

SPORTS ANTI-DOPING RESEARCH FUNDING PROPOSAL

Development of New Methodology to detect Corticosteroids

R. Kazlauskas, G. J. Trout, A. Lisi and C. Goebel

AUSTRALIAN SPORTS DRUG TESTING LABORATORY
AUSTRALIAN GOVERNMENT ANALYTICAL LABORATORIES
1 Suakin St, Pymble, NSW, 2073 Australia

SPORTS ANTI-DOPING RESEARCH FUNDING PROPOSAL

Application Form and Information Requirements

Organisational Details

Legal name of organisation **Commonwealth of Australia, Department of Industry, Tourism and Resources**

Short name or trading name **Australian Government Analytical Laboratories**

Type of organisation

Non-profit organisation	Regional organisation
For profit organisation	Educational institution
Registered charity/charitable organisation	Aboriginal or Torres Strait Islander organisation
Health institution	<input checked="" type="checkbox"/> Government
Community group	Private individual

Postal address

Street name & number/PO box **1 Suakin St.**

Suburb/Town **Pymble**

City **Sydney** State/Territory **NSW** Postcode **2073**

Nominated contact for project/program

Title **Dr**

First name **Rymantas**

Last name **Kazlauskas**

Position **Director, Australian Sports Drug Testing Laboratory**

Phone **02 94490111**

Facsimile **02 94498080**

Email address **Ray.kazlauskas@agal.gov.au**

Organisation Identification

Australian Business Number (ABN) or Australian Company Number (ACN)

5 1 8 3 5 4 3 0 4 7 9 0 0 2

Is the organisation

GST registered? Yes ☒ No ☐

Incorporated? Yes ☐ No ☒

If yes, please provide the incorporation number and year of incorporation

Incorporation number

Date of incorporation

Purpose/objective/mission statement of organisation (5 lines max)

The Australian Government Analytical Laboratories (AGAL) is the Australian Government's principal agency for the provision of analytical services in chemistry, microbiology, and materials and building science

Information requirements

The following information must be provided with this application

<p>1. Details of any ethical consideration for the project.</p> <ul style="list-style-type: none">– Include a copy of National Health and Medical Research Council approved ethical committee application form, informed consent form, and documentation of the ethical approval process. <p><i>See attached papers</i></p>
<p>2. A detailed budget for the project. This should include:</p> <ul style="list-style-type: none">– A detailed cost item breakdown;– Details of where the funds will be spent; and– Details of any other capital or in-kind support secured for the project. <p><i>See attached papers</i></p>
<p>3. Consultation and/or collaboration arrangements.</p> <ul style="list-style-type: none">– Identify the International Olympic Committee accredited laboratory(ies) that you will communicate or collaborate with to ensure that the new or modified detection protocols and methodologies developed by your research can be implemented by IOC accredited laboratories. <p><i>Australian Sports Drug Testing Laboratory is the applicant. The methods used are those utilised by IOC accredited laboratories. Regular discussion with IOC laboratories occurs especially at the Cologne Workshop where progress will be reported.</i></p>
<p>4. Project summary, suitable for publication (maximum 1000 words)</p> <p><i>See attached papers</i></p>
<p>5. Project description.</p> <ul style="list-style-type: none">– This should focus on the expected outcome of the project and the selection criteria (max 5 pages). <p><i>See attached papers</i></p>
<p>6. Project timetable, including proposed milestones.</p> <p><i>See attached papers</i></p>
<p>7. Project management plan, including reporting and evaluation plans.</p> <p><i>See attached papers</i></p>
<p>8. Other enclosures.</p> <ul style="list-style-type: none">a. Curriculum vitae of principal investigator with 10 relevant, recent publicationsb. Curriculum vitae of main collaborating investigators with 5 relevant, recent publicationsc. List of literature relevant to the project (max 10 publications) <p><i>See attached papers</i></p>

DECLARATION: I declare that, to the best of my knowledge, the information provided in this application is true and complete, and that I have read, understand, and agree to comply with the Guidelines for Applicants.

Signature of CEO or equivalent office holder:

Date:

Project Title

Development of New Methodology to detect Corticosteroids

1. Ethical Considerations

For some of the studies no administration of drugs is required. Where excretion studies are needed to investigate the analysis of metabolites single dose administrations will be carried out. The Human Research Ethics Committee of Southern Cross University, which is listed with the Australian Health Ethics Committee (AHEC) as being compliant with N.H. & M.R.C. Guidelines has given approval for single dose administration of pharmaceutical and related nutritional products in general for laboratory metabolite analysis (ECN –02-68).

