SPORTS ANTI-DOPING RESEARCH FUNDING PROPOSAL

Improved Method for the Detection of Erythropoietin Isoforms in Urine.

R. Kazlauskas, G. J. Trout, C. Howe, and N.H. Packer

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: **AGAL**

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	❖ 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 94490144

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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***
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***
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.** ***see attached***
4. Project summary, suitable for publication (maximum 1000 words) ***see attached***
5. Project description. – **This should focus on the expected outcome of the project and the selection criteria (max 5 pages).** ***see attached***
6. Project timetable, including proposed milestones. ***see attached***
7. Project management plan, including reporting and evaluation plans. ***see attached***
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***

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:

1. Details of any ethical consideration for the project.

Ethics approval for all samples which will be used in this study is covered by research proposal "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.

2. 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	 **43,000**	 **11,000**
Salary, technical personnel: 100% of technician plus on costs	 **45,000**	
Consumables: approximately 25 IEF gels at $1800 per gel dye affinity, lectin, and ion exchange columns, visualisation reagents, EPO test kits and reference standards.	 **45,000** **10,000**	 **12,000**
Overheads (indirect costs including support infrastructure, equipment maintenance and repairs, services etc)	**31,900**	**4,500**
Total budget year 1:	**174,900**	**27,500**
Year 2: 2004 **Salary, scientific personnel:** 10% of SPOA plus on costs 50% of SPOC plus on costs	 **45,000**	 **11,500**
Salary, technical personnel: 100% of technician plus on costs	 **47,000**	
Consumables: 10 IEF gels at $1800 per gel 30 IPG gels at $600 per gel 500 cleanup columns Test kits for 1000 EPO assays Capillary HPLC columns Nanospray tips for electrospray	 **14,400** **15,000** **12,000** **10,000** **4,000** **3,000**	 **3,600** **3,000** **3,000** **3,000** **2,000** **1,000**
Overheads (indirect costs including support infrastructure, equipment maintenance and repairs, services etc)	**33,000**	**5,000**
Total budget year 2:	**183,400**	**32,100**
Total budget all years	**358,300**	**59,600**

Additional budget information

It is planned that the two main phases of the project set out below should run concurrently Whilst Proteome Systems are developing the IPG gels and the necessary hardware none of the ADRP funds will be allocated to them as they already have funding from the Biotechnology Innovation Fund. In fact it is anticipated that for the first year of the project at least that all IPG gels will be provided at no cost to the project. However there is still a high cost of consumables associated with the double blotting procedure and the final visualisation which will still be required. In addition a considerable number of ampholyte gels will be needed for comparative purposes. The project will require a scientist to spend approximately 50% of their time on the project along with a full-time highly skilled technician. The running expenses are high because of the initial high cost of running the gels for the EPO urine test. In the later stages of the project the cost per sample will markedly reduce but more samples will need to be run to validate the new method and the mass spectral component of the project will begin incurring costs.

3. Consultation and/or collaboration arrangements.

The Australian Sports Drug Testing Laboratory which is IOC accredited is the applicant. The methods used are those utilised by our, and some other IOC accredited laboratories. Regular discussion with IOC laboratories occurs especially at the Cologne Workshop where progress will be reported.

4. Project summary.

The purpose of this project is to simplify and improve the existing urine test for human recombinant EPO which was developed by Dr Francoise Lasne of the Paris IOC laboratory. The test, which is based on using gel electrophoresis to separate the isoforms of EPO, is being used in approximately one third of the 27 laboratories accredited by the IOC. There are a number reasons why not all IOC laboratories have adopted the method. One is the high cost of acquiring methodology which is quite different to that normally used in other drug detection methods both in equipment and personnel. Another is the complexity of the process requiring many steps several of which demand a high degree of manual dexterity and skill. This means the process is slow and very expensive to perform. Because of the high cost very few federations or drug control agencies are willing or able to fund the testing of a large number of samples and are using inadequate screening procedures in a attempt to reduce the number of urine samples tested.

