants (98%) reported on the identity of the cutting agents. Laboratories **12** and **13** correctly reported all cutting agents used.

Most laboratories did not successfully determine the cutting agent for Sample S1, potentially due to its inorganic nature. Two participants correctly identified baking soda. Additionally, one participant correctly indicated that there was an inorganic sodium salt, and another participant correctly indicated that there was a bicarbonate salt, hypothesising this to be baking soda. One participant incorrectly reported lactose in this sample.

For Sample S2, thirty-five participants correctly reported caffeine as the cutting agent. One participant identified caffeine, but also incorrectly reported lactose.

For Sample S3, six participants correctly identified both glucodin and niacinamide as the cutting agents, three identified glucodin only, and twenty-six identified niacinamide only. One participants identified niacinamide, but reported an incorrect sugar.

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 3 5 8 10 11 12 13 15 16 17 18 19 20 22 23 25 26 30 31 32 33 34 35 36 37 38 40
Yes to ANAB and ASCLD/LAB	1 2 6 14 18 24 25 27 29 33 38
No	4 7 9 21 28 39

Sample Mass Used (mg)	Laboratory Code
5-30	1 2 3 6 7 8 9 10 11 13 14 15 17 18 19 20 21 23 24 25 26 27 28 29 31 33 34 35 36 37 38 39 40
31-50	4 5 12 18 22 30 32 38
51-100	26
101-150	5 16

Instrument Used for quantification	Laboratory Code
GC-FID or GC-MS	1 2 5 6 8 11 12 14 16 20 24 25 27 29 33 34 36 40
UPLC (w/ DAD, PDA, or MSMS)	4 13 23 31 32 37
HPLC (w/ UV, DAD, or MS)	3 9 10 15 17 18 19 21 22 26 28 30 35 38 39
QNMR	7

Sources of Calibration Standard	Laboratory Code
NMI Australia	4 5 11 13 23 26 28 31 37 40
Lipomed	3 8 10 18 20 21 22 30 32 36 38
Sigma Aldrich	7 34 35
LGC	15 19
Johnson Matthey	9 17
Toronto Research Chemicals	2 6 29
Other	1 12 14 16 24 25 27 33 39

Plots of the measurement instrument used vs z-score, extraction solvent vs z-score, and calibration standard vs z-score are presented in Figures 7, 8 and 9 respectively. No trends were identified.

