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RECENT ADVANCES IN DOPING ANALYSIS (9)

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An Overview of Testing for EPO at the Sydney 2000 Olympic Games and Beyond
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An Overview of Testing for EPO at the Sydney 2000 Olympic Games and Beyond

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Introduction

Some years before the Sydney 2000 Olympic Games a decision was made to instigate a series of research programmes to ensure that the Sydney laboratory would be ready for any additional drug testing requirements that could arise before September 2000. One of the areas which was targeted was the detection of peptide hormone abuse particularly erythropoietin (EPO). In 1997 a program was begun in collaboration with the Australian Institute of Sport (AIS) to investigate the possibility of detecting EPO doping using a series of blood and serum measurements. An EPO administration trial was carried out in 1999 and the results indicated that the detection of current or recent EPO doping was possible using a five parameter ON model and a three parameter OFF model (1). If a test for EPO was to be implemented then a validation of the proposed method was required. This required repeating the original study, carrying out additional administrations of EPO at lower doses, and determining reference ranges in elite athletes for the parameters used in the blood models. At the same time as ASDTL and the AIS had been developing an indirect blood test for EPO the Paris IOC laboratory (LNDD) had been developing their direct urine test (2). In order to have both tests ready for the Sydney Games a consortium of researchers was established (EPO2000) and a proposal for funding made to the IOC and the Australian Government. The scientific collaborators who took part in this work came from Australia, Canada, China, France, and Norway. Funds of $2,000,000 USD were provided and the work began in late 1999 to have a validated test for EPO in place for the Sydney Games in September 2000.

Results and Discussion

Apart from the tasks that made up the EPO2000 project there were a number of important administrative tasks that had to be carried out before the results of the research were known so that should a test be approved it could be implemented immediately. These included:

- Preparing a protocol for blood testing so that it could be circulated to the international sporting federations prior to the Games and be incorporated into the Sydney 2000 Doping Control Guide.
- Modifying a section of the laboratory so that it was suitable for handling blood samples.
- Arranging for a Bayer Advia 120 Haematology Analyser to be available at short notice.
- Purchasing blood testing kits.
- Duplicating the equipment used at LNDD for urinary EPO analysis.
- Sending two ASDTL staff to Paris to be trained at LNDD.
- Arranging for Francoise Lasne and two of her staff to come to Sydney for four to six weeks prior to and during the Games.

Fortunately most of the EPO2000 research work went according to plan and a report was prepared on the results in July 2000. As there was no time for the usual scientific review process of publication of the results in peer reviewed journals, the IOC had arranged for a scientific panel to review the data. A panel consisting of the IOC Doping Sub-Commission and a number of independent experts was convened at Lausanne on the 31st July and the 1st August 2000, and the Australian and French teams made presentations on the results achieved.

The blood test is an indirect method which looks for changes in up to five blood and serum parameters which indicate stimulated or depressed erythropoiesis (ON and OFF models) following EPO doping (3), whilst the urine method is a direct method which detects the presence of the injected recombinant EPO in the athletes urine. Each test has its advantages and disadvantages as shown in Table 1. The blood and urine methods were presented as valid independent tests for the detection of EPO doping. It was proposed that both tests be used in combination as the most effective means of deterring EPO abuse.

Table 1

Direct	Indirect
Can only detect relatively recent EPO use.	Can detect EPO abuse for up to 21 days after the last injection.
Gives a result that proves the presence of the injected recombinant EPO.	Relies on population statistics to determine whether a result is positive.
Is thus able to confirm suspicious results from the blood ON model.	No direct confirmation possible for OFF model.

The original proposed blood test was:

- The ON model score (>2.5 for men or >2.4 for women) is used to select those requiring two additional blood tests.
- In the event of a positive OFF model score two additional blood samples will also be required.
- Confirmation is either by:
  - A decrease in ON model score of more than 0.6 over two samples and an OFF model positive from one sample.
  - Two consecutive samples being positive for the OFF model and the third sample not positive.

