SPORTS ANTI-DOPING RESEARCH FUNDING PROPOSAL

Statistical Population Studies to Support New Analytical Methodologies using the EPO2000 Project Urine Samples.

R. Kazlauskas, G. J. Trout, C. Howe and J. Rogerson

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

SPORTS ANTI-DOPING RESEARCH FUNDING PROPOSAL

Application Form and Information Requirements

Organisational Details

Legal name of organisation	**Australian Government Analytical Laboratories**
Short name or trading name	**Australian Sports Drug Testing Laboratory**

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	X	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 ASDTL
Phone	02 94490111
Facsimile	02 94498080
Email address	Ray.kazlauskas@agal.gov.au

Organisation Identification

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

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

1. Details of any ethical consideration for the project. – **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.** ***See attached papers***
2. A detailed budget for the project. This should include: – **A detailed cost item breakdown;** – **Detailes of where the funds will be spent; and** – **Details of any other capital or in-kind support secured for the project.** ***See attached papers***
3. Consultation and/or collaboration arrangements. – **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.** ***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.***
4. Project summary, suitable for publication (maximum 1000 words) ***See attached papers***
5. Project description. – **This should focus on the expected outcome of the project and the selection criteria (max 5 pages).** ***See attached papers***
6. Project timetable, including proposed milestones. ***See attached papers***
7. Project management plan, including reporting and evaluation plans. ***See attached papers***
8. Other enclosures. **a. Curriculum vitae of principal investigator with 10 relevant, recent publications** **b. Curriculum vitae of main collaborating investigators with 5 relevant, recent publications** **c. List of literature relevant to the project (max 10 publications)** ***See attached papers***

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

Statistical Population Studies to Support New Analytical Methodologies using the EPO2000 Project Urine Samples.

1. Ethical Considerations

The samples to be used for this study have been 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 specified per calendar year		
Expense category	**From ADRP Amount**	**From AGAL Amount**
Year 1: 2003 **Salary, scientific personnel:** 10% of SPOA plus on costs 50% of SPOC plus on costs for EPO 50% of PO2 plus on costs for CIR **Salary, technical personnel** 100% of technician plus on costs for EPO 30% of technician plus on costs for CIR **Consumables:** EPO – approximately 60 gels at $1800 per gel CIR – 1100 SPE extractions with GC/MS consumables including columns and CIR furnaces. **Overheads** (indirect costs including support infrastructure, equipment maintenance and repairs, services etc)	 **44,000** **36,000** **48,000** **13,000** **90,000** **40,000** **30,500**	 **10,000** **20,000** **10,000** **30,500**
Total budget year 1:	**301,500**	**70,500**
Year 2: 2004 **Salary, scientific personnel** 5% of SPOA plus on costs. 25% of SPOC plus on costs for EPO 50% of PO2 plus on costs for CIR **Salary, technical personnel** 70% of technician plus on costs for CIR **Consumables** CIR – 200 SPE extractions and 1000 diol extractions with GC/MS consumables including columns and CIR furnaces. **Overhead** (indirect costs including support infrastructure, equipment maintenance and repairs and services etc)	 **23,000** **38,000** **31,000** **50,000** **17,500**	 **5,000** **10,000** **17,500**
Total budget year 2:	**159,500**	**32,500**
Total budget all years	**461,000**	**103,000**

BUDGET JUSTIFICATION

The details of each year's budget is set out above. In 2003 it is proposed that the ADRP funds be used to carry out the analysis of some 600 urine samples for their urinary EPO isoforms. Due to the high cost of consumables and the complexity of the test this will require a scientist for 50% of their time, a technician full time, and approximately 70% of the running expenses. The remainder of the funds will be used to carry out the carbon isotope ratio analysis of androsterone and etiocholanolone in approximately 1100 urine samples. This will require a scientist for 50% of their time and a technician for 30% of their time.

In 2004 it is proposed that the CIRMS analysis of 1000 urine samples for diols and 200 samples for keto steroids be carried out requiring a scientist for 50% of their time and a technician for 70% of their time. The diol extraction procedure is much more complex requiring significantly more time per sample and more consumables. It is anticipated that laboratory work associated with the EPO aspect of the project would be completed very early in 2004 and the CIR aspect completed late in 2004. Final results and publications would be prepared for the EPO work in early 2004. The ketosteroid CIR results would be evaluated and prepared for publication in early 2004 and the diol results prepared for publication in late 2004. ASDTL is proposing to fund half the overheads and supply a significant proportion of the consumables.

4. Project Summary

The aims of this project are twofold –

1. to determine the variability in the isotope ratio of some selected endogenous steroid metaboliites and evaluate whether the delta ^{13}C values are significantly affected by factors such as gender, ethnicity and geographical location, and

2. to determine the variability of the natural isoform pattern of urinary EPO and evaluate whether the patterns are significantly affected by factors such as gender, ethnicity and altitude.