There are two obvious ways by which the existing process could be improved. The first is the use of commercial Immobilised pH gradient (IPG) gels for the electrophoretic separation of the EPO isoforms. Such gels once developed would give more reproducible results, simplify the method and ultimately reduce costs. The second is to improve the selectivity of the

processes used to extract and concentrate the EPO from the urine sample. At present the EPO is concentrated simply by ultra-filtration which means that many other proteins in the urine are also present in the concentrate applied to the gel. These proteins such as albumen are at much higher concentrations than the EPO which means that the double blotting process is needed to detect a trace of EPO in the presence of these other proteins. The transfers involved in the double blotting are responsible for much of the complexity of the test. Thus improved methods for extracting and concentrating EPO from urine would initially give better separation of the isoforms of EPO and improve the sensitivity of the method and may ultimately lead a major simplification of the method by eliminating the double blotting process. This would result in major cost savings.

If both aspects of the project succeed then the method for the detection of recombinant EPO in urine would be greatly simplified. This would result in significant cost savings both in the setting up of the method in new laboratories and in its routine application.

Project description

Improved Method for the Detection of Erythropoietin Isoforms in Urine.

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Phone 61 2 94490111 Fax 61 2 94498080 email ray.kazlauskas@agal.gov.au or graham.trout@agal.gov.au
Researchers: R. Kazlauskas, G. J. Trout, C. Howe, and N.H. Packer

INTRODUCTION

At present the detection of doping with human recombinant erythropoietin (EPO) relies on the detection of abnormal blood parameters such as those reported by Parisotto (Parisotto et al 2001) coupled with the presence of recombinant human EPO in a corresponding urine sample. The urine test developed by LNDD uses isoelectric focussing and a patented double blotting technique (Lasne 2001) to separate the EPO isoforms into a series of bands. A positive cannot be declared unless the urine has bands which correspond to those found in human recombinant EPO. Data collected so far indicate 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 is greater than 80%. Whilst the method has been introduced into about one third of the IOC laboratories so far, the cost of applying the method more widely is very high due to the cost of the specialised equipment, the long training needed to acquire the specialised skills, and the high cost of consumables and labour that routine application of the test requires. Thus for more effective detection of doping with recombinant EPO and related products such as NESP a simplified and more cost effective method is required.

AIMS

To simplify the methodology used so that the French EPO urine test can be applied to more samples at substantially lower cost. This will take place in a number of stages:

1. In collaboration with Proteome Systems develop a robust immobilised pH gradient (IPG) gel that can be used in all IOC laboratories to improve reproducibility of results, particularly between laboratories, obtained using the gel electrophoresis test for recombinant EPO developed by the French IOC laboratory. The gel developed will also have the capability to resolve the isoforms of the new EPO replacement Novel Erythrocyte Stimulating Peptide (Egrie and Browne 2001);
2. Investigate means of selectively extracting recombinant EPO from urine to improve the sensitivity of the current method and remove the need for the complicated double blotting process.

3. Investigate whether improved the extraction methodology can lead to the measurement of EPO isoforms in blood.
4. Investigate how mass spectrometry can be applied to the analysis of the purified extracts for recombinant EPO.

SIGNIFICANCE

If no improvements are made the existing method for the detection of recombinant EPO in urine then it will remain a method unsuitable for high volume routine screening. Successful completion of the steps outlined above should simplify and automate the methodology so that it can be economically applied to high volume screening. This would mean that poor strategies using one or two blood parameters currently used to select urines for EPO testing would no longer be required and the EPO test could become one of the routine urine tests.

Before any significant improvements can be made a more reliable source of gels is needed. The French urine test for EPO, which uses a gel electrophoresis method for the detection of recombinant EPO, uses gels that are prepared within the laboratory. Such gels lack robustness and the results obtained on one gel will not necessarily be exactly the same as those from another gel even prepared in the same laboratory. The inter-laboratory trial of the French urine EPO method funded by the IOC (Pascual et al 2001) has confirmed the problems that are encountered with the laboratory prepared gels. The newer immobilised pH gradient gels are now used for most electrophoretic separation of proteins. AGAL does not have the technical expertise to produce such gels but an Australian company, Proteome Systems, is a world leader in this technology and is collaborating in this work.

BACKGROUND

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 heavily glycosylated and has fewer sialic acid residues than normal urinary EPO. The test for recombinant EPO in urine developed by our colleagues at LNDD in Paris, whilst able to detect the abuse of EPO, has a number of significant limitations. They all relate to the complexity of the method, which means that:

- the production of results is slow and cannot always be guaranteed
- the reproducibility between laboratories is not as high as desired
- the cost of the method is so high that it is impractical to test as many samples as required.