For the second year of the study using carbon isotope ratio mass spectrometry (CIRMS) it is proposed to use some of the urine samples which were collected with full ethical approval for use in both CIR, EPO and HGH testing ("EPO 2000 validation study: incorporating blood profiling – development of reference ranges for blood parameters in elite athletes and the EPO administration trial" which was approved by the Australian Institute of Sport Ethics Committee on 21st September 1999.). No further ethics approval will be sought or needed.

2. Budget - Request

Project budget	
Expense category	From ADPR Amount
Year 2004 Salary, scientific personnel: 20% of SPOC plus on costs 17,600 50% of PO2 plus on costs 35,500 Salary, technical personnel 30% of technician plus on costs 18,600 Consultant statistical analysis 5,000 Consumables: SPE cartridges, HPLC columns, solvents for analysis of excretion studies. 5,000 SPE cartridges, HPLC columns, solvents for analysis of 300 urine samples for natural corticosteroids. 4,000 CIRMS consumables including SPE cartridges, columns and CIR furnaces for initial method dev. 12,000 5,000 Other direct costs and overheads (including support infrastructure, equipment maintenance and repairs, services etc) 61,700	
Total budget year 1	164,400
Possible Year 2005 Salary, scientific personnel: 2% of PRS plus on costs 2,200 5% of SPOA plus on costs 5,500 20% of PO2 plus on costs 14,200 Salary, technical personnel 20% of technician plus on costs 12,400 Consumables: CIRMS consumables including SPE cartridges, columns and CIR furnaces for 300 samples. 15,000 Travel to Cologne Workshop to present and discuss findings with other WADA laboratories 3,200 Other direct costs and overheads (including support infrastructure, equipment maintenance and repairs, services etc) 29,000	
Indicative total budget year 2	81,500

BUDGET JUSTIFICATION

The details of the budget are set out above. It is proposed that AGAL develop the initial method sufficient to meet ASDTL's immediate commercial needs and to meet the WADA April 2004 deadline for compliance. The ADRP funds will be used for further development of the method to more fully understand the analysis of corticosteroids within the antidoping context which will include study of metabolites and the naturally occurring (endogenous) corticosteroids. Should external funding not be available, we believe this further research will still need to be carried out to ensure the future maintenance of ASDTL's accreditation status. The expenses incurred will then be recovered through increased costs of testing to clients.

The methodology finally developed will be applied to the analysis of some 300 athlete urine samples with a view to determining criteria for detecting the exogenous intake of natural corticosteroids. A further study will be undertaken to investigate the feasibility of using carbon isotope ratio analysis of natural corticosteroids to distinguish endogenous from exogenous material.

The funds shown in the second year will only be needed to validate the CIRMS method should it show potential. By analysing a subset of the EPO2000 urine profiling samples it should be possible to set criteria to confirm doping with natural corticosteroids.

4. Project Summary

The aims of this project are to develop and validate methods for the analysis of corticosteroids which will be adequate to meet the current and future requirements of the World Anti-Doping Agency (WADA). There is an urgent need to develop such methods because of the sudden inclusion of glucocorticosteroids in the WADA 2004 prohibited list. WADA has mandated that corticosteroids will be part of the analytical suite of substances routinely tested by the laboratory during competition testing. It will include corticosteroids in its routine Proficiency Testing programme which will have implications upon the laboratory accreditation status.

It is proposed to develop methodology using LC/MS/MS which is capable of detecting the fourteen synthetic corticosteroids currently specified by WADA. This aspect of the project has already begun. This will only allow the detection of the parent substance to the WADA specification but will not represent the best procedure to identify corticosteroid administration.

The main needs for the methodology to be practical will be to investigate the metabolites of synthetic corticosteroids with a view to including them in the screening method and thus enhance ASDTL's capability to detect corticosteroid abuse. Until that has been done there will not be an understanding of the biological modification and excretion of these substances

nor an understanding of the difference between allowed routes of administration and that procedures that are banned. There is little if any literature available that is useful to the laboratory for antidoping purposes. WADA has already indicated that the initial laboratory requirement to detect fourteen synthetic corticosteroids is only the first step in the detection of corticosteroid abuse. In the near future WADA will require that laboratories be able to detect a much wider range of corticosteroids and their metabolites including the natural corticosteroids.