Both objectives will be achieved by analysing urine samples already collected from a large cohort of ethnically and geographically diverse elite athletes. When the blood test for the indirect detection of recombinant EPO was being developed (Parisotto et al 2001) it was necessary to collect blood samples from a wide range of elite athletes around the world to establish the normal ranges for the parameters being measured. This activity formed a major part of the EPO2000 project, which was jointly funded by the International Olympic Committee and the Australian Government. At the same time as the blood samples were collected the opportunity was also taken to collect urine samples which could be used for the development of future tests. Ethics approval was obtained from each subject to permit the samples to be used in research on the detection of doping with EPO and endogenous steroids.

In the last two years the application of two new urine based tests has been extended into a wider range of IOC laboratories. The first is the use of carbon isotope ratio mass spectrometry (CIRMS) for the confirmation of doping with synthetic endogeous steroids and the second is the use of gel electrophoresis to confirm the presence of human recombinant erythropoietin (EPO). The methods are quite dissimilar in the techniques used and the analytes detected but they have one major thing in common. Both rely on detecting the use of a banned drug, which is naturally occurring, by comparing a measured parameter or parameters in the suspect sample with the values found in the normal population.

The detection of endogenous steroid abuse using CIRMS relies on detecting differences in the properties of steroid metabolites (Aguilera et al 2000). This difference is the ratio of carbon 13 to carbon 12 and is expressed as a delta ^{13}C value. The absolute ^{13}C delta values are measured and may be compared to the values found for other endogenous compounds in determining whether the source of the metabolite is exogenous or endogenous. It is known that the carbon isotopic composition affected by diet (Morrison et al 2000) and hence it is desirable to establish the range of delta values that exist in a statistically significant number of elite athletes from around the world.

The urine test developed by LNDD uses isoelectric focussing and a patented double blotting technique (Lasne 2001) to separate the isoforms of EPO into a series of bands. Data collected so far indicates that normal urinary EPO has isoforms which are more acidic than those found in human recombinant EPO although there is some overlap (Lasne and De Ceaurriz 2000). A positive is declared if the percentage of basic isoforms (recombinant EPO) is greater than 80%. This value was statistically determined on the basis of the range of values found in a relatively small number (a few hundred) of normal subjects. There should in fact be a survey of a statistically significant number of elite athletes from around the world to support the premise that the range of urinary EPO isoform distributions is outside the values found for recombinant human EPO.

6. Project Description

Statistical Population Studies to Support New Analytical Methodologies using the EPO2000 Project Urine Samples.

Australian Sports Drug Testing Laboratory
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Researchers: R. Kazlauskas, G. J. Trout, C. Howe and J. Rogerson

INTRODUCTION

When the blood test for the indirect detection of recombinant EPO was being developed (Parisotto et al 2001) it was necessary to collect blood samples from a wide range of elite athletes around the world to establish the normal ranges for the parameters being measured. This activity formed a major part of the EPO2000 project, which was jointly funded by the International Olympic Committee and the Australian Government. At the same time as the blood samples were collected the opportunity was also taken to collect urine samples for future studies. Ethics approval was obtained from each subject to permit the samples to be used in any future research on the detection of doping with EPO and endogenous steroids.

In the last two years the application of two new urine based tests has been extended into a wider range of IOC laboratories. The first is the use of carbon isotope ratio mass spectrometry (CIRMS) for the confirmation of doping with synthetic endogeous steroids and the second is the use of isoelectric focussing to confirm the presence of human recombinant erythropoietin (EPO). The methods are quite dissimilar in the techniques used and the analytes detected but they have one major thing in common. Both rely on detecting the use of a banned drug, which is naturally occurring, by comparing a measured parameter or parameters in the suspect sample with the values found in the normal population.

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The urine test developed by LNDD uses isoelectric focussing and a patented double blotting technique (Lasne 2001) to separate the isoforms of EPO into a series of bands. Data collected so far indicates that normal urinary EPO has isoforms which are more acidic than those found in human recombinant EPO although there is some overlap (Lasne and De

Ceaurriz 2000). A positive is declared if the percentage of basic isoforms (recombinant EPO) is greater than 80%. This value was statistically determined on the basis of the range of values found in a relatively small number (a few hundred) of normal subjects. There should in fact be a survey of a statistically significant number of elite athletes from around the world to support the premise that the range of urinary EPO isoform distributions is outside the values found for recombinant human EPO.

AIMS

Using urine samples already collected from a large cohort of ethnically and geographically diverse elite athletes to:

A. Determine the variability in the isotope ratio of some selected endogenous steroid metaboliites and evaluate whether the delta ^{13}C values are significantly affected by factors such as gender, ethnicity and geographical location.

B. Determine the variability of the natural isoform pattern of urinary EPO and evaluate whether the patterns are significantly affected by factors such as gender, ethnicity and altitude.

