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National Measurement Institute

Proficiency Test Report
AQA 19-18
Cocaine

July 2020

ACKNOWLEDGMENTS

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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.

Jenny Xu

Raluca Iavetz

Manager, Chemical Reference Values

105 Delhi Rd, North Ryde NSW 2113, Australia

Phone: +61 2 9449 0178

Email: raluca.iavetz@measurement.gov.au

Accredited for compliance with ISO/IEC 17043

TABLE OF CONTENTS

SUMMARY 1
1 INTRODUCTION 2
1.1 NMI Proficiency Testing Program 2
1.2 Study Aims 2
1.3 Study Conduct 2
2 STUDY INFORMATION 3
2.1 Study Timetable 3
2.2 Participation 3
2.3 Test Material Specification 3
2.4 Laboratory Code 3
2.5 Test Sample Homogeneity 3
2.6 Sample Dispatch 3
2.7 Instructions to Participants 4
2.8 Interim Report 4
3 PARTICIPANT LABORATORY INFORMATION 5
3.1 Test Methods Reported by Participants 5
3.2 Reported Basis of Participants' Measurement Uncertainty Estimates 6
3.3 Details of Participant Calibration Standard 9
3.4 Participants' Comments 10
4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS 11
4.1 Results Summary 11
4.2 Assigned Value 11
4.3 Robust Average and Robust Between Laboratory Coefficient of Variation 11
4.4 Performance Coefficient of Variation (PCV) 11
4.5 Target Standard Deviation 11
4.6 z-Score 12
4.7 E_n-Score 12
4.8 Traceability and Measurement Uncertainty 12
5 TABLES AND FIGURES 13
6 DISCUSSION OF RESULTS 20
6.1 Assigned Value 20
6.2 Measurement Uncertainty Reported by Participants 20
6.3 z-Score 20
6.4 E_n-Score 22
6.5 Identification of Cutting Agent 23
6.6 Duplicate Samples S2 and S3 23
6.7 Participants' Analytical Methods 25
6.8 Comparison with Previous Cocaine PT Studies 28
7 REFERENCES 29
APPENDIX 1 – MEASUREMENT UNCERTAINTY OF THE ASSIGNED VALUE 30
APPENDIX 2 - ACRONYMS AND ABBREVIATIONS 31

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SUMMARY

AQA 19-18 Cocaine commenced in October 2019. Three test samples of cocaine hydrochloride were sent to thirty laboratories, with one laboratory requesting two sets of test samples to be analysed by different analysts. All participants returned results.

Samples were prepared at the NMI laboratory in Sydney using an illicit seizure of cocaine hydrochloride, approximately 84% base (m/m) supplied by the Australian Federal Police.

The assigned values were the robust averages 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 cocaine in samples typical of a routine seizure;*

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

Of 93 z-scores, 72 (77%) returned $|z| \leq 2.0$ (indicating a satisfactory performance).

Of 93 E_n-scores, 73 (78%) returned $|E_n| \leq 1.0$ (indicating agreement of the participant's results with the assigned value within their respective expanded uncertainties).

Laboratories **1**, **3**, **4**, **5**, **6**, **8**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **24**, **26**, **28** and **30** returned satisfactory z- and E_n-scores for all samples.

Laboratories **7** and **22** returned unsatisfactory z- and E_n-scores for all samples.

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

Of 93 results, 90 (97%) were reported with an associated expanded measurement uncertainty. Laboratory **23** did not report uncertainties for their results; this laboratory was not accredited. The magnitude of reported uncertainties was within the range 0% to 15% relative.

The metrological traceability of the assigned values has not been established as they were the consensus of participants' results.

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

Sample S1 was cut with levamisole, and Samples S2 and S3 were blind duplicates cut with caffeine.

All participants reported on the identity of the cutting agents of at least one sample. Laboratories **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, **19**, **21**, **22**, **23**, **25**, **26**, **27**, **28** and **29** correctly identified all cutting agents in the test samples.

For Sample S1, 26 participants (84%) correctly identified levamisole as the only cutting agent. For Sample S2, all participants correctly identified caffeine as the cutting agent. For Sample S3, 30 participants (97%) correctly identified caffeine as the cutting agent.

After the release of the interim report, one participant stated they had mixed the reporting of results for Samples S1 and 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 the: '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; and
- allergens in food.

1.2 Study Aims

The aims of the study were to:

- assess the proficiency of laboratories measuring cocaine 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 cutting agents commonly found in controlled drug preparation.

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

1.3 Study Conduct

The conduct of NMI proficiency tests is described in the NMI Study Protocol for Proficiency Testing.[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/IEC 17043[1] and The International Harmonized Protocol for Proficiency Testing of Analytical Chemistry Laboratories.[4]

NMI is accredited by the National Association of Testing Authorities, Australia (NATA) to ISO/IEC 17043 as a provider of proficiency testing schemes. This study is within the scope of NMI's accreditation.

2 STUDY INFORMATION

2.1 Study Timetable

The timetable of the study was:

Invitation issued	8 October 2019
Samples dispatched	21 January 2020
Results due	26 June 2020
Interim report issued	10 July 2020

2.2 Participation

Thirty laboratories participated in this study. One laboratory requested two sets of test samples to be analysed by different analysts. All participants returned results.

2.3 Test Material Specification

Three test samples were prepared in November 2019. The starting material was cocaine hydrochloride approximately 84% base (m/m) supplied by the Australian Federal Police. Caffeine purchased from Sigma Aldrich and levamisole purchased from Acros Organics were used as cutting agents. Sample S1 was cut with levamisole, and Samples S2 and S3 were blind duplicates cut with caffeine.

The cocaine was ground and sieved through a 180 μm sieve. The cutting agents were processed similarly to the cocaine. Test samples were then 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 ~65% cocaine base (m/m).

Sample S2 was prepared to contain ~46% cocaine base (m/m).

Sample S3 was identical to Sample S2 (duplicate).

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 test samples.

2.6 Sample Dispatch

A set of three test samples, each containing approximately 150 mg of test material, were dispatched to participants in January 2020. 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 cocaine base;
- an estimate of the expanded uncertainty associated with the result as % m/m cocaine base at the 95% confidence level;
- brief detail on how the uncertainty was calculated e.g. uncertainty budget method, repeatability precision;
- the identity of the cutting agents in all three samples, if part of routine analysis;
- source and stated purity of the analytical reference standard used;
- brief summary of the quantitative method used; and
- return the completed results sheet by 27 March 2020, as late results may not be included in the report.

The results due date was changed from 27 March 2020 to 26 June 2020. Due to the exceptional international circumstances occurring over the course of this study, some laboratories were not able to report results as originally scheduled, and therefore the results turnaround time was extended by 3 months.

2.8 Interim Report

An interim report was emailed to all participants on 10 July 2020.

3 PARTICIPANT LABORATORY INFORMATION

3.1 Test Methods Reported by Participants

Participants were requested to provide information about their test methods. Responses are presented in Table 1.

Table 1 Participant Summary of Test Methods*

Lab. Code	Extraction Solvent	Internal Standard	Calib. Points	Technique	Detector	Column
1	acetonitrile/water (80/20)	external standard	2	HPLC	DAD	C8
2	Acetonitrile/water (60/40)	None	5	HPLC	UV	Column C8
3	Methanol	Tetracosane	4	GC	FID	SGE 12 x 0.22 mm
4	Methanol	None	5	HPLC	DAD	Kinetex 2.6 µ XB-C18
5	Acetonitrile	Strychnine	6	GC	FID	HP1
6	Ethanol	Tetracosane	6	GC	FID	HP5
7	Acetonitrile/Methanol (95:5)	Pholcodine 1mg/ml	3	UPLC	PDA	ACQUITY C-18
8	Ethanol	Triphenylacetophenone (TPAP)	3	GC	FID	HP1
9	Methanol		4	HPLC	DAD	Zorbax Eclipse XDB-C-18
10	mobile phase	N/A	4	HPLC	UV	uBondapak
11	Acetonitrile/water/ trifluoroacetic acid (25/75/0.1)	N/A	3	HPLC	DAD	ODS2 Inertpak column
12	Acetonitrile:water (75:25)	Diethylphthalate	3	UPLC5	DAD	Acquity BEH C18 1.7µm (2.1 x 100mm)
13	Ethanol	Tribenzylamine	6	GC	FID	HP5
14	Methanol	No	7	HPLC	DAD	ZORBAX ECLIPSE XDB-C18 (5µm pore size, 4.6mmx150mm)
15	Methanol	Vanillin	1	LC	DAD	Lichrospher 60 RP-select B, 25cm x 4 mm id, 5um
16	CDCl3	1,4-bis(trimethylsilyl)benzene		QNMR		NA
17	deuterium oxide	Maleic acid		1H QNMR	Bruker AVIII 600 with BBFO probe	N/A
18	Methanol	none	3	GC	FID	CP sil5CB
19	Methanol	n/a	6	UPLC	PDA	Acquity UPLC BEH 1.7um 2.1 x 100mm
20	Acetonitrile/Water	None	5	HPLC	UV	Kinetex 5u C18

Lab. Code	Extraction Solvent	Internal Standard	Calib. Points	Technique	Detector	Column
21	ACN/MeOH/H2O	Analogue of cocaine	7	UPLC	MSMS	C-18 coloumn
22	Chloroform	octacosane	5	GC	MS	ZB 5 plus
23	Ethanol	Propylparaben	7	UPLC	DAD	BEH Shield RP18
24	Sodiumphosphate (pH4.5)	None	4	HPLC	UV-DAD	Hypersil GOLD C8
25	Dichloromethane/ methanol 50/50		5	HPLC	UV	C8
26	ethanol	tribenzylamine	4	GC	FID	HP-Ultra1
27	water/acetonitrile/n10 sulphuric acid 90:10:1	None	3	HPLC	Diode array	Shimpack XR-ODS
28	Methanol	Vanilin	1	LC	DAD	Lichrospher 60 RP-select B, 25cm x 4 mm id, 5um
29	Acetonitrile/water	none	1	HPLC	UV	Kromasil
30	acetonitrile/water (80/20)	none	3	HPLC	DAD	C8
31	Methanol	Methadone	4	GC	FID	RXI-5MS

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

3.2 Reported Basis of Participants' Measurement Uncertainty Estimates

Participants were requested to provide information about their basis of measurement uncertainty (MU). Responses are presented 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		Duplicate analysis	Standard purity	ISO/GUM
2	Professional judgement	Control samples - RM		
3	Top Down - precision and estimates of the method and laboratory bias	Control samples	Instrument calibration Matrix effects Laboratory bias from PT studies Recoveries of SS Standard purity	EA-4/16: 2003 and ILAG G-17: 2002
4	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples - CRM	Instrument calibration Standard purity	Eurachem/CITAC Guide
5	Top Down - precision and estimates of the method and laboratory bias	Control samples - CRM Duplicate analysis	Instrument calibration Masses and volumes Matrix effects Standard purity	ISO/GUM
6	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Matrix effects Recoveries of SS Standard purity	ISO/GUM

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
7	Top Down - precision and estimates of the method and laboratory bias	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	Nata Technical Note 33
8	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Matrix effects Laboratory bias from PT studies Recoveries of SS Standard purity	Eurachem/CITAC Guide
9				
10				
11	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - SS	Instrument calibration Masses and volumes Recoveries of SS	ISO/GUM
12	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Standard purity	Eurachem/CITAC Guide
13	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Standard purity	
14	Top Down - precision and estimates of the method and laboratory bias	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Laboratory bias from PT studies Standard purity	Nordtest Report TR537, Measurement Uncertainty for Weight Determination in Seized Drug Analysis
15	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
16	Top Down - precision and estimates of the method and laboratory bias	Control samples - previously analysed real seizure samples Duplicate analysis	Instrument calibration Matrix effects	Eurachem/CITAC Guide
17	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples - CRM Duplicate analysis	Instrument calibration Masses and volumes Standard purity	
18	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Instrument calibration Recoveries of SS	ISO/GUM
19	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	Nata Technical Note 33

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
20	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	Eurachem/CITAC Guide
21	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM		
22	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis		
23				
24	Top Down - precision and estimates of the method and laboratory bias	Standard deviation from PT studies only		Nordtest Report TR537
		Control samples - Samples from police case Duplicate analysis	Laboratory bias from PT studies	
25		Control samples - CRM Duplicate analysis		
26	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis		internal document based on Eurachem/CITAC, ISO/GUM
27	Professional judgement	Control samples - CRM Duplicate analysis	Standard purity	ISO/GUM
28	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
29	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - RM		ISO/GUM
30	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Laboratory bias from PT studies Standard purity	NF V03-110
31	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis	Masses and volumes	ISO/GUM

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

3.3 Details of Participant Calibration Standard

Participants were requested to provide information about their calibration standard used. Responses are presented in Table 3.