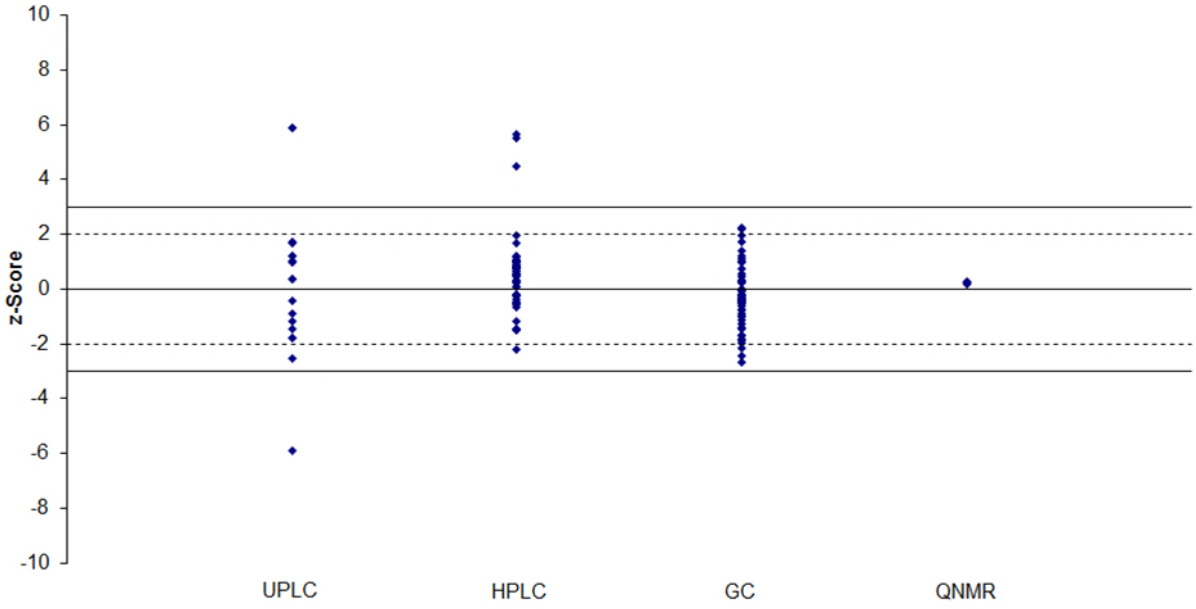


Figure 7 Measurement instrument vs z-score

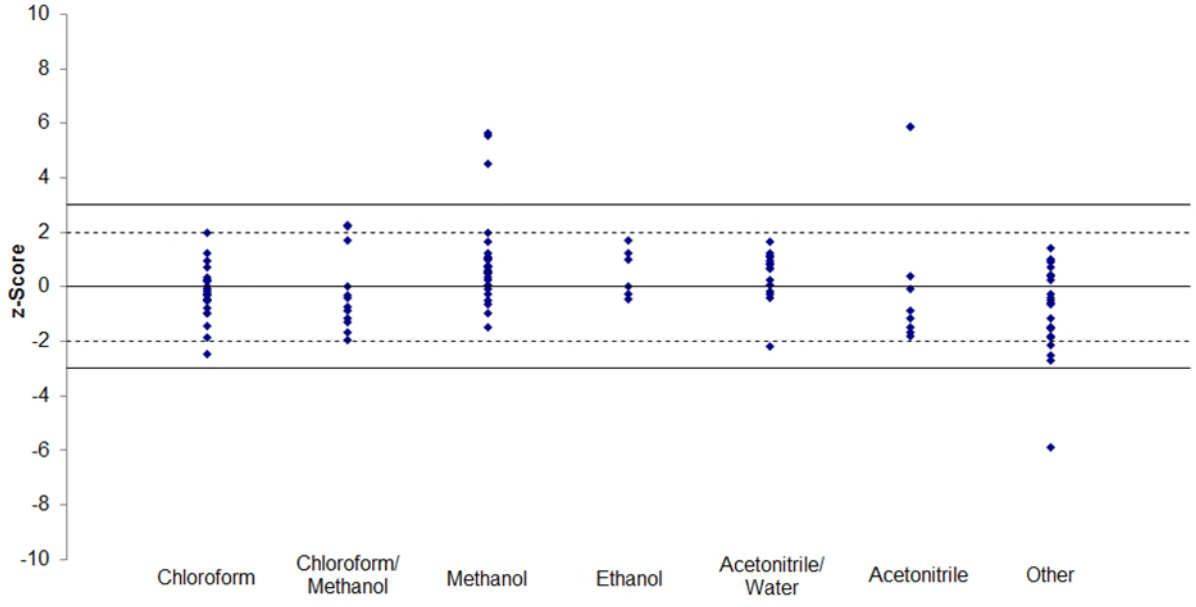


Figure 8 Extraction solvent vs z-score

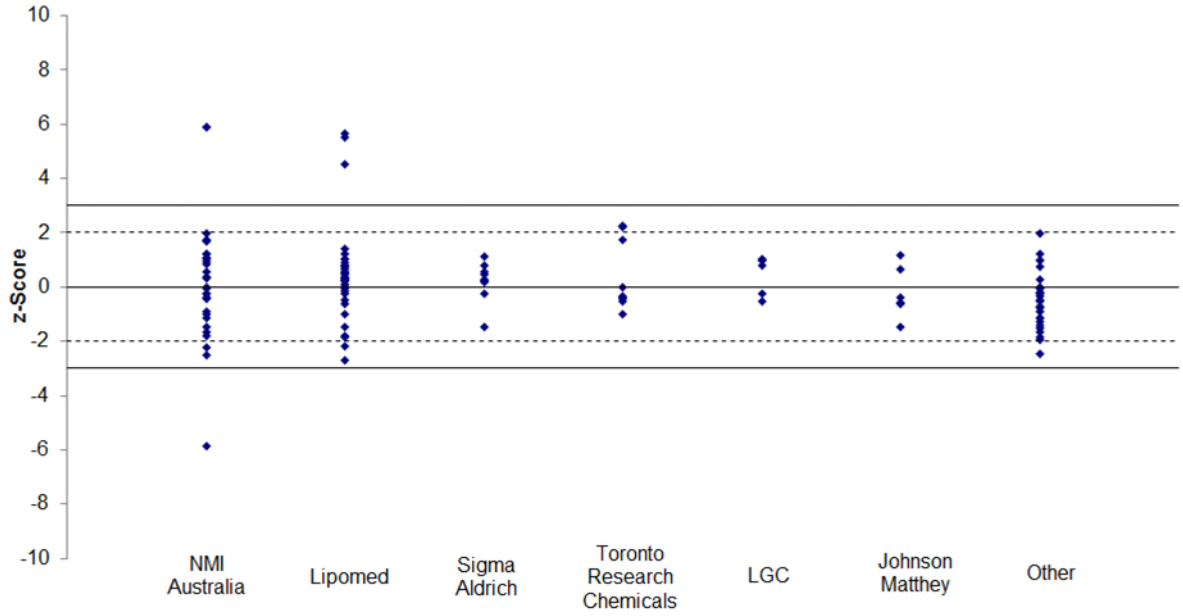


Figure 9 Calibration standard vs z-score

6.7 Summary of participation and performance in Heroin Studies

Overall percentages of satisfactory z-scores and E_n-scores obtained by laboratories since 2008 are presented in Figure 10. The proportion of satisfactory z-scores and E_n-scores over 12 years on average is 77% and 73% respectively.

