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RECENT ADVANCES IN DOPING ANALYSIS (10)

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WAADS QA Programme 2001/2002
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WAADS QA PROGRAMME 2001/2002

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INTRODUCTION

The World Association of Anti-Doping Scientists (WAADS) was formed in 2001. The constitution of the Association states that the objectives of the Association shall be to:

- Maintain excellence in the science and practice of anti-doping programs in the interest of all athletes;
  This shall include, but not be limited to, sponsorship of scientific meetings, and development of a proficiency-testing program for members to assist in promulgation of ISO and other accreditation.
- Facilitate harmonization of modern scientific methodology for effective anti-doping control;
  A proficiency-testing scheme, as noted above, will serve as an effective means of promoting consistency of laboratory performance. The Association favors the establishment of performance standards rather than the adoption of standard methods as a means of achieving harmonization of results.
- Provide reliable information concerning the scientific aspects of anti-doping programs in the public interest;
  The Association wishes to lend its expertise to the governing bodies to assist in the further development of anti-doping programs. The Association will be empowered to speak to the press about anti-doping science. It will refrain from discussing the specifics of cases.
- Foster good will and co-operation among members;
  The Association should facilitate communication including, but not limited to, intelligence information about changes to substance use among athletes; new methods to detect substance use; other information of importance to members, e.g. court (panel or tribunal judgements), management or automation advances, etc.

The WAADS Quality Assurance (proficiency testing) programme is designed to help laboratories to meet ISO 17025 requirements. All laboratories accredited by the IOC as well as some laboratories close to accreditation have joined the Association and are most willing to undertake the studies from WAADS. This paper describes the operation of the QA programme

and summaries the results of the first two studies. The QA programme is organised by the WAADS QA Committee currently chaired by RK. The aim is to have three studies per year, which simulate conditions expected in the field.

The mechanism established for selection of studies at present is:

⇒ Executive committee input

⇒ QA chair prepared proposal

⇒ Approval of executive

⇒ Preparation of protocol

⇒ Approval of protocol

⇒ QA preparation & circulation

⇒ Results collation & evaluation

⇒ Report

WAADS QA01_01

The principle of this first study was to try to determine the uniformity and accuracy of determination of 19-norandrosterone (NA) and 19-noretiocholanolone (NE) at low concentration.

The study design utilised a urine that did not contain any NA or NE to which sufficient NA-glucuronide and NE-glucuronide were added to produce expected concentrations of NA of 4 ng/mL and NE of 3 ng/mL. These samples were to be analysed by the usual in-house method. Laboratories were asked to include in their report provided the following:

- An estimate of the concentration of substances;
- Description of the confirmation extraction procedure;
- Internal standard used and its concentration;
- Description of the quantification procedure;
- Description of instrumental method;
- The wording that would be used in a laboratory report for this case if it were a real sports sample.

All laboratories provided a response in a timely fashion.

RESULTS

Table 1. Summary statistics for sample QA01_01

	NA	NE
Expected value (ng/mL)	4	3
Mean (ng/mL)	2.8	3.4
Standard Deviation (ng/mL)	0.89	0.93
Relative standard deviation (CV%)	32	28
N	24	19

Z-SCORE

A useful way to compare results between laboratories and between studies is by use of the Z-score (Figure 1). The Z-score (|Z|) is calculated via the formula: |Z| = (result - mean)/SD. This parameter allows an estimate of the closeness of a laboratory result to the consensus mean.

Generally the Z-score can be interpreted as:

$\|Z\| <= 2$	Satisfactory
$2 < \|Z\| < 3$	Questionable
$3 <= \|Z\|$	Unsatisfactory

The information was provided to the laboratories, who can thus interpret their contributions and use the information to improve the overall results.

METHODOLOGY VARIATIONS

In most aspects the sample preparation procedures used by the laboratories were similar but there were two different approaches for determining the overall results:

- Full quantification in which a calibration curve was obtained and the sample results compared to the line of best fit; or
- Comparison of 3 sets of sample to 3 sets of standard at 2 ng/mL and allowing for measurement uncertainty using:
  - the mean -3 standard deviations; or
  - student t-test with $p < 0.001$; or
  - >2 ng/mL

OVERALL RESULT

- Six laboratories gave a negative result of which 3 asked for targeting of the athlete. The highest value obtained for which a negative result was reported because of the protocol required within that laboratory was 2.7 ng/mL.
- Eighteen laboratories gave a positive finding for which the lowest value was 2.7 ng/mL.