Table 3 Participant Calibration Standard*

Lab. Code	Reference Standard	Purity (%)
1	NMI	96.10
2	Lipomed	100
3	Merck	100
4	Lipomed	>98.5
5	NMI	95.7
6	ALCALIBER	100
7	NMI	95.7
8	NMI	96.1
9	LGC	1 mg/mL (U=0.003 mg/mL)
10	MacFarlan Smith	100.2
11	Johnson Matthey (MacFarlan Smith)	100.2
12	NMI	99.8
13	NMI (LGC NMIAD747-500MG)	99.8 +- 1.0
14	LIPOMED	99.55
15	Lipomed	99.247 ± 0.012
16	NA	
17	Sigma Aldrich	99.98±0.065
18	Duchefa	>99
19	NMI	96.1
20	Sigma	98.7
21	Unikem	100
22		
23	NMI	96
24	Cocaine HCl (C5776), Sigma Aldrich	100
25	SIGMA	99
26	Fagron	99.5
27	LGC	99.7
28	Lipomed	99.247 ± 0.012
29	Sigma-Aldrich	98.7
30	Lipomed	99.566
31	LGC	100

* Some responses 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 participants' comments are listed as received in Table 4 along with the study coordinator's response where appropriate.

Table 4 Participant Comments

Lab. Code	Participants' Comments	Study Coordinator's Response
4	We would wish to have amphetamine in future portfolio, and not only metamphetamine Methodology: Average of two determinations	We will take this into consideration for the planning of future amphetamine-type stimulant PT studies.
8	Methodology: A small amount of dichloromethane was used to dissolve the TPAP pior to the addition of ethanol.	
11	Uncertainty: The reported result (in routine case samples) is defined as the average of the individual results multiplied by the uncertainty correction factor and is rounded down to the nearest whole number (unless <1%w/w). The uncertainty correction factor is defined as (mean-2SD)/mean expressed as a percentage using the relevant standard control chart. E.g. a result of 62.0% would give a reported result of 62.0*0.9613 = 59.60 therefore rounded down to 59%.	
14	Qualitative analysis was carried out by GC-MS Methodology: External Standard	
15	Methodology: Multiple point calibration used during validation of the method. Routinely, a 1 point calibration is used every run and checked with 2 QC samples.	
16	Methodology: No reference standard involved	
17	Methodology: Simultaneous observation of analyte and IS peaks in 1H NMR spectrum acquired using QNMR conditions.	
18	Methodology: External standard	
25	Our analytical method is based on analysis weight of 10mg. Methodology: External standard	
27	Uncertainty: MuM determined from 3 x std deviation of multiple injections expanded by professional judgement. No analysis carried out for inert bulking agents	
28	Methodology: Multiple point calibration used during validation of the method. Routinely, a 1 point calibration is used every run and checked with 2 QC samples.	
30	Please send 3 samples of different concentrations (e.g. 20%, 40% and 60%) and not 2 samples of the same concentration.	Samples were prepared to be of levels which could cater to the needs of different laboratories. Occasionally, identical samples are prepared to assess laboratories' performance for blind duplicate samples.

4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

4.1 Results Summary

Participant results are listed in Tables 5 to 7 with the summary statistics: robust average, mean, median, maximum (Max.), minimum (Min.), 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.

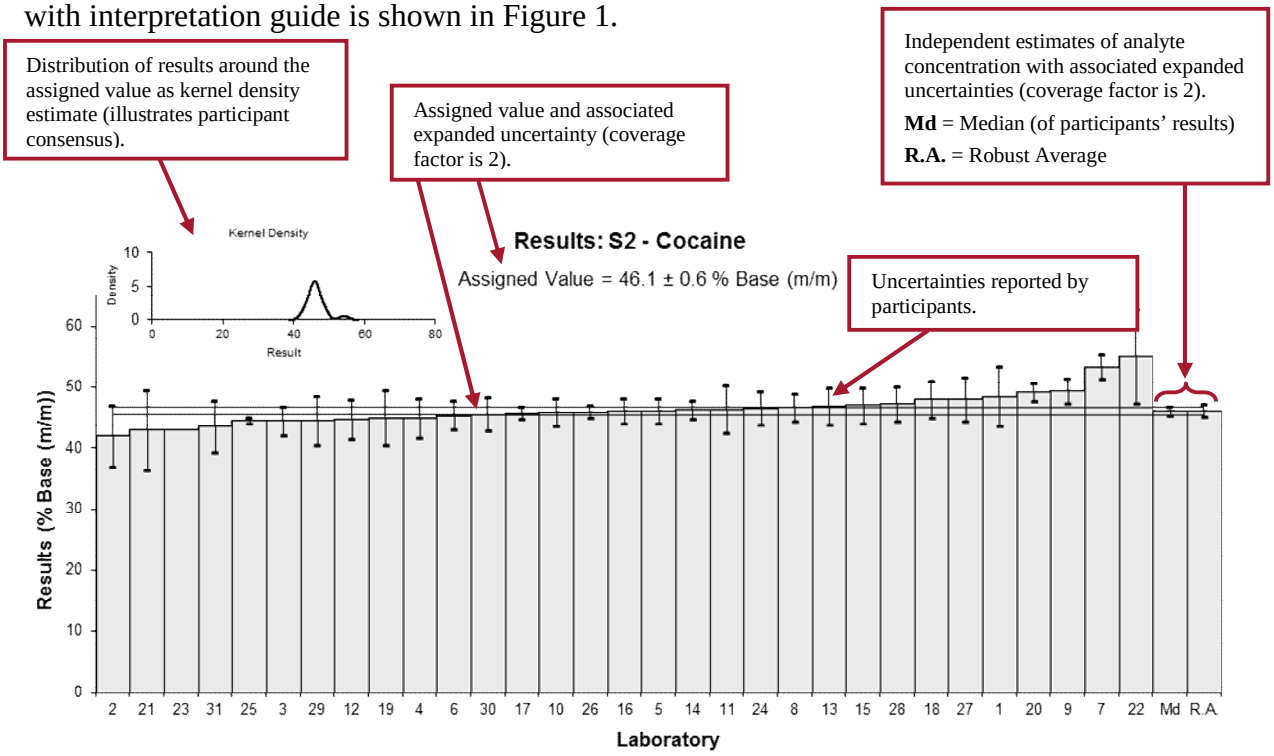


Figure 1 Guide to Presentation of Results

4.2 Assigned Value

The assigned value is defined as the: 'value attributed to a particular property of a proficiency test item'.[1] In this study, the property is the concentration of the analyte in the test samples. Assigned values were the robust averages of participants' results and the expanded uncertainties were estimated from the associated robust SDs (Appendix 1).

4.3 Robust Average and Robust Between Laboratory Coefficient of Variation

The robust averages and associated expanded MUs, and robust CVs (a measure of the variability of participants' results) were calculated using the procedure described in ISO 13528:2015.[5]

4.4 Performance Coefficient of Variation (PCV)

The performance coefficient of variation (PCV) is a measure of the between laboratory variation that in the judgement of the study coordinator would be expected from participants given the sample concentration. It is important to note that this is a performance measure set by the study coordinator; it is not the CV of participants' results.

4.5 Target Standard Deviation

The target standard deviation (σ) is the product of the assigned value (X) and the PCV, as presented in Equation 1. This value is used for calculation of z-scores.

$$\sigma = X \times PCV \qquad \textit{Equation 1}$$

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 a participant's result

X is the assigned value

σ is the target standard deviation from Equation 1

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

- $|z| \leq 2.0$ is satisfactory;
- $2.0 < |z| < 3.0$ is questionable;
- $|z| \geq 3.0$ 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 participant's result

X is the assigned value

U_χ is the expanded uncertainty of the participant's result

U_X is the expanded uncertainty of the assigned value

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

- $|E_n| \leq 1.0$ is satisfactory;
- $|E_n| > 1.0$ is unsatisfactory.

4.8 Traceability and Measurement Uncertainty

Laboratories accredited to ISO/IEC 17025 must establish and demonstrate the traceability and measurement uncertainty associated with their test results.[6]

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	Cocaine
Units	% Base (m/m)

