Australian Government
Department of Industry, Science and Resources

National Measurement Institute

# Proficiency Test Final Report
# AQA 23-13
# Heroin

December 2023

## ACKNOWLEDGMENTS

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

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

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

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## SUMMARY

AQA 23-13 Heroin commenced in May 2023. Sets of heroin hydrochloride, each containing three test samples, were sent to 33 laboratories, with one laboratory requesting two sample sets to be analysed independently by different analysts. All participants submitted results. Samples were prepared at the Sydney NMI laboratory using heroin hydrochloride samples supplied by the Australian Federal Police.

The assigned values in this study 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 participants measuring heroin in samples typical of a routine seizure.*

Participant performance was assessed by *z*-scores and $E_n$-scores.

Of 102 *z*-scores, 86 (84%) returned $|z| \leq 2.0$, indicating an acceptable performance.

Of 102 $E_n$-scores, 93 (91%) returned $|E_n| \leq 1.0$, indicating agreement of the participant's result with the assigned value within their respective expanded uncertainties.

Laboratories **1**, **3**, **6**, **9**, **10**, **11**, **12**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **23**, **25**, **26**, **27**, **28**, **29**, **30**, **33** and **34** returned acceptable *z*-scores and $E_n$-scores for all results.

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

Of 102 reported results, 96 (94%) were reported with an associated expanded measurement uncertainty. The magnitude of reported uncertainties was within the range 2.9% to 26% relative.

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

Sample S1 was cut with niacinamide, Sample S2 was cut with sucrose, and Sample S3 was cut with procaine hydrochloride.

All participants reported on the identity of at least one sample's cutting agent. Laboratories **3**, **4**, **7**, **9**, **10**, **16**, **17**, **22**, **25**, **29**, **30** and **31** correctly reported all cutting agents used.

- *Produce materials that can be used in method validation and as control samples.*

The test samples produced for this study are homogeneous and well characterised. Surplus samples are available for purchase and can be used for quality control and for method validation purposes.

# 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, vegetables and herbs, soil and water;
- petroleum hydrocarbons in soil and water;
- per- and polyfluoroalkyl substances in water, soil, biota and food;
- inorganic analytes in soil, water, filters, food and pharmaceuticals;
- controlled drug assay, drugs in wipes and clandestine laboratory; and
- allergens in food.

## 1.2 Study Aims

The aims of the study were to:

- assess the proficiency of participants 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;
- test the ability of participants to identify cutting agents commonly found in controlled drug preparation; and
- produce materials that can be used in method validation and as control samples.

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 the 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.[1] This controlled drug study is within the scope of NMI's accreditation.

## 2 STUDY INFORMATION

### 2.1 Study Timetable

The timetable of the study was:

| | |
|---|---|
| Invitations sent | 22/05/2023 |
| Samples sent | 27/07/2023 |
| Results due | 3/11/2023 |
| Interim Report | 8/11/2023 |
| Preliminary Report | 8/11/2023 |

The study timeline was extended to accommodate sample delivery delays to some international participants.

### 2.2 Participation and Laboratory Code

Thirty-three laboratories registered to participate, with one laboratory requesting two sets of samples each to be analysed independently by different analysts. All participants were assigned a confidential laboratory code number for this study. All participants submitted results.

### 2.3 Test Material Specification

Three test samples were prepared in July 2023. The starting material was heroin hydrochloride (approximately 80% heroin base (m/m)) supplied by the Australian Federal Police.

Niacinamide (nicotinamide), sucrose and procaine hydrochloride purchased from Sigma-Aldrich were used as cutting agents. Sample S1 was cut with niacinamide, Sample S2 was cut with sucrose, and Sample S3 was cut with procaine hydrochloride.

The heroin hydrochloride was ground and sieved through a 180 µm sieve. The cutting agents were processed similarly. Test samples were prepared by adding a known mass of sieved cutting agent to a known mass of sieved drug material, and then mixing this 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 approximately 15% heroin base (m/m).

Sample S2 was prepared to contain approximately 30% heroin base (m/m).

Sample S3 was prepared to contain approximately 66% heroin base (m/m).

### 2.4 Test Sample Homogeneity and Stability

The preparation of homogeneous test samples is an important part of a PT study. Given the small (usually < 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, and no additional homogeneity testing was conducted in this proficiency study. Results returned by the participants also gave no reason to question the homogeneity of the test samples.

To assess stability of the samples, results returned by participants were compared to the date of analysis. The results gave no reason to question the stability of the test samples (Section 6.7).

### 2.5 Sample Dispatch and Receipt

Sets of three test samples, with each sample containing approximately 150 mg of material, were dispatched to participants on 27 July 2023.

The following items were also sent with the samples:

- a covering letter which included a description of the test samples and 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 emailed to participants.

### 2.6 Instructions to Participants

Participants were instructed as follows:

- Analyse each sample for amount of heroin base by your routine test method.
- Identify and report the diluent(s) and/or adulterant(s) in all samples if this is within your normal scope of analysis.
- For each sample, report % m/m heroin as base. Report this figure as if reporting to a client.
- For each result, report an estimate of your expanded uncertainty as % m/m heroin as base.
- Give brief details of your:
  - basis of uncertainty estimate (e.g. uncertainty budget, repeatability precision)
  - analytical method (e.g. sample treatment, instrument type, calibration method)
  - reference standard (e.g. source, purity)

  as requested by the results sheet.
- Please complete the results sheet by Wednesday 20 September 2023 and return by email to jenny.xu@measurement.gov.au. Late results may not be included in the study report.

The results due date was extended to 3 November 2023 for all participants. This was due to significant sample delivery delays to several international participants, caused by delays with receiving some export permits.

### 2.7 Interim Report and Preliminary Report

An Interim Report was emailed to all participants on 8 November 2023.

A Preliminary Report was emailed to all participants on 8 November 2023. This report included a summary of the results reported by participants, assigned values, performance coefficients of variation (PCVs), *z*-scores and $E_n$-scores for each analyte in this study. No data from the Preliminary Report has been changed in the present Final Report.

## 3 PARTICIPANT LABORATORY INFORMATION

### 3.1 Test Methods Reported by Participants

Participants' reported test methods are presented in Table 1. Responses may have been modified so that the participant cannot be identified.

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 | Ethanol | Propylparaben | 8 | UPLC | DAD | BEH Shield RP18 |
| 3 | Acetonitrile/Water | None | 5 | HPLC | UV | Kinetex 5u C18 |
| 4 | water/acetonitrile/2.5M sulphuric acid 90:10:1 | None | 3 | HPLC | Diode Array | Shimpack XR-ODS |
| 5 | Methanol | none | 2 | HPLC | DAD | Luna 3 µm PFP 100 Å 150x4.6 mm |
| 6 | Chloroform:methanol (9:1) | Triphenylacetophenone | 1 | GC | FID | HP5 |
| 7 | Acetonitrile | Strychnine | 6 | GC | FID | Phenyl |
| 8 | Chloroform | octacosane | 5 | GC | MS | Rxi-5Sil-MS |
| 9 | Water:Acetonitrile | - | 3 | HPLC | UV/Vis | Luna C18 |
| 10 | acetonitrile/H20 (80/20) | External standard | 3 | HPLC | DAD | NH2 |
| 11 | methanol | NO | 7 | HPLC | DAD | Poroshell 120 C18 (4.6X150mm, 2.7 microns particle size) |
| 12 | Chloroform | Nortriptyline | 1 | GC | FID | HP5 |
| 13 | ethanol:dimethylformamide (9:1) | tribenzylamine | 6 | GC | FID | HP1 |
| 14 | acetonitrile / water | none | 1 | HPLC | UV/Vis | Kromasil |
| 15 | Acetonitrile:Water (75:25) | Benzocaine | 3 | UPLC | DAD | Acquity UPLC BEH C18 1.7um (2.1x100mm) |
| 16 | Chloroform | 2,2,2-triphenylacetophenone | S1, S2: 1 S3: 4 | GC | FID | HP-1 |

| Lab. Code | Extraction Solvent | Internal Standard | Calib. Points | Technique | Detector | Column |
|---|---|---|---|---|---|---|
| 17 | Acetonitrile, acetic acid, water | NO ISTD | 4 | HPLC | UV DAD | Poroshell 120 Ec-18 |
| 18 | Methanol | N/A | 3 | HPLC | PDA | Silica 15cm |
| 19 | Chloroform | Octacosane | 5 | GC | FID | HP5 |
| 20 | Chloroform | Nortriptyline | 1 | GC | FID | HP5 |
| 21 | ACN/MeOH/H2O | Analog of heroin | 7 | UPLC | MS/MS | C-18 column |
| 22 | Ethanol absolute | Tribenzylamine | 6 | GC | FID | DB5 |
| 23 | Methanol | Diazepam | 6 | GC | FID | J&W 128-5512 |
| 24 | METHANOL | LOXAPINE | 5 | HPLC | DAD | XTERRA |
| 25 | Acetonitrile | None | 7 | HPLC | UV/Vis | Luna 3µm C8(2) 100Å, 100x2mm |
| 26 | Acetonitrile/Methanol (95:5) | Pholcodine 1mg/ml | 3 | UPLC | PDA | ACQUITY C-18 |
| 27 | Methanol | Mepivacaine | 4 | UPLC | DAD | Kinetex Evo C18 |
| 28 | Methanol | none | 5 | HPLC | DAD | Kinetex C-18-XB |
| 29 | Ethanol | Triphenylacetophenone (TPAP) | 3 | GC | FID | HP1-MS |
| 30 | Methanol | Methadone | 4 | GC | FID | Rxi-5ms |
| 31 | acetonitrile/water (86/14) | none | 4 | HPLC | DAD | NH2 |
| 32 | Ethanol | Eicosane | 6 | GC | FID | HP5 |
| 33 | HPLC Methanol | - | 1 | UPLC | DAD | Thermo Scientific Hypersil-5-ODS |
| 34 | HPLC Methanol | - | 1 | UPLC | DAD | Thermo Scientific Hypersil-5-ODS |

### 3.2 Details of Participant Calibration Standards

Participants' responses regarding their calibration standard are presented in Table 2. Responses may have been modified so that the participant cannot be identified.

Table 2 Participant Calibration Standard

| Lab. Code | Reference Standard | Purity (%) |
|---|---|---|
| 1 | Toronto Research Chemicals | 98 |
| 2 | NMI | 99.4 |
| 3 | British Pharmacopoeia | 99.3 |
| 4 | LGC (Mikromol) | 99.7 |
| 5 | Lipomed (M-29-FB-1LA) | 1 mg/mL |
| 6 | in-house reference material | 98 |
| 7 | NMI | 99.3 |
| 8 | Lipomed | 99.912 |
| 9 | British Pharmacopeia | 99.3 |
| 10 | NMI | 99.4 |
| 11 | Lipomed | 99.879 |
| 12 | Inhouse Reference Material | 98 |
| 13 | Lipomed | 99.912 |
| 14 | Lipomed | 99.91 |
| 15 | NMI | 99.3 |
| 16 | In-house synthesis | 97.3 |
| 17 | Lipomed | 99.88 |
| 18 | Johnson Matthey | 99.4 |
| 19 | NMI | 99.3 |
| 20 | Inhouse Reference Material | 99.8 |
| 21 | Lipomed | 100 |
| 22 | Lipomed | 99.912%+/-0.018% free base content 86.4% |
| 23 | Lipomed | 99.1 |
| 24 | LGC STANDARDS | >99.9% |
| 25 | NMI | 99.4 |
| 26 | NMI | 99.3 |
| 27 | Lipomed | 99.600+/-0.020 |
| 28 | Chiron | 99.8 (±4.0) |
| 29 | NMI | 99.3 +/- 1.3 |
| 30 | LGC | 1.011mg/ml |
| 31 | Lipomed | 99.912 |
| 32 | Alcaliber | 98.4 |
| 33 | Lipomed | 99.912 ± 0.018 |
| 34 | Lipomed | 99.912 ± 0.018 |

### 3.3 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 3. Responses may have been modified so that the participant cannot be identified.