CONCLUSION FOR STUDY QA01_01

The overall result at this very low concentration was very good. The consensus mean suggested a substantially smaller NA concentration than that of the expected value. This probably means that NA concentration is reduced under the conditions currently used to send samples worldwide and that there may be a general underestimation of the NA levels in doping samples (which would normally benefit the athlete).

WAADS QA01_02 and 03

The principle of this second study was to investigate the uniformity and accuracy of determination of ephedrines as a mixture. The study design was to provide two urine samples with different ephedrine levels and to try to use a Youden style analysis of the results to determine the systematic and random error components of the analysis. The urines provided were:

- Urine 1 with an ephedrine concentration above the IOC reporting threshold and pseudoephedrine below;
- Urine 2 with a pseudoephedrine concentration above the IOC reporting threshold and ephedrine below.

In both these samples the **total** concentration of ephedrine and pseudoephedrine in each sample was to be similar and each laboratory was to use their in-house method for the analysis.

REPORTING

The instructions for reporting of the results requested, as in the previous study, the following:

- An estimate of the concentration of substances;
- Description of the confirmation extraction procedure;
- Internal standard used and its concentration;
- Description of the quantification procedure;

- Description of the instrumental method;
- The wording that would be used in a laboratory report for this case if it were a real sports sample.

RESULTS

Table 2 - Summary statistics for sample QA01_02

	Ephedrine	Pseudoephedrine
Expected value (µg/mL)	14.03	22.22
Mean (µg/Ml	13.38	21.96
Standard Deviation (µg/mL)	1.93	2.34
Relative standard deviation (CV%)	14	11
N	25	25
Difference from expected (µg/mL)	0.65	0.26

Table 3 - Summary statistics for sample QA01_03

	Ephedrine	Pseudoephedrine
Expected value (µg/mL)	7.66	27.95
Mean (µg/mL)	6.12	26.08
Standard Deviation (µg/mL)	1.17	2.89
Relative standard deviation (CV%)	19	11
N	25	25
Difference from expected (µg/mL)	1.54	1.87

VARIATION IN METHODS

There were considerable variation in the methods and procedures used. These appear to reflect the relevant expertise within each laboratory. However the overall results showed that in general that regardless of the method used an acceptable result was obtained. Some variations noted are summarised below:

- Derivatisation (number of laboratories reporting each is shown in parenthesis)
  MSTFA/MBTFA (4); MBTFA (1); PFBA (1); TFAA (2); MSTFA (2); PFPA (2); NONE (13).
- Internal standards
  Pholedrine (2); methylephedrine (5); bemegride; diphenylamine (6); D3-ephedrine (5); etafedrine; mephentermine; methoxyphenamine; ethylephedrine; 3-phenylpropanolamine; 2-amino-1,2-diphenylethanol.
- Measurement Technique
  - GCMS (11);

- GC/NPD (10);
- HPLC/UV (3); and
- HPLC/ESI (1).

YOUDEN PLOT

This study design enabled the use of Youden analysis of the results to help ascertain sources of variation. A type of Youden plot of the results for this study is shown in Figure 1. The laboratories close to the 45 degree line have a spread caused by systematic variation while those away from this axis have a random contribution. All values were within 2 SD of the consensus value with most clustered close to the consensus value. The few laboratories (5) with Z-scores in the 2-3 range appear to have a major random contribution to the measurement, that is their values are away from the 45 degree line.