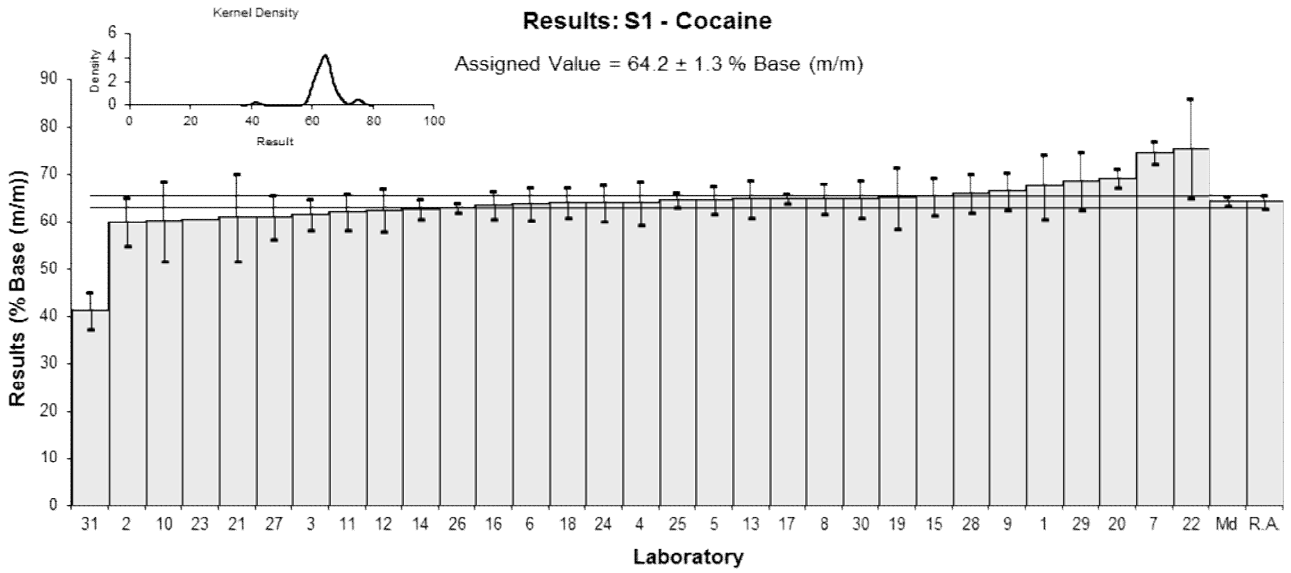
Participant Results

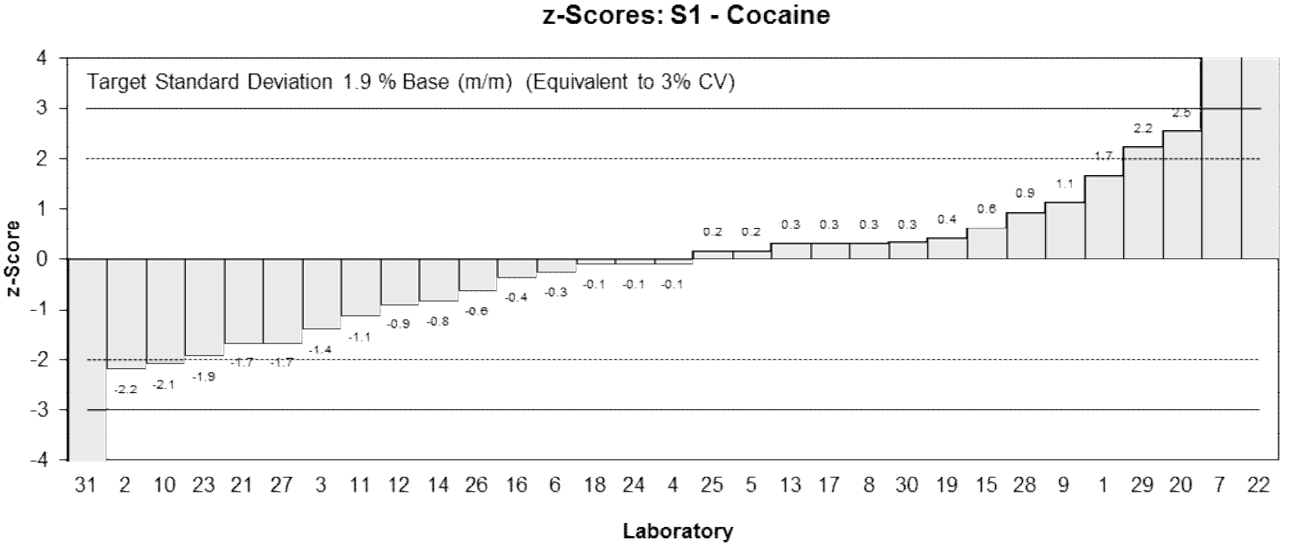
Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	67.4	6.8	1.66	0.46
2	60	5	-2.18	-0.81
3	61.5	3.2	-1.40	-0.78
4	64	4.6	-0.10	-0.04
5	64.5	2.8	0.16	0.10
6	63.7	3.3	-0.26	-0.14
7	74.7	2.4	5.45	3.85
8	64.8	3.2	0.31	0.17
9	66.4	4	1.14	0.52
10	60.2	8.4	-2.08	-0.47
11	62.0	3.87	-1.14	-0.54
12	62.47	4.47	-0.90	-0.37
13	64.8	4	0.31	0.14
14	62.6	2.0	-0.83	-0.67
15	65.4	4.0	0.62	0.29
16	63.5	2.9	-0.36	-0.22
17	64.8	0.9	0.31	0.38
18	64	3	-0.10	-0.06
19	65	6.5	0.42	0.12
20	69.1	2.07	2.54	2.00
21	61	9.2	-1.66	-0.34
22	75.5	10.6	5.87	1.06
23	60.5	NR	-1.92	-2.85
24	64.0	3.8	-0.10	-0.05
25	64.5	1.5	0.16	0.15
26	63	1	-0.62	-0.73
27	61	4.6	-1.66	-0.67
28	66.0	4.0	0.93	0.43
29	68.5	6.1	2.23	0.69
30	64.83	3.89	0.33	0.15
31	41.41	3.94	-11.83	-5.49

Statistics*

Assigned Value	64.2	1.3
Robust Average	64.2	1.3
Median	64.3	0.9
Mean	64.7	
N	30	
Max.	75.5	
Min.	60	
Robust SD	2.8	
Robust CV	4.4%	

* Result from Laboratory 31 was omitted from all statistical calculations. After the interim report was released, the participant reported they had switched results for Samples S1 and S3.


Kernel Density
Density
Result
Results: S1 - Cocaine
Assigned Value = 64.2 ± 1.3 % Base (m/m)
Results (% Base (m/m))
Laboratory
31 2 10 23 21 27 3 11 12 14 26 16 6 18 24 4 25 5 13 17 8 30 19 15 28 9 1 29 20 7 22 Md R.A.


z-Scores: S1 - Cocaine
Target Standard Deviation 1.9 % Base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
31 2 10 23 21 27 3 11 12 14 26 16 6 18 24 4 25 5 13 17 8 30 19 15 28 9 1 29 20 7 22

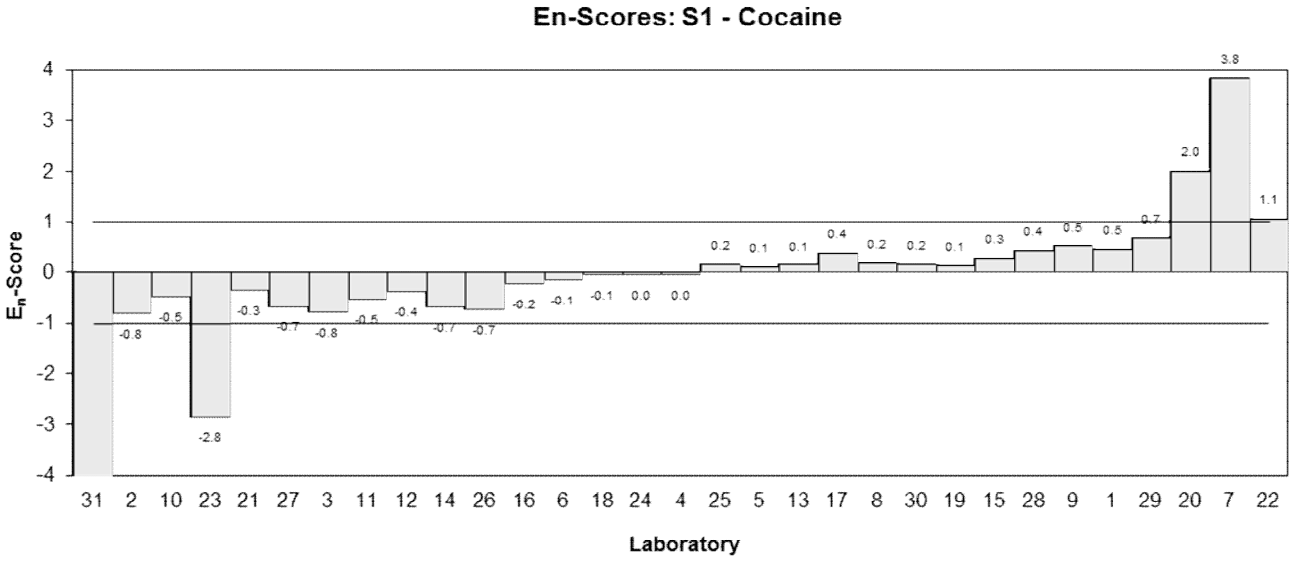

En-Scores: S1 - Cocaine
En-Score
Laboratory
31 2 10 23 21 27 3 11 12 14 26 16 6 18 24 4 25 5 13 17 8 30 19 15 28 9 1 29 20 7 22

