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RECENT ADVANCES
IN DOPING ANALYSIS
(11)

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WAADS QA PROGRAMME 2002

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INTRODUCTION

The World Association of Anti-Doping Scientists (WAADS) has conducted Quality Assurance (QA) studies since its formation two years ago. The studies last year (nandrolone and ephedrine/pseudoephedrine) produced reasonable results (Kazlauskas and Cowan, 2002). The studies this year showed improvements and this confirming the value of QA programmes to the laboratories and as a necessary part of a quality system. This paper describes the samples studied during 2002, the results obtained and provides some concluding comments.

Three studies were undertaken during 2002. These were nandrolone (QA2002_01), cannabis (carboxy-THC) (QA2002_05) and caffeine (QA2002_6). The participation was excellent with all laboratories providing feedback.

WAADS QA2002_01

This study was undertaken to investigate the questions raised from the study in 2001 relating to the traceability of the standards and their stability in urine samples.
The study consisted of:
- A urine representing a nandrolone (19-norandrosterone) positive sample QA2002_01
- A blank urine QA2002_2
- A standard methanolic 19-norandrosterone glucuronide solution QA2002_03
- A standard methanolic solution of 19-norandrosterone QA2002_04

Methodology

The labs were requested to perform the following testing protocol:

- Analyse the sample QA2002_01 and the blank QA2002_02
- Spike the 19-norandrosterone glucuronide QA2002_03 into the blank at a fixed specified amount
- Directly analyse a specified amount of the 19-norandrosterone solution QA2002_04 against your standard

Output

The protocol allows the following to be measured and to determine some quality aspects:

1. Concentration of 19-norandrosterone in QA2002_01
   - Checks stability of NA in urine
   - Checks reproducibility
2. Concentration of 19-norandrosterone in spike from QA2002_03
   - Checks reproducibility
3. Concentration of 19-norandrosterone in QA2002_04
   - Checks in-house standard for all laboratories

Outcome 1

The following results were found for the nandrolone positive urine (spiked at 4 ng/mL):

- The 19-norandrosterone urine QA2002_01 was stable for the period of this study which was for about 1 month;
- The reproducibility data were:

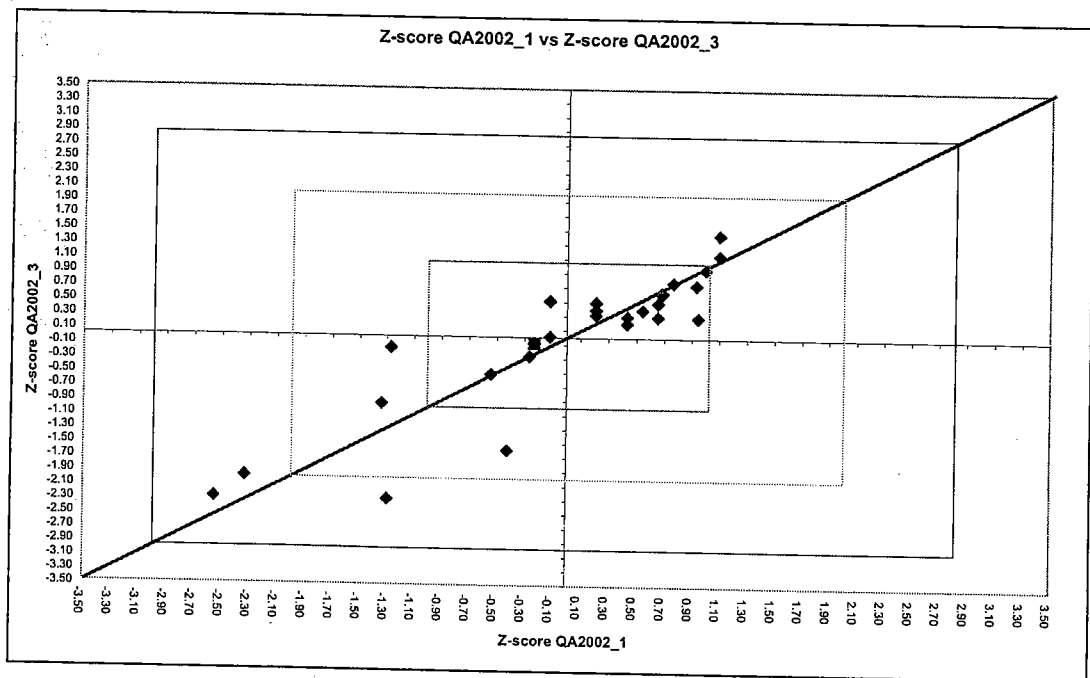| Expected value (ng/mL)	4.00
Mean (ng/mL)	4.20
Standard Deviation (ng/mL)	0.92
Relative standard deviation (CV%)	21.9 %
N	26
Difference from expected	0.20 ng/mL