SIGNIFICANCE

The use of CIRMS has been introduced as a means of detecting and confirming the abuse of steroids such as testosterone that occur naturally in the body. The detection of such doping has been based on measuring the testosterone to epi-testosterone ratio and ratios above 6 are indicative of doping. CIRMS provides a means of confirming doping by measuring the delta ^{13}C values of steroid metabolites and comparing these with other naturally occuring steroid precursors. As it is known that diet has a significant effect on the delta values it is important to have more data on the natural variation of carbon isotopic composition to help set legally defensible criteria for the application of the technique.

At present the confirmation of the presence of human recombinant EPO depends on the detection of abnormal blood parameters in conjunction with the presence of a urinary EPO profile consistent with that of recombinant EPO. Thus it is essential to have data demonstrating that the range of normal isoform patterns is such that a false positive is extremely unlikely in the general athlete population. Without such data it will be difficult to successfully defend court challenges based on arguments that a specific athlete has a naturally occuring but unusual EPO isoform pattern. Currently the presence of abnormal parameters from the additional blood test can be used as additional proof. However if the urinary EPO test is to ever stand alone as proof of EPO doping then the additional data from a large population of elite athletes will be needed. Whilst some data has been obtained showing that the distribution of urinary EPO isoforms is not significantly affected by external

factors such as altitude there has been no large systematic study on factors such as gender and ethnicity. It is only a matter of time before the technical aspects of the urinary EPO test are legally challenged and hence it is essential that data be collected and published in advance to establish whether there are any conditions under which the pattern of isoforms present naturally can more closely resemble those found in recombinant EPO.

It should be noted that long term users of low dose recombinant EPO may not exhibit any detectable abnormalities in their blood parameters other than an elevated but not necessarily abnormal haematocrit. The urine test is the only method available to detect such users and hence until the urine test can be accepted as proof of EPO doping by itself then such chronic doping cannot be sanctioned.

BACKGROUND

It was recognised in the early stages of the development of an indirect test for detecting recombinant human EPO that it was essential to establish the range of variability expected for all the relevant parameters in a large group of elite athletes. Multiple samples of blood and urine were collected from over 1100 athletes and are currently stored frozen. At the time of the collection it was recognised that the samples represented a valuable resource which could be needed to validate future tests for endogenous compounds. Thus the ethics approval obtained from each subject permits the samples to be used in research on the detection of doping with EPO and endogenous steroids. The samples were collected in 12 countries with representation of all major ethnic groups. Because of repeat urine collections there are some 2000 urine samples in total. The factors related to each sample which have been recorded include gender, ethnicity, age, sport, altitude and time since exercise. The sample size and diversity is such that it should be possible to determine if there are any significant effects of gender, ethnicity, exercise, sporting discipline, altitude and biological variation.

The detection and confirmation of the presence of ingested or injected endogenous steroids using CIRMS is based on the fact that the synthetic versions of the steroids have a lower proportion of carbon 13 resulting in more negative delta ^{13}C values compared with naturally produced steroids. Typically synthetic steroids have delta values close to –30 whilst the delta value of natural circulating steroids are typically –22 to –24. The CIRMS methods used to detect doping measure the delta values of steroid metabolites and compares them to the values found for precursors. The premise on which the method is based is that in a normal individual the delta values of the precursors and the metabolites will be similar. Actual measurement of these values in a wide range of subjects will provide the sound statistical basis on which to base the criteria used for determining a positive doping case.

The current method used to confirm doping with human recombinant EPO is based on the fact that recombinant EPO is significantly less acidic than urinary EPO despite the recombinant product being produced from mammalian cells containing the human gene. This difference in acidity arises from the fact that the recombinant product is less glycosylated and has fewer sialic acid residues than normal urinary EPO. In the normal human body EPO is produced in the kidney in response to low levels of oxygen in the blood. The glycosylation of the EPO protein is needed to allow it to circulate to the bone marrow where it stimulates the production of new red blood cells. The EPO protein is rapidly destroyed and is not effective if injected in the deglycosylated form. The protective effect of the sugar moieties has been extended in the new Amgen product Aranesp, which has the EPO protein modified so that two additional sugar chains are introduced. This has the effect of significantly increasing the half life of the circulating material (Egrie and Browne 2001). Although much is known of the structure of the isoforms of recombinant EPO from mass spectral and other measurements (Ohta et al 2001) what data there is on the structure of natural EPO comes from a very few subjects. In fact virtually all the data available on the variability of natural EPO isoforms has come from the few IOC laboratories that are currently routinely performing the EPO urine test. Since the urinary EPO test is critically dependent on the difference in glycosylation between natural and recombinant EPO, it is essential to be aware of the extent of natural variability of this glycosylation in the general population of elite athletes.