Figure 2

Table 6

Sample Details

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

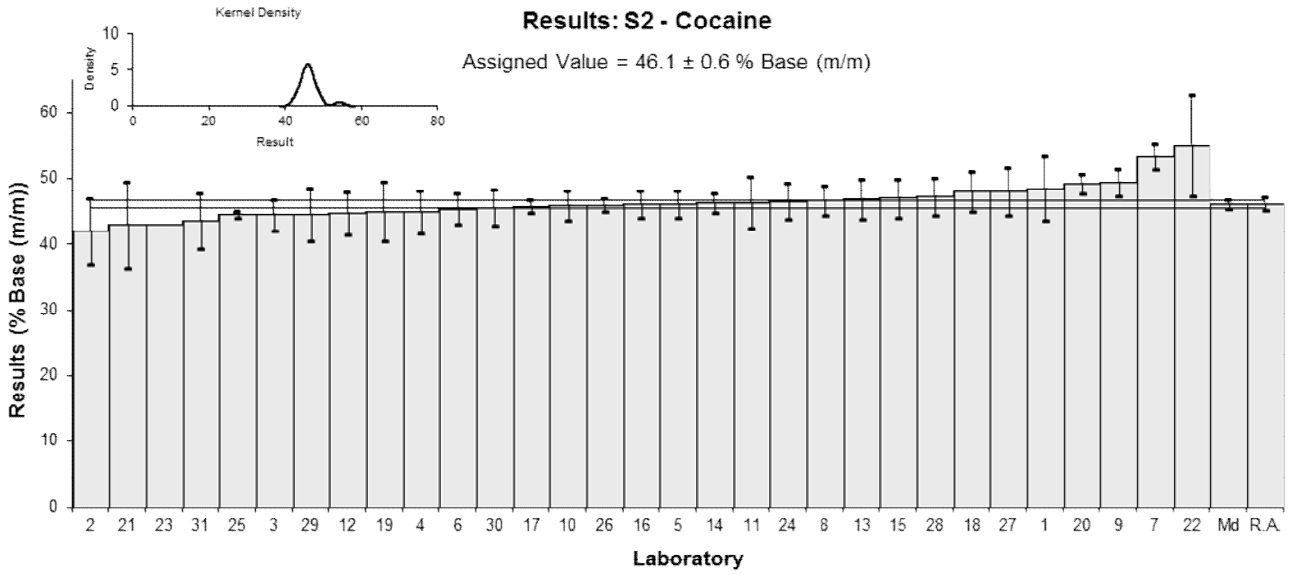
Participant Results

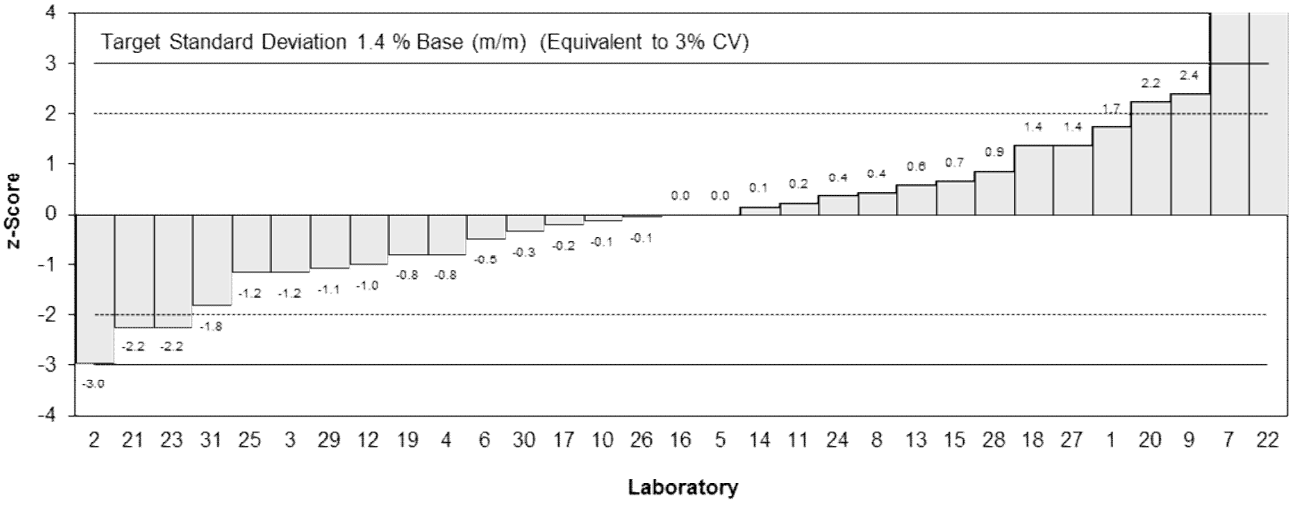
Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	48.5	4.9	1.74	0.49
2	42	5	-2.96	-0.81
3	44.5	2.3	-1.16	-0.67
4	45	3.2	-0.80	-0.34
5	46.1	2.0	0.00	0.00
6	45.4	2.4	-0.51	-0.28
7	53.4	1.9	5.28	3.66
8	46.7	2.3	0.43	0.25
9	49.4	2	2.39	1.58
10	45.9	2.2	-0.14	-0.09
11	46.4	3.87	0.22	0.08
12	44.70	3.26	-1.01	-0.42
13	46.9	3	0.58	0.26
14	46.3	1.5	0.14	0.12
15	47.0	2.9	0.65	0.30
16	46.1	2.1	0.00	0.00
17	45.8	1.0	-0.22	-0.26
18	48	3	1.37	0.62
19	45	4.5	-0.80	-0.24
20	49.2	1.48	2.24	1.94
21	43	6.5	-2.24	-0.47
22	55.0	7.7	6.44	1.15
23	43.0	NR	-2.24	-5.17
24	46.6	2.8	0.36	0.17
25	44.5	0.5	-1.16	-2.05
26	46	1	-0.07	-0.09
27	48	3.6	1.37	0.52
28	47.3	2.9	0.87	0.41
29	44.6	4.0	-1.08	-0.37
30	45.62	2.74	-0.35	-0.17
31	43.58	4.15	-1.82	-0.60

Statistics

Assigned Value*	46.1	0.6
Robust Average	46.2	1.0
Median	46.1	0.7
Mean	46.4	
N	31	
Max.	55.0	
Min.	42	
Robust SD	2.1	
Robust CV	4.6%	

* Assigned value calculated as the robust average of the combined results of duplicate pair Samples S2 and S3.


Results: S2 - Cocaine
Assigned Value = 46.1 ± 0.6 % Base (m/m)
Kernel Density
Density
Result
Results (% Base (m/m))
Laboratory
2 21 23 31 25 3 29 12 19 4 6 30 17 10 26 16 5 14 11 24 8 13 15 28 18 27 1 20 9 7 22 Mid R.A.


z-Scores: S2 - Cocaine
Target Standard Deviation 1.4 % Base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
2 21 23 31 25 3 29 12 19 4 6 30 17 10 26 16 5 14 11 24 8 13 15 28 18 27 1 20 9 7 22

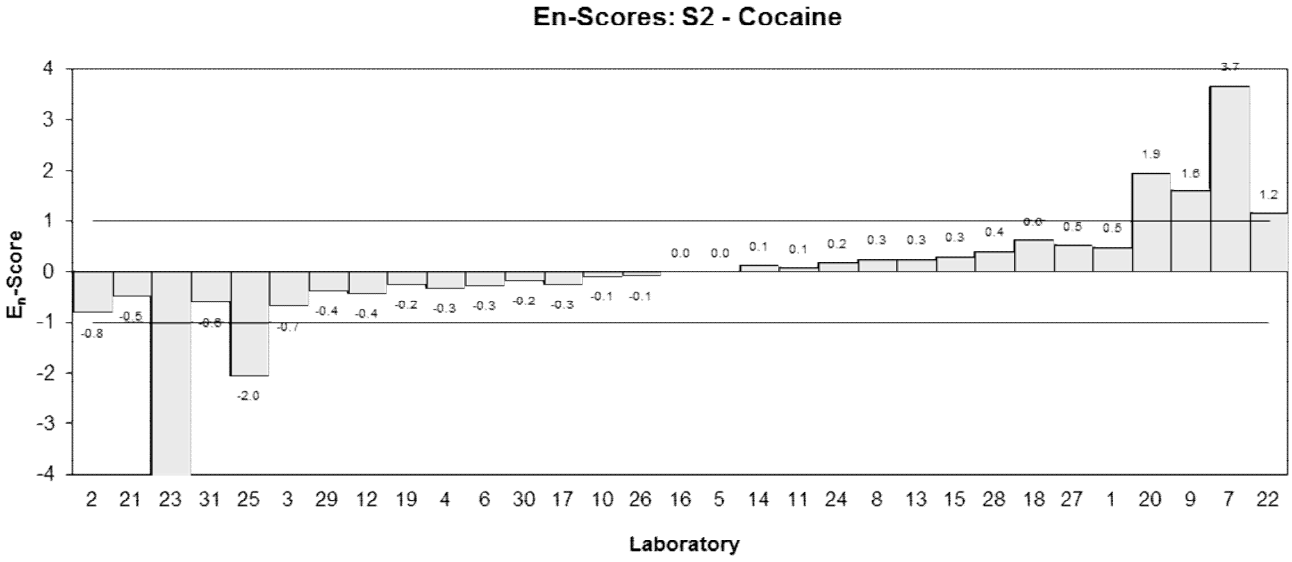

En-Scores: S2 - Cocaine
En-Score
Laboratory
2 21 23 31 25 3 29 12 19 4 6 30 17 10 26 16 5 14 11 24 8 13 15 28 18 27 1 20 9 7 22

Figure 3

Table 7

Sample Details

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

Participant Results

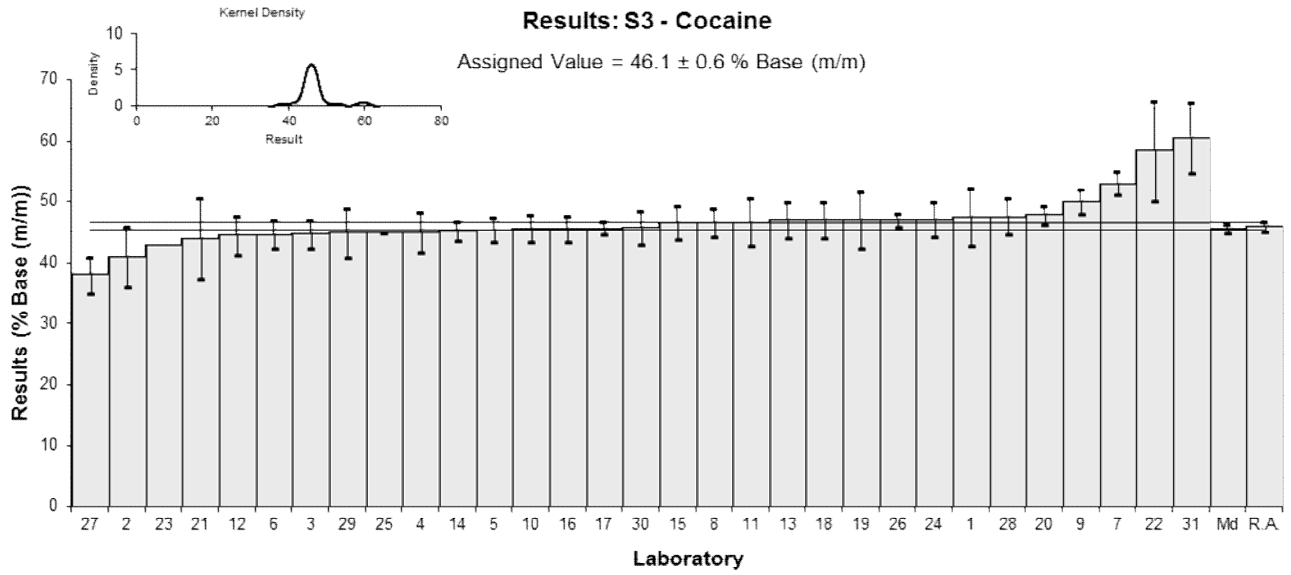
Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	47.5	4.8	1.01	0.29
2	41	5	-3.69	-1.01
3	44.7	2.3	-1.01	-0.59
4	45	3.2	-0.80	-0.34
5	45.5	2.0	-0.43	-0.29
6	44.6	2.3	-1.08	-0.63
7	53.0	1.9	4.99	3.46
8	46.6	2.3	0.36	0.21
9	50.0	2	2.82	1.87
10	45.6	2.2	-0.36	-0.22
11	46.7	3.87	0.43	0.15
12	44.54	3.19	-1.13	-0.48
13	47.0	3	0.65	0.29
14	45.2	1.5	-0.65	-0.56
15	46.6	2.8	0.36	0.17
16	45.6	2.1	-0.36	-0.23
17	45.7	1.1	-0.29	-0.32
18	47	3	0.65	0.29
19	47	4.7	0.65	0.19
20	47.9	1.44	1.30	1.15
21	44	6.6	-1.52	-0.32
22	58.4	8.2	8.89	1.50
23	42.9	NR	-2.31	-5.33
24	47.1	2.8	0.72	0.35
25	45	0	-0.80	-1.83
26	47	1	0.65	0.77
27	38	2.9	-5.86	-2.74
28	47.6	2.9	1.08	0.51
29	44.9	4.0	-0.87	-0.30
30	45.76	2.75	-0.25	-0.12
31	60.50	5.77	10.41	2.48