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Outcome 2

The reproducibility data for blank urine spiked in each laboratory with QA2002_03 was:

Expected value (ng/mL)	4.00
Mean (ng/mL)	4.07
Standard Deviation (ng/mL)	1.1
Relative standard deviation (CV%)	26.1 %
N	26
Difference from expected	0.07 ng/mL

→Student-t test not significant (p=0.32 for QA2002_01 against QA2002_03).

The plot of the Z-score for QA2002_01 against QA2020_3 is shown below.

Outcome 3

The reproducibility data for the provided standard QA2002_04 compared to the laboratory standard:

Expected value (ng/mL)	3.98
Mean (ng/mL)	3.71
Standard Deviation ng/mL	0.93
Relative standard deviation (CV%)	25.0 %
N	25
Difference from expected	-0.27 ng/mL

→Large CV for a simple comparison

→Variations in laboratory 19-norandrosterone standards

The plot of the Z-scores for sample QA2002_03 against QA2002_04 is shown below.

Z-score of QA2002_3 Vs QA2002_4
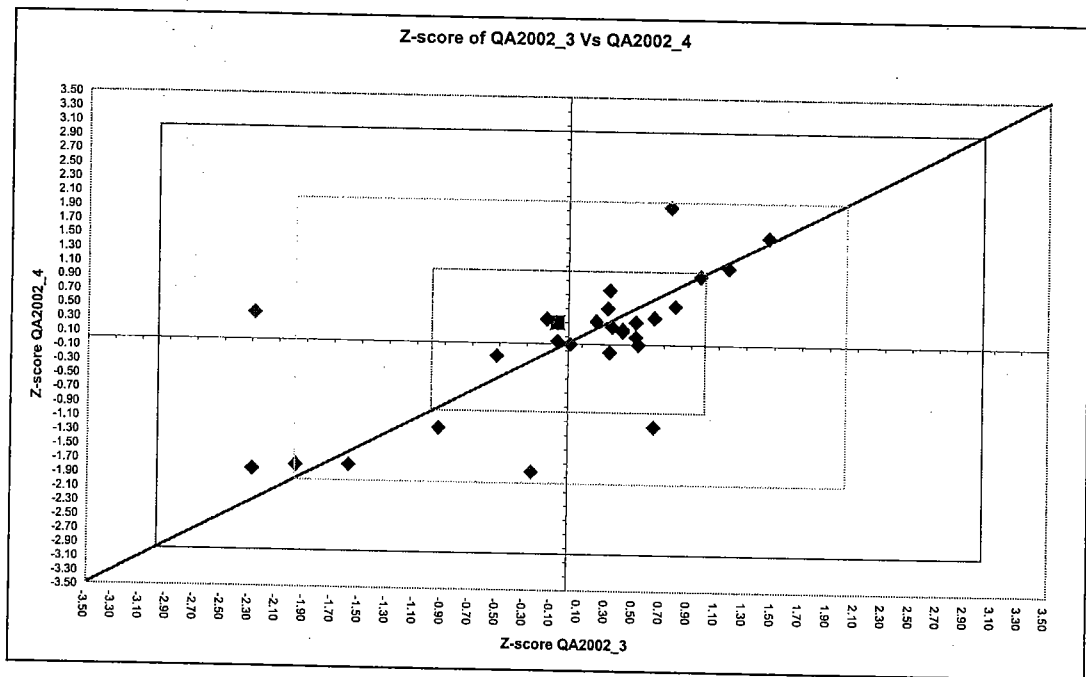
Overall Summary of results