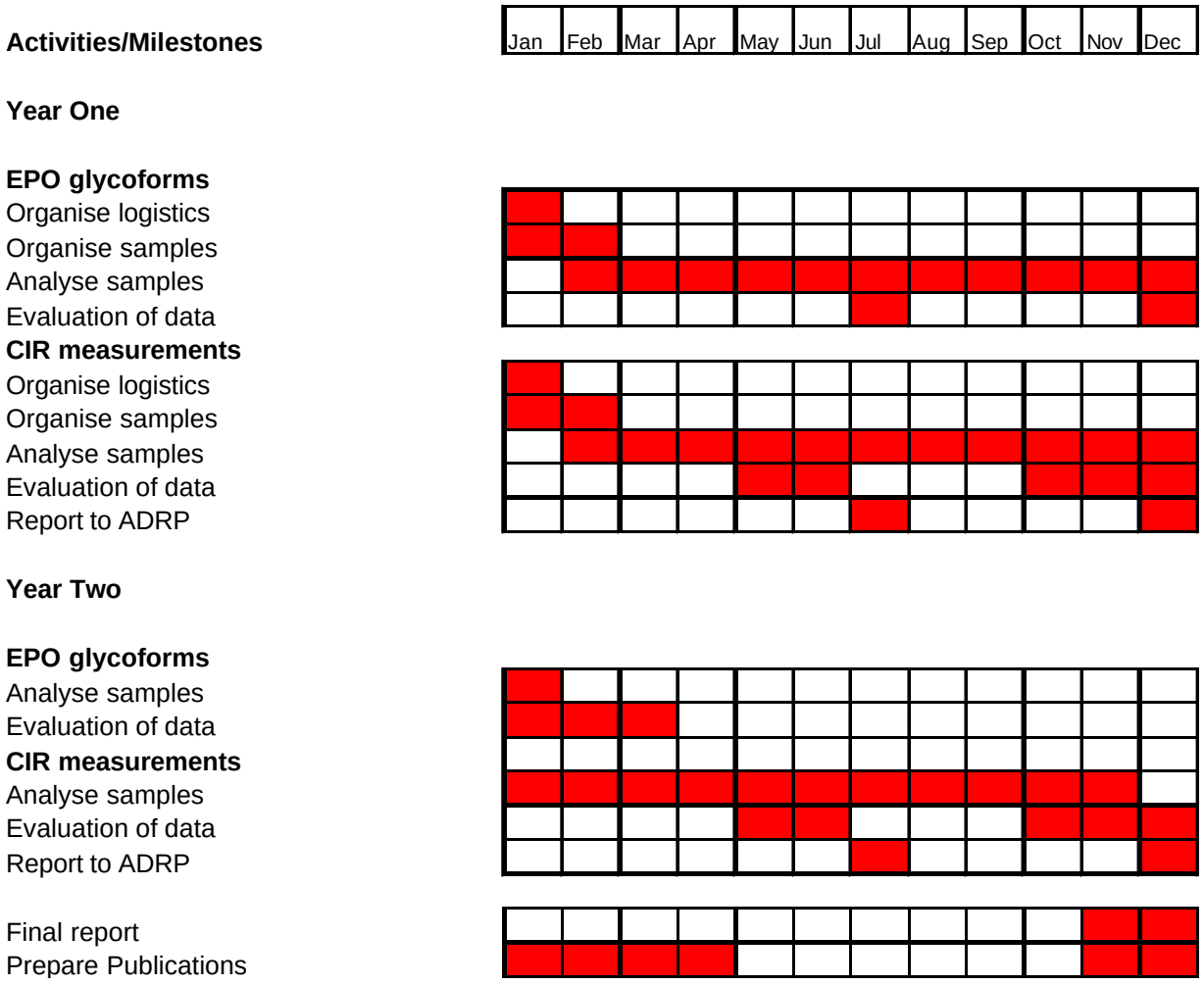
RESEARCH PLAN

Some 500 samples from four countries have already been analysed and this funding will permit the analysis of most of the remaining 1500 samples. The samples will be extracted and the delta values measured for androsterone, etiocholanolone and 11-ketoetiocholanolone. The differences in the values found for all three compounds will be determined as will the ratio of the values found. It is these differences and ratios that are currently used in determining whether a sample is positive. Once these measurements are complete it is proposed to carry out a statistical analysis of the results to determine the variability found in a large population and assess whether factors such as ethnicity and geographical location, which will predominantly effect the diet of the subjects, can affect the delta values of the analytes. This data will be used to provide the statistical evidence needed to support the criteria used to determine whether the steroids in a sample are endogenous or exogenous in origin. Whilst some laboratories rely on measuring the delta values of androsterone and etiocholanolone compared to a precursor to confirm doping others use a more complex procedure involving the extraction and analysis of a number of diols (Shackleton et al 1997). Once the analyses of the androsterone and etiocholanolone extracts are complete it is proposed to begin the second phase which requires the extraction and analysis of the diols to produce the statistical data required to support the diol procedure.