Table 3 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 | Homogeneity of sample<br>Masses and volumes<br>Matrix effects<br>Standard purity | ASCLD/LAB Guidance On The Estimation Of Measurement Uncertainty, AL-PD-3061 |
| 2 | | | | |
| 3 | Top Down - precision and estimates of the method and laboratory bias | Control samples<br>Duplicate analysis | Instrument calibration<br>Homogeneity of sample<br>Masses and volumes<br>Matrix effects | Eurachem/CITAC Guide |
| 4 | Standard deviation of replicate analyses multiplied by 2 or 3 | Control samples - RM<br>Duplicate analysis | Instrument calibration<br>Standard purity | ISO/GUM |
| 5 | Top Down - precision and estimates of the method and laboratory bias | Control samples - Samples from case<br>Duplicate analysis | Laboratory bias from PT studies | Nordtest Report TR537 |
| 6 | Top Down - precision and estimates of the method and laboratory bias | Control samples - RM | Homogeneity of sample<br>Matrix effects<br>Standard purity | ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty, AL-PD-3061 |
| 7 | Top Down - precision and estimates of the method and laboratory bias | Control samples - CRM<br>Duplicate analysis | Instrument calibration<br>Homogeneity of sample<br>Masses and volumes<br>Laboratory bias from PT studies | ISO/GUM |
| 8 | Top Down - precision and estimates of the method and laboratory bias | Control samples | Laboratory bias from PT studies | ISO/GUM |
| 9 | Precision and Bias | Control samples - Known Value Samples | Instrument calibration<br>Homogeneity of sample<br>Masses and volumes<br>Recoveries of SS | ISO/GUM |
| 10 | Top Down - precision and estimates of the method and laboratory bias | Duplicate analysis | Instrument calibration<br>Standard purity | ISO/GUM |
| 11 | Top Down - precision and estimates of the method and laboratory bias | Control samples - CRM<br>Duplicate analysis | Instrument calibration<br>Homogeneity of sample<br>Masses and volumes<br>Laboratory bias from PT studies<br>Standard purity | |

| Lab. Code | Approach to Estimating MU | Information Sources for MU Estimation* | | Guide Document for Estimating MU |
|---|---|---|---|---|
| | | Precision | Method Bias | |
| 12 | Top Down - precision and estimates of the method and laboratory bias | Duplicate analysis | Instrument calibration | ISO/GUM |
| 13 | Top Down - precision and estimates of the method and laboratory bias | Control samples - RM | Standard purity | |
| 14 | Standard deviation of replicate analyses multiplied by 2 or 3 | Control samples - RM | | ISO/GUM |
| 15 | Top Down - precision and estimates of the method and laboratory bias | Control samples - RM<br>Duplicate analysis | Masses and volumes<br>Standard purity | Eurachem/CITAC Guide |
| 16 | Validation | | | |
| 17 | Top Down - precision and estimates of the method and laboratory bias | | | ISO 5725-2 and ISO/TS 21748 |
| 18 | Standard deviation of replicate analyses multiplied by 2 or 3 | Control samples - SS | Instrument calibration<br>Masses and volumes<br>Recoveries of SS | ISO/GUM |
| 19 | Top Down - precision and estimates of the method and laboratory bias | Control samples - previously analysed police seizures<br>Duplicate analysis | Instrument calibration<br>Homogeneity of sample<br>Masses and volumes<br>Matrix effects<br>Standard purity | Eurachem/CITAC Guide |
| 20 | Top Down - precision and estimates of the method and laboratory bias | Duplicate analysis | Masses and volumes<br>Recoveries of SS | Eurachem/CITAC Guide |
| 21 | Top Down - precision and estimates of the method and laboratory bias | | | |
| 22 | Black Box | Control samples - CRM Duplicate analysis | Instrument calibration<br>Homogeneity of sample<br>Masses and volumes<br>Matrix effects<br>Laboratory bias from PT studies<br>Standard purity | Eurachem/CITAC Guide |
| 23 | Estimating Measurement Uncertainty by black box with pairs of values | Standard deviation from PT studies only | | ISO/GUM<br>ENAC G 09 or ISO 21748 |
| 24 | | | | |
| 25 | Top Down - precision and estimates of the method and laboratory bias | Control samples - In House Control<br>Duplicate analysis | Instrument calibration<br>Homogeneity of sample<br>Masses and volumes<br>Standard purity | ISO/GUM |
| 26 | Top Down - precision and estimates of the method and laboratory bias | Control samples - CRM<br>Duplicate analysis | Instrument calibration<br>Homogeneity of sample<br>Masses and volumes | NATA GAG Estimating and Reporting Measurement |

| Lab. Code | Approach to Estimating MU | Information Sources for MU Estimation* | | Guide Document for Estimating MU |
|---|---|---|---|---|
| | | Precision | Method Bias | |
| | | | | Uncertainty of Chemical Test Results |
| 27 | Top Down - precision and estimates of the method and laboratory bias | Control samples<br>Duplicate analysis | Instrument calibration<br>Homogeneity of sample<br>Matrix effects<br>Laboratory bias from PT studies<br>Recoveries of SS<br>Standard purity | EA-04/16 EA guidelines on the expression of uncertainty in quantitative testing. |
| 28 | Standard deviation of replicate analyses multiplied by 2 or 3 | Control samples - RM<br>Duplicate analysis | | Eurachem/CITAC Guide |
| 29 | Bottom Up (ISO/GUM, fish bone/cause and effect diagram) | Control samples - CRM<br>Duplicate analysis | Instrument calibration<br>Homogeneity of sample<br>Masses and volumes<br>Matrix effects<br>Recoveries of SS<br>Standard purity | Eurachem/CITAC Guide |
| 30 | Standard deviation of replicate analysis multiplied by 2 or 3 | Duplicate analysis | Masses and volumes | ISO/GUM |
| 31 | Top Down - precision and estimates of the method and laboratory bias | Control samples - RM | Laboratory bias from PT studies | NF ISO 11352 and NF V03-110 |
| 32 | Top Down - precision and estimates of the method and laboratory bias | Control samples - RM<br>Duplicate analysis | Matrix effects | ISO/GUM |
| 33 | Top Down - precision and estimates of the method and laboratory bias | Control samples - SS<br>Duplicate analysis | Laboratory bias from PT studies<br>Recoveries of SS | Eurachem/CITAC Guide |
| 34 | Top Down - precision and estimates of the method and laboratory bias | Control samples - SS<br>Duplicate analysis | Laboratory bias from PT studies<br>Recoveries of SS | Eurachem/CITAC Guide |

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

### 3.4 Participants' Comments

Participants were invited to comment on the samples, their methodology, the PT study in general and suggestions for future PT studies. Such feedback allows for the improvement of future studies. Participants' comments are presented in Table 4, along with the study coordinator's response where appropriate. Some responses may be modified so that the participant cannot be identified.

Table 4 Participant Comments

| Lab. Code | Participants' Comments | Study Coordinator's Response |
|---|---|---|
| 1 | Methodology: Quantitation of heroin and monoacetylmorphines by GC-FID | |
| 4 | Uncertainty: MuM determined from multiple injections of reference material. 3x(Std Dev/mean)x100. | |

| Lab. Code | Participants' Comments | Study Coordinator's Response |
|---|---|---|
| 6 | Methodology: Quantitation for heroin and monoacetylmorphines by GC | |
| 9 | Methodology: The sample is weighed and extracted in duplicate and the components separated by HPLC with UV detection. The percentage purity is then determined by comparison with a standard calibration curve. | |
| 11 | Methodology: External standard | |
| 15 | Heroin and acetylcodeine detected in S1, S2 & S3. | |
| 17 | Methodology: 0 ; 5 ; 20 ; 100 mg/l | |
| 18 | Routine case samples would always be round down i.e -3.92% for example 15.3*0.9608=14<br>Uncertainty: The reported result (in routine case samples) is defined as the mean of the individual results multiplied by the uncertainty correction factor and is rounded down to the nearest whole number (unless<1%w/w). E.g a mean result of 15.3% with an uncertainty correction value of 96.08% would give a reported result of 15.3*0.9608=14.7 therefore rounded down to 14%. | |
| 20 | Methodology: Quantification of heroin by GC-FID | |
| 22 | Methodology: Dilution of sample in 10 mL of iSTD (0.25 mg/mL of TBA in abs. ETOH) | |
| 25 | It is acknowledged that Acetylcodine and Monoacetyl morphine are not diluents/adulterants but it is standard laboratory practice to report these. Niacinamide was tentatively identified based on comparison of the compound's mass spectrum and FTIR spectrum with that of a literature source. Unequivocal identification of this compound can be made on receipt of a certified reference material at the laboratory. | |
| 29 | Methodology: Dichloromethane (30ml/L of ethanol) was used to dissolve the TPAP | |
| 30 | Could the sample vials be submitted without the name of the analyte being present - this would enable a full blind test by the analyst rather than them being prompted straight away as to what the sample is. | This PT is not a qualitative study. All participants are informed what analyte they are assessing for, on the sample label as well as the sample dispatch letter provided with the samples. |
| 31 | we would like to receive 3 samples of very different concentration for example 3%, 30% and 80%<br>Methodology: Eluant acetonitrile/water (86/14) + 2.25ml picA/litre | A range of drug purities are selected to cater for the needs of different laboratories. In this study, the samples contained 14.8%, 29.3% and 65.3% heroin base (m/m). |

# 4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

## 4.1 Results Summary

Participant results are listed in Tables 5 to 7 with summary statistics: robust average, median, mean, number of numerical results (N), 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.

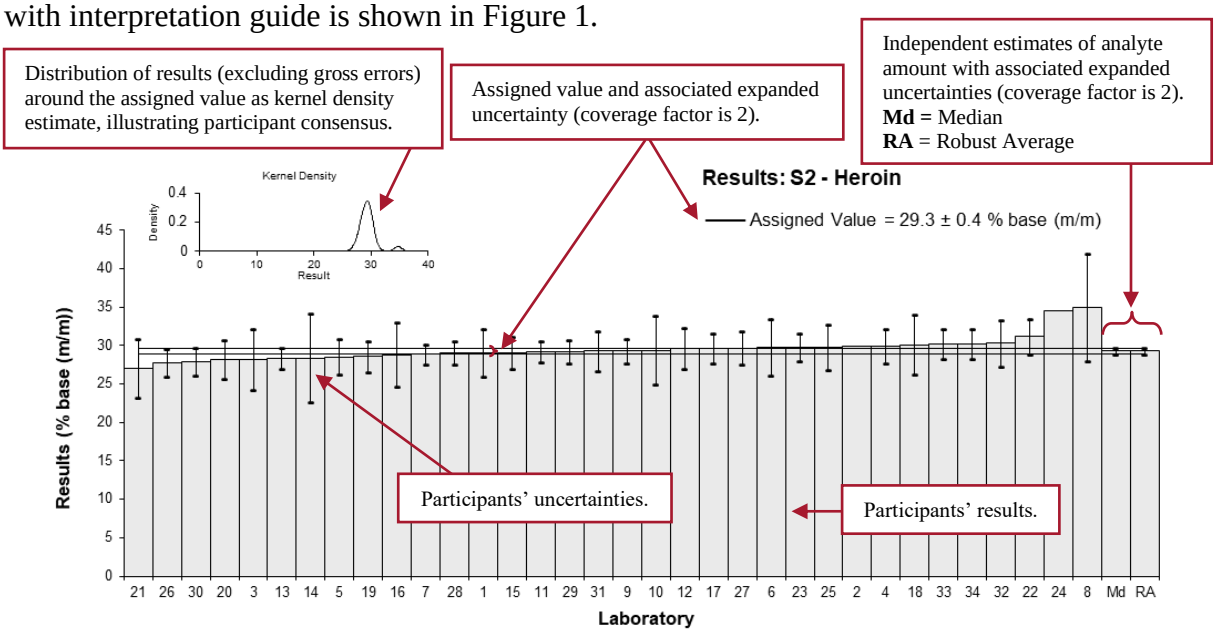



Figure 1 Guide to Presentation of Results

## 4.2 Outliers and Extreme Outliers

Outliers were results less than 50% and greater than 150% of the robust average, and these were removed before the calculation of the assigned value, if applicable.[3,4] Extreme outliers were obvious blunders, e.g. results reported with incorrect units or for a different analyte or sample, and such results were removed for the calculation of all summary statistics.[3,4]

## 4.3 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 % heroin base (m/m) 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).

## 4.4 Robust Average and Robust Standard Deviation

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

## 4.5 Performance Coefficient of Variation (PCV)

The PCV is a fixed measure of the between-laboratory variation that in the judgement of the study coordinator would be expected from participants given the levels of analytes present, and is supported by mathematical models such as the Thompson-Horwitz equation.[6] It is important to note that this is a performance measure set by the study coordinator and it is not the robust CV of participants' results. By setting a fixed and realistic value for the PCV, a participant's performance does not depend on other participants' performances, and can be compared from study to study.

### 4.6 Target Standard Deviation for Proficiency Assessment

The target standard deviation for proficiency assessment (σ) is the product of the assigned value ($X$) and the PCV, as presented in Equation 1.

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

### 4.7 *z*-Score

For each participant's result, a *z*-score is calculated according to Equation 2.

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

where:

$z$ is *z*-score

$\chi$ is a participant's result

$X$ is the assigned value

$\sigma$ is the target standard deviation for proficiency assessment from Equation 1

For the absolute value of a *z*-score:

- $|z| \leq 2.0$ is acceptable;
- $2.0 < |z| < 3.0$ is questionable; and
- $|z| \geq 3.0$ is unacceptable.

### 4.8 $E_n$-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.

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

where:

$E_n$ is $E_n$-score

$\chi$ is a participant's result

$X$ is the assigned value

$U_\chi$ is the expanded uncertainty of the participant's result

$U_X$ is the expanded uncertainty of the assigned value

For the absolute value of an $E_n$-score:

- $|E_n| \leq 1.0$ is acceptable; and
- $|E_n| > 1.0$ is unacceptable.