Once an expanded method has been developed and validated it is proposed to use the method to measure the natural corticosteroids and their metabolites and precursors in approximately 300 urine samples from elite athletes. The aim is to determine normal levels and attempt to find means such as ratios of corticosteroid levels to precursor or related compounds which are indicative of corticosteroid abuse. Extensive statistical analysis will need to be carried out with the aim of developing robust models for the detection of corticosteroid abuse.

The use of CIRMS to detect the exogenous intake of natural corticosteroids has been proposed. The final stage of this project will be to develop and apply such methodology to a small number of samples to investigate its potential for confirming the abuse of natural corticosteroids. Should the CIRMS method show its expected potential then validation of the method could begin in 2005 should funding be available. It is anticipated that because of the large amount of data that has been generated relating to the use of CIRMS for natural anabolic steroid detection that a relatively small sample of 300 from selected countries will be sufficient to establish reliable criteria for confirming doping with natural corticosteroids. Once validated the CIRMS method would only be used in doping control to confirm suspect samples detected in the corticosteroid screen.

5. Project Description

Development of New Methodology to detect Corticosteroids

Australian Sports Drug Testing Laboratory

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Researchers: R. Kazlauskas, G. J. Trout, A. Lisi and C. Goebel

INTRODUCTION

Prior to 2004 corticosteroids were on the IOC restricted list and were only routinely tested for by one IOC laboratory. The main reason for this was the difficulty of determining route of administration for compounds which were allowed under some circumstances.

Corticosteroids have never been included in any IOC or WADA accreditation or proficiency testing samples. However the new 2004 WADA list (WADA 2004) now has glucocorticosteroids listed as a class in the substances prohibited in-competition. With the development of the Therapeutic Use Exemption guide a mechanism for determining allowed use now exists. This means that all WADA accredited laboratories will need to have methods in place to detect corticosteroids.

The corticosteroids are prohibited when administered orally, rectally, or by intravenous or intramuscular administration. All other administration routes require a medical notification in accordance with a therapeutic use exemption. As there is no means by which the laboratories can confirm the route of administration this means that ASDTL will need to test all in-competition samples for corticosteroids and report the results to the client who can determine whether a therapeutic use exemption exists. Unfortunately the corticosteroids cannot be readily detected using any of the existing drug screens and so a whole new screening program will need to be developed.

At present the laboratories have been advised by WADA that only fourteen specified synthetic corticosteroids must be tested for. However WADA has advised that the list will be extended and natural corticosteroids are to be included. This will raise the extremely difficult problem of distinguishing endogenous and exogenous sources such as currently occurs with naturally occurring anabolic steroids (Aguilera et al 2000). Some work has been published on distinguishing endogenous and exogenous corticosteroids using CIRMS (Bourgogne et al 2000).

There is thus an urgent need for the development of a rapid cost effective screen for the 14 specified corticosteroids and a longer term need to expand this screen to include metabolites, other synthetic corticosteroids, and endogenous compounds. The latter will require population studies as well as the application of confirmatory techniques such as CIRMS.

AIMS

The main aims of the project are to:

- A. Develop a cost effective screen for the detection of fourteen specified synthetic corticosteroids in urine. This work has already begun with funding from AGAL.
- B. Extend this screen to include metabolites of synthetic corticosteroids as well as other corticosteroids such as the naturally occurring corticosteroids and their metabolites.
- C. Measure the endogenous corticosteroid concentrations in a selection of urine samples collected from a large cohort of elite athletes to determine whether concentrations or ratios can be used to distinguish endogenous from exogenous origin.
- D. Investigate the application of CIRMS for confirming the presence of synthetic endogenous corticosteroids.
- E. Validate a CIRMS method for confirming the abuse of natural corticosteroids.