The second part of the project involves measuring the urinary EPO isoform distribution using the gel electrophoresis method in those urine samples that comprise the EPO2000 project

collection which have a sufficient concentration of urinary EPO. It is anticipated that will involve up to 600 samples. These samples will cover both males and females from all major ethnic groups involved in a wide range of sports and will provide a much wider statistical base than currently exists. To carry out these measurements will require the preparation and analysis of some 60 gels. The very high cost of performing the EPO urine test is reflected in the high consumables cost.

Project Timeline



Equipment and resources already available for use in this project

DPC Immulite

Amersham Biosciences and Biorad Gel Electrophoresis and Western Blotting Equipment

Fuji LAS1000 Camera and Image Analysis Software

Finnigan MAT Delta Plus Isotope Ratio Mass Spectrometer

2000 Urine samples collected in the EPO2000 project.

References

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

Morrison, D.J., Dodson, B., Slater, C., Preston, T. (2000) Carbon 13 natural abundance in the British diet: implications for 13C breath tests. Rapid Communications in Mass Spectrometry, 14:1321-1324.

Lasne. F, Double-blotting: a solution to the problem of non-specific binding of secondary antibodies in immunoblotting procedures. (2000) J Immunol Methods. 253(1-2):125-31.

Lasne. F, and J. de Ceaurriz, (2000) Recombinant erythropoietin in urine. Nature. 405: 635.

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.

Shackleton, C.H., Phillips, A., Chang, T., Li, Y., (1997), Confirming testosterone administration by isotope ratio mass spectrometric analysis of urinary androsatanediols. Steroids, 62:379-387.

Egrie, J.C., Browne J.K., (2001), Development and characterisation of novel erythropoiesis stimulating protein (NESP). Brit J. Cancer 84(Suppl 1):3-10.

Ohta, M., Kawasaki, N., Hyuga, S., Hyuga, M., Hayakawa, T., (2001) Selective glycopeptide mapping of erythropoietin by on-line high-performance liquid chromatography-electrospray ionization mass spectrometry.

Curriculum Vitae of principal researchers .

Curriculum Vitae – R. Kazlauskas
Director Australian Sports Drug Testing Laboratory
Australian Government Analytical Laboratory

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.

Recent publications

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.

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

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Lisi, A.M., Kazlauskas, R. and Trout, G.J.. (1997). Gas chromatographic-mass spectrometric quantitation of urinary buprenorphine and norbuprenorphine after derivatization by direct extractive alkylation, *J Chromatogr B Biomed Appl*. 692:67-77.

Published Conference Proceedings

Trout, G., Emslie, KR., Howe, C., Kazlauskas, R. and Lasne, F. (2001). An Overview of testing for EPO at the Sydney 2000 Olympic Games and beyond. *Proceed. Manfred Donike Workshop 19th Cologne Workshop on Dope Analysis,* pp191-200..

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Yap, B. and Kazlauskas, R. (2001). Rapid HPLC screening of chlorothiazide, torasemide, xipamide and benzthiazide in the Sydney 2000 Olympic Games. *Proceed. Manfred Donike Workshop 19th Cologne Workshop on Dope Analysis* pp209-214.

Fouracre, C., Kazlauskas, R. (2001). Diuretics screening and confirmation using LC/MS/MS, *Proceed. Manfred Donike Workshop 19th Cologne Workshop on Dope Analysis* pp321-326.

Trout, G.J., Soo S. and Kazlauskas, R. (1999). Single screen for steroids using high resolution mass spectrometry, *Proceed. Manfred Donike Workshop 17th Cologne Workshop on Dope Analysis.*

Trout, G., Murby, J. and Kazlauskas, R. (1998) High Resolution Mass Spectrometry in the Antipodes. *Proceed. Manfred Donike Workshop 16th Cologne Workshop on Dope Analysis*, pp269-276.

Kazlauskas, R., Lisi, A. and Trout, G. (1998). *Chiral Derivatisation, Proceed. Manfred Donike Workshop 16th Cologne Workshop on Dope Analysis* pp431-441.

Kazlauskas, R. (1997). Effects of Dehydroepiandrosterone on Urinary Steroids, *Proceed. Manfred Donike Workshop 15th Cologne Workshop on Dope Analysis,* pp83-90.

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 late1999 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 20 publications in peer reviewed journals or books, plus numerous conference presentations.

Journal Articles:

A novel method utilising markers of altered erythropoiesis for the detection of recombinant human erythropoietin abuse in athletes. Haematologica, 85, 2000, 564-572. R. Parisotto, C. J. Gore, K. R. Emslie, M. J. Ashenden, C. Brugnara, C. Howe, D. T. Martin, G. J. Trout and A. G. Hahn.

Detection of recombinant human eryhtropoietin abuse in athletes utilizing markers of altered erythropoiesis. Haematologica, 86, 2001, 128-137. R. Parisotto, M. Wu, M. J. Ashenden, K. R. Emslie, C. J. Gore, C. Howe, R. Kazlauskas, K. Sharpe, G. J. Trout, M, Xie and A. G. Hahn.