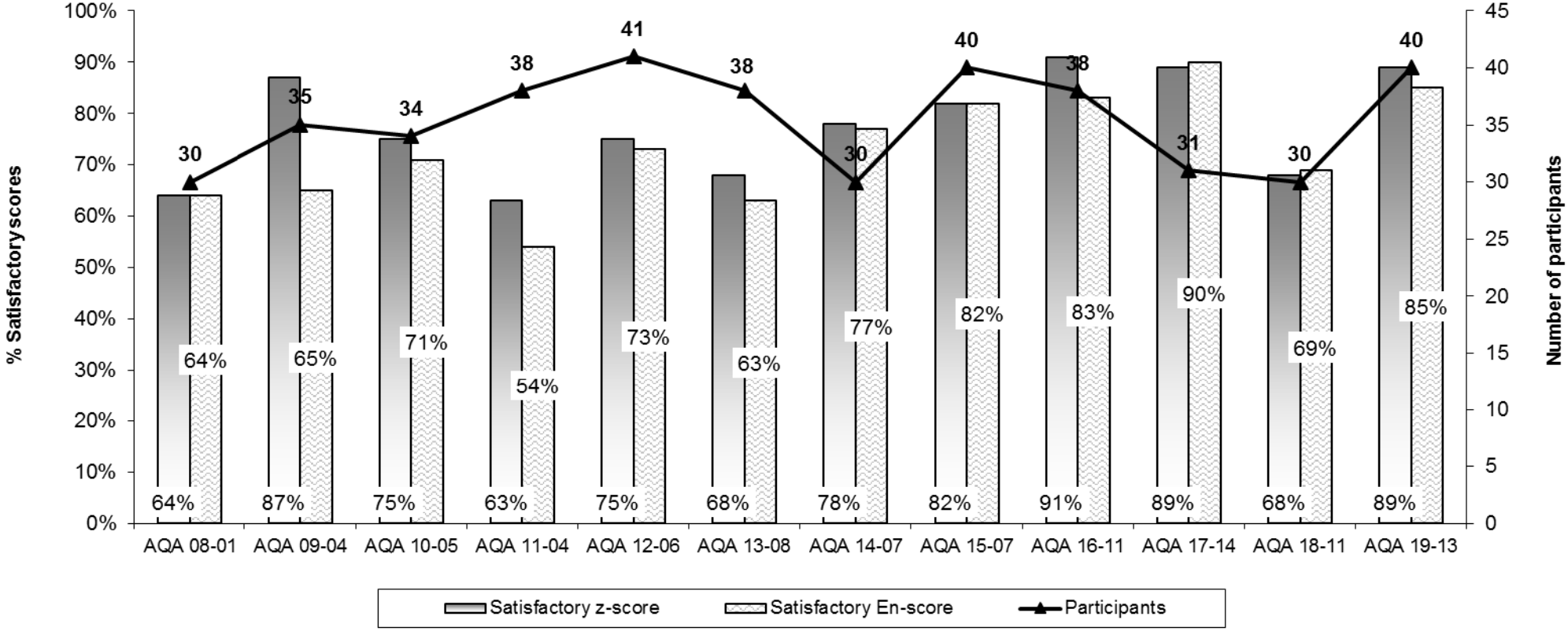


Figure 10 Summary of participants' performance since 2008

7 REFERENCES

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

[2] NMI, *Chemical Proficiency Testing Study Protocol*, viewed 19 June 2019, <http://www.measurement.gov.au>.

[3] NMI, *Chemical Proficiency Testing Statistical Manual*, viewed 19 June 2019, <http://www.measurement.gov.au>.

[4] Thompson, M., Ellison, S.L.R. and Wood, R. 2006. 'The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories', *Pure Appl. Chem.* 78, pp 145-196.

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

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

[7] Eurachem/CITAC Guide 2012, *Quantifying uncertainty in analytical measurement*, 3rd Edition, <http://eurachem.org/images/stories/guides/pdf/quam2012_P1.pdf>.

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

APPENDIX 1 - MEASUREMENT UNCERTAINTY OF THE ASSIGNED VALUE

When the assigned value is calculated as the robust average using the procedure described in 'ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons – Annex C',[5] the uncertainty is estimated as:

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

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 S1 as % base (m/m)

No. results (p)	40
Robust Average	51.8
$S_{rob\ average}$	2.0
$u_{rob\ average}$	0.4
k	2
$U_{rob\ average}$	0.8

The robust average for Sample S1 is 51.8 ± 0.8% heroin base (m/m).

APPENDIX 2 - ACRONYMS AND ABBREVIATIONS

ASCLD	American Society of Crime Laboratory Directors
ANAB	ANSI (American National Standards Institute) National Accreditation Board
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
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
MS	Mass Spectrometry
MSMS	Tandem Mass Spectrometry
MU	Measurement Uncertainty
NATA	National Association of Testing Authorities
NMI	National Measurement Institute Australia
NR	Not Reported
NT	Not Tested
PCV	Performance Coefficient of Variation
PDA	Photodiode array
PT	Proficiency Test
QNMR	Quantitative Nuclear Magnetic Resonance
R.A.	Robust Average
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