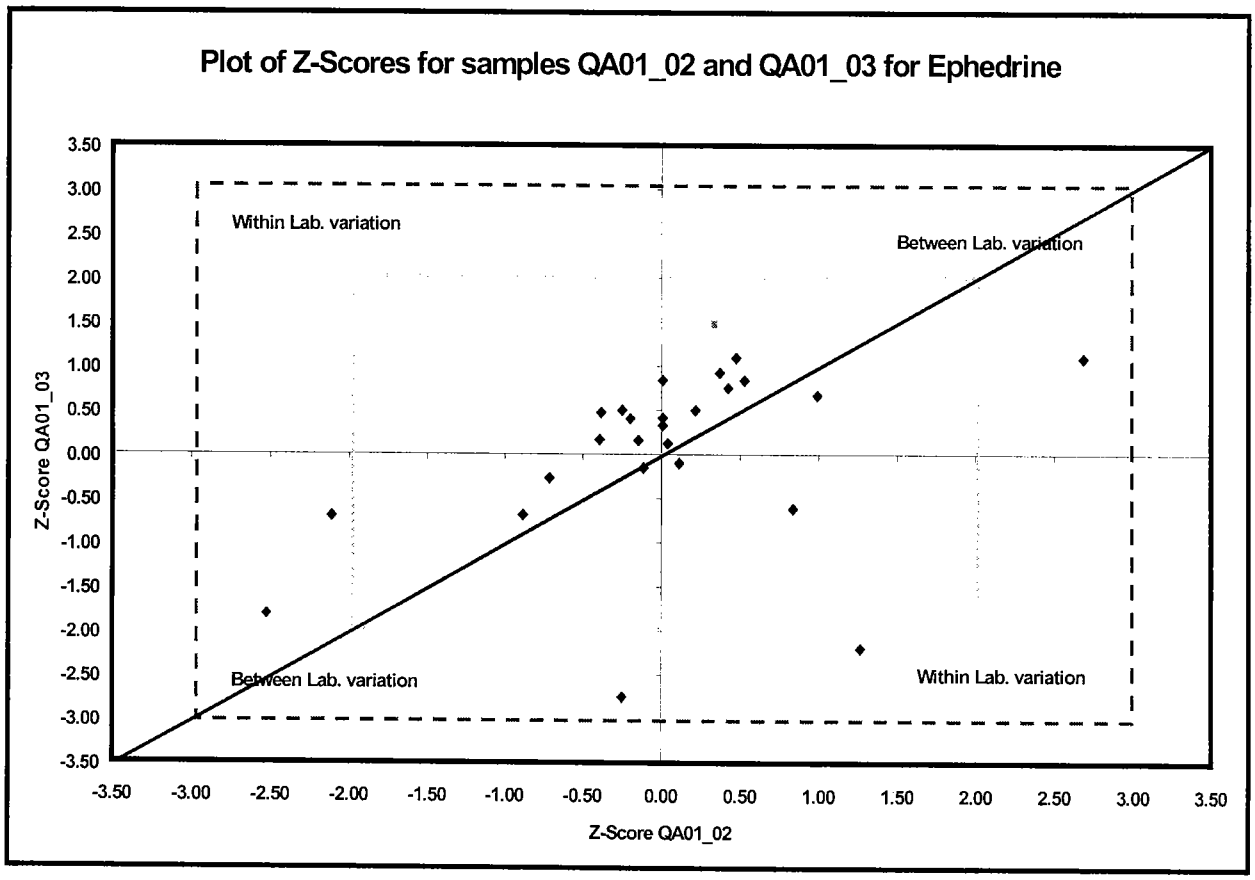

Figure 1. Plot of Z-scores for the two ephedrine samples facilitating identification of source of variation.

There appears to be considerable variation in the method used for the estimation of the error for these measurements. Most laboratories use the within experiment standard deviation as the estimate. A few report uncertainties as the cumulative variation from many measurements over a period of time within their laboratory.

REPORTING OF RESULTS

The reporting of results varies between laboratories. This is a particularly difficult aspect of the final result since it has to correctly reflect the data found. Under ISO 17025 opinion is not to be offered but the facts need to be provided in the report. Some particularly good wording is provided below:

- "No significant quantity of any substance banned by the Medical Commission of the International Olympic Committee was detected in any of the samples"
- Following procedure (xxxx) the mentioned samples have been analysed in this laboratory without any substance being detected in them either qualitatively or quantitatively which would give rise to a positive doping result.
- No prohibited compounds were detected at concentrations above the reporting limits. Remarks: Ephedrine and pseudoephedrine were detected, but taking the error estimates into consideration, the concentrations did not meet the IOC criteria for a positive sample definition (10 and 25 μg/mL, respectively).
- "Ephedrine was detected at a concentration significantly above the reporting limit (10 μg/mL). Remarks: Pseudoephedrine was also detected, but the concentration did not meet the IOC criteria for a positive sample definition (25 μg/mL)."
- "Ephedrine and pseudoephedrine are present in urine sample coded QA01_02 at high concentration, and the concentration of ephedrine significantly exceeds the maximum permissible limit set in the latest Olympic Movement Anti Doping Code."

CONCLUSIONS

The overall results are good for the first of a series of interlaboratory studies. Further studies have now been completed and, as expected, improvements in consensus have been observed as well as preparation of QA urine samples with better defined concentrations.

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