SIGNIFICANCE

The sudden need to detect corticosteroids has placed a great strain on the resources of ASDTL. Without such a screen in place ASDTL will lose its WADA accreditation and be unable to analyse samples for clients such as ASDA and the NZSDA. As there was no indication prior to September 2003 that corticosteroids were to be added to the WADA List neither ASDTL, ASDA nor NZSDA have budgeted for the cost of corticosteroid analysis. In fact the technical advice that was circulated by WADA prior to the adoption of the List was that corticosteroids should be dropped from the List completely. Thus there now exists an urgent need to develop cost effective screening and confirmation methodology for a series of synthetic corticosteroids. The intention of WADA to include more corticosteroids including endogenous compounds requires that methods be developed to distinguish naturally occurring levels from those arising from exogenous application. By spending research funds now it is hoped that the methods developed will be the most cost effective available and thus minimise the additional ongoing cost to sport of introducing a completely new class of compounds in the prohibited list.

BACKGROUND

Corticosteroids are a class of steroids which are not amenable to the normal methods of analysis used for anabolic steroids. Whilst they can be analysed by GC/MS after extraction and derivitisation (Yap et al 1992, Courtheyn et al 1994), a completely different GC/MS procedure would be required for their detection. Whilst some natural and synthetic corticosteroids can be detected by such an extension of the anabolic steroid screen it does not work for all the required corticosteroids at the required proficiency level (30 ng/mL) and usually gives rise to multiple derivatives which occur in amounts which vary with each analysis. Most recent papers on the detection of corticosteroids have concentrated on the use of LC/MS and LC/MS/MS (Fluri et al 2001, Antignac et al 2002). This methodology requires less preparation time by avoiding the need for complex derivitisation procedures but does require access to state of the art instrumentation. ASDTL has LC/MS/MS capability but the requirement for a completely new drug screen will stretch its physical resources. Despite this it is felt that the cost of developing and implementing an effective screen for corticosteroids will be significantly lower than the cost of developing and implementing a new GC/MS screen.

There is no currently published screening method for the detection of all the fourteen corticosteroids specified by WADA. It is proposed to take a recently published LC/MS/MS method for seven synthetic corticosteroids (Deventer and Delbeke 2003) which uses labour intensive solvent extraction and modify as needed it to include all the required compounds using automated solid phase extraction. Without such automation the cost per analysis would

be considerably higher. Most published methods for the detection of corticosteroids have concentrated on the detection of the parent compounds. Whilst it is relatively straightforward to develop such methodology for the parent compounds it is not so simple for those compounds which undergo extensive metabolism. For most of the corticosteroids the metabolites are not readily available as pure compounds and so excretion studies will have to be used. For some corticosteroids it will be very difficult to detect and characterise the metabolites because they are given in very low doses by such means as nasal inhalation. Single doses will be given to volunteers in conjunction with Southern Cross University and the current ethics approval for administration of therapeutic drugs.

Once this methodology has been validated it is proposed to further develop the method to include other corticosteroids including the endogenous compounds cortisone and hydrocortisone and their metabolites tetrahydrocortisol and tetrahydrocortisone. If it not possible to include all the corticosteroids in one method a separate method will be developed for the natural corticosteroids and related compounds in the cholesterol and corticosteroid metabolic pathways. It is hoped that this will not be necessary as it will greatly increase the cost of analysis for the overall urine screening.

Application of this method to a wide range of samples will help establish what levels are typical and whether the application of concentrations or ratios of concentrations may be useful in detecting doping with these compounds. The analysis of this data will require the services of a consultant statistician and it is proposed that this be done by Dr Ken Sharpe of the University of Melbourne who played a major role in the development of models for the detection of EPO doping (Sharpe et al 2002).

The method with the greatest potential for differentiating between endogenous and exogenous corticosteroids is carbon isotope ratio mass spectrometry (CIRMS). It is proposed to determine the applicability of the method published by the Paris IOC laboratory (Bourgogne et al 2000) to our samples. Assuming this methodology can be successfully developed and validated then a large number of urine samples will need to have their corticosteroid isotope ratios measured before such a method could be applied for doping control purposes. It is proposed to use a selection of the urines collected in the EPO2000 profiling study which were collected from an ethnically and geographically diverse group of athletes.