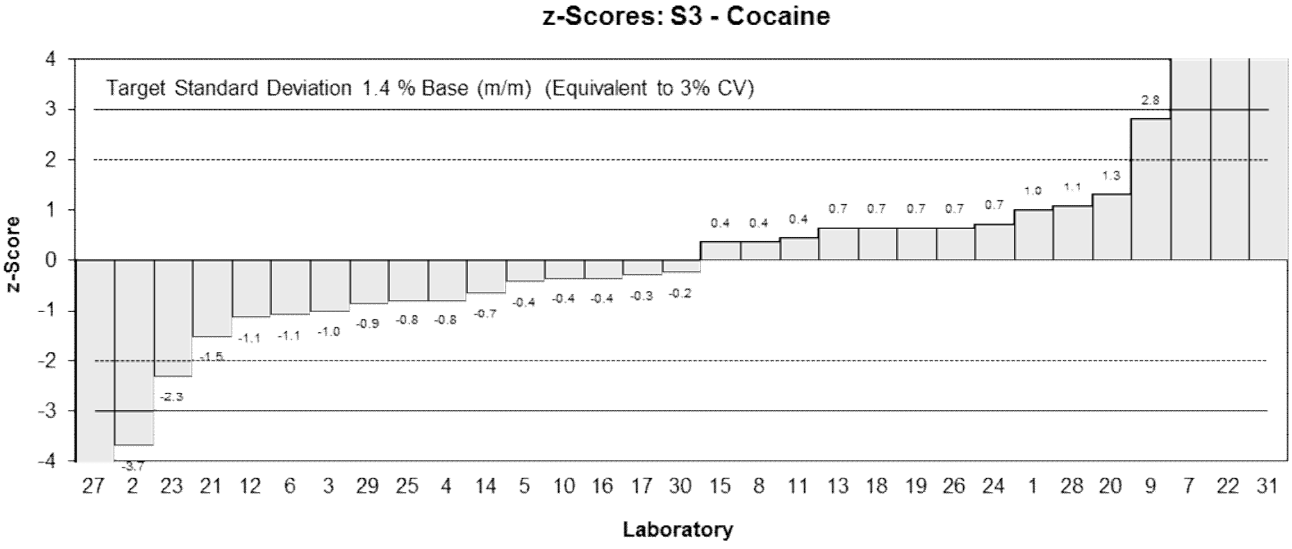
Statistics*

Assigned Value**	46.1	0.6
Robust Average	46.0	0.8
Median	45.7	0.7
Mean	46.2	
N	30	
Max.	58.4	
Min.	38	
Robust SD	1.8	
Robust CV	3.9%	

* Result from Laboratory 31 was omitted from all statistical calculations. After the interim report was released, the participant reported they had switched results for Samples S1 and S3.

** Assigned value calculated as the robust average of the combined results of duplicate pair Samples S2 and S3.


Results: S3 - Cocaine
Assigned Value = 46.1 ± 0.6 % Base (m/m)
Kernel Density
Density
Result
Results (% Base (m/m))
Laboratory
27 2 23 21 12 6 3 29 25 4 14 5 10 16 17 30 15 8 11 13 18 19 26 24 1 28 20 9 7 22 31 Md R.A.


z-Scores: S3 - Cocaine
Target Standard Deviation 1.4 % Base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
27 2 23 21 12 6 3 29 25 4 14 5 10 16 17 30 15 8 11 13 18 19 26 24 1 28 20 9 7 22 31

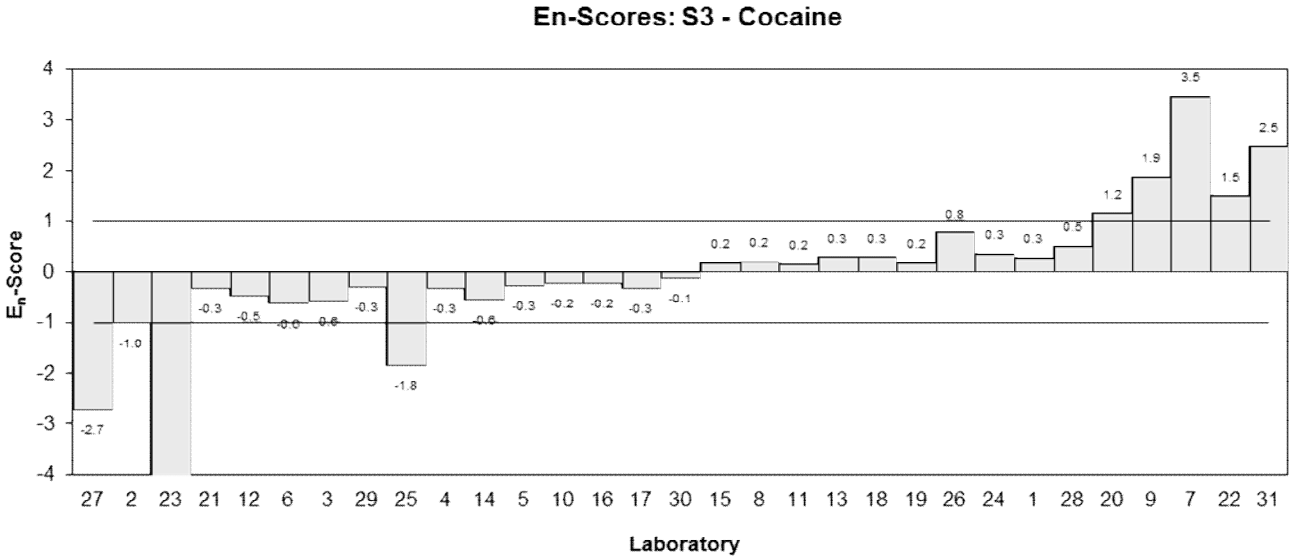

En-Scores: S3 - Cocaine
En-Score
Laboratory
27 2 23 21 12 6 3 29 25 4 14 5 10 16 17 30 15 8 11 13 18 19 26 24 1 28 20 9 7 22 31

Figure 4

Table 8 Participants' Identification of Cutting Agents*

Lab. Code	Cutting Agents		
	S1	S2	S3
1	Levamisole	Caffeine	Caffeine
2	Levamisole	Caffeine	Caffeine
3	Levamisole	caffeine	caffeine
4	Levamisole	Caffeine	Caffeine
5	Levamisole	Caffeine	Caffeine
6	Levamisole	Caffeine	Caffeine
7	Levamisole	Caffeine	Caffeine
8	Tetramisole The name tetramisole has been used because the analysis cannot discriminate between the stereoisomers levamisole and dexamisole.	Caffeine	Caffeine
9	Tetramisole	Caffeine	Caffeine
10	phenylimidothiazole	caffeine	caffeine
11	Levamisole	Caffeine	Caffeine
12	Levamisole indicated	Caffeine detected	Cafeine detected
13	24% levamisole.HCl	46% caffeine	45% caffeine
14	Levamisole	Caffeine	Caffeine
15	Levamisole/tetramisole (specific isomer not determined	Caffeine	Caffeine
16	levamisole	caffeine	caffeine
17	Tetramisole 20.2%	Caffeine 45.5%	Caffeine 45.7%
18	Levamisole, caffeine	Caffeine	Caffeine
19	Levamisole/Dexamisole	Caffeine	Caffeine
20	None detected	Caffeine	Caffeine
21	Levamisole	Caffeine	Caffeine
22	levamisole	caffeine	caffeine
23	Levamisole = 17.8 %	Caffeine = 42.4 %	Caffeine = 43.0 %
24		Caffeine	Caffeine
25	levamisole	caffeine	caffeine
26	(a mixture of) levamisole and/of dexamisole	caffeine	caffeine
27	Levamisole	Caffeine	Caffeine
28	Levamisole/tetramisole (specific isomer not determined	Caffeine	Caffeine
29	levamisole	caffeine	caffeine
30	levamisole, caffeine	caffeine	caffeine
31	Caffeine	Caffeine	Tetramisole

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

6 DISCUSSION OF RESULTS

6.1 Assigned Value

The assigned values are the robust averages of the results reported by participants. The robust averages and associated expanded uncertainties were calculated using the procedure described in ISO 13528:2015.[5] Results less than 50% and greater than 150% of the robust average were removed before calculation of the assigned value.[3,4] The calculation of the expanded uncertainty for robust averages is presented in Appendix 1, using Sample S1 as an example.

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.

After the release of the interim report, Laboratory **31** reported they had switched results for Samples S1 and S3. These results were excluded from the calculation of the assigned values.

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/IEC 17025:2017 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.[6] From July 2012 this is also a requirement of the ASCLD/LAB-International accreditation program.

Of 93 results, 90 (97%) were reported with an associated expanded measurement uncertainty. Laboratory **23** did not report uncertainties for their results; this laboratory was not accredited.

The magnitude of reported uncertainties was within the range 0% to 15% relative.

Of 90 expanded measurement uncertainties, 68 (76%) 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 62.47 ± 4.47%, it is better to report this as 62.5 ± 4.5%).[7]

6.3 z-Score

A target SD equivalent to 3% PCV was used to calculate z-scores. The CVs predicted by the Thompson-Horwitz equation,[8] target SDs, and between laboratories CVs obtained in this study are presented in Table 9.

Table 9 Comparison of Target SDs, Thompson-Horwitz CVs and Between Laboratories CVs

Sample	Analyte	Assigned value (% base (m/m))	Thompson-Horwitz CV (%)	Target SD (as PCV) (%)	Between Laboratories CV (%)
S1	Cocaine	64.2	1.2	3	4.4
S2	Cocaine	46.1	1.5	3	4.6
S3					3.9

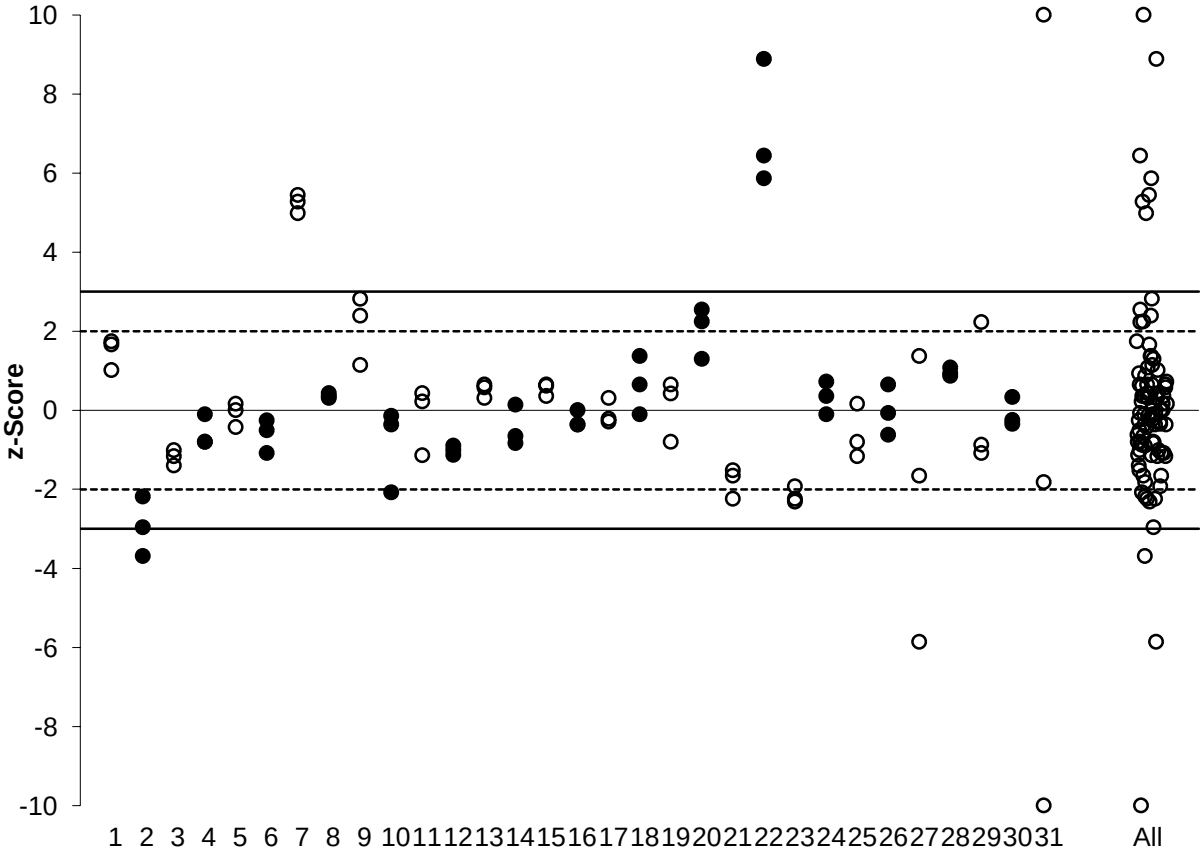
Of 93 results for which z-scores were calculated, 72 (77%) returned a satisfactory z-score of $|z| \leq 2.0$.