A more robust and simpler method is needed. The year one program is the first stage in a series of steps to simplify and automate the methodology so that it can be economically applied to high volume screening. The first step in this long process is to obtain a reliable source of IPG gels that can be used in the first stage of the method. Proteome Systems have obtained a Biotechnology Innovation Fund grant to develop a gel or gels specially designed for the detection of recombinant EPO. ASDTL is providing the knowledge and skills needed to test the gels provided and incorporate them into the urine test for recombinant EPO but are not funded to do so. Proteome Systems have now developed a commercial IPG gel that can resolve the isoforms of both recombinant EPO and NESP. The resolution and reproducibility is superior to the ampholyte gels currently used. The availability of such a reliable commercial gel will not only significantly improve the robustness of the method but will also reduce the overall cost and complexity of the method.

The double blotting process used in the method is a major source of the method's complexity and cost. Alternative means of separating the recombinant EPO from other proteins will be examined. The methods which have shown the most promise so far are extraction methods using dye affinity chromatography and another using lectin affinity chromatography (Trout 2002). Dye affinity chromatography preferentially removes albumen (Gianazza and Arnaud, 1982), which is the main protein present in urine and thus has the potential to remove much of the interfering protein. Lectins react with glycoproteins including EPO (Rudzki et al., 1978) and thus can be used to retain only the glycoproteins whilst discarding all other proteins. The use of ion-exchange media will also be investigated (Morimoto et al., 1996). The aim is to remove more than 90% of the interfering proteins whilst retaining at least 50% of the EPO. If this approach is successful it will not only simplify the method but also mean that it could be applied to blood as well as to urine. It is known that serum EPO concentrations correlate much more closely with EPO use than do urinary EPO concentrations.

Once a purified EPO can be extracted from urine or blood then detection will be simplified. it should also be possible to use mass spectrometry (MS) to more specifically characterise the extracted EPO. At present the confirmation of the presence of peptide hormones and other large bioactive molecules is done using techniques that rely on specific antibody reactions to large molecules. All banned drugs that are detected in IOC laboratories, other than peptide hormones, must be confirmed by the use of MS. The reason the peptide hormones were excluded from this requirement was that it was not practicable to attempt mass spectrometric analysis of large bio-molecules both because of their high molecular weight and because of the very low concentrations found in blood and urine. However with the ever increasing demands of proteomics research the use and capabilities of MS using electrospray and liquid chromatography for the analysis of bio-molecules has increased dramatically in the last few years and will continue to do so. As electrospray MS has already been used in this laboratory for the confirmation of the presence of haemoglobin based oxygen carriers (Trout, 2002a) and it is planned to extend the technology for the detection and confirmation of recombinant EPO.

The existing equipment will be able to demonstrate whether such mass spectral analysis is possible for EPO.