### 4.9 Traceability and Measurement Uncertainty

Laboratories accredited to ISO/IEC 17025 must establish and demonstrate the traceability and measurement uncertainty associated with their test results.[7]

Guidelines for quantifying uncertainty in analytical measurement are described in the Eurachem/CITAC Guide.[8]

## 5 TABLES AND FIGURES

Table 5

**Sample Details**

| | |
|---|---|
| **Sample No.** | S1 |
| **Matrix** | Powder |
| **Analyte** | Heroin |
| **Unit** | % base (m/m) |

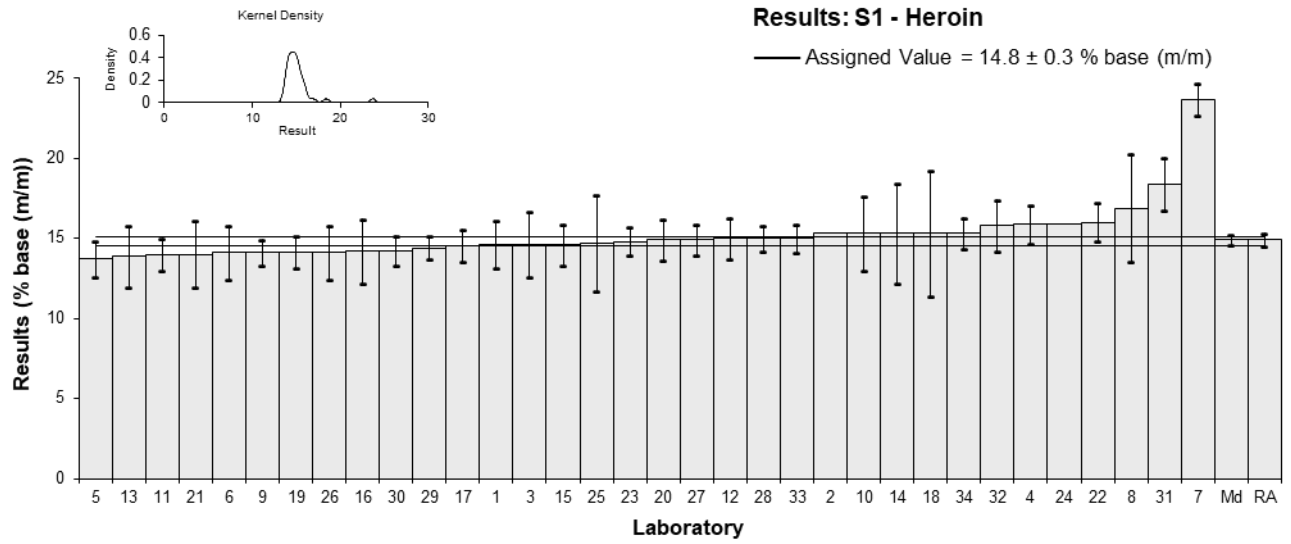
**Participant Results**

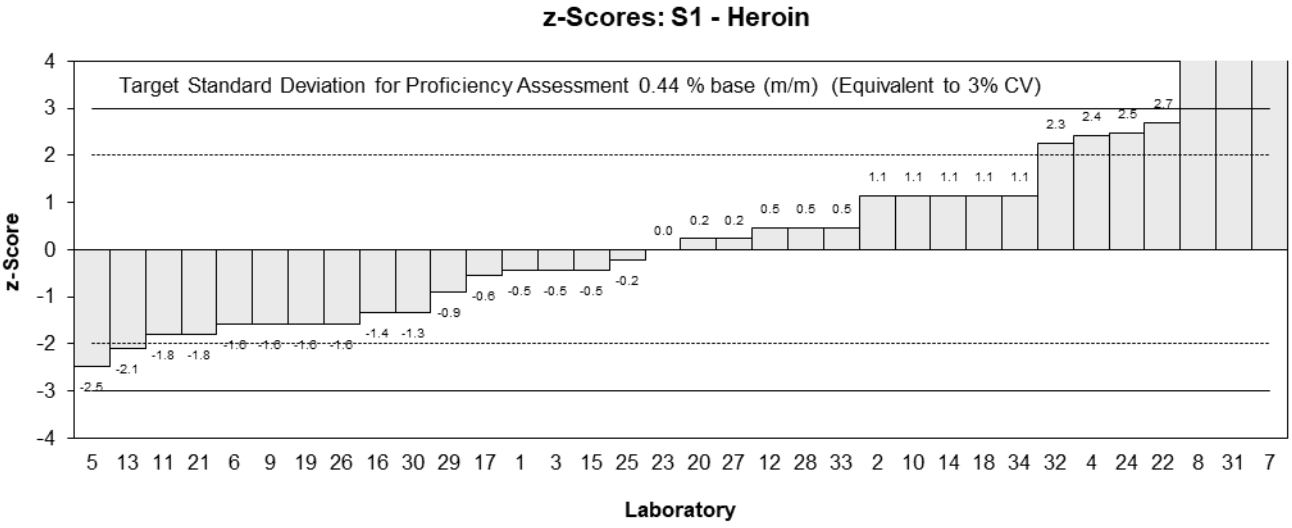
| Lab. Code | Result | Uncertainty | z | $E_n$ |
|---|---|---|---|---|
| **1** | 14.6 | 1.5 | -0.45 | -0.13 |
| **2** | 15.3 | NR | 1.13 | 1.67 |
| **3** | 14.60 | 2.04 | -0.45 | -0.10 |
| **4** | 15.87 | 1.18 | 2.41 | 0.88 |
| **5** | 13.7 | 1.1 | -2.48 | -0.96 |
| **6** | 14.1 | 1.7 | -1.58 | -0.41 |
| **7*** | 23.7 | 1 | 20.05 | 8.52 |
| **8** | 16.9 | 3.4 | 4.73 | 0.62 |
| **9** | 14.1 | 0.8 | -1.58 | -0.82 |
| **10** | 15.3 | 2.3 | 1.13 | 0.22 |
| **11** | 14 | 1 | -1.80 | -0.77 |
| **12** | 15 | 1.3 | 0.45 | 0.15 |
| **13** | 13.87 | 1.9 | -2.09 | -0.48 |
| **14** | 15.3 | 3.1 | 1.13 | 0.16 |
| **15** | 14.6 | 1.3 | -0.45 | -0.15 |
| **16** | 14.2 | 2.0 | -1.35 | -0.30 |
| **17** | 14.55 | 1.03 | -0.56 | -0.23 |
| **18** | 15.3 | 3.92 | 1.13 | 0.13 |
| **19** | 14.1 | 1.0 | -1.58 | -0.67 |
| **20** | 14.9 | 1.3 | 0.23 | 0.07 |
| **21** | 14 | 2.1 | -1.80 | -0.38 |
| **22** | 16.0 | 1.2 | 2.70 | 0.97 |
| **23** | 14.8 | 0.9 | 0.00 | 0.00 |
| **24** | 15.9 | NR | 2.48 | 3.67 |
| **25** | 14.7 | 3 | -0.23 | -0.03 |
| **26** | 14.1 | 1.7 | -1.58 | -0.41 |
| **27** | 14.9 | 1.0 | 0.23 | 0.10 |
| **28** | 15 | 0.8 | 0.45 | 0.23 |
| **29** | 14.4 | 0.7 | -0.90 | -0.53 |
| **30** | 14.21 | 0.94 | -1.33 | -0.60 |
| **31** | 18.39 | 1.66 | 8.09 | 2.13 |
| **32** | 15.8 | 1.6 | 2.25 | 0.61 |
| **33** | 15.0 | 0.9 | 0.45 | 0.21 |
| **34** | 15.3 | 1.0 | 1.13 | 0.48 |

* Outlier, see Section 4.2

**Statistics**

| | | |
|---|---|---|
| **Assigned Value** | 14.8 | 0.3 |
| **Robust Average** | 14.9 | 0.4 |
| **Median** | 14.9 | 0.3 |
| **Mean** | 15.2 | |
| **N** | 34 | |
| **Max** | 23.7 | |
| **Min** | 13.7 | |
| **Robust SD** | 0.83 | |
| **Robust CV** | 5.6% | |


Kernel Density
Density
Result
Results: S1 - Heroin
Assigned Value = 14.8 ± 0.3 % base (m/m)
Results (% base (m/m))
Laboratory
5 13 11 21 6 9 19 26 16 30 29 17 1 3 15 25 23 20 27 12 28 33 2 10 14 18 34 32 4 24 22 8 31 7 Md RA


z-Scores: S1 - Heroin
Target Standard Deviation for Proficiency Assessment 0.44 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
5 13 11 21 6 9 19 26 16 30 29 17 1 3 15 25 23 20 27 12 28 33 2 10 14 18 34 32 4 24 22 8 31 7

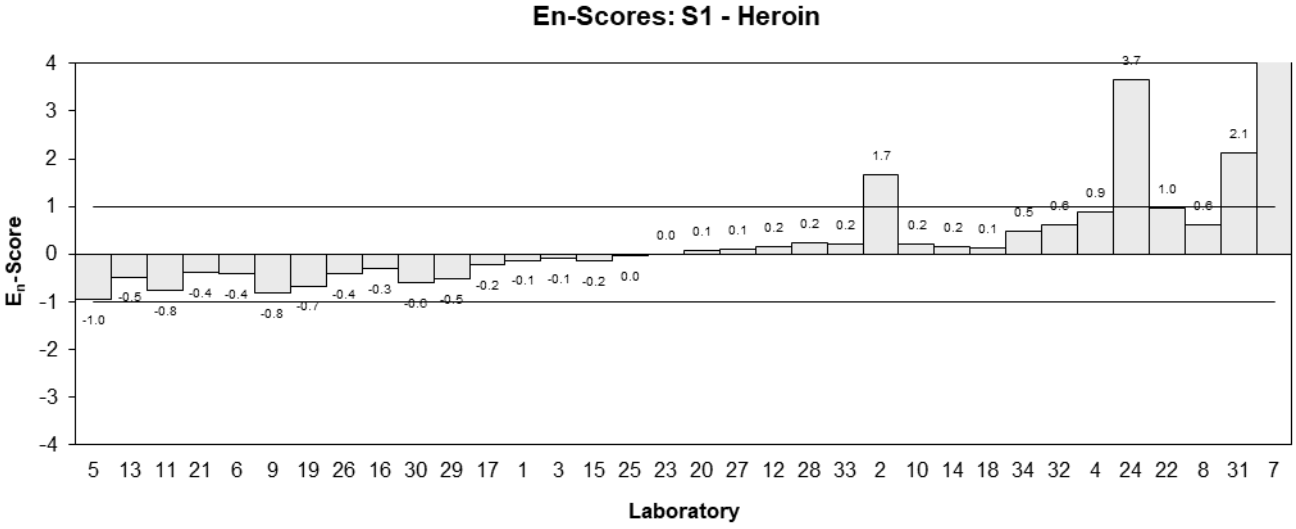

En-Scores: S1 - Heroin
En-Score
Laboratory
5 13 11 21 6 9 19 26 16 30 29 17 1 3 15 25 23 20 27 12 28 33 2 10 14 18 34 32 4 24 22 8 31 7

Figure 2

Table 6

**Sample Details**

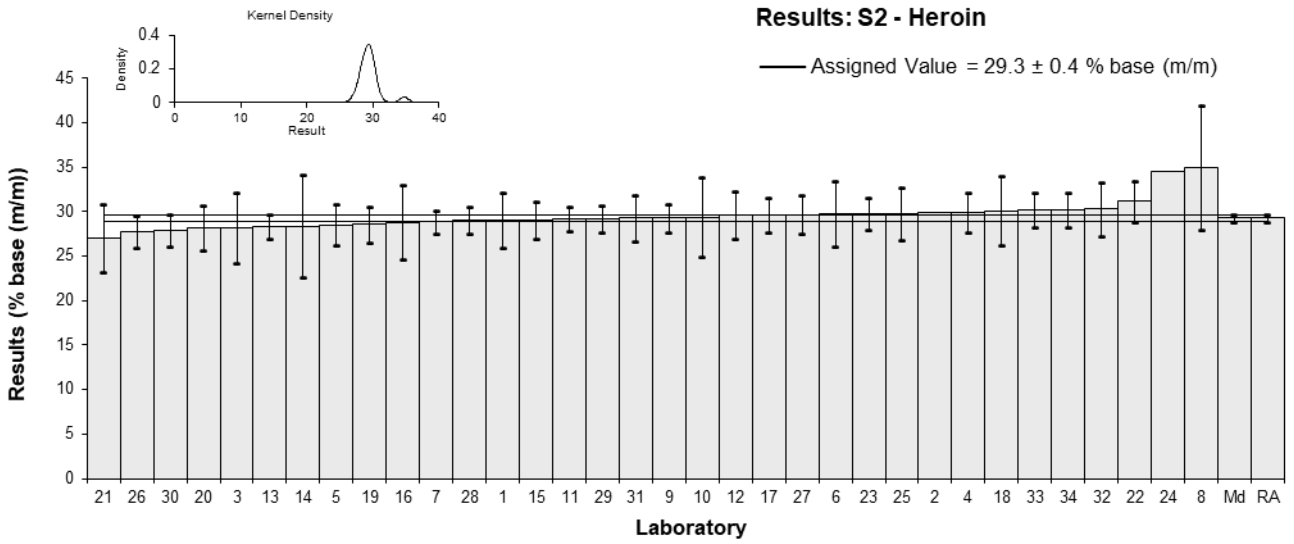
| | |
|---|---|
| **Sample No.** | S2 |
| **Matrix** | Powder |
| **Analyte** | Heroin |
| **Unit** | % base (m/m) |