Physiological and pharmacological regulation of 20-kDA qrowth hormone. Am. J. Physiol. Endocrinol. Metab., 283(4), 2002, E838-43. Keung K.C., Howe C., Gui L.Y., Trout G., Veldhuis J.D., Ho K.K.

Drugs in sports: analytical trends. Ther. Drug Monit., 22(1), 2000, 103-109. Kazlauskas R, Trout G.

Simulated moderate altitude elevates serum erythropoietin does not increase reticulocyte production in well-trained runners. Eur. J. Appl. Physiol., 81(5), 2000, 428-35. Ashenden M.J., Gore C.J., Dobson G.P., Boston T.T., Parisotto R., Emslie K.R., Trout G.J., Hahn A.G.

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, 2002, 191-200. Trout G.J., Emslie K.R., Howe C., Kazlauskas R., Lasne F.

Strategies for rhEPO detection in sport. Clin. J. Sport Med., 12(4), 2002, 229-35. Kazlauskas R., Howe C., Trout G.

Recent Conference Presentations

A Single Screen for Steroids using High Resolution Mass Spectrometry, 17th Cologne Workshop on Dope Analysis, 1999. G.J. Trout, S. Soo and R. Kazlauskas.

GC-IRMS Detection of Steroid Metabolites – some Hardware Considerations. 17th Cologne Workshop on Dope Analysis, 1999. J.H. Rogerson and G.J. Trout.

GC-IRMS in Sports Drug Testing, Australian International Symposium on Analytical Science, July 1999, J.H. Rogerson, G.J. Trout, and R Kazlauskas.

EPO – Can its Use in Sport be Detected? Drugs in Sport Symposium, Fifth World IOC Congress on Sports Sciences, Sydney November 1999, G. Trout

Am Overview of Testing for EPO at the Sydney 2000 Olympic Games and Beyond. 19th Cologne Workshop on Dope Analysis, G. J. Trout, K.R. Emslie, C. Howe, R. Kazlauskas, and F. Lasne.

Testing for Recombinant EPO at the Sydney Olympics, invited plenary lecturer at the New Zealand Institute of Medical and Laboratory Scientists Conference, Auckland New Zealand, September 14th 2001. G. J. Trout

Development of a test for recombinant EPO and its use during the Sydney 2000 Olympic Games, invited keynote speaker at workshop for China Sports Bureau "An update on Recent Advances of EPO abuse detection in Sports Games", Guangzhou China, September 28th September 2001. G. J. Trout

Detection of haemoglobin based oxygen carriers. invited keynote speaker at Interact 2002, Sydney, Australia, July 25th 2002. G.J. Trout, C. Alma, N. Woodland

IRMS detection of banned endogenous steroid use: an interlaboratory study, Interact 2002, Sydney, Australia, July 25th 2002. J. Rogerson, G.J. Trout, R. Kazlauskas.

The detection of DHEA administration in athletes by GC-MS and GC-C-IRMS, Interact 2002, Sydney, Australia, July 25th 2002, A. Cawley, J. Rogerson, G.J. Trout, R. Kazlauskas.

The detection of haemoglobin based oxygen carriers. invited speaker at the 1st Annual USADA Symposium on Anti-Doping Science, Atlanta, USA, Oct 4-6 2002. G.J. Trout

Christopher John HOWE

E-mail: c.howe@agal.gov.au
Current Appointment: Manager Peptide Hormones, Australian Sports Drug Testing Laboratory

Qualifications: University of NSW. Bachelor of Science in biochemistry. Graduated 1978.

University of Sydney. Master of Science in Medicine, by research and thesis in the Department of Obstetrics and Gynaecology, Faculty of Medicine. Project: Development of methods for the measurement of the androgens nandrolone and testosterone in human body fluids, and application of the assays to the study of the pharmacokinetics and pharmacodynamics of nandrolone in man. Graduated April, 1996.

Member, Endocrine Society of Australia.

EMPLOYMENT HISTORY

CURRENT APPOINTMENT

January 1998-Present. Research Officer, Australian Sports Drug Testing Laboratory.

This position was created as a part of the Olympics Research Program, funded by the Australian Commonwealth Government, to improve detection of the misuse of recombinant and naturally derived protein hormones. Two main projects were conducted:

A. Erythropoietin (EPO): A major series of studies was conducted to identify and validate a set of haematological parameters which indicate EPO misuse. In 1999, a pilot study in collaboration with the Department of Physiology at the Australian Institute of Sport identified a combination of factors in whole blood and serum which, suitably weighted and combined, distinguished current and recent use of EPO.