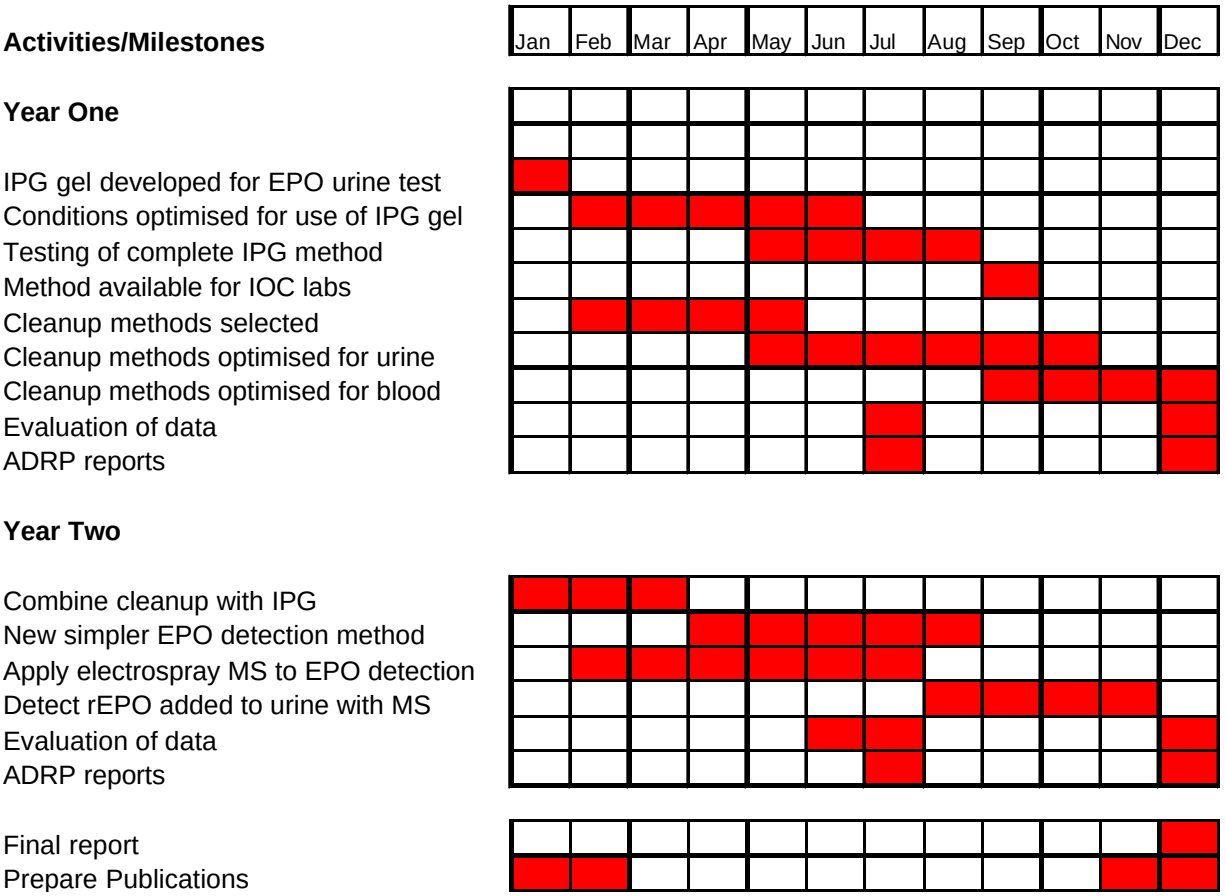
RESEARCH PLAN

This project was begun in late 2001 in collaboration with Proteome Systems with the initial objective of obtaining a reliable IPG gel for the electrophoretic separation isoforms. Thus far Proteome Systems have produced a gel having the desired pH range which can effectively resolve the isoforms of EPO and of NESP. The resolution is clearly superior to that obtainable with the laboratory prepared ampholyte gels that are currently used for the detection of recombinant EPO. The next phase of this IPG gel method development is to optimise the blotting conditions so that the IPG gel can be used in the EPO detection method. This requires ASDTL to provide spiked and incurred samples to Proteome Systems and also carry out the specialised blotting and visualisation procedures needed to measure the EPO isoforms. This will take approximately six months and will involve running a number of gels both ampholyte and IPG for optimisation of the new method. It is anticipated that in this timeframe Proteome Systems will have completed development of the prototype gel electrophoresis and blotting system that will enable laboratories to simply adopt the new IPG technology. The complete system will be extensively evaluated by ASDTL before it is released for general use in the IOC laboratory community.

Investigation of the replacement of the simple ultra-filtration procedure with a more selective method for the removal of and concentration of the EPO in urine has begun. The three processes to be actively investigated are the use of dye affinity chromatography, lectin chromatography and ion exchange chromatography. Investigation and optimisation of these methods will require numerous gels to be run to determine the degree of purification that is being achieved and should be complete within year one of the project.

In year two it is proposed to combine the new IPG gel technology and the cleanup methods in a concerted effort to reduce the complexity and cost of the existing recombinant EPO detection method, whilst simultaneously increasing its robustness and sensitivity. The development will include any other cleanup procedures that become available including the use of immunoafffinity chromatography. It is also proposed to apply the cleanup methods to blood in an attempt to measure the EPO isoform distribution therein. Cleaned extracts and extract from gels will also be analysed by electrospray mass spectrometry to further characterise the extracted EPO.

PROJECT TIMELINE



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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.

