Development of an External Quality Assessment Scheme for Urine Drugs of Abuse

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Introduction

Urine testing for both prescribed and illicit drugs is increasingly used in both Laboratory and Point of Care settings. The aim of the study was to develop and validate material for use in an EQA scheme for Urine Drugs of Abuse testing and to assess the material's stability and commutability.

Method

Each drug or metabolite was gravimetrically added to base urine from a negative healthy donor to provide two panels at high target concentrations. The pools were then mixed with the negative base urine to produce a panel of intermediate pools. The "weighed-in" value and purity of the spiked drug was used to calculate the target value and was used in the interpretation of the qualitative result. All microbalances were calibrated using certified weights allowing full gravimetric traceability.

Table 1 – Range covered for each analyte

Analyte	"Cut off"	Range Covered	Units
Amphetamine	1000	0 - 3000	µg/L
Benzodiazepine	300	0 - 1000	µg/L
Barbiturate	300	0 - 1000	µg/L
Buprenorphine	10	0 - 50	µg/L
Cocaine	300	0 - 1000	µg/L
Cannabis	50	0 - 150	µg/L
6-Acetylmorphine (heroin)	10	0 - 50	µg/L
Ketamine	1000	0 - 3000	µg/L
Methadone	300	0 - 1000	µg/L
EDDP	300	0 - 1000	µg/L
Methamphetamine	1000	0 - 3000	µg/L
Opiates	300	0 – 3000	µg/L
Phencyclidine (PCP)	25	0 - 100	µg/L
Tricyclic antidepressants	1000	0 - 3000	µg/L
MDMA	1000	0 – 3000	µg/L
Amphetamines Group Screen	1000	Qualitative only	

Over 60 sites were recruited to take part in the study. Each site was sent 3 samples per month with negative and positive samples covering 16 drugs. Three of the pools were analysed by LC-MS/MS and assessed for their stability at 4°C, ambient and at -20°C.

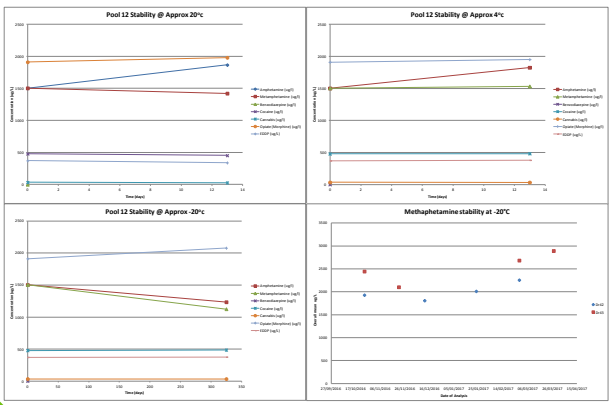
Long term stability of pools stored at -20°C was also assessed from participants data where pools had been distributed on more than one occasion. The results for the mid concentration pool is provided in Figures 1a , 1b and 1c for all the drugs at all temperatures and a further long term stability for methamphetamine using participants data is provided in Figure 1d.

Performance assessment

Assessment of the qualitative results were based on the cut-offs in Table 1 and reflect those used by the majority of participants in the UK, and are higher than the European Guidelines for Workplace Drug Testing. Examples of the performance of the methods providing qualitative results (pos or neg) are shown in Figures 2a and 2b. The quantitative results were compared with the gravimetric values and the performance specification calculated from precision profiles established during the pilot (Figures 3a & 3b). Uncertainty of the gravimetric value was calculated from the Guide to the Expression of Uncertainty in Measurement (GUM).

Results

Fig 1a & 1b – Short Term Stability. Fig 1c & 1d – Long term Stability.



Good stability was observed for the majority of analytes, although a decrease of -18% was observed for the extended stability at -20° C for amphetamine and methamphetamine (Figure 1c) which was not confirmed from the participant data (Figure 1d).

Table 2 – Recovery against gravimetric value

Analyte	Gravimetric	LC-MS/MS	Recovery %
Amphetamine (ug/L)	3000	2936	97.9
Cannabis (ug/L)	300	227	75.7
Opiate (Morphine) (ug/L)	5000	5483	109.7
Benzodiazepine (ug/L)	800	749	93.6
Cocaine (ug/L)	800	842	105.3
Methadone (EDDP) (ug/L)	600	720	120.0
Methamphetamine (ug/L)	3000	3193	106.4
6-Acetylmorphine (ug/L)	30	25	83.3
Buprenorphine (ug/L)	30	31	103.3
Ketamine (ug/L)	3000	2786	92.9
Barbiturates (ug/L)	800	820	102.5
MDMA (ug/L)	2500	2766	110.6
Phencyclidine (ug/L)	73.4	78*	106.3

Traceability

Good agreement was observed between the 'gravimetric' weighed in target and the LC-MS/MS data for the majority of analytes, however a decreased recovery of 76% and 83% was observed for cannabis and acetylmorphine respectively.

* Overall mean – no LC-MS data

Method Performance / Interferences / Specificity of Methods

Amphetamine / Methamphetamine / MDMA

It was identified early in the pilot that a number of immunoassay methods for amphetamines also measured methamphetamine and MDMA. Methamphetamine was added to a base urine to a concentration of 1500 ug/L, and of the 14 immunoassay results received, 9 users reported positive result for amphetamine. A separate analyte called 'amphetamines group screen' was set up which took into account the total amount of amphetamine, methamphetamine and MDMA in the sample for interpretation purposes.

Benzodiazepines

For the benzodiazepine both Oxazepam and Temazepam were used. Negative results were observed for the Alere Triage for Oxazepam at a target concentration of 400 µg/L, however positive results were observed for Temazepam at the same concentration.. The method has low specificity for Oxazepam and requires x10 fold increase in concentration to give a positive result.

Figure 2a & 2b –Assessment of positive "cut off"

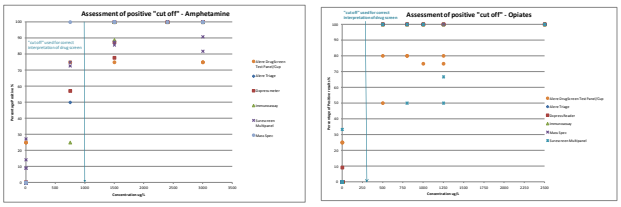
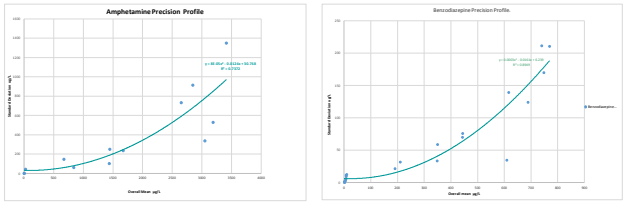


Figure 3a & 3b Performance Specification



Conclusion

The use of Gravimetric 'weighed in' values as a performance target in EQA data provides a stable, reliable target that is not influenced by the overall or method mean. The stable linear panel of samples allows the distribution of repeat samples on a number of occasions facilitating the assessment of linearity, within and between batch precision and traceability. The programme provides laboratories with on going evidence for their compliance to ISO 15189.