The panel of independent scientific experts convened by the IOC questioned both the Australian and French groups about the data presented and made the following decisions on August 1st:

- Taking additional samples from suspect positives was not logistically practical.
- Only those athletes that were positive on both the blood ON model and the urine test would be sanctioned.
- Athletes that produced an abnormal result on the OFF blood model would be noted and their relevant sporting federation advised for further unannounced out of competition testing.

As a result of this decision blood testing was to be carried out at a summer Olympics for the first time. To carry out the testing the following additional facilities were required:

- A dedicated laboratory for handling blood.
- A Bayer ADVIA120 Haematology System.
- A DPC Immulite automated chemiluminescent immunoassay analyser.
- A Dade Behring BN2 automated nephelometric immunoassay analyser.
- Gel electrophoresis and Western blotting equipment.
- A Fuji 1000 cooled CCD camera.

Over 300 paired blood and urine samples were collected from athletes prior to their competing. The athletes were selected from sports likely to benefit from EPO and on the basis of their standing in that sport.

- 10mL of blood was taken in two tubes (one for whole blood and one for serum).
- There was no provision for a B sample as it was not possible to store the whole blood for more than a few hours. In the case of a suspect result repeat measurements were made on the A sample.
- All urines were tested for steroids and diuretics and EPO concentration using a simplified version of the method previously published (4).
- Selected urines were tested for recombinant EPO.

The results for the urinary EPO concentrations are shown in Figure 1. Our EPO administration trials had shown that serum EPO concentration rises during the course of administration but that urinary EPO concentrations did not correlate well with the serum EPO levels. The results from the Olympic samples shown in Figure 2 confirmed the lack of correlation between serum and urinary EPO concentrations.