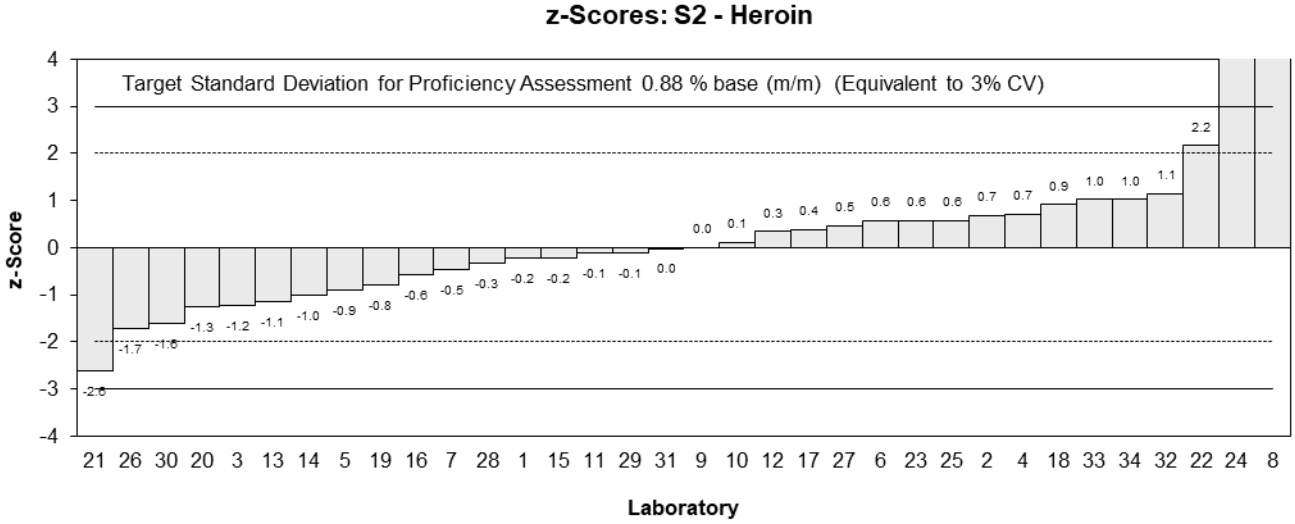
**Participant Results**

| Lab. Code | Result | Uncertainty | z | $E_n$ |
|---|---|---|---|---|
| **1** | 29.1 | 3.1 | -0.23 | -0.06 |
| **2** | 29.9 | NR | 0.68 | 1.50 |
| **3** | 28.22 | 3.95 | -1.23 | -0.27 |
| **4** | 29.92 | 2.22 | 0.71 | 0.27 |
| **5** | 28.5 | 2.3 | -0.91 | -0.34 |
| **6** | 29.8 | 3.7 | 0.57 | 0.13 |
| **7** | 28.9 | 1.3 | -0.46 | -0.29 |
| **8** | 35.0 | 7.0 | 6.48 | 0.81 |
| **9** | 29.3 | 1.6 | 0.00 | 0.00 |
| **10** | 29.4 | 4.5 | 0.11 | 0.02 |
| **11** | 29.2 | 1.4 | -0.11 | -0.07 |
| **12** | 29.6 | 2.7 | 0.34 | 0.11 |
| **13** | 28.3 | 1.4 | -1.14 | -0.69 |
| **14** | 28.4 | 5.7 | -1.02 | -0.16 |
| **15** | 29.1 | 2.1 | -0.23 | -0.09 |
| **16** | 28.8 | 4.2 | -0.57 | -0.12 |
| **17** | 29.64 | 1.98 | 0.39 | 0.17 |
| **18** | 30.1 | 3.92 | 0.91 | 0.20 |
| **19** | 28.6 | 2.0 | -0.80 | -0.34 |
| **20** | 28.2 | 2.5 | -1.25 | -0.43 |
| **21** | 27 | 3.8 | -2.62 | -0.60 |
| **22** | 31.2 | 2.3 | 2.16 | 0.81 |
| **23** | 29.8 | 1.8 | 0.57 | 0.27 |
| **24** | 34.5 | NR | 5.92 | 13.00 |
| **25** | 29.8 | 3 | 0.57 | 0.17 |
| **26** | 27.8 | 1.8 | -1.71 | -0.81 |
| **27** | 29.7 | 2.1 | 0.46 | 0.19 |
| **28** | 29 | 1.5 | -0.34 | -0.19 |
| **29** | 29.2 | 1.5 | -0.11 | -0.06 |
| **30** | 27.89 | 1.85 | -1.60 | -0.74 |
| **31** | 29.29 | 2.64 | -0.01 | 0.00 |
| **32** | 30.3 | 3 | 1.14 | 0.33 |
| **33** | 30.2 | 1.9 | 1.02 | 0.46 |
| **34** | 30.2 | 1.9 | 1.02 | 0.46 |

**Statistics**

| | | |
|---|---|---|
| **Assigned Value** | 29.3 | 0.4 |
| **Robust Average** | 29.3 | 0.4 |
| **Median** | 29.3 | 0.4 |
| **Mean** | 29.5 | |
| **N** | 34 | |
| **Max** | 35 | |
| **Min** | 27 | |
| **Robust SD** | 0.95 | |
| **Robust CV** | 3.2% | |


Kernel Density
Density
Result
Results: S2 - Heroin
Assigned Value = 29.3 ± 0.4 % base (m/m)
Results (% base (m/m))
Laboratory
21 26 30 20 3 13 14 5 19 16 7 28 1 15 11 29 31 9 10 12 17 27 6 23 25 2 4 18 33 34 32 22 24 8 Md RA


z-Scores: S2 - Heroin
Target Standard Deviation for Proficiency Assessment 0.88 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
21 26 30 20 3 13 14 5 19 16 7 28 1 15 11 29 31 9 10 12 17 27 6 23 25 2 4 18 33 34 32 22 24 8

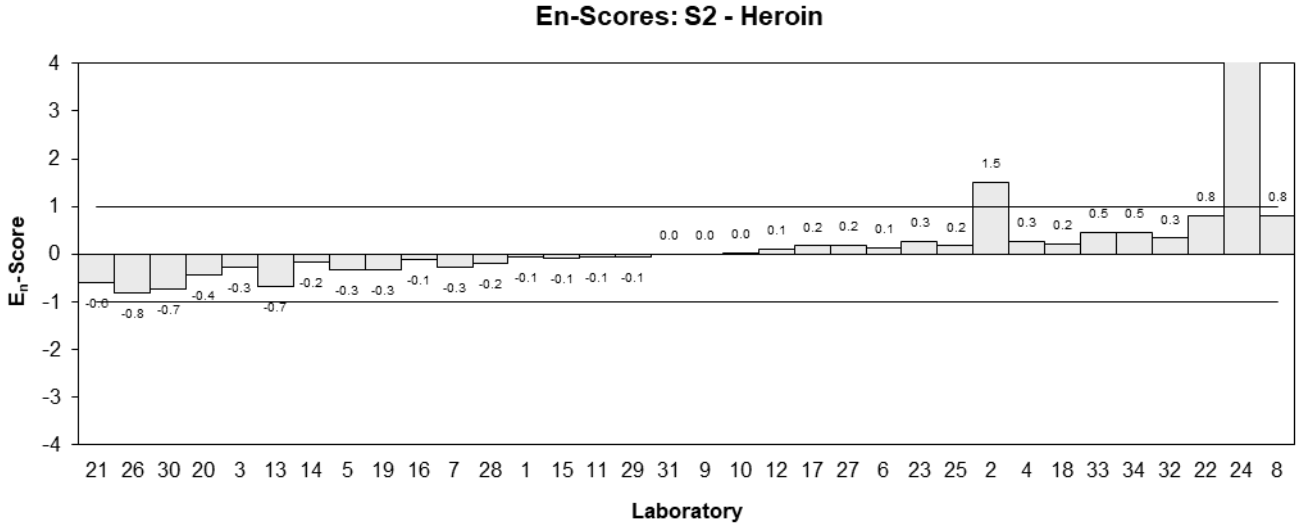

En-Scores: S2 - Heroin
En-Score
Laboratory
21 26 30 20 3 13 14 5 19 16 7 28 1 15 11 29 31 9 10 12 17 27 6 23 25 2 4 18 33 34 32 22 24 8

Figure 3

Table 7

**Sample Details**

| | |
|---|---|
| **Sample No.** | S3 |
| **Matrix** | Powder |
| **Analyte** | Heroin |
| **Unit** | % base (m/m) |

