

Analytical errors in quantification of drugs of abuse in urine

J.F. Wilson

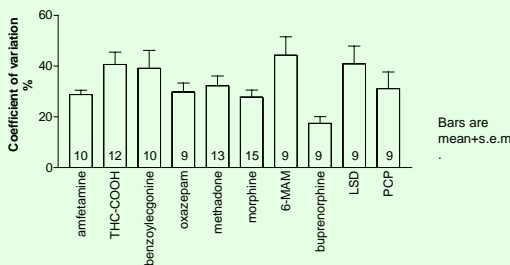
On behalf of the Steering Committee for the UKNEQAS for Drug Assays*

Department of Pharmacology, Therapeutics & Toxicology, University of Wales College of Medicine, Heath Park, Cardiff, CF14 4XN, U.K. (wilsonjf@cardiff.ac.uk)

INTRODUCTION

The United Kingdom National External Quality Assessment Scheme (UKNEQAS) for drugs of abuse in urine circulates 12 freeze-dried samples of urine per year to over 200 laboratories world-wide to monitor their performance in detecting the major groups of abused drugs. The required laboratory report for each drug group is 'present' or 'not found' relative to clinical or workplace-testing thresholds. In addition, participating laboratories are asked to report the results of the individual analytical tests used in sample assessment and a proportion of these data are in the form of quantitative measures of drug compounds. The inter-laboratory variation of the latter data is relatively high with coefficients of variation of measurements ranging from 17 to 44%.

Inter-laboratory precision 1999-2002



We assess here possible sources of the between-laboratory differences by analysis of the data for the individual laboratories.

MATERIALS AND METHODS

- Data from 33 samples distributed during 1999-2002 were analysed for 10 drugs.
- The number of samples containing each drug is given in the bar graph above.
- Data for a laboratory were included where there was at least 4 measurements for a drug by the same technique.
- A mathematical function describing the variation in the inter-laboratory standard deviation (SD) with respect to drug concentration was calculated from a linear fit to the square root of SD for each drug.
- Weighted linear and quadratic regression was undertaken for each laboratory using SD squared (variance) calculated from the linear functions as weights.
- The weighted regressions were repeated after correction of the weights to bring the total population variance across all laboratories for a drug to a value of unity.
- The resulting sums of squares statistics are distributed as chi-square allowing tests for individual laboratories for 1) assay bias, 2) slope errors, 3) non-linearity, and 4) random errors relative to the group performance for a drug as a whole. See: Wilson et al., (1984) *Clin. Chim. Acta*, 143: 203-216 for full details.

RESULTS

The total number of laboratories analysing each drug and the numbers identified that produced significant ($P < 0.05$) errors of the 4 types and of any type are tabulated.

Drug	bias	slope	non-linearity	random	any error	total labs analysed
amfetamine	8	5	0	5	9	24
THC-COOH	8	4	2	3	9	24
benzoylecgonine	4	3	1	4	9	26
oxazepam	5	2	2	1	7	13
methadone	2	2	0	4	7	20
morphine	4	2	1	4	7	25
6-MAM	2	1	1	1	3	11
buprenorphine	2	0	0	0	2	4
LSD	3	0	0	1	4	10
PCP	2	0	0	1	2	5
Total	40	19	7	24	59	162

Examples of data from individual laboratories that performed well and from laboratories showing significant errors ($P < 0.05$) of the most frequent types are illustrated. Significant analytical errors were detected in 36% of laboratories. The high incidence of significant errors was the result of a divergence in laboratory performance. The standard was set by the majority of laboratories performing with good accuracy whilst a smaller number produced errors ranging from 30 to 70% or higher. The most frequent errors were of bias though 13 bias errors occurred simultaneously with, and were the result of, slope errors.

There was no significant correlation in error frequency with analytical technique in a comparison between GCMS, LCMS, HPLC/GC and immunoassay groups (χ^2 $P > 0.05$).

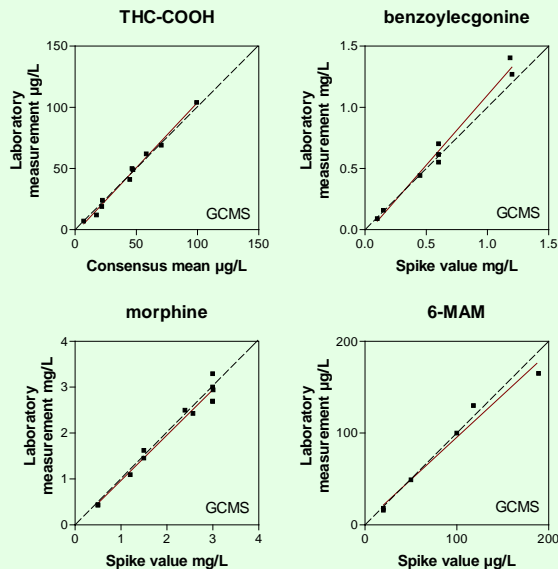
DISCUSSION

Observed inter-laboratory errors were traced to consistent and random intra-laboratory sources. In several cases, the scale of the errors was such that laboratories need to improve their technique for measurement of certain drugs of abuse in urine. Bias errors may often be reduced by improvement in external calibration. Random errors will require wide-ranging attention to laboratory procedures for their solution whilst slope errors are suggestive of problems specifically with internal standardisation.

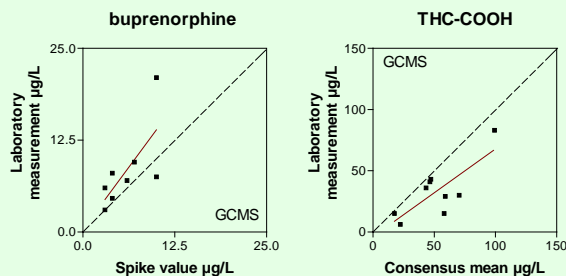
*Membership of UKNEQAS steering committee for drug assays

DG Bullock, NE Capps, S George, DW Holt, JD Ramsey, G Sweeney, BL Smith, AH Thomson, A Trewick, ID Watson, J Williams & JF Wilson.

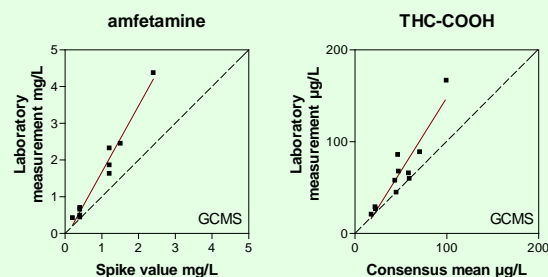
Good performers



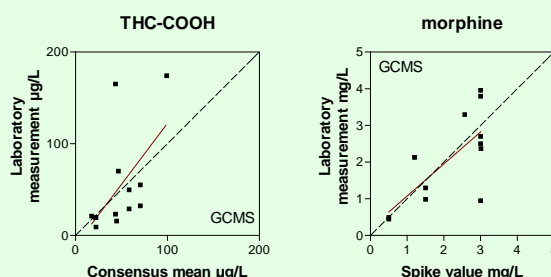
Laboratories with bias errors



Laboratories with slope + bias errors



Laboratories with random errors



Linear regression fits (solid line) to data from individual laboratories plotted against the spike or consensus mean value. Dashed lines show the expected result.