In 2000, a study was funded by the Australian Commonwealth Government and the International Olympic Committee to validate the procedure. This study involved a collaboration between ASDTL and the AIS as well as active collaborators in China, France, Norway, Canada and Italy. Blood samples were collected from 1200 elite athletes in 14 countries, as baseline data to test the validity of the test model. I collected samples over a three week period from elite athletes in Paris. EPO administration studies were conducted in 150 recreational athletes in Sydney, Canberra, Beijing and Norway, to confirm the results obtained in the pilot study. The entire study, including data reduction, was conducted between December 1999 and July 2000, for presentation to the Medical Commission of the IOC in the beginning of August. The test procedure was approved for use at the Sydney Olympic Games, together with a procedure for identifying recombinant EPO in urine. I was sent to Paris in July 2000 to transfer this method to Sydney. These procedures are now being established as part of the service assays of the laboratory.

B. Human Growth Hormone (hGH). A smaller study was conducted in collaboration with the Pituitary Research Unit of the Garvan Institute of Medical Research, to investigate the use of molecular weight isoforms of hGH as markers of recombinant human growth hormone abuse. Sera from normal volunteers and patients suffering from a variety of relevant clinical conditions were examined and both the 20 kilodalton and 17 kilodalton forms were confirmed as having potential as markers of hGH abuse.

PREVIOUS APPOINTMENTS

1988-January 1998. Technical Officer and Hospital Scientist (May 1997-January 1998), Andrology Unit, Royal Prince Alfred Hospital, Central Sydney Area Health Service.

Originally employed as scientific support (immunoassay, sperm function analyses and computer support) for the Sydney centre in two multicentre contraception studies conducted by the World Health Organisation Human Reproduction Program. Further developed interests in the physiology and pharmacology of androgens, especially as applied to the development of male contraceptive methods. Completed a Master's degree in the development of methods for the analysis of endogenous and exogenous androgens in human serum, with application to the pharmacology of nandrolone esters in man.

December, 1977-1982. Scientific Officer, Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW 2050.

1982-1988. Senior Scientific Officer, Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW 2050.

SELECTED PUBLICATIONS

A: Peer-Reviewed Papers .

Handelsman, DJ, Conway, AJ, Howe, C, Turner, L, Mackey, MA (1996). Establishing the minimum effective dose and additive effects of depotprogestin in suppression of human spermatogenesis by a testosterone depot.. Journal of Clinical Endocrinology and Metabolism 81:4113-4121.

Minto, C, Howe, C, Wishart, S, Conway, AJ, Handelsman, DJ (1997). Pharmacokinetics and pharmacodynamics of nandrolone esters in oil vehicle: Effects of ester, injection site and volume. Journal of Pharmacology and Experimental Therapeutics 281:93-102.

Handelsman, DJ, Mackey, MA, Howe, C, Turner, L, Conway, AJ (1997). An analysis of testosterone implants for androgen replacement therapy. Clinical Endocrinology 47:311-316.

Howe, C, Handelsman, DJ (1997). The use of filter paper for sample collection and transport in steroid pharmacology. Clinical Chemistry 43:1408-1415.

Kicman, AT, Coutts, SB, Cowan, DA, Handelsman, D J, Howe, CJ, Burring, S, Wu, FCW (1999) Adrenal and gonadal contributions to urinary excretion and plasma concentration of epitestosterone in men - effect of adrenal stimulation and implications for detection of testosterone abuse. Clinical Endocrinology 50:661-668.

Parisotto, R, Gore, CJ, Emslie, KR, Ashenden, MJ, Brugnara, C, Howe, CJ, Martin, DT, Trout, GJ, Hahn, AG (2000) A novel method utilising markers of altered erythropoiesis for the detection of recombinant human erythropoietin abuse in athletes. Haematologica 85:564-572.

Parisotto, R, Wu, M, Ashenden, MJ, Emslie, KR, Gore, CJ, Howe, C, Kazlauskas, R, Sharpe, K, Trout, GJ, Xie, M & Hahn, A (2001). Detection of recombinant human erythropoietin abuse in athletes utilizing markers of altered erythropoiesis. Haematologica 86:128-137.

Physiological and pharmacological regulation of 20-kDA qrowth hormone. Am. J. Physiol. Endocrinol. Metab., 283(4), 2002, E838-43. Keung K.C., Howe C., Gui L.Y., Trout G., Veldhuis J.D., Ho K.K.

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, 2002, 191-200. Trout G.J., Emslie K.R., Howe C., Kazlauskas R., Lasne F.

Strategies for rhEPO detection in sport. Clin. J. Sport Med., 12(4), 2002, 229-35. Kazlauskas R., Howe C., Trout G.

B. Selected recent reports and conference presentations.

Howe, CJ, Leung, K-C, Emslie, K, Trout, G & Ho, K (1998) Development of a radioimmunoassay for a 17kDa human growth hormone fragment in serum. Proceedings of the 8th St. Vincent's Campus Research Symposium, Sydney, September 1998.