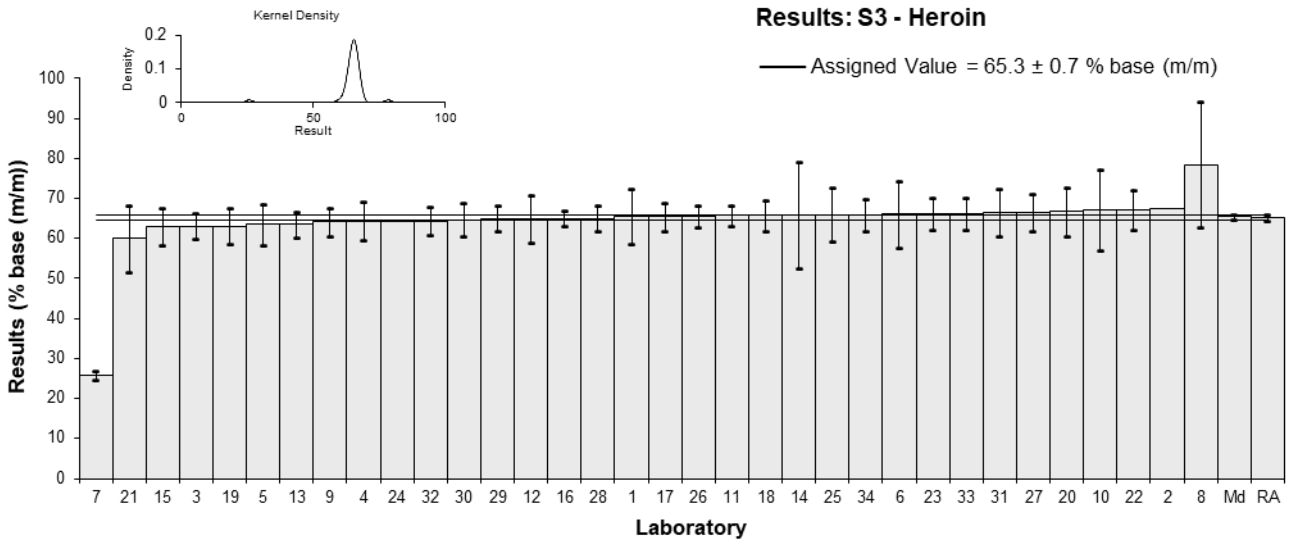
**Participant Results**

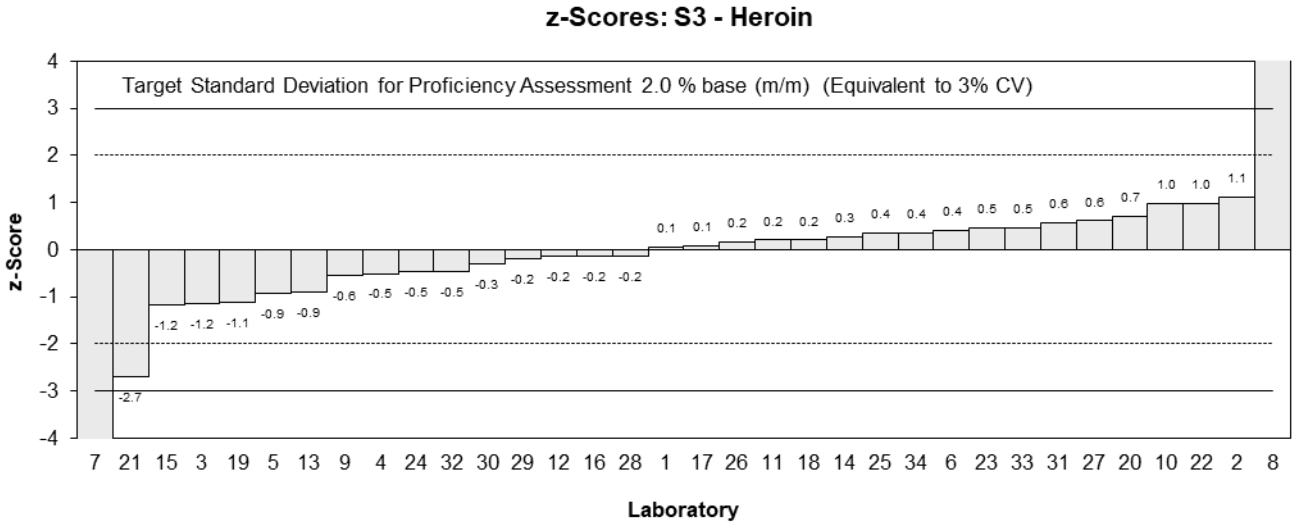
| Lab. Code | Result | Uncertainty | z | $E_n$ |
|---|---|---|---|---|
| **1** | 65.4 | 6.9 | 0.05 | 0.01 |
| **2** | 67.5 | NR | 1.12 | 3.14 |
| **3** | 63.04 | 3.15 | -1.15 | -0.70 |
| **4** | 64.31 | 4.77 | -0.51 | -0.21 |
| **5** | 63.5 | 5.1 | -0.92 | -0.35 |
| **6** | 66.1 | 8.3 | 0.41 | 0.10 |
| **7*** | 25.8 | 1.1 | -20.16 | -30.30 |
| **8** | 78.5 | 15.7 | 6.74 | 0.84 |
| **9** | 64.2 | 3.5 | -0.56 | -0.31 |
| **10** | 67.2 | 10.1 | 0.97 | 0.19 |
| **11** | 65.7 | 2.7 | 0.20 | 0.14 |
| **12** | 65 | 5.9 | -0.15 | -0.05 |
| **13** | 63.54 | 3.2 | -0.90 | -0.54 |
| **14** | 65.8 | 13.2 | 0.26 | 0.04 |
| **15** | 63.0 | 4.6 | -1.17 | -0.49 |
| **16** | 65.0 | 1.9 | -0.15 | -0.15 |
| **17** | 65.44 | 3.46 | 0.07 | 0.04 |
| **18** | 65.7 | 3.92 | 0.20 | 0.10 |
| **19** | 63.1 | 4.4 | -1.12 | -0.49 |
| **20** | 66.7 | 6.1 | 0.71 | 0.23 |
| **21** | 60 | 8.4 | -2.71 | -0.63 |
| **22** | 67.2 | 5 | 0.97 | 0.38 |
| **23** | 66.2 | 4.0 | 0.46 | 0.22 |
| **24** | 64.4 | NR | -0.46 | -1.29 |
| **25** | 66.0 | 6.6 | 0.36 | 0.11 |
| **26** | 65.6 | 2.8 | 0.15 | 0.10 |
| **27** | 66.5 | 4.7 | 0.61 | 0.25 |
| **28** | 65 | 3.3 | -0.15 | -0.09 |
| **29** | 64.9 | 3.2 | -0.20 | -0.12 |
| **30** | 64.72 | 4.3 | -0.30 | -0.13 |
| **31** | 66.41 | 5.98 | 0.57 | 0.18 |
| **32** | 64.4 | 3.5 | -0.46 | -0.25 |
| **33** | 66.2 | 4.0 | 0.46 | 0.22 |
| **34** | 66.0 | 4.0 | 0.36 | 0.17 |

* Outlier, see Section 4.2

**Statistics**

| | | |
|---|---|---|
| **Assigned Value** | 65.3 | 0.7 |
| **Robust Average** | 65.2 | 0.7 |
| **Median** | 65.4 | 0.6 |
| **Mean** | 64.4 | |
| **N** | 34 | |
| **Max** | 78.5 | |
| **Min** | 25.8 | |
| **Robust SD** | 1.6 | |
| **Robust CV** | 2.4% | |


Kernel Density
Density
Result
Results: S3 - Heroin
Assigned Value = 65.3 ± 0.7 % base (m/m)
Results (% base (m/m))
Laboratory
7 21 15 3 19 5 13 9 4 24 32 30 29 12 16 28 1 17 26 11 18 14 25 34 6 23 33 31 27 20 10 22 2 8 Md RA


z-Scores: S3 - Heroin
Target Standard Deviation for Proficiency Assessment 2.0 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
7 21 15 3 19 5 13 9 4 24 32 30 29 12 16 28 1 17 26 11 18 14 25 34 6 23 33 31 27 20 10 22 2 8

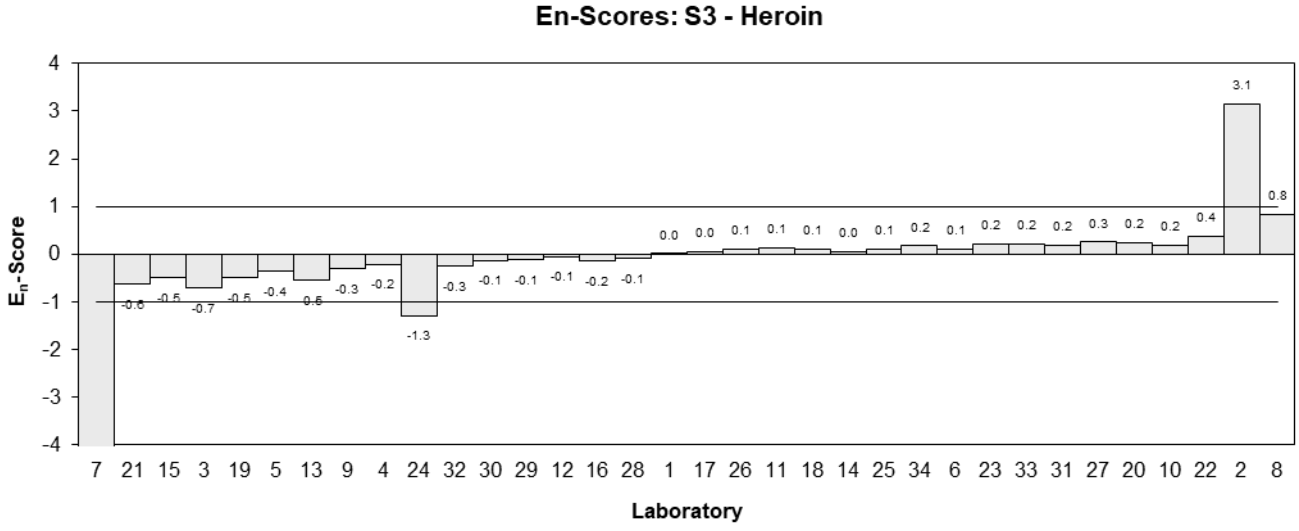

En-Scores: S3 - Heroin
En-Score
Laboratory
7 21 15 3 19 5 13 9 4 24 32 30 29 12 16 28 1 17 26 11 18 14 25 34 6 23 33 31 27 20 10 22 2 8

Figure 4

Table 8 Reported Cutting Agents*

| Lab. Code | Cutting Agents | | |
|---|---|---|---|
| | S1 | S2 | S3 |
| Preparation | Niacinamide (nicotinamide) | Sucrose | Procaine hydrochloride |
| 1 | Niacinamide | - | Procaine |
| 2 | Nicotinamide | | Procaine: 14.5 % |
| 3 | Niacinamide | Sucrose | Procaine, Acetyl codeine |
| 4 | Niacinamide | Sucrose | Procaine |
| 5 | Nicotinamide | | Procaine |
| 6 | niacinamide | - | procaine |
| 7 | Niacinamide (Nicotinamide) | Sucrose | Procaine |
| 8 | acetylcodeine / niacinamide | acetylcodeine / 6-MAM | acetylcodeine / 6-MAM / procaine |
| 9 | Niacinamide | Sucrose | Procaine |
| 10 | Nicotinamide | Sucrose | Procaine |
| 11 | Nicotinamide | | Procaine |
| 12 | Niacinamide | - | Procaine |
| 13 | Nicotinamide | Sugars | Procaine |
| 14 | Nicotinamide | | Procaine |
| 15 | Nicotinamide indicated | | Procaine indicated |
| 16 | Acetylcodeine, Nicotinamide | Acetylcodeine, Sucrose | Acetylcodeine, Procaine |
| 17 | Nicotinamide | Saccharose | Procaine |
| 18 | Niacinamide | | Procaine |
| 19 | niacinamide | | |
| 20 | Niacinamide | No | Procaine |
| 21 | none | none | procaine |
| 22 | 6-MAM, Niacinamide, Acetylcodeine | 6-MAM, Acetylcodeine, Sucrose | 6-MAM, Acetylcodeine, Procaine |
| 23 | niacinamide, acetylcodeine, 6-monoacetylmorphine. | acetylcodeine, 6-monoacetylmorphine. | procaine, acetylcodeine, 6-monoacetylmorphine. |
| 24 | NICOTINAMIDE | | PROCAINE |
| 25 | Acetylcodeine, Monoacetylmorphine, Niacinamide* (Tentative Identification) | Acetylcodeine, Monoacetylmorphine, Sucrose | Acetylcodeine, Monoacetylmorphine, Procaine |
| 26 | Nicotinamide | | Procaine |
| 27 | Nicotinamide | | Procaine |
| 28 | Nicotinamide, acetylcodeine | Acetylcodeine | Procaine, acetylcodeine |
| 29 | Nicotinamide | Sucrose | Procaine |
| 30 | Niacinamide | Sucrose | Procaine |
| 31 | nicotinamide | saccharose | procaine |
| 32 | Nicotinamide | | Procaine |
| 33 | Nicotinamide | - | Procaine |
| 34 | Nicotinamide | - | Procaine |

* Responses may have been modified so that the participant cannot be identified.

# 6 DISCUSSION OF RESULTS

## 6.1 Assigned Value

The robust averages and associated expanded uncertainties were calculated using the procedure described in ISO 13528.[5] The assigned values for all scored analytes were the robust averages of participants' results, after results less than 50% and greater than 150% of the robust average had been removed.[3,4] The calculation of the expanded uncertainty for a robust average, using Sample S2 as an example, 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 MU associated with their results and the basis of this uncertainty estimate (Table 3). One participant reported using the NATA GAG Estimating and Reporting MU as their guide; NATA no longer publishes this.[9]

It is a requirement of ISO/IEC 17025 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.[7]

Of 102 reported results, 96 (94%) were reported with an associated expanded MU. Laboratories **2** and **24** did not report any uncertainties; these participants reported that they were not accredited.

The magnitude of reported uncertainties was within the range 2.9% to 26% relative. In general, an expanded uncertainty of less than 3% may be unrealistically small for the routine measurement of illicit drugs, while over 10% may be too large and not fit for purpose. Of the 96 expanded MUs, 66 (69%) were between 3% and 10% relative to the result, one was less than 3% and 29 were greater than 10%.

Uncertainties associated with results returning a satisfactory *z*-score but an unsatisfactory $E_n$-score may have been underestimated.

In some cases, results were reported with an inappropriate number of significant figures. Including too many significant figures may inaccurately reflect measurement precision. 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 reporting 65.44 ± 3.46%, the recommended format is 65.4 ± 3.5%.[8]

## 6.3 *z*-Score

Target SDs equivalent to 3% PCV were used to calculate *z*-scores. CVs predicted by the Thompson-Horwitz equation,[6] target SDs (as PCVs) and between-laboratory CVs obtained in this study are presented for comparison in Table 9.

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

| Sample | Analyte | Assigned Value (% base (m/m)) | Thompson-Horwitz CV[a] (%) | Between-Laboratory CV[b] (%) | Target SD (as PCV) (%) |
|---|---|---|---|---|---|
| S1 | Heroin | 14.8 | 2.6 | 5.3 | 3 |
| S2 | Heroin | 29.3 | 1.8 | 3.2 | 3 |
| S3 | Heroin | 65.3 | 1.2 | 2.3 | 3 |

[a] Calculated from the assigned value.

[b] Robust between-laboratory CV with outliers removed, if applicable.

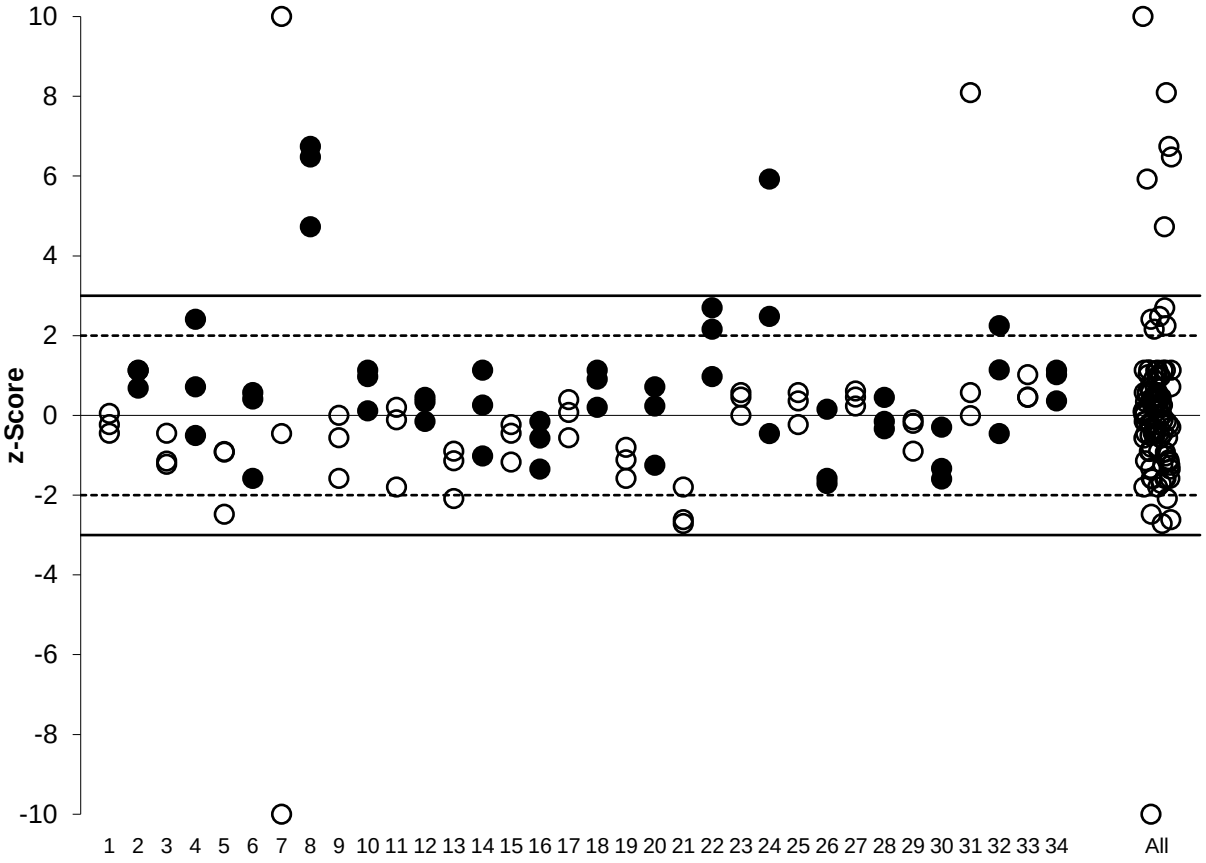
Of 102 results for which *z*-scores were calculated, 86 (84%) returned a *z*-score with $|z| \leq 2.0$, indicating an acceptable performance.