Figure 1

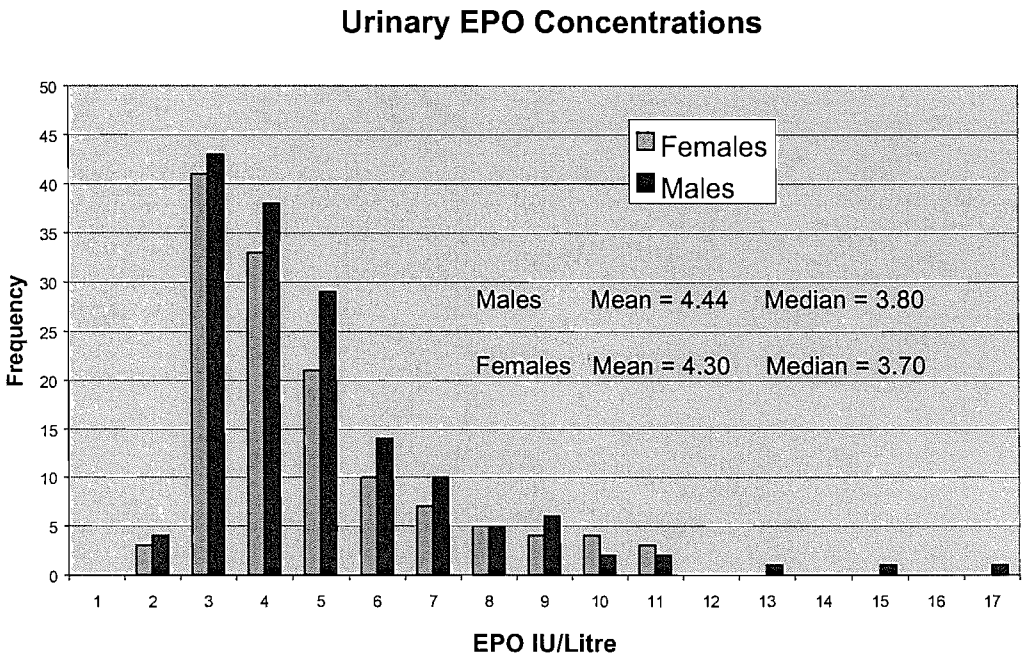
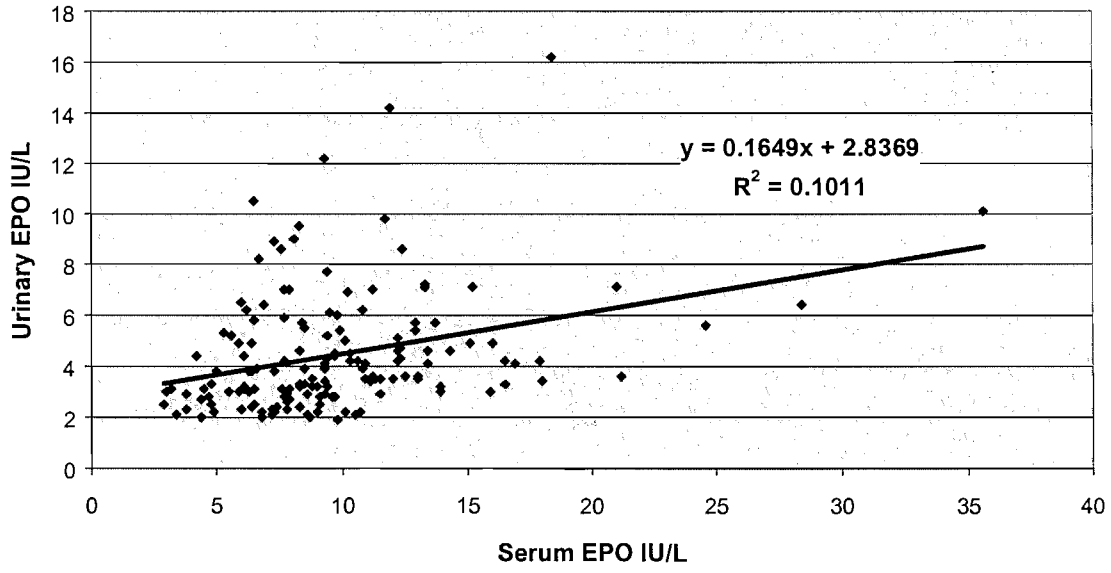
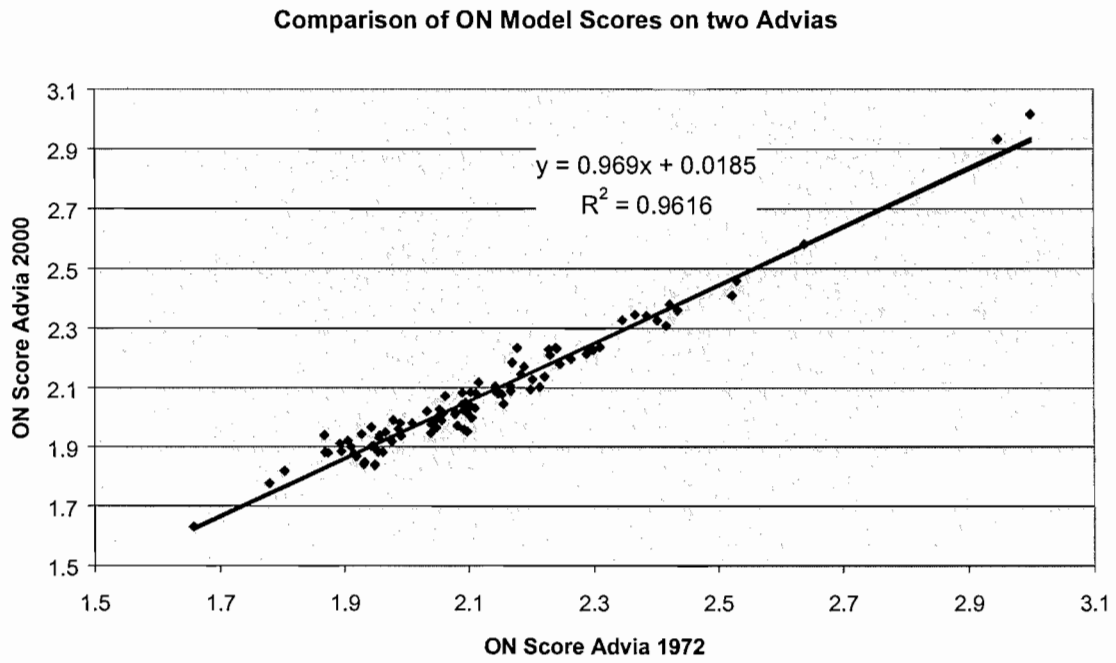