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

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Trout, G.J. (2002a) The detection of haemoglobin based oxygen carriers. Presented at the 1st Annual USADA Symposium on Anti-Doping Science October 4-6, 2002.

Gianazza, E and Arnaud, P (1982) A general method for fractionation of plasma proteins. Biochemical Journal 201:129-136.

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Morimoto, K, Tsuda, E, Said, AA, Uchida, E, Hatakeyama, S, Ueda, M and Hayakawa, T (1996) Biological and physicochemical characterization of recombinant human erythropoietins fractionated by Mono Q chromatography and their modification with sialyltransferase. Glycoconjugate Journal 13: 1013-1020.

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

Family name Kazlauskas
First name Rymantas
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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

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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.

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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). Currently my major responsibility is the management of the $3,000,000 Olympics research program whose aim is to develop new testing techniques for the Sydney 2000 Olympics. Problems under active investigation include 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 have 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 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 post 1997:

Gas chromatographic-mass spectrometric quantitation of urinary buprenorphine and nor-buprenorphine after derivitization by direct extractive alkylation. Journal of Chromatography Biomedical Applications, 692, 1997, 67. A.M Lisi, R Kazlauskas, and G.J. Trout.

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Recent Conference Presentations

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Drugs in Sport: Advances in the Fight. Seminar for Sports Medicine Australia, 1998. G.J. Trout

Drugs in Sport – Research and Development Update. Australian Conference of Science and Medicine in Sport, 1998. G.J. Trout and R. Kazlauskas.

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.

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Measurement of Urinary Erythropoietin Levels in Athletes. 17th Cologne Workshop on Dope Analysis, 1999. K.R. Emslie, C. Howe and G.J. Trout.

Evaluation of individual EPO and sTFR levels during the 6-day "Tour Down Under" cycling race, Workshop on the Detection of Abnormal Activation of Erythroiesis by Pharmacological Doses of human Recombinant EPO, 11th May 1999 – Rosny-sous-Bois – France. K.R. Emslie, G.J. Trout, T Boston, P Mangin, and D.T. Martin.

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

Erythropoietin Administration Trial in Athletes: Preliminary Results, Australian International Symposium on Analytical Science, July 1999, K.R. Emslie, C. Howe, T. Boston, A. Hahn, C. Gore, R. Parisotto, R. Kazlauskas, and G.J. Trout.

Steroid Analysis using Electrospray Mass Spectrometry, Australian International Symposium on Analytical Science, July 1999, P. Darmos and G.J. Trout.

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

Investigation of 17kDa human growth hormone fragment is serum as a marker for human growth hormone doping, Fifth World IOC Congress on Sports Sciences, Sydney November 1999, C. Howe, K. Leung, K. emslie, G. Trout, and K .Ho.

Screening results for Nandrolone Metabolites in Australia, 18th Cologne Workshop on Doping Analysis, 2000. G. J. Trout, S. Soo, and R. Kazlauskas.

Quality Assurance Processes at the Australian Sports Drug Testing Laboratory, AGAL, 18th Cologne Workshop on Doping Analysis, 2000. R. Kazlauskas, R. Millar, A. Stenhouse, G. J. Trout, and B. Yap.

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.

Sydney 2000 and the EPO Test, invited keynote speaker at the Australian Institute of Medical Scientists National Scientific Meeting, Melbourne Australia, September 5th 2001. 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. 20th Cologne Workshop on Dope Analysis, C. Alma, G.J. Trout, N. Woodland.

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 development and application of the "ON" blood model for EPO doping. invited speaker at the 1st Annual USADA Symposium on Anti-Doping Science, Atlanta, USA, Oct 4-6 2002. G.J. Trout

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.

NAME: Nicolle Hannah PACKER

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North Ryde, Sydney, 2113 NSW,
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QUALIFICATIONS: B.Sc. Sydney University 1971
Ph.D. Sydney University 1978

EMPLOYMENT:

1998: Senior Research Scientist engaged in the establishment of the Australian Proteome Analysis Facility at Macquarie University.
Consultant to CRC for Eye Research Technology on wettability by sugars of contact lenses

1999 – present: Founding shareholder of Proteome Systems Ltd, a biotechnology company using proteomics to solve biological problems. Currently the company is a privately owned public company comprising 70 employees, situated in laboratories in North Ryde.