Twenty-four participants: **1**, **2**, **3**, **6**, **9**, **10**, **11**, **12**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **23**, **25**, **26**, **27**, **28**, **29**, **30**, **33** and **34** returned acceptable *z*-scores for all three samples. Ten participants returned at least one questionable or unacceptable *z*-score.

Laboratory **7**'s results were similar across all three samples, resulting in a very high *z*-score for Sample S1 and a very low *z*-score for Sample S3. This participant should check that they have reported results for the correct sample.

Laboratory **8** returned unacceptable *z*-scores across all reported results, with all results being higher than the assigned value (positive bias). This participant may have reported results as % salt (m/m) instead of % base (m/m) as requested for this PT study; otherwise, this participant should check their methodology for the cause of this positive bias.

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




*z*-Scores greater than 10.0 or less than -10.0 have been plotted at 10.0 or -10.0 respectively.

Figure 5 *z*-Score Dispersal by Laboratory

### 6.4 $E_n$-Score

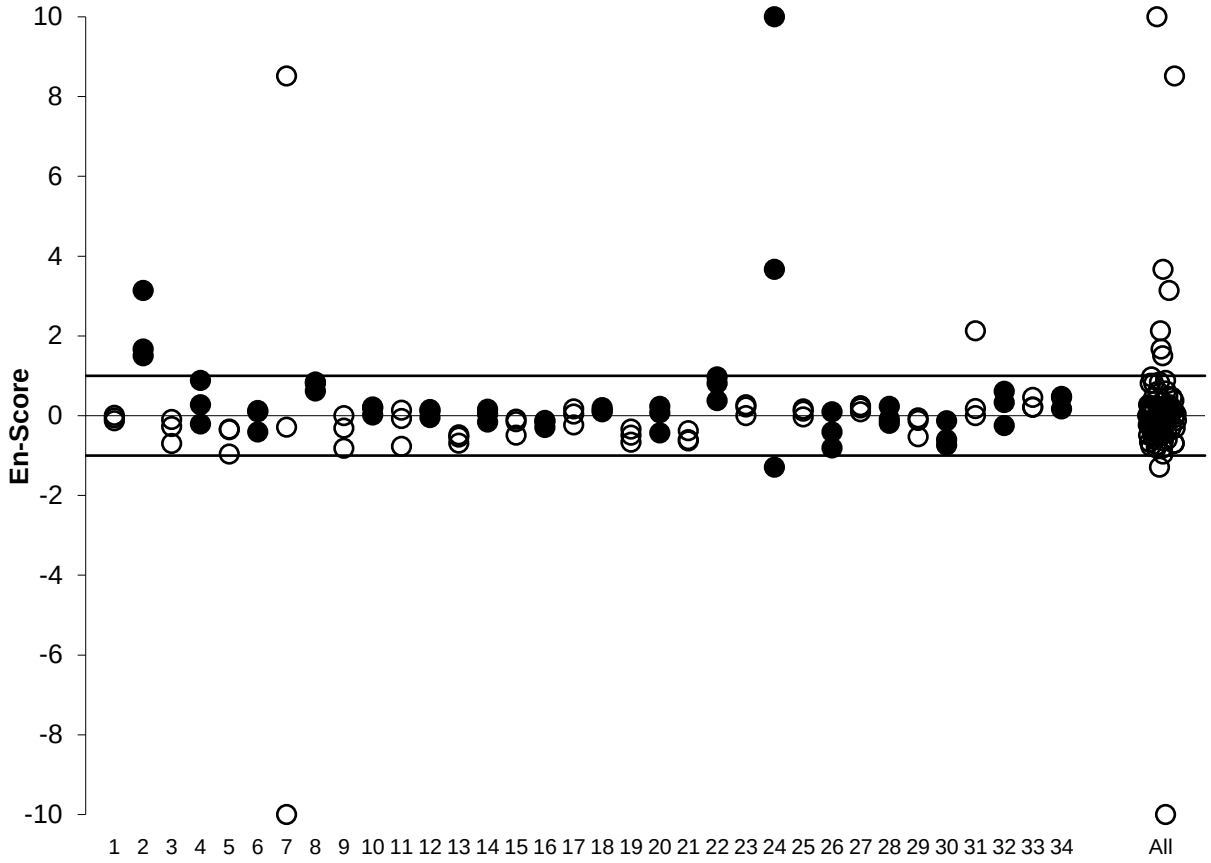
$E_n$-Scores can be interpreted in conjunction with *z*-scores, as an unsatisfactory $E_n$-score can be caused by an inappropriate measurement, or uncertainty, or both. If a participant did not report an uncertainty with a result, an expanded uncertainty of zero (0) was used to calculate the $E_n$-score.

Of 102 results for which $E_n$-scores were calculated, 93 (91%) returned an acceptable $E_n$-score of $|E_n| \leq 1.0$, indicating agreement of the participant's result with the assigned value within their respective expanded uncertainties.

Thirty participants: **1**, **3**, **4**, **5**, **6**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **23**, **25**, **26**, **27**, **28**, **29**, **30**, **32**, **33** and **34** returned acceptable $E_n$-scores for all three samples. Four participants returned at least one unacceptable $E_n$-score.

Laboratories **2** and **24** returned unacceptable $E_n$-scores across all reported results; these participants did not report any uncertainties.

The dispersal of participants' $E_n$-scores is presented graphically in Figure 6.




$E_n$-Scores greater than 10.0 or less than -10.0 have been plotted at 10.0 or -10.0 respectively.

Figure 6 $E_n$-Score Dispersal by Laboratory

### 6.5 Identification of Cutting Agents

Cutting agents were added to each sample: niacinamide for Sample S1, sucrose for Sample S2, and procaine hydrochloride for Sample S3.

All participants reported on the identity of at least one sample's cutting agent. Results reported by participants are presented in Table 8.

Laboratories **3**, **4**, **7**, **9**, **10**, **16**, **17**, **22**, **25**, **29**, **30** and **31** correctly reported all cutting agents.

For Sample S1, all participants except Laboratory **21** reported on the identity of the cutting agent; all participants who did report for the cutting agent were correct.

For Sample S2, Laboratories **3**, **4**, **7**, **9**, **10**, **16**, **17**, **22**, **25**, **29**, **30** and **31** correctly identified sucrose as the cutting agent. Laboratory **13** identified 'sugars' however did not specify what type of sugar was present. Twenty-one participants did not report on the identity of the cutting agent.

For Sample S3, all participants except Laboratory **19** reported on the identity of the cutting agent; all those who did report for the cutting agent were correct.

## 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 methodologies 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 | 1, 4, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 33, 34 |
| | Not Accredited / Not Reported | 2, 3, 5, 10, 17, 24, 32 |
| Average Sample Mass Used per Analysis (mg) | < 20 | 3, 9, 13, 14, 23, 24, 29, 31, 32 |
| | 20 – 30 | 1, 4, 5, 6, 8, 10, 12, 17, 18, 19, 20, 22, 25, 26, 28, 30, 33, 34 |
| | 31 – 50 | 2, 11, 15, 21, 27 |
| | 51 - 100 | 7 |
| | > 100 | 16 |
| Conversion to Base? | Yes | 1, 3, 6, 12, 13, 16, 18, 19, 20, 22, 23, 24, 25, 27, 32 |
| | No | 2, 4, 5, 7, 8, 9, 11, 14, 15, 17, 26, 28, 29, 30, 31, 33, 34 |
| | Not Reported | 10, 21 |
| Instrument Used for Quantification | HPLC-DAD | 4, 5, 10, 11, 17, 18, 24, 28, 31 |
| | HPLC-UV/Vis | 3, 9, 14, 25 |
| | UPLC-DAD | 2, 15, 26, 27, 33, 34 |
| | UPLC-MS/MS | 21 |
| | GC-FID | 1, 6, 7, 12, 13, 16, 19, 20, 22, 23, 29, 30, 32 |
| | GC-MS | 8 |
| Solvent | Acetonitrile | 7, 25 |
| | Acetonitrile/Water | 3, 9, 10, 14, 15, 31 |
| | Acetonitrile/Water/Acid | 4, 17 |
| | Chloroform | 8, 12, 16, 19, 20 |
| | Chloroform/Methanol | 1, 6 |
| | Ethanol | 2, 22, 29, 32 |
| | Methanol | 5, 11, 18, 23, 24, 27, 28, 30, 33, 34 |
| | Other | 13, 21, 26 |
| Source of Calibration Standard | NMI Australia | 2, 7, 10, 15, 19, 25, 26, 29 |
| | Lipomed | 5, 8, 11, 13, 14, 17, 21, 22, 23, 27, 31, 33, 34 |
| | LGC | 4, 24, 30 |
| | British Pharmacopoeia | 3, 9 |
| | Other | 1, 6, 12, 16, 18, 20, 28, 32 |

Plots of *z*-scores against various parameters are presented in Figures 7 to 11 (outliers have not been plotted). One participant used GC-MS for analysis, and they returned unacceptable *z*-scores across all samples (positive bias).