Figure 2



A number of problems were encountered in implementing the testing for EPO at the Games. The first problem was finding the specified athletes in the large Olympic complex without giving undue warning of the testing as the intention was for the test to be unannounced. The second was getting the blood samples to the laboratory within seven hours of collection. The laboratory was about 30 minutes by car from the Olympic complex but problems with tight security at the village meant that samples had to be carried by hand for up to a kilometer. The longest time delays occurred when the one or more of the athletes selected were unable to produce a urine sample at the time of blood collection. The protocol was modified so that when such delays occurred the blood samples were transported first and the urine sent later. The only problem that related to the laboratory testing procedure was the known variability in the calibration of the Bayer Advia instruments. There were two instruments installed in the laboratory one being a backup in case the first instrument failed. Both instruments were calibrated by the same engineer. The ON model scores calculated for 100 of the samples run on the two instruments are shown in Figure 3. Whilst the correlation is good (R^2 =0.96) a few samples had differences of 0.1 in the ON scores.

Figure 3



The ON scores obtained in the profiling study from over 700 male athletes around the world are shown in Figure 4 along the results for the male samples from the Sydney 2000 Olympic Games. Whilst all serum measurements for EPO and sTfr were made in the one laboratory the whole blood measurements were made on 14 different ADVIA instruments which means that inter-laboratory variation is a confounding factor when attempting to look at whether the ON score varies significantly with country or racial origin. All results were used for determining cut-off values for use in EPO detection. The mean ON score from the male Olympic samples is slightly lower than that found in the profiling study. The breakdown of male ON score by sport is shown in Figure 5.

As a result of carrying out the blood testing a number of points became apparent. The first was that rapid results were obtained with minimal sample preparation. This means that the blood test is an excellent screening procedure. However this only achieved by a large expenditure on capital equipment which is then under utilised. The capacity of each of the clinical analysers is hundreds of samples per day. A related problem is the high cost and short life of controls for the Bayer Advia 120, which mean the ongoing costs are high. The short time between sample collection and analysis also restricts a more general application of the test. For confirmation of positives serial testing and preferably urine testing is required.

There were some problems encountered with applying the urine test. The first is due to the complex nature of the procedure itself as it demands great skill and takes at least 48 hours to complete. Hence it is unsuitable for screening purposes and is really a confirmation method. The second problem encountered was the low level of EPO in some urines and the need to use larger volumes of urine. In some cases there was insufficient urine to permit a determination which could be repeated. The third problem was the occasional difference in the quality of the results obtained in Paris and Sydney and was due to the short time available for implementation of the test. This occurred despite training of our staff in Paris and having staff from the Paris laboratory at ASDTL during the Sydney Games. We have since solved this problem which related to air flow and drying out of gels during electrofocusing in our laboratory. The urine test is one that needs considerable time for implementation and minor problems can take considerable time to solve.

The results from the testing for EPO are summarised below:

- No positives were reported.
- The vast majority of samples had no indication at all of EPO use.
- A small number of samples produced results that warrant follow up due to high OFF model scores.
- The decision by the expert scientific panel to require a result from both the blood and urine tests before a positive was declared was the correct decision for the Sydney Games.

Following the successful application of the combined blood and urine test at the Sydney Games a number of questions arise. Does the test work? The answer is clearly yes both from the laboratory's perspective and from the obvious deterrent effect of the testing. Is the test only useful for an Olympic Games? Here the answer is no as the test is now being applied routinely by the Australian Sports Drug Agency for targeted out of competition testing. However this would not be happening if the equipment and expertise gained during the Games were not available. Can the test be set up in any IOC laboratory tomorrow? Yes it can but this raises the next question. Should the test be set up in every IOC laboratory? The answer is no at least not until the problems with the blood test such as the high capital and running costs of the equipment, availability of reference standards, and time limitations for the testing are overcome. Also the urine test should be simplified and made as robust as possible before general implementation.