RESEARCH PLAN

Development of and LC/MS/MS method for the detection of fourteen specified synthetic corticosteroids has already begun. When this method has been developed and validated it is proposed to put it into operational use. It is then proposed to carry out a number of excretion studies on volunteers to investigate the detection of metabolites. Often with anabolic steroids the metabolites are excreted at much higher concentration to the parent drug and these metabolites must be used for detecting use. Once the metabolites have been identified and

characterised it is proposed to expand the original method to include these major metabolites if they assist in better detection of corticosteroid administration. At the same time it is proposed to develop a method, which may be an extension of the original method or a new method, to measure the concentrations of the natural corticosteroids, their metabolites, precursors, and other related compounds. Measurements of concentrations will be made on 300 urine samples with a view to determining normal ranges. These results will be compared to excretion studies from subjects who have been given synthetic and natural corticosteroids. A statistical analysis of the results will be carried out with the aim of developing models which can be used to detect corticosteroid abuse particularly of natural compounds.

A separate and initially smaller part of the project will be to investigate the use of CIRMS for confirming the abuse of natural corticosteroids. This will require a separate cleanup procedure to meet the special needs of compound purity imposed by the CIRMS methodology. The CIRMS protocols needed to measure the carbon isotopic ratios of natural corticosteroids and their metabolites will be developed. A small number of urine samples will be analysed by this technique to assess whether it has the potential to be an effective doping control procedure. Should this be shown, then a similar though smaller validation to that which has been carried out for the application of CIRMS to the detection of anabolic steroids will be required, because of the known confounding effects of diet. This would be carried out in the second year of the project.

ETHICS Approvals.

ASDTL through Southern Cross University has obtained ethics approval for administration of standard doses of therapeutic drugs given as a single dose only to a volunteer. These studies are often carried out by Dr Robert Weatherby from Southern Cross University using student volunteers or at ASDTL using volunteers from staff members. A list of proposed studies will be provided to the Ethics Committee, for information, prior to the work commencing.

Project Timeline

Activities

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
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2004

Corticosteroids by LC/MS/MS

Develop and validate screening method for synthetics

Perform excretion studies

Develop and validate method for naturals
and related compounds

Identify major metabolites and include in method for synthetics

Analyse 300 urine samples for natural corticosteroids and related compounds

Statistical evaluation of data

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			■	■	■														
			■	■															
				■	■	■													
							■	■	■										
								■	■	■	■								
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CIR measurements

Develop methodology for extraction and purification

Develop method for measuring isotope ratios

Analyse selection of samples

Evaluation of data

Report to ADRP

[illegible]

2005

CIR validation

CIR method validation

Extraction of 300 samples for CIRMS analysis

CIRMS analysis of 300 samples

Prepare paper for Cologne Workshop

Evaluation of data

Report to ADRP

Equipment and resources available for use in this project

Micromass Quattro Micro MS/MS with Waters Alliance 2795 HPLC.

Finnigan MAT Delta Plus Isotope Ratio Mass Spectrometer

Gilson ASPEC XL4 Automated SPE System.

References

- World Anti-Doping Agency (2004). The 2004 prohibited list. International standard. <http://www.wada-ama.org/en/t3.asp?p=30639&pp=29645>. Accessed 14/1/2004.
- Aguilera R., Chapman T.E., Catlin D.H., (2000). A rapid screening assay for measuring urinary androsterone and etiocholanolone delta 13C values by gas chromatography/combustion/isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry*, 14:2294-2299.
- Bourgogne E., Herron V., Mathurin J-C., Becchi M., de Ceaurriz J. (2000) Detection of exogenous intake of natural corticosteroids by gas chromatography/combustion/isotope ratio mass spectrometry: application to misuse in sport. *Rapid Communications in Mass Spectrometry*, 14:2343-2347.
- Yap B., Johnston G. A., Kazlauskas R. (1992) Routine screening and quantitation of urinary corticosteroids using bench-top gas chromatography-mass-selective detection. *J Chromatogr.* 573(2):183-90.
- Courtheyn D., Vercammen J., De Brabander H., Vandereyt I., Batjoens P., Vanoothuyze K., Van Peteghem C. (1994). Determination of dexamethasone in urine and faeces of treated cattle with negative chemical ionization-mass spectrometry. *Analyst*, 119:2557-2564.
- Fluri K., Rivier L., Dienes-Nagy A, You C., Maitre A., Schweizer C., Saugy M., Mangin P. (2001). Method for confirmation of synthetic corticosteroids in doping urine samples by liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. A*, 926:87-95.
- Antignac J-P., Le Bizec B., Monteau F., Andre F. (2002). Study of natural and artificial corticosteroid phase II metabolites in bovine urine using HPLC-MS/MS. *Steroids*, 67:873-882.
- Deventer K., Delbeke F.T., (2003). Validation of a screening method for corticosteroids in doping analysis by liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, 17:2107-2114.
- Sharpe K., Hopkins W., Emslie K. R., Howe, C., Trout, G. J., Kazlauskas, R., Ashenden, M. J., Gore C. J., Parisotto, R., Hahn, A. G. (2002). Development of reference ranges in elite athletes for markers of altered erythropoiesis. *Haematologica*, 87: 1248-1257.