Twenty participants: **1**, **3**, **4**, **5**, **6**, **8**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **24**, **25**, **26**, **28** and **30** returned satisfactory z-scores for all three samples.

Eleven participants returned at least one questionable or unsatisfactory z-score.

Laboratories **2**, **7** and **22** returned questionable or unsatisfactory z-scores for all test samples, demonstrating an unsatisfactory performance. Laboratory **2** reported all results lower than the assigned value (negative bias), and therefore may need to investigate their sources of bias. Laboratories **7** and **22** reported all results higher than the assigned value (positive bias); these laboratories should check if they reported results as % salt (m/m) instead of % base (m/m), or investigate potential sources of bias.

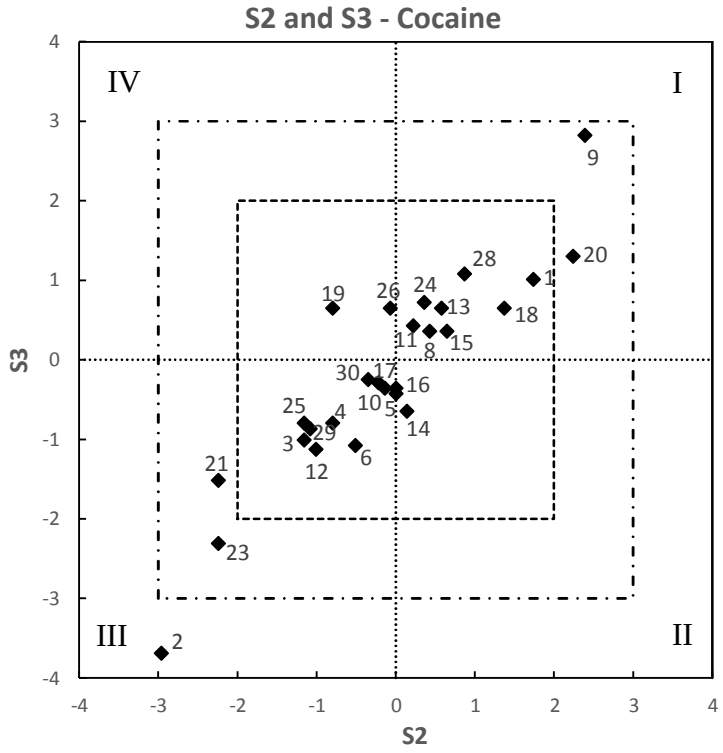
The dispersal of participants' z-scores is presented graphically by laboratory in Figure 5.



Scores greater than 10 or less than -10 have been plotted at 10 and -10 respectively.

Figure 5 z-Score Dispersal by Laboratory

A scatter plot of z-scores for blind duplicate Samples S2 and S3 is presented in Figure 6. Scores are predominantly in quadrants I and III, indicating that laboratory bias is the major contributor to the variability of results.



Laboratories 7, 22, 27 and 31 are off scale.

Figure 6 z-Score Scatter Plot – Cocaine in S2 and S3

6.4 E_n-Score

Where a participant did not report an expanded uncertainty with a result, an expanded uncertainty of zero (0) was used to calculate the E_n-score.

Of 93 results for which E_n-scores were calculated, 73 (78%) returned a satisfactory E_n-score of $|E_n| \leq 1.0$.

Twenty-two participants: **1**, **3**, **4**, **5**, **6**, **8**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **21**, **24**, **26**, **28**, **29** and **30** returned satisfactory E_n-scores for all three samples.

Nine participants returned at least one unsatisfactory E_n-score.

Laboratories **7**, **20**, **22** and **23** returned questionable or unsatisfactory E_n-scores for all test samples.

The dispersal of participants' E_n-scores is presented graphically in Figure 7.

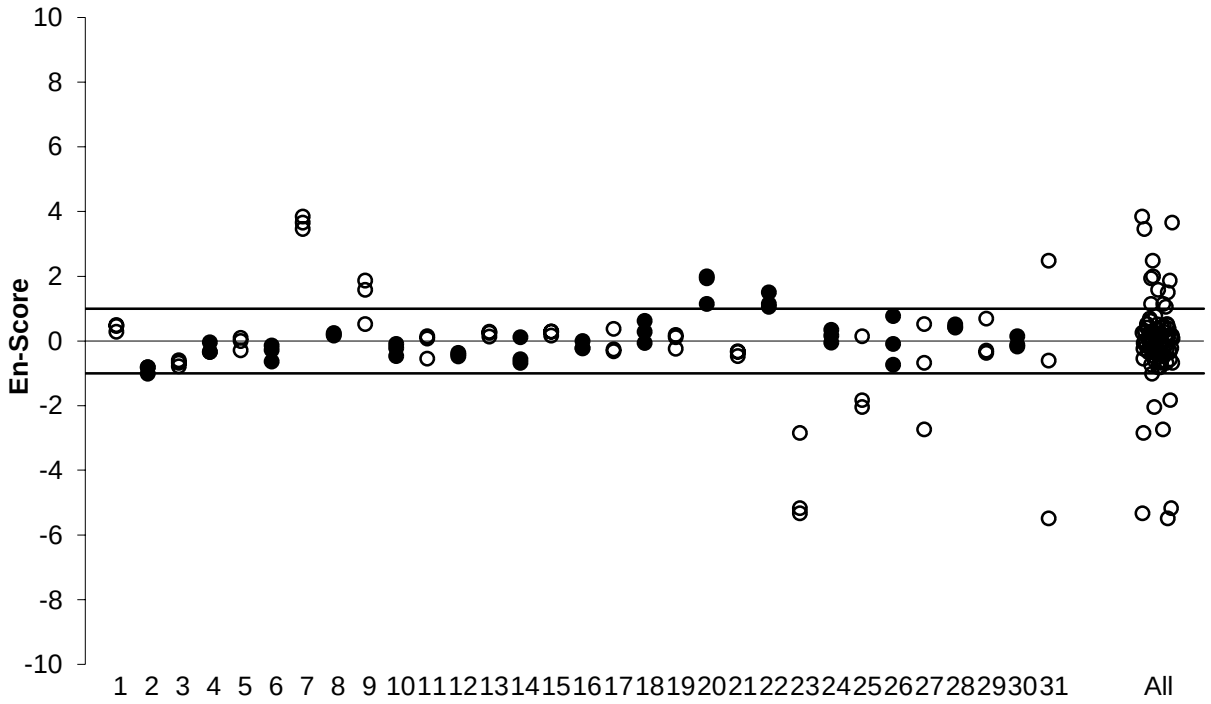


Figure 7 E_n-score Dispersal by Laboratory

6.5 Identification of Cutting Agent

Samples were prepared using a seizure of cocaine hydrochloride, approximately 84% base (m/m) supplied by the Australian Federal Police. The study coordinator added levamisole to Sample S1 and caffeine to duplicate Samples S2 and S3. All participants reported on the identity of the cutting agents of at least one sample (Table 8). Laboratories **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, **19**, **21**, **22**, **23**, **25**, **26**, **27**, **28** and **29** correctly identified all cutting agents in the test samples.

For Sample S1, 26 participants (84%) correctly identified that levamisole was used as the cutting agent, two participants incorrectly reported caffeine as an additional cutting agent to the levamisole, and one participant incorrectly reported caffeine only as the cutting agent. Two participants did not report any cutting agents for this sample. For Sample S2, all participants correctly identified that caffeine was used as the cutting agent. For Sample S3, 30 participants (97%) correctly identified that caffeine was used as the cutting agent, with one participant incorrectly reporting tetramisole.

After the interim report was released, Laboratory **31** stated they had mixed up the reporting for Samples S1 and S3.

6.6 Duplicate Samples S2 and S3

Samples S2 and S3 were blind duplicate samples. The results for these samples are presented in Figure 8. The majority of participants' results for Samples S2 and S3 were in agreement with each other within their reported uncertainties.

Results for Samples S2 and S3 for laboratories **23**, **27** and **31** were not in agreeance. For laboratory **23** this was because they did not report any uncertainties and their results were very close but not exactly the same. Laboratories **27** and **31** reported significantly different results for these duplicate samples. After the release of the interim report, Laboratory **31** stated they had mixed up the results for Samples S1 and S3; if these results were switched, their results for Samples S2 and S3 would be in agreement with each other within their reported uncertainties.