Conclusions

The combined blood and urine test for the detection of doping with recombinant EPO worked well at the Sydney 2000 Olympic Games. The blood test is fast and relatively simple but currently requires expensive equipment not normally present in an IOC accredited laboratory. The relatively short time allowed between collection and analysis of the whole blood restricts the more general application of the blood test at present. The urine test in its current form is really a confirmation procedure and not suitable for high volume screening. However with further work it is very likely that a positive urine result alone will be sufficient to confirm doping with recombinant EPO. There is thus an urgent need for a simple screen, be it blood or urine, to be developed which can be used in an effective manner to select those urines on

which to run the full electrophoretic method. We are currently evaluating our EPO administration and profiling data to determine whether simpler, yet more sensitive blood screening models, can be developed.

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Appendix: Members of the EPO2000 consortium.

Country	Institution	Members
Australia	ASDTL	R. Kazlauskas, G. Trout, K. Emslie, C. Howe
Australia	AIS	A. Hahn, R. Parisotto, C. Gore, P Davis
Canada	University of Quebec	R. Gareau
China	China Doping Control Centre	M. Wu
France	LNDD	J. de Ceaurriz, F. Lasne
France	University of Montpellier	M. Audran
Norway	Norway University of Physical Education and Sport	J. Stray-Gundersen

Figure 4

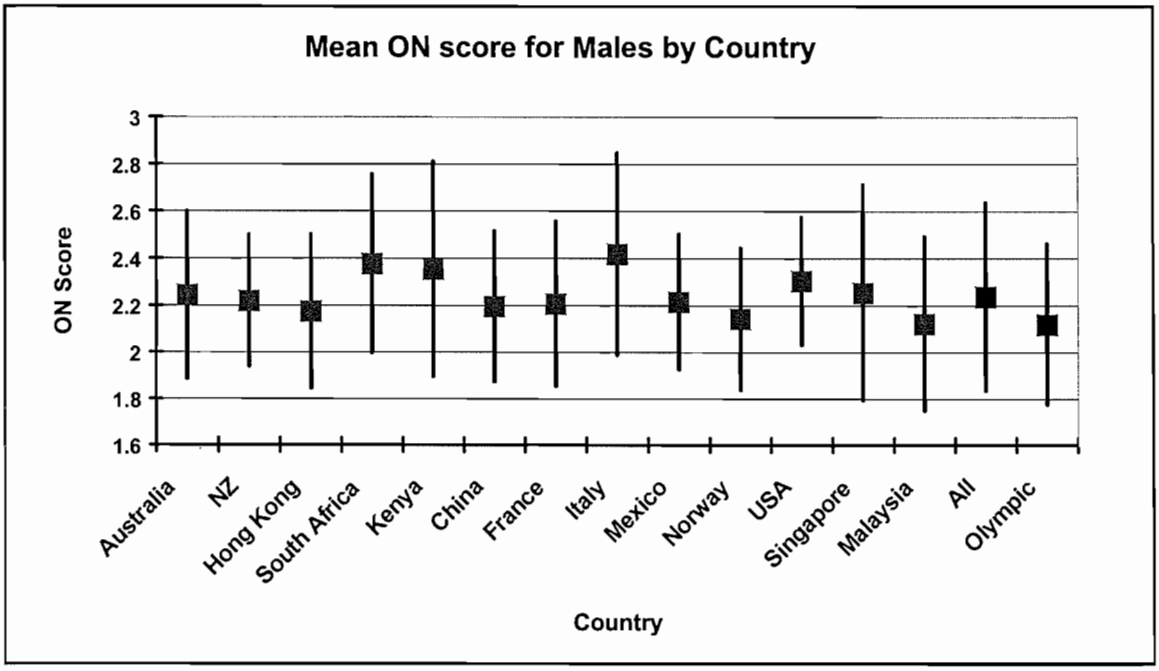

Figure 5