Curriculum Vitae of principal researchers.

<p>Curriculum Vitae – R. Kazlauskas Director Australian Sports Drug Testing Laboratory Australian Government Analytical Laboratory</p>

Family name Kazlauskas

First name Rymantas

Title Dr

Department/school/other

Organisation Australian Government Analytical Laboratories

Postal address 1 Suakin St, Pymble, NSW, 2073, Australia.

QUALIFICATIONS:

B.Sc. 1st Class Honours, University of Sydney. 1968.

Ph.D. University of Sydney, 1972.

Post Doctoral work with Professor A.R. Battersby, University Chemical Laboratory,

EMPLOYMENT HISTORY:

1988-present Director, Australian Sports Drug Testing Laboratories, Pymble

1986-1988 Research and Development, Australian Government Analytical Laboratories, Pymble.

1982-1986 Senior Research Officer, Department of Pharmacology, University of Sydney.

1981-1982 Visiting Fellow, Research School of Chemistry, A.N.U.

1973-1981 Senior Scientist, Roche Research Institute of Marine Pharmacology

Has been involved in many aspects of research in analytical chemistry since 1971, with a concentration on aspects related to doping in sport since 1988. During this period has published more than 70 papers in refereed journals.

As Director of the only IOC accredited laboratory in the Australasian area since the facility was established at AGAL in 1990 has had responsibility for ensuring that methods were introduced and developed to ensure compliance with the ideals and needs of the IOC anti-doping code. The laboratory was able to obtain IOC accreditation very quickly because of the expertise held within the laboratory. ASDTL are considered the Australian experts, as evidenced by the fact that ASDTL was responsible for analysis of all samples for the 2000 Sydney Olympics.

To maintain this pre-eminent position ASDTL needs to be actively engaged in research into new methodologies both to improve tests for existing drugs and to combat new drugs as they appear. During the past three years has been a contributor to grants totalling in excess of \$3 million from the IOC and the Australian Government "Backing Australia's Sporting Ability" initiatives. The World Anti-doping Agency has also agreed to provide funding for a number of projects within ASDTL and for partnerships with other expert groups.

Awards

2000 Australian Sports Medal

2001 Australia Day Achievement Medallion (joint)

Adjunct Professorship – Southern Cross University.

Recent publications

Trout G. J. and Kazlauskas R. (2004) Sports drug testing – an analyst's perspective. Chemical Society Reviews. 33:1-13.

Goebel C., Trout G. J., Kazlauskas R. (2004) Rapid screening method for diuretics in doping control using automated solid phase extraction and liquid chromatography-electrospray mass spectrometry. *Analytica Chimica Acta* 502:65-74.

Kazlauskas, R. (2002), Analysing the Olympic Games: A case study within the Anti-Doping Programme: An Overview. *Clin. Biochemist Reviews*, 2002; 23(ii): 35

Kazlauskas, R., Howe, C. and Trout, G. (2002), Strategies for rhEPO detection in sport. *Clin. J. Sports Med.* 12(4):2002;229-235.

Gore C. J., Parisotto R., Ashenden M. J., Stray-Gundersen J., Sharpe K., Hopkins W., Emslie K., Howe C., Trout G. J., Kazlauskas R., Hahn A. G. (2003). Second generation blood tests to detect erythropoietin abuse in athletes. *Haematologica* 88:333-344.

Trout, G., Kazlauskas R. and Westwood, S. (2001). The Role of Reference Standards in the Sydney 2000 Olympic Games Drug Testing Program. *CITAC Newsletter*.

Parisotto, R., Wu, M., Ashinton, M.J., Emslie, K.R., Gore, C.J., Howe, C., Kazlauskas, R., Sharpe, K., Trout, G.J., Xie, M. and Hahn, A.G. (2001). Detection of recombinant human erythropoietin abuse in athletes utilising markers of altered erythropoiesis. *Haematologica* 86: 128-137.