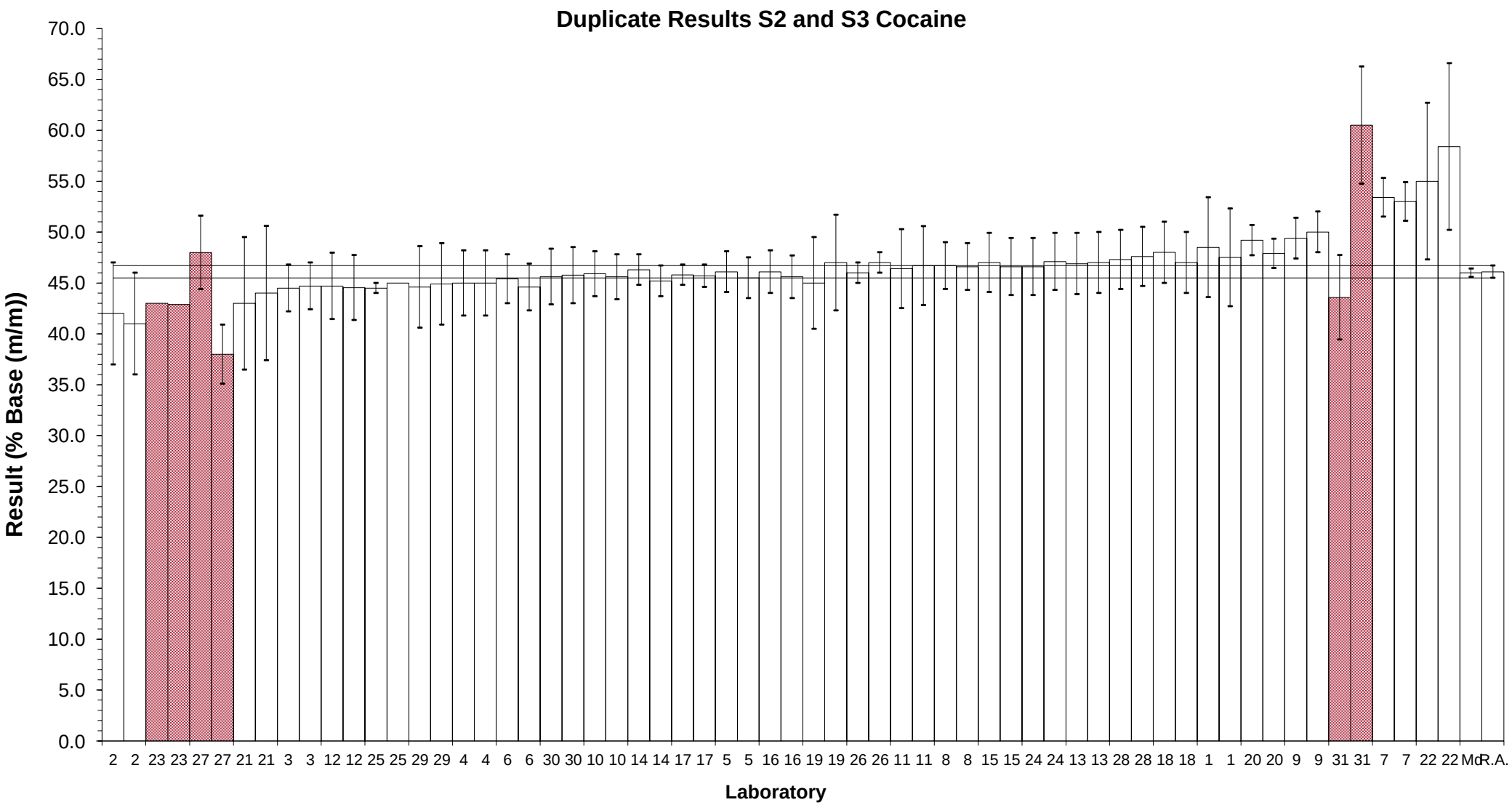


Figure 8 Results for Blind Duplicate Samples S2 and S3. Horizontal lines are the upper and lower 95% confidence interval of the assigned value. Participants' results which are not in agreement with each other within reported uncertainties are shaded.

6.7 Participants' Analytical Methods

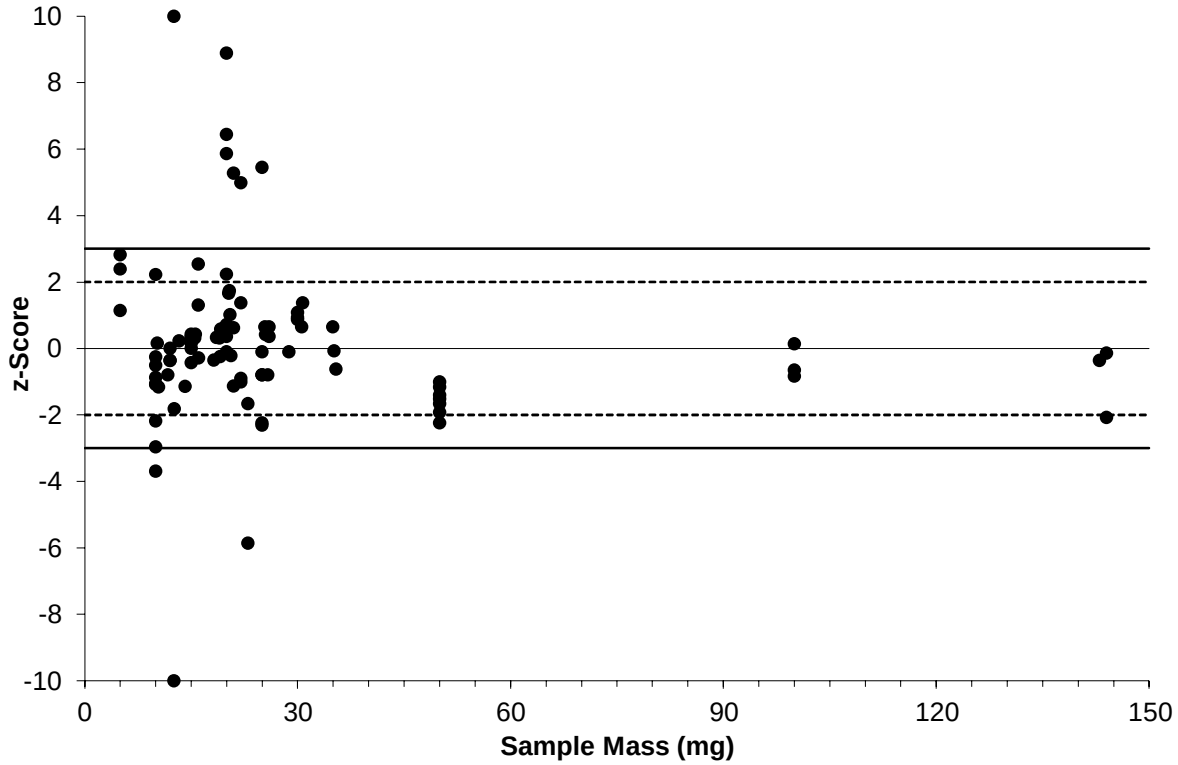
Participants were requested to analyse the samples using their normal test methods and to report a single result for each sample as they would normally report to a client. Results reported in this way reflect the true variability of results reported to laboratory clients. The method descriptions provided by participants are presented in Table 1.

A summary of accreditation status, participants' methods and reference standards is presented in Table 10.

Table 10 Summary of Participants' Analytical Methods

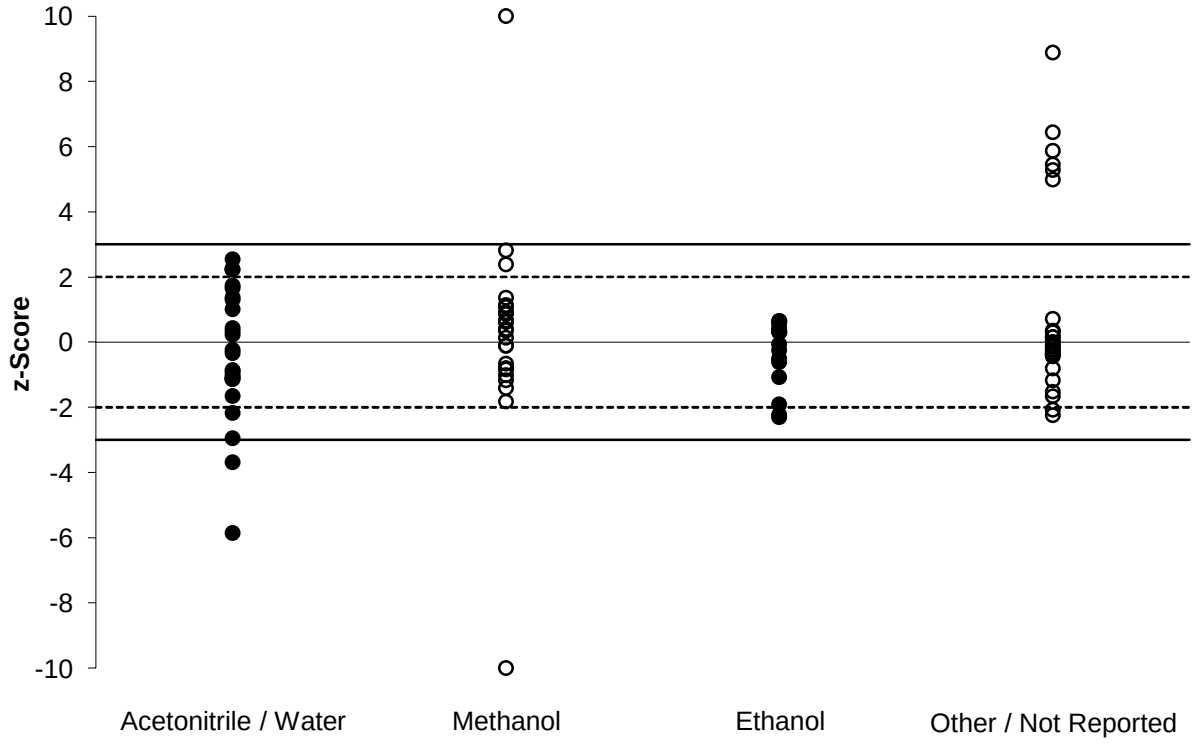
		Lab. Code
Accreditation	Yes to ISO/IEC 17025	3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 18 (currently inactive), 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 31
	Yes to ANAB and ASCLD/LAB	15, 28
	Not accredited / Not reported	1, 2, 9, 17, 23, 30
Sample Mass Used (mg)	5 – 10	2, 6, 9, 25, 29
	11 – 30	1, 4, 5, 7, 8, 11, 12, 13, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 27, 28, 30, 31
	31 – 50	3, 18, 21, 23, 26
	51 – 100	14
	101 – 150	10
Instrument Used for Quantification	HPLC-DAD	1, 4, 9, 11, 14, 27, 30
	HPLC-UV	2, 10, 20, 25, 29
	HPLC-UV-DAD	24
	UPLC-PDA	7, 19
	UPLC-DAD	12, 23
	UPLC-MS/MS	21
	LC-DAD	15, 28
	GC-FID	3, 5, 6, 8, 13, 18, 26, 31
	GC-MS	22
	QNMR	16, 17
Solvent	Acetonitrile/Water	1, 2, 11, 12, 20, 27, 29, 30
	Methanol	3, 4, 9, 14, 15, 18, 19, 28, 31
	Ethanol	6, 8, 13, 23, 26
	Other / Not reported	5, 7, 10, 16, 17, 21, 22, 24, 25
Sources of Calibration Standard	NMI Australia	1, 5, 7, 8, 12, 13, 19, 23
	Lipomed	2, 4, 14, 15, 28, 30
	Merck / Sigma Aldrich	3, 17, 20, 24, 25, 29
	LGC	9, 27, 31
	Johnson Matthey / MacFarlan Smith	10, 11
	Other	6, 18, 21, 26
	Not reported	16, 22

Plots of the z-score versus the sample mass used, measurement instrument, solvent and source of calibration standard are presented in Figures 9 to 12. No trends were observed.