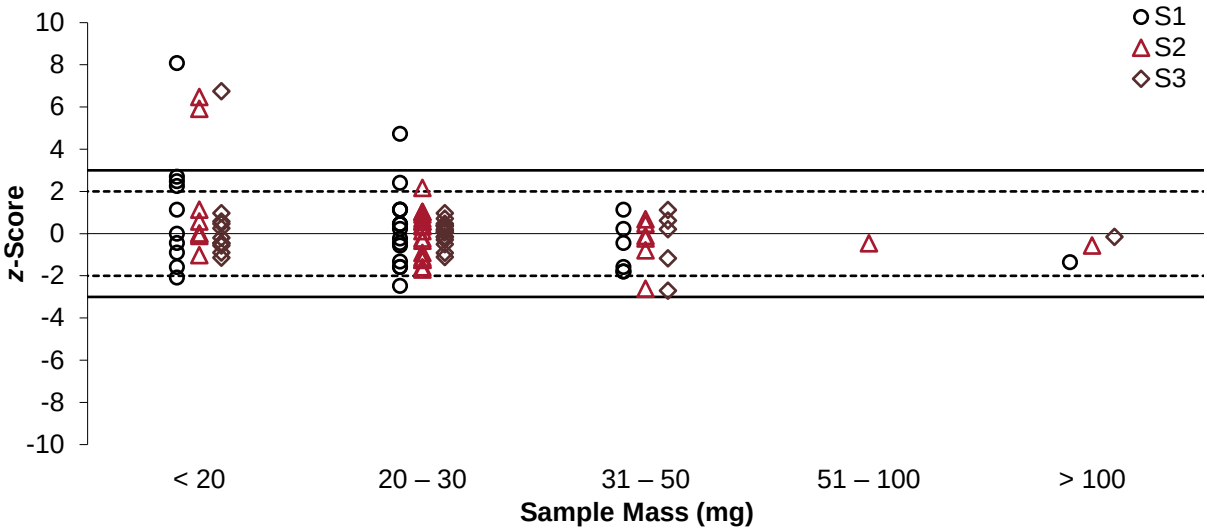



Figure 7 *z*-Score vs Sample Mass Used per Analysis

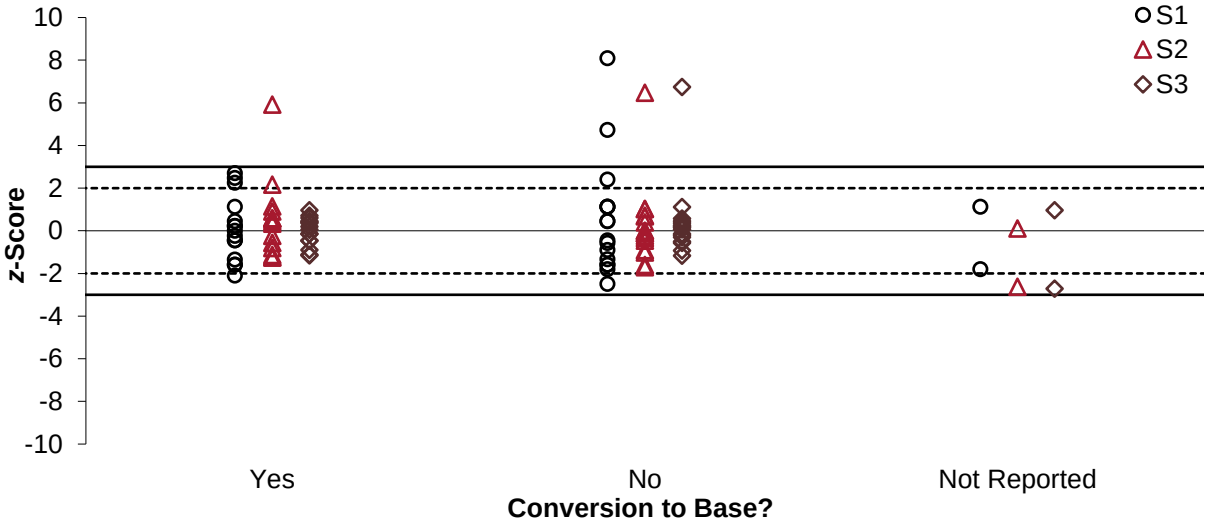



Figure 8 *z*-Score vs Sample Processing

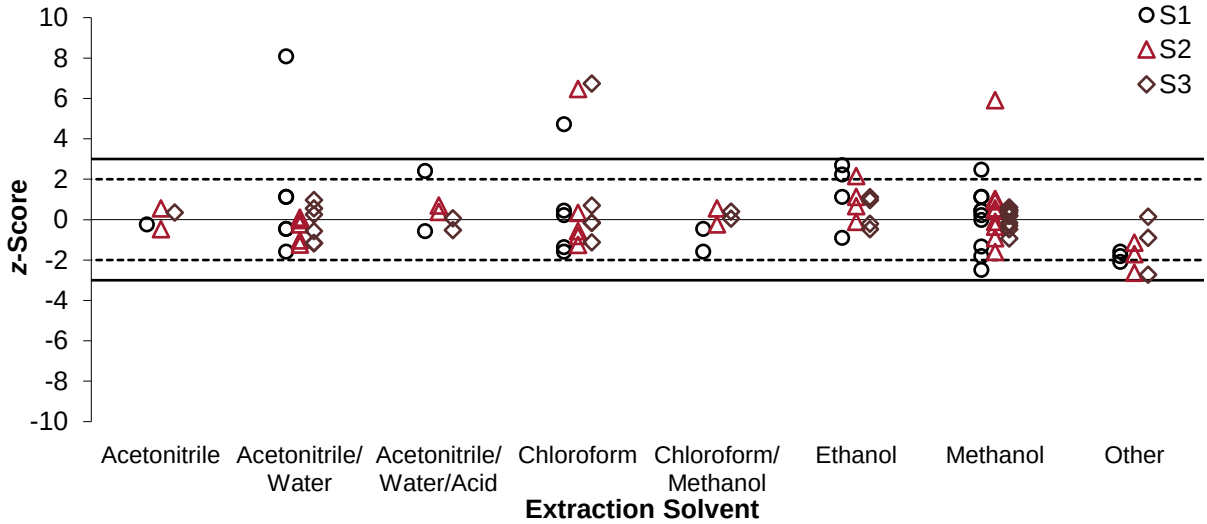



Figure 9 *z*-Score vs Extraction Solvent

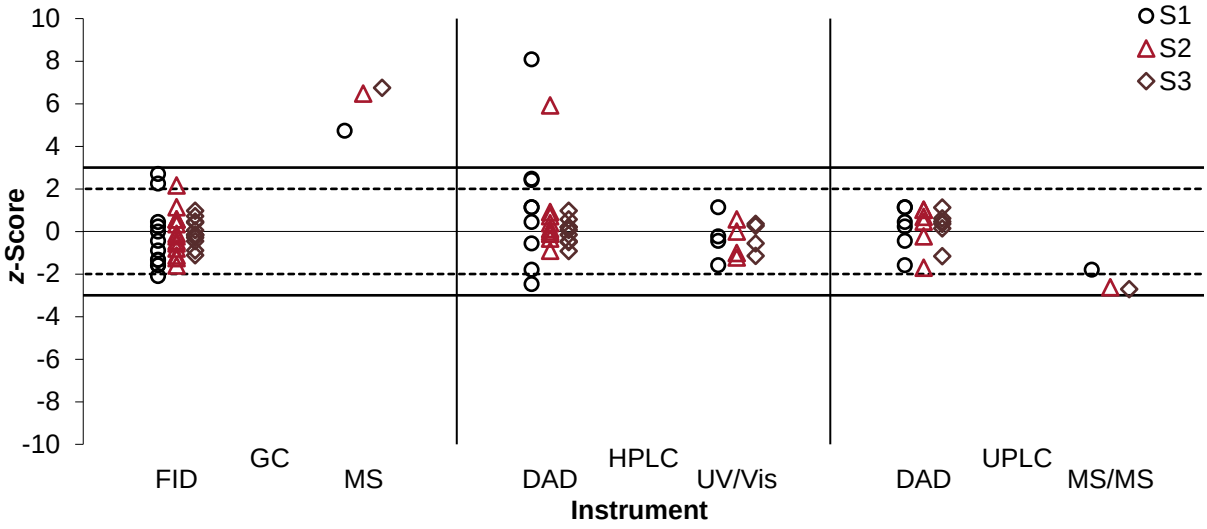



Figure 10 *z*-Score vs Measurement Instrument

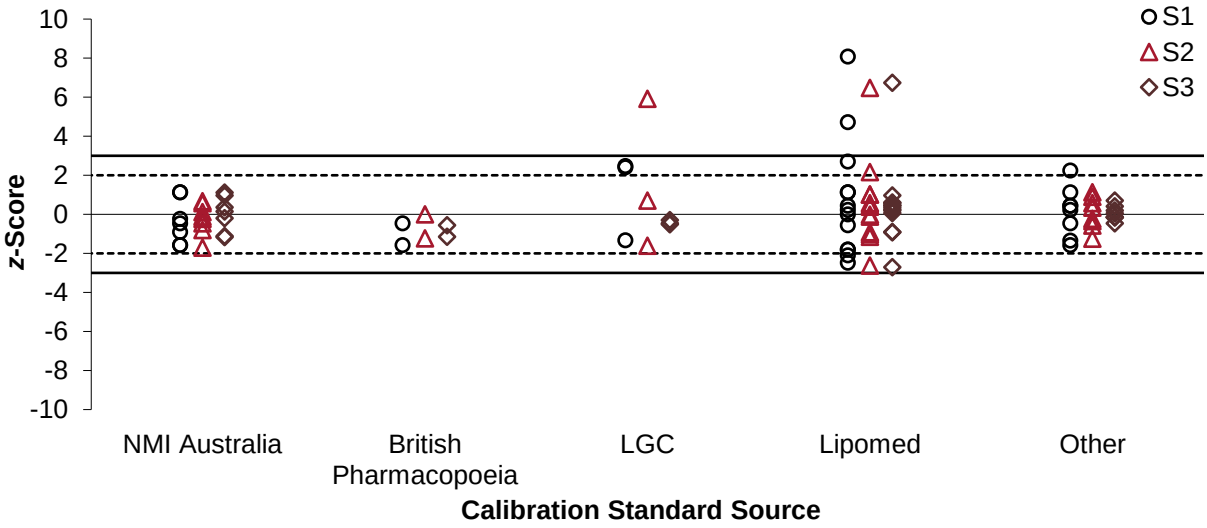



Figure 11 *z*-Score vs Calibration Standard Source

### 6.7 Comparison of Results and Date of Analysis

As there were delays with sample delivery to some participants, the test samples were analysed over the course of approximately three months. There was no evidence of sample degradation over this period (Figure 12).

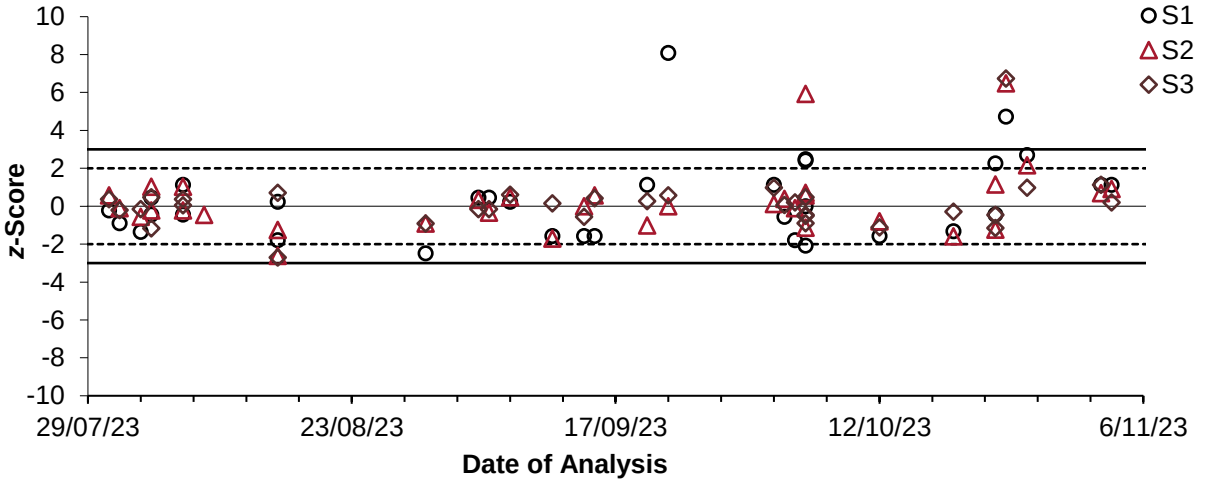



Figure 12 *z*-Score vs Sample Analysis Date

### 6.8 Comparison with Previous Heroin PT Studies

To enable direct comparison with previous NMI heroin 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 2014 to 2023 (last ten studies) is presented in Figure 13. The proportion of satisfactory *z*-scores and $E_n$-scores over this period on average is 84% for both.

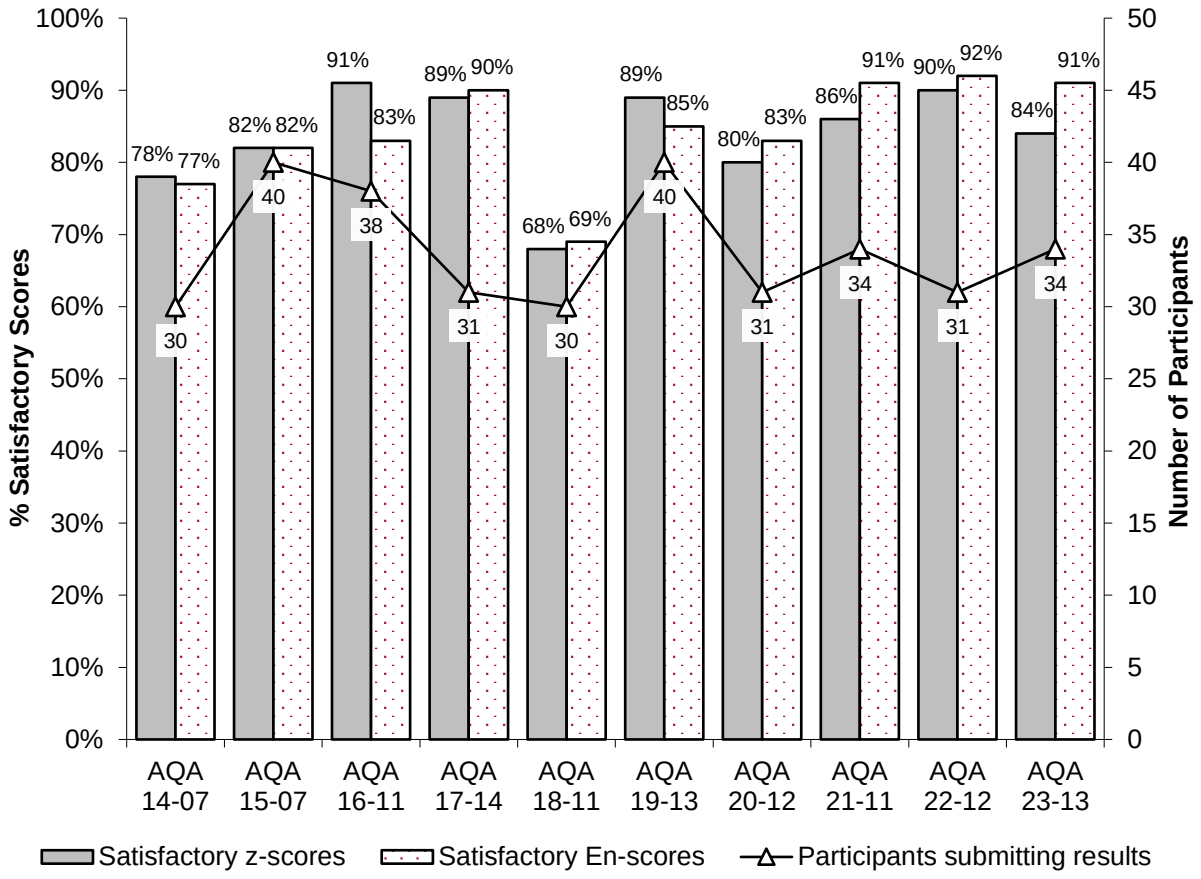



Figure 13 Summary of Participants' Performance in NMI Heroin PT Studies

A number of participants have consistently participated in NMI heroin PT studies, and individual performance history reports are emailed to each participant at the end of each 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.