Howe, CJ, Leung, K-C, Emslie, K, Trout, G & Ho, K (1999) Investigation of 17kDa human growth hormone fragment in serum as a marker for human growth hormone doping. Fifth IOC Conference on Sports Science, Sydney, October 1999.
This presentation was awarded the Prince Alexandre de Merode Award, Clinical Sciences division

Hahn, A, Parisotto, R, Gore, C, Ashenden, M, Kazlauskas, R, Trout, G, Emslie, K, Howe, C, Sharpe, K , de Ceaurriz, J, Lasne, F, Audran, M, Gareau, R, Stray-Gundersen, J, Wu, M, Smith, R, Davis, P (2000) Validation of an Indirect Test for EPO Use in Sport. Report to the Medical Commission of the International Olympic Committee.

K-C Leung, K-C, Howe, C, Gui, L, Trout, G & Ho, KKY (2001). Physiological and pharmacological regulation of 20-kilodalton growth hormone in humans. Submitted for the annual meeting of the US Endocrine Society. Denver, Colorado, June 2001.

Jill Rogerson

Qualifications

Tertiary Education: University of Melbourne

Postgraduate Course:

PhD in Chemistry, 1987 - 1994
Title of PhD thesis:
Lipids of an Extreme Environment: Organic Lake, Antarctica.

Undergraduate Course:

Bachelor of Science with Honours (First Class) 1983-1986

Publications

Posters:

Rogerson, J. H., G. J. Trout and R. E. Kazlauskas (2000). CIR in Doping Analysis: A New Background Marker. Manfred Donike Workshop, 18th Cologne Workshop on Dope Analysis, Cologne, Germany

Rogerson, J. H., G. J. Trout and R. E Kazlauskas (2001). Carbon Isotope Ratios and Doping Control. 18th Australian and New Zealand Society of Mass Spectrometry Meeting, Gold Coast, Australia, 4th-8th February.

Oral Presentations:

Rogerson, J. H. & G. J. Trout (1999). GC-C-IRMS Detection of Steroid Metabolites - Some Hardware Considerations. Manfred Donike Workshop, 17th cologne workshop on Dope Analysis, Cologne, Germany.

Rogerson, J. H., G. J. Trout & R. Kazlauskas (1999). GC-IRMS in Sports Drug Testing. 15th Australian International Symposium on Analytical Science, 4-9th July, Melbourne, Australia.

Rogerson, J. H., A. J. Bryce, A. M. Stenhouse, K. Skelton, D. Auer, G. J. Trout, A. Andrew, R. Kazlauskas (2001). Sodium bicarbonate doping in racehorses: An IRMS approach to detection. 7th Conference on Isotopes in the Environment, Robertson, NSW, Australia, 3th-6th September.

Rogerson, J.H., G.J. Trout, R. Kazlauskas (2002) IRMS detection of banned endogenous steroid use: an interlaboratory study, Interact 2002, Sydney, Australia, July 25th

Papers:

Rogerson, J. H. & G. J. Trout (2000). GC-C-IRMS Detection of Steroid Metabolites - Some Hardware Considerations. Recent Advances in Doping Analysis (7) - Proceedings of the Manfred Donike Workshop, 17th Cologne Workshop on Dope Analysis, 14th-19th March,1999, 223-232.

Employment History

1998 – present**, Research Chemist (PO2):** Australian Sports Drug Testing Laboratory, AGAL, Pymble,

The main requirement of this position is the development of a method for isotope ratio assessment of the presence of synthetic versions of endogenous steroids in athletes urine. This has involved developing appropriate clean-up procedures for the sample, and investigation of the urinary steroid composition. A Finnigan delta plus GC-C-IRMS system was installed at ASDTL in 1999 and I have been responsible for its operation and maintenance, and for method development using this system. Prior to this I had been working on a Finnigan-MAT 252 IRMS with GC, dual and multiport inlet facilities and have considerable experience constructing combustion interfaces for this system. The final part of this latter work involved the production of an instruction manual concerning the construction of a combustion interface for this system.

1997-1998 **Analytical Chemist:** AGAL, South Melbourne.

Purge and Trap Unit, AGAL, South Melbourne A contract position in the Purge and Trap Unit gave me valuable experience of routine analysis of volatile compounds from a variety of media (soil, water, sludge, effluent and air), the international conventions, methods and NATA requirements regarding these analyses. I was also involved in routine operation and maintenance of the equipment, development of Excel programs to facilitate reporting and measurement of matrix effects of different soils.

1994-1997 **Dairyfarmer:** Lynwood,

An illness in the family has allowed me to gain valuable experience in primary production by assisting my brother operate our family dairy farm. This experience also provided many opportunities to develop my problem solving skills.

1991-1992 **Analytical Chemist:** Biochemistry School, University of Melbourne,.

A part time position which involved supervising and training a laboratory assistant in the technical aspects of the operation and maintenance of gas chromatographs and a mass selective detector, for the purposes of lipid and sugar analysis.

Memberships

Royal Australian Chemical Institute
Australian National Antarctic Research Expedition Club.
Australian Trust for Conservation Volunteers.