Research Interests:

- Analysis of site - specific glycosylation of mucins with a view to determining peptide motifs which direct O-glycosylation. The approach includes chemical analysis of native and recombinant mucin domains.
- Characterisation of glycoforms on proteins separated by 2-D electrophoresis
- Development of new methodologies for the analysis of sugars: fluorescent tagging of released oligosaccharides, desalting of sugars for mass spectroscopy, chromatography and electrophoresis
- Collaboration on a number of other projects (University, CRC and CSIRO) which involve glycosylation and the techniques used for their analysis.
- Development of curated and annotated relational database on published glycan structures on proteins.

Other Activities:

- Course co-ordinator, editor and part-presenter of an advanced training course involving theory and practicals in the "Analysis of Glycoconjugates and Complex Sugars" at the Macquarie University Centre of Analytical Biotechnology (MUCAB).
- Guest Lecturer in undergraduate courses in Schools of Biological Sciences and Chemistry at Macquarie University.
- Invited speaker at conferences on “Cancer – associated mucins” in Cambridge, UK and “Proteomics” in Siena, Italy

- Consultation on various sugar related problems with biotechnology companies (Novogen, Trace BioSystems, Bureau of Sugar Experimental Stations, Polartechnics, Cooperative Research Centre for Eye Research Technology)
- Negotiation with overseas companies (Beckman, Bio-Rad, Oxford GlycoSystems, Glyko Inc) on technology collaborations.
- Supervision of honours and post graduate students in both the technical aspects and the direction of the research.
- On organisational committee for establishment of the Australian Proteome Analysis Facility, a Major National Research Facility.
- On editorial Board of Electrophoresis journal, serving as Guest Editor for Special Issue on post translational modifications in proteomics.

Publications: 53 papers in refereed journals
10 book chapters
6 patent applications

Relevant Recent Publications:

1. **Packer N.H. and Harrison, M.J. (1998). Glycobiology and Proteomics: Is Mass Spectrometry The Holy Grail?** *Electrophoresis* 19**, 1872-1882.**
2. **Packer N.H., Lawson, M.A., Jardine, D.R. and Redmond, J.W. (1998). A general approach to desalting oligosaccharides released from glycoprotein.** *Glycoconj. J.* 15**, 737-747.**
3. **Hermann, A, Davies, J.R., Lindell, G., Martensson, S., Packer N.H., Swallow, D., and Carlstedt, I. (1999). Studies on the 'insoluble' glycoprotein complex from human colon** *JBC 274,.* **15828-15836**
4. **Mreyen, M., Champion, A, Srinivasan, S., Karuso, P., Williams, KL. and Packer, N.H. (2000). Multiple O-Glycoforms on the Spore Coat Protein SP96 in *Dictyostelium discoideum*** *JBC* 275**, 12164-12174**
5. **Harry J.L., Wilkins, M.R, Herbert, B.R., Packer, N.H., Gooley, A.A. Williams, K.L. et al Proteomics: capacity vs utility (2000).** *Electrophoresis* 21**, 1071–1081**
6. **Cooper, C.A., Harrison , M.J., Wilkins, M.R., Packer, N.H. (2001). GlycoSuiteDB: a new curated relational database of glycoprotein glycan structures and their biological sources.** *Nucleic Acids Research* 29**, 332-335**
7. **Herbert BR, Harry JL, Packer NH, Gooley AA, Pedersen SK, Williams KL. (2001)What place for polyacrylamide in proteomics?** *Trends Biotechnol.*;**19**, S3-9.
8. **Karlsson, N.G. & Packer, N.H. (2002) Analysis of O-linked reducing oligosaccharides released by an in-line flow system** *Anal.Biochem*. accepted for publication.
9. **Harrison, M.J., Wathugala, I.M., Tenkanen, M., Packer, N.H. and Nevalainen K.M.H. (2002). Glycosylation of Acetyl-Xylan Esterase from Trichoderma reesei**. *Glycobiology***,** accepted for publication**.**
10. **Schulz, B., Oxley D, Packer, N.H., Karlsson N, (2002) Identification of Two Highly Sialylated Human Tear Fluid DMBT1 Isoforms: Major High Molecular Weight Glycoproteins in Human Tears.** *Biochemical J.* accepted for publication.