Kazlauskas, R. and Trout, G. (2000). Drugs in sports: analytical trends. *Ther. Drug Monit.* 22:103-109.

Corrigan, B. and Kazlauskas, R. (2000). Drug testing at the Sydney Olympics. *Med. J. Austral.* 173:312-313.

Numerous presentations published in the proceedings from the Cologne Workshop, Recent Advances in Doping Analysis, W. Schanzer et al (ed.), Sport und Buch Strauss.

Graham John Trout

Born 6th March 1944.

Tertiary Education:

University of Sydney:

B.Sc. 1965

M.Sc. 1967 "Radiolytic and Non-Radiolytic Decomposition of Methanol"

Ph.D. 1972 "Triboluminescence and Associated Decomposition of Solid Methanol"

Grad. Dip. Ed. 1980

Employment:

1. From 1966 to 1971 I was a full-time Teaching Fellow at the University of Sydney in the Pharmacy Department.
2. From early 1972 to late 1974 I was a Research Scientist in the Central Research Department of Unilever Australia Pty Ltd.
3. From late 1974 to early 1978 I was a Senior Research Chemist and Deputy Research Manager of the Research and Development Division of Australian Newsprint Mills.
4. From 1978 to early 1988 I was a Lecturer of Chemical Instrumentation in the School of Applied Science in the NSW Department of TAFE.
5. From 1988 to 1996 I was the officer in charge of the Gas Chromatography / Mass Spectrometry (GC/MS) Section at the Australian Government Analytical Laboratories (AGAL) at Pymble.. I was also the second in charge of the team developing methods for drug testing in athletes. This work mainly involved the development of GC/MS methods for such testing
6. Since early 1996 I have been the Deputy Director of ASDTL with the primary responsibility of developing new methods including high resolution mass spectrometry (HRMS). This included the management of the \$3,000,000 Olympics research program whose aim was to develop new testing techniques for the Sydney 2000 Olympics. Problems under investigation included the detection of low level steroids by instrumental techniques such as HRMS, the detection of administered endogenous steroids by isotope ratio mass spectrometry, and the detection of peptide hormone administration. Our efforts in the peptide hormone field concentrated on the detection of EPO abuse with some effort on measuring growth hormone isoforms in collaboration with the Garvan Institute. In late 1999 the International Olympic Committee awarded a competitive research grant of US \$1,000,000 to an international consortium led by the ASDTL and the AIS for the development and validation of a test for recombinant EPO. Matching funds were provided by the Australian Government. As a result of these efforts a test was developed and approved for use at the Sydney 2000 Olympic Games. On-going research includes the improved detection of EPO, the detection of growth hormone and the detection of haemoglobin based oxygen carriers

Awards

1990 Department of Administrative Services Award for Excellence (joint)

1991 Australia Day Achievement Medallion

1995 Department of Administrative Services Award for Excellence

2000 Australian Sports Medal

2001 Australia Day Achievement Medallion (joint)

Publications

Co-author of over 25 publications in peer reviewed journals or books, plus numerous conference presentations.

Journal Articles:

Trout G. J. and Kazlauskas R. (2004) Sports drug testing – an analyst's perspective. Chemical Society Reviews. 33:1-13.

Goebel C., Trout G. J., Kazlauskas R. (2004) Rapid screening method for diuretics in doping control using automated solid phase extraction and liquid chromatography-electrospray mass spectrometry. *Analytica Chimica Acta* 502:65-74.

Alma C., Trout G., Woodland N., Kazlauskas R. (2002). The detection of haemoglobin based oxygen carriers., in *Recent Advances in Doping Analysis* (10), W. Schanzer et al (ed.), Sport und Buch Strauss, Koln, 2002, 169-178.

Keung K.C., Howe C., Gui L.Y., Trout G., Veldhuis J.D., Ho K.K. (2002). Physiological and pharmacological regulation of 20-kDA growth hormone. *Am. J. Physiol. Endocrinol. Metab.*, 283: E838-43.

Trout G.J., Emslie K.R., Howe C., Kazlauskas R., Lasne F. (2002). An overview of testing for EPO at the Sydney 2000 Olympic Games and beyond. in *Recent Advances in Doping Analysis* (9), W. Schanzer et al (ed.), Sport und Buch Strauss, Koln, 191-200.