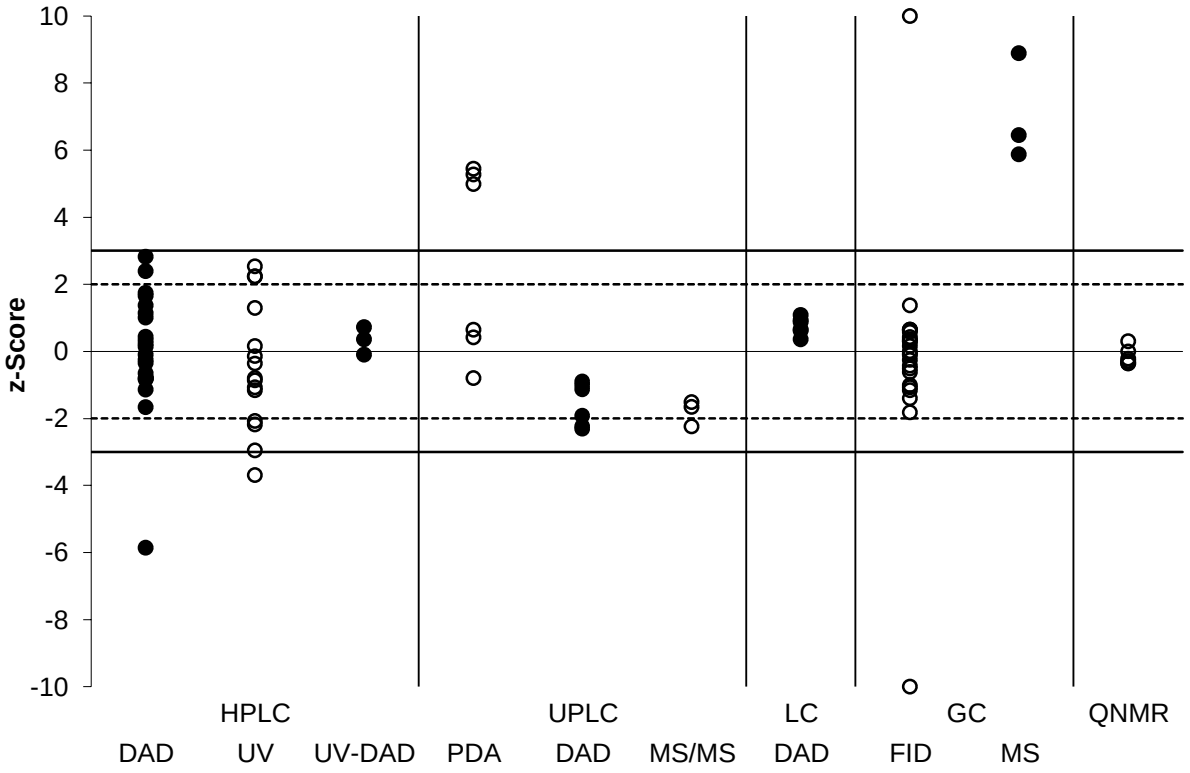
Scores greater than 10 or less than -10 have been plotted at 10 and -10 respectively.

Figure 9 z-Score vs Sample Mass Used



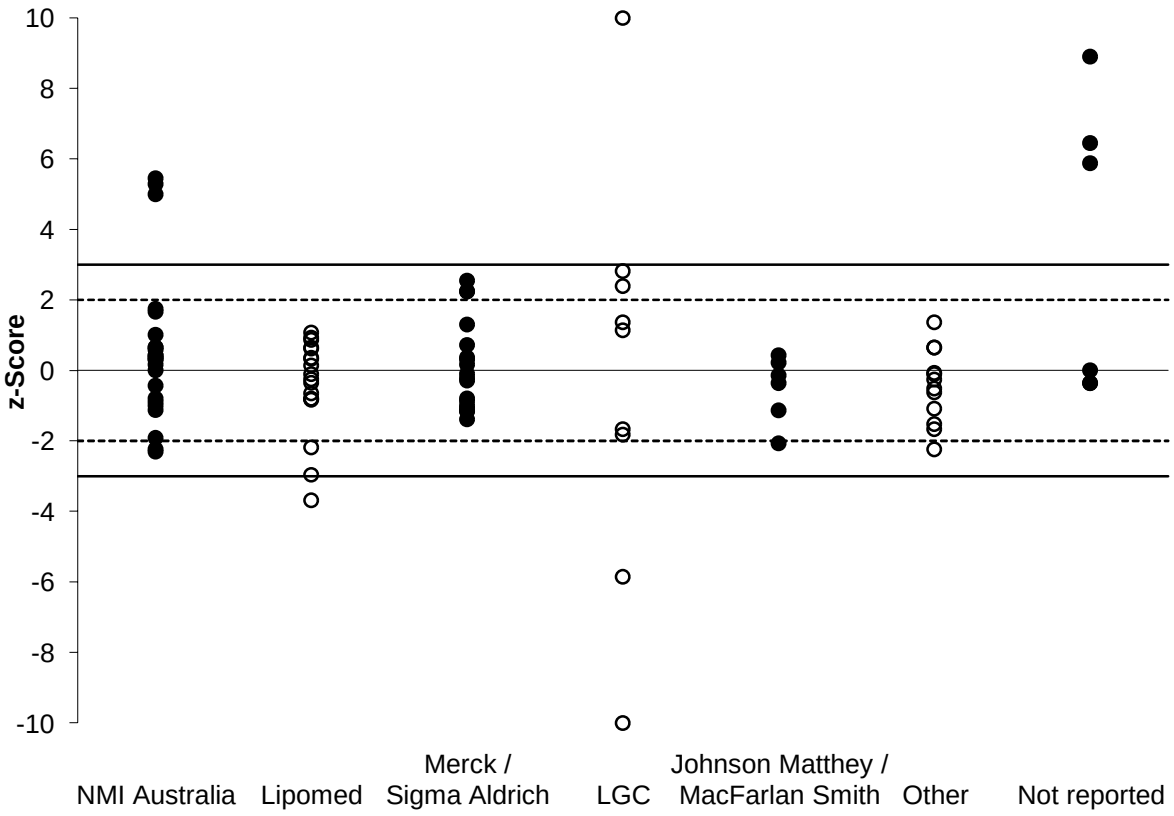
Scores greater than 10 or less than -10 have been plotted at 10 and -10 respectively.

Figure 10 z-Score vs Extraction Solvent



Scores greater than 10 or less than -10 have been plotted at 10 and -10 respectively.

Figure 11 z-Score vs Measurement Instrument



Scores greater than 10 or less than -10 have been plotted at 10 and -10 respectively.

Figure 12 z-Score vs Measurement Instrument

6.8 Comparison with Previous Cocaine PT Studies

To enable direct comparison with previous Cocaine PT studies, the target SD used to calculate z-scores has been kept constant at 3% PCV.

A summary of the satisfactory performance, presented as a percentage of the total number of scores, obtained by participants from 2010 to 2019 (last 10 studies) are presented in Figure 13. The average proportion of satisfactory z-scores and E_n-scores over this period is 79% and 82% respectively.

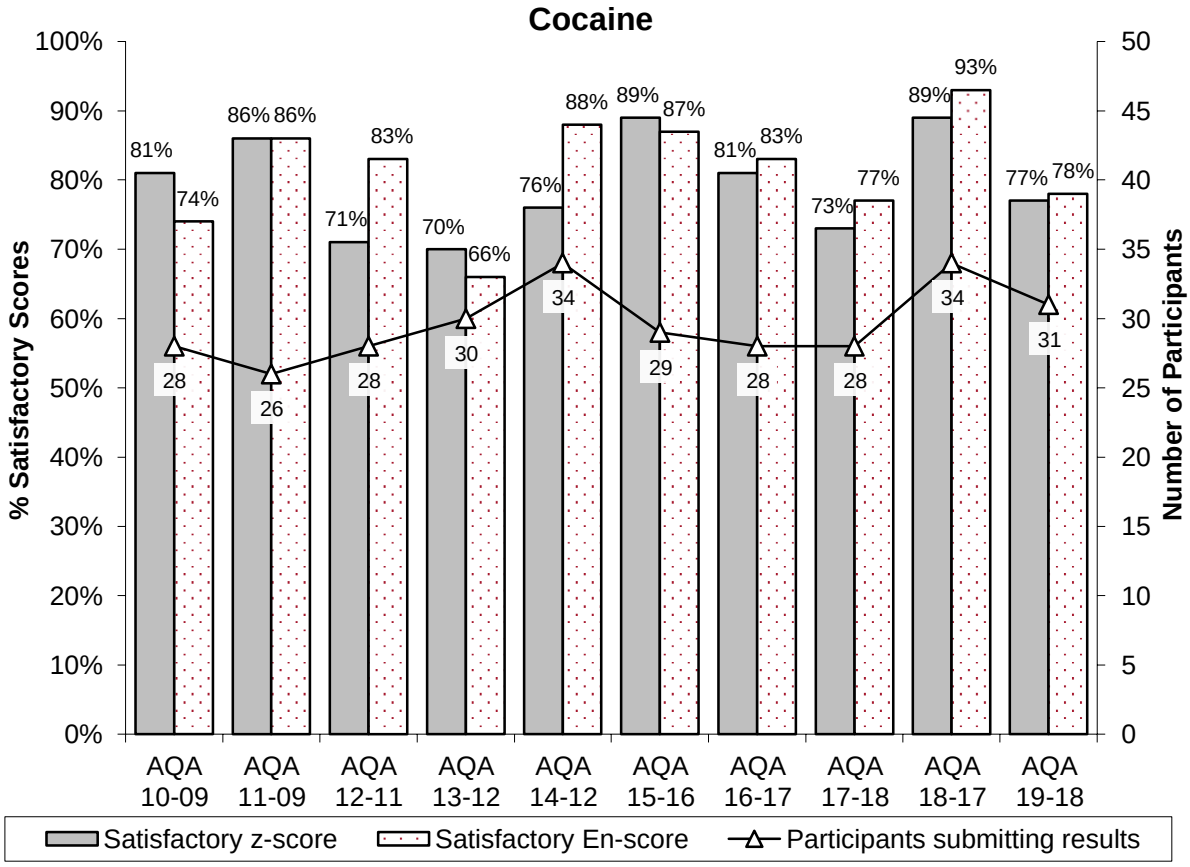


Figure 13 Summary of Participants' Performance in Cocaine PT Studies

Individual performance history reports are emailed to each participant at the end of the study; the consideration of z-scores for an analyte over time provides much more useful information than a single z-score. Over time, laboratories should expect at least 95% of their scores to lie within the range $|z| \leq 2.0$. Scores in the range $2.0 < |z| < 3.0$ can occasionally occur, however, these should be interpreted in conjunction with the other scores obtained by that laboratory. For example, a trend of z-scores on one side of the zero line is an indication of method or laboratory bias.

7 REFERENCES

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

[2] NMI, *Study Protocol for Proficiency Testing*, viewed November 2019, <https://www.industry.gov.au/client-services/chemical-and-biological-measurement-services/proficiency-testing-services>

[3] NMI, *Chemical Proficiency Testing Statistical Manual*, viewed November 2019, <https://www.industry.gov.au/client-services/chemical-and-biological-measurement-services/proficiency-testing-services>

[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.*, vol 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 CG 4, *Quantifying Uncertainty in Analytical Measurement*, 3rd Edition, <http://eurachem.org/images/stories/guides/pdf/quam2012_P1.pdf>

[8] Thompson, M., 2000, 'Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing', *Analyst*, vol 125, pp 385-386.

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:

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

where:

$u_{rob\ average}$	is the standard uncertainty of the robust average
$S_{rob\ average}$	is the standard deviation of the robust average
p	is the 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 for Sample S1 is set out below in Table 11.

Table 11 Uncertainty of Assigned Value for Sample S1 as % base (m/m)

No. results (p)	30
Robust average	64.2
$S_{rob\ average}$	2.8
$u_{rob\ average}$	0.64
k	2
$U_{rob\ average}$	1.28

Therefore, the assigned value for Sample S1 is 64.2 ± 1.3% base (m/m).

APPENDIX 2 - ACRONYMS AND ABBREVIATIONS

ANAB	ANSI (American National Standards Institute) National Accreditation Board
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
EA	European Accreditation
FID	Flame Ionization Detector
GC	Gas Chromatography
GUM	Guide to the expression of Uncertainty in Measurement
HPLC	High Performance Liquid Chromatography
IEC	International Electrotechnical Commission
ILAC	International Laboratory Accreditation Cooperation
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
MS/MS	Tandem Mass Spectrometry
MU	Measurement Uncertainty
NATA	National Association of Testing Authorities, Australia
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
RM	Reference Material
SD	Standard Deviation
SI	International System of Units
SS	Spiked Samples
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet

END OF REPORT