- Need for a certified 19-norandrosterone standard solution for calibration
- Expanded inter-laboratory Uncertainty (k = 2) is in the order of 50 %
- This is a reasonable result for measurements at 4 ng/mL.
- Results will improve on further QA studies, as this is already an improvement on last year's findings.
- The blank was found to contain a small concentration of 19-norandrosterone (<0.5 ng/mL) by many of the laboratories.

This study involved the analysis of two carboxyTHC (standard purchased from Radian Laboratories) spiked samples for quantification and then the analysis repeated one month later after storage at -20 °C. The purpose of this study was to:

- Check reproducibility
- Check stability of carboxyTHC in urine.

The reproducibility data is shown in the table below, this shows that there is instability in storage of carboxyTHC urine samples;

Sample QA2002_5A	CarboxyTHC A1	CarboxyTHC A2
Expected value (ng/mL)	42	42
Mean (ng/mL)	30.4	27.4
Standard Deviation (ng/mL)	7.3	6.4
Relative standard deviation (CV%)	24 %	23 %
N	26	24
Difference from expected (ng/mL)	11.6	14.6

Sample QA2002_5	CarboxyTHC B1	CarboxyTHC B2
Expected value (ng/mL)	31	31
Mean (ng/mL)	24.8	23.1
Standard Deviation (ng/mL)	6.5	4.9
Relative standard deviation (CV%)	26 %	21 %
N	26	24
Difference from expected (ng/mL)	6.3	7.9

The plot of the Z-scores for sample A against sample B is shown below.


Z-score A1 vs B1
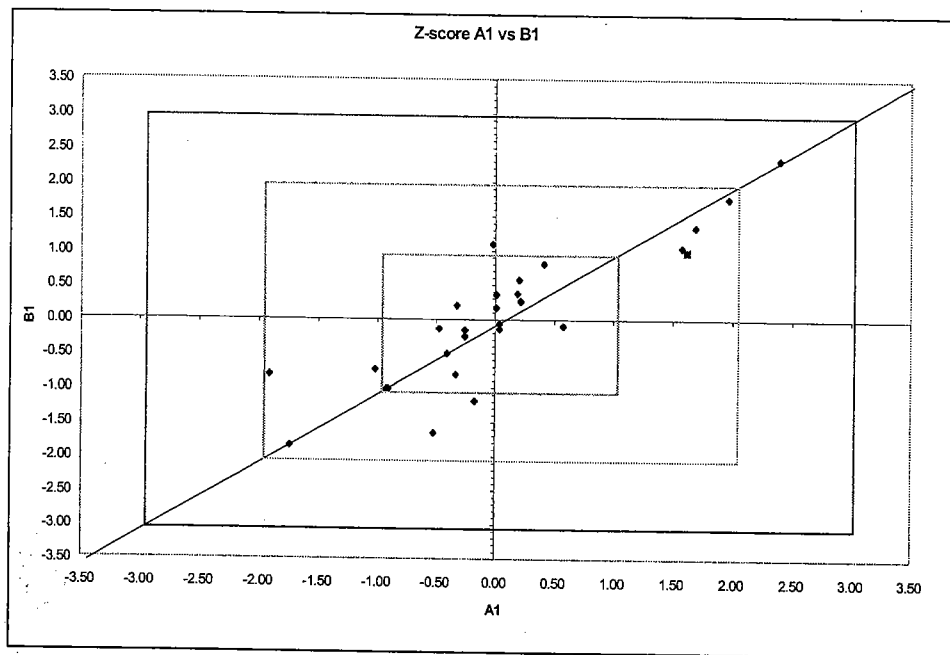
WAADS QA_06

This study involved the analysis of two Caffeine samples with caffeine concentrations for one above the set threshold and one at a "normal" level. Both samples were to be analysed and concentration estimated using the screening protocol for each laboratory and the quantification undertaken using the confirmation protocol. The rationale for this study was to anticipate the possibility that WADA may decide to redefine the status of caffeine even though caffeine is performance enhancing at low levels as well as high levels. It may be required by WADA for laboratories to obtain statistics on screen results now and later if status is changed which meant there was a need to know the variability in the screen data between labs. It was also important to obtain the inter-laboratory variability of caffeine quantification.