A summary of individual laboratories' performances over the last ten studies is presented in Figures 14 and 15 for Australian and international laboratories respectively. *z*-Scores greater than 10.0 or less than -10.0 have been plotted at 10.0 or -10.0 respectively. Two Australian and four international laboratories have achieved acceptable *z*-scores across all samples in all heroin PT studies participated in over this period.

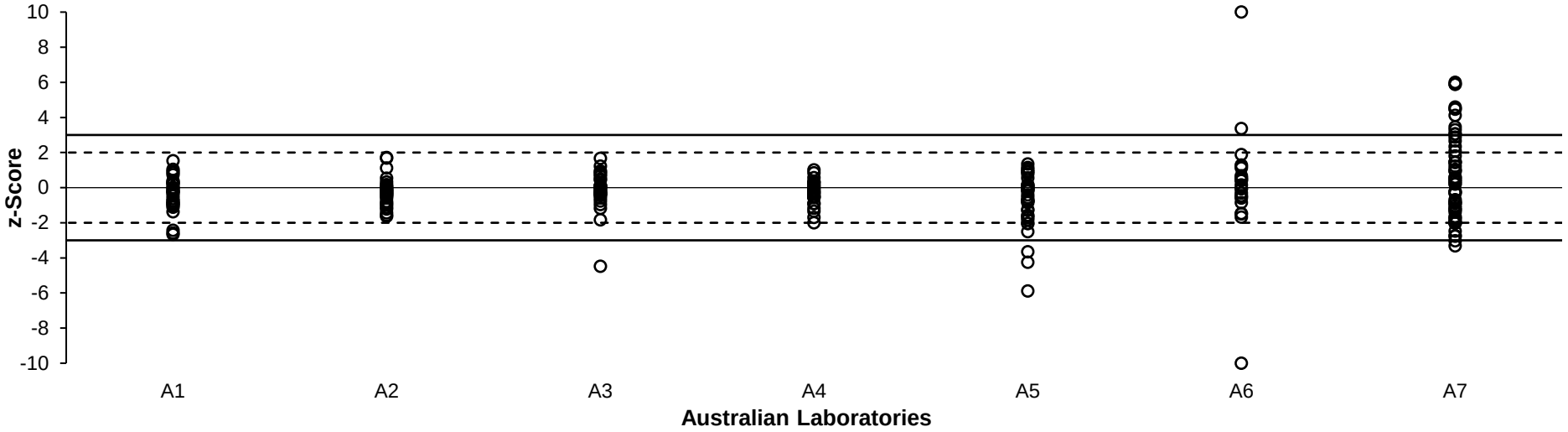



Figure 14 Summary of Australian Participants' z-Scores in NMI Heroin PT Studies

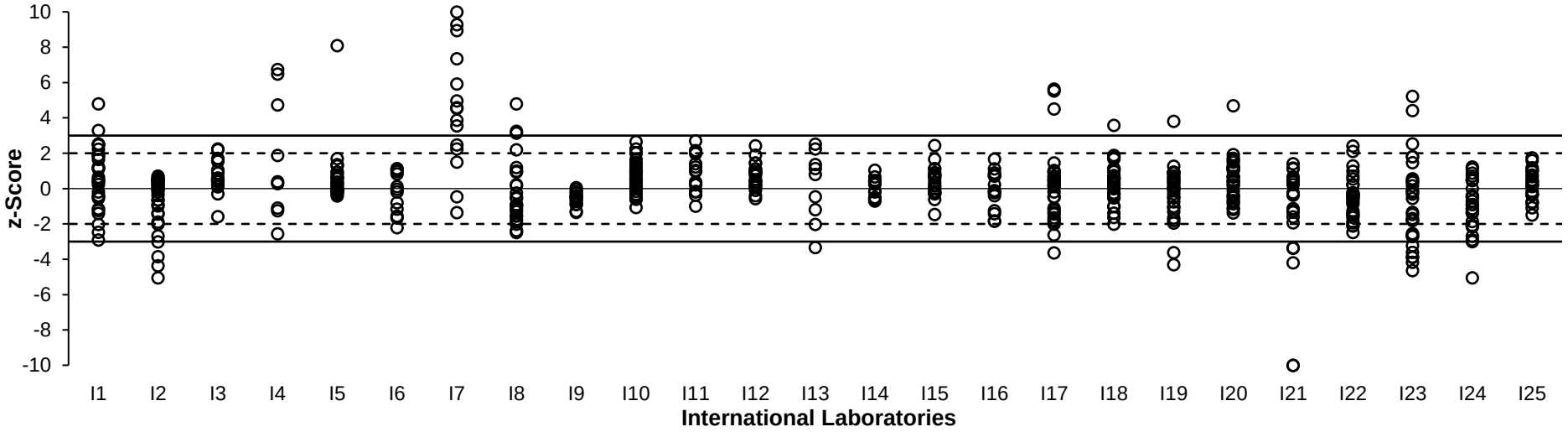



Figure 15 Summary of International Participants' z-Scores in NMI Heroin PT Studies

A comparison of all results from Australian and international laboratories in NMI heroin PT studies over the last ten studies is presented in Figure 16. Overall, both groups have performed very similarly, with Australian and international laboratories both achieving 84% acceptable *z*-scores respectively over this period.

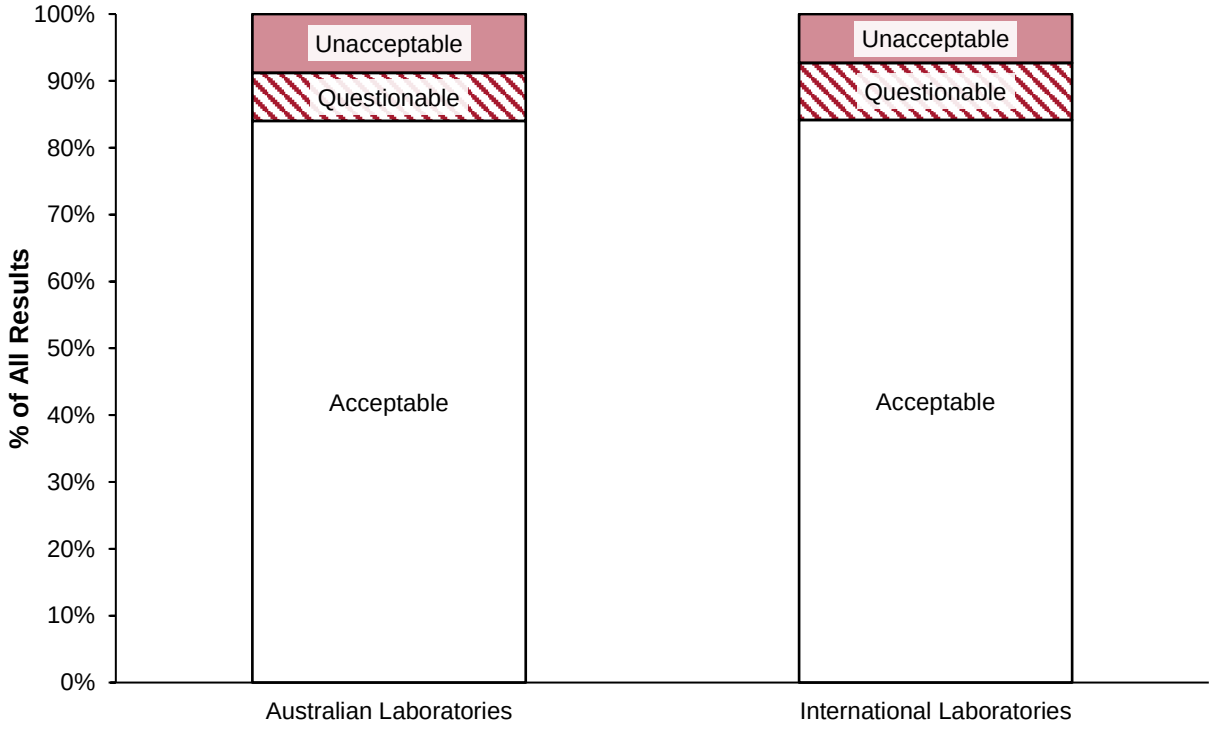



Figure 16 Comparison of Australian and International Laboratories in NMI Heroin PT Studies

## 7 REFERENCES

Please note that for all undated references, the latest edition of the referenced document (including any amendments) applies.


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

[2] NMI, 2023, *Study Protocol for Proficiency Testing*, viewed December 2023, <https://www.industry.gov.au/sites/default/files/2020-10/cpt_study_protocol.pdf>.

[3] NMI, 2023, *Chemical Proficiency Testing Statistical Manual*, viewed December 2023, <https://www.industry.gov.au/sites/default/files/2019-07/cpt_statistical_manual.pdf>.

[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, *Statistical methods for use in proficiency testing by interlaboratory comparison*.

[6] 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.

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

[8] Eurachem/CITAC Guide CG 4, QUAM:2012.P1, *Quantifying Uncertainty in Analytical Measurement*, 3rd ed., viewed December 2023, <http://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf>.

[9] NATA, 2020, *Update to Measurement Uncertainty resources*, viewed December 2023, <https://nata.com.au/news/update-to-measurement-uncertainty-resources/>

## APPENDIX 1 ROBUST AVERAGE AND ASSOCIATED UNCERTAINTY, *z*-SCORE AND $E_n$-SCORE CALCULATIONS

### A1.1 Robust Average and Associated Uncertainty

Robsut averages were calculated using the procedure described in ISO 13528.[5] The associated uncertainties were estimated as according to Equation 4.

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

where:

$u_{rob\ av}$ is the standard uncertainty of the robust average

$S_{rob\ av}$ is the standard deviation of the robust average

$p$ is the number of results

The expanded uncertainty ($U_{rob\ av}$) 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 11.

Table 11 Uncertainty Estimate for Robust Average of Sample S2

| | |
|---|---|
| Number of Results ($p$) | 34 |
| Robust Average | 29.3% base (m/m) |
| $S_{rob\ average}$ | 0.95% base (m/m) |
| $u_{rob\ average}$ | 0.20% base (m/m) |
| $k$ | 2 |
| $U_{rob\ average}$ | 0.40% base (m/m) |

Therefore, the robust average for Sample S2 is 29.3 ± 0.4% base (m/m).

### A1.2 *z*-Score and $E_n$-Score Calculations

For each participant's result, a *z*-score and $E_n$-score are calculated according to Equations 2 and 3 respectively (Section 4).

A worked example is set out below in Table 12.

Table 12 *z*-Score and $E_n$-Score Calculation for Sample S1 Result Reported by Laboratory 1

| Participant Result (% base (m/m)) | Assigned Value (% base (m/m)) | Target Standard Deviation | z-Score | $E_n$-Score |
|---|---|---|---|---|
| 14.6 ± 1.5 | 14.8 ± 0.3 | 3% as PCV, or: 0.03 × 14.8 = 0.444% base (m/m) | z-Score = $\frac{14.6-14.8}{0.444}$ = -0.45 | $E_n$-Score = $\frac{14.6-14.8}{\sqrt{1.5^2+0.3^2}}$ = -0.13 |

## APPENDIX 2 ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| ASCLD/LAB | American Society of Crime Laboratory Directors – Laboratory Accreditation Board |
| CITAC | Cooperation on International Traceability in Analytical Chemistry |
| CRM | Certified Reference Material |
| CV | Coefficient of Variation |
| DAD | Diode Array Detection |
| EA | European Accreditation |
| FID | Flame Ionisation Detection |
| GAG | General Accreditation Guidance (NATA) |
| GC | Gas Chromatography |
| GUM | Guide to the expression of Uncertainty in Measurement |
| HPLC | High Performance Liquid Chromatography |
| IEC | International Electrotechnical Commission |
| ISO | International Organization for Standardization |
| Max | Maximum |
| Md | Median |
| Min | Minimum |
| MS | Mass Spectrometry |
| MS/MS | Tandem Mass Spectrometry |
| MU | Measurement Uncertainty |
| N | Number of numeric results |
| NATA | National Association of Testing Authorities, Australia |
| NMI | National Measurement Institute, Australia |
| NR | Not Reported |
| PCV | Performance Coefficient of Variation |
| PDA | Photodiode Array Detection |
| PT | Proficiency Testing |
| RA | Robust Average |
| RM | Reference Material |
| SD | Standard Deviation |
| SI | International System of Units |
| SS | Spiked Samples |
| UPLC | Ultra Performance Liquid Chromatography |
| UV/Vis | Ultraviolet/Visible Detection |

**END OF REPORT**