Caffeine quantification

Sample QA2002_6B	Caffeine screen	Caffeine screen	Caffeine quantification
Expected value (μg/mL)	2.57	12.85	12.85
Mean (μg/mL)	2.31	13.04	12.7
Standard Deviation (μg/mL)	0.8	3.1	0.6
Relative standard deviation (CV%)	34	24	5.1
N	27	27	27
Difference from expected (μg/mL)	0.26	0.19	0.15

The plot of the Z-scores for sample A against sample B is shown below.

Plot of Z-score for QA_6A vs QA_6B (screen results)
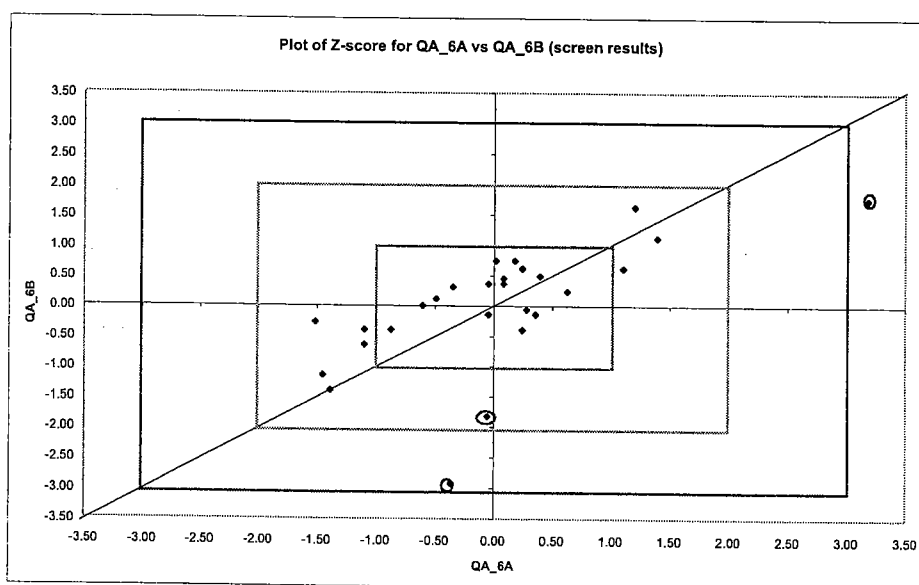
CONCLUSIONS

The results for the year 2002 showed that there was a good improvement in 19-norandrosterone analysis with Inter-laboratory U for non-standardised methods such as 19-norandrosterone and carboxy-THC about 50 % (k=2). The inter-laboratory uncertainty for standardised methods such as caffeine is about 10 % (k=2). The high uncertainty for non-standardised methods may be due in part to the variation in the quality of the standards

available but is well within the level expected for the analysis of substances at very low ng/mL levels. As expected analysis of analytes at the high concentration needed for caffeine results in low inter-laboratory uncertainty.

The result for carboxy-THC is in agreement with published data and the experience of laboratories wherein the stability of the metabolite is not good and variation (lowering) between the A sample and the B sample analysed a month apart can be expected to be different.

ACKNOWLEDGMENTS

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References

R. Kazlauskas and D. Cowan, "WAADS QA Programme 2001/2002", Recent Advances in Doping Analysis (10), Proceedings of the Manfred Donike Workshop, 20th cologne Workshop on Dope Analysis, 10th to 15th March 2002, Ed W. Schänzer et al., Sport & Buch Strauss, Köln 2002.