Institution: King's College London

Unit of Assessment: C26 - Sport, Exercise, Leisure and Tourism

Title of case study: Catching the Drug Cheats: The Science Behind Anti-Doping for the London 2012 Olympic and Paralympic Games

1. Summary of the impact

King's College London (KCL), operating a state-of-the-art Drug Control Centre (DCC) in collaboration with GlaxoSmithKline (GSK), delivered the anti-doping analysis at the London 2012 Olympic and Paralympic Games. This operation, undertaken in their World Anti-Doping Agency's (WADA) accredited laboratories, was characterised by unprecedented scale, speed and accuracy. It succeeded in protecting the health of athletes and the integrity of the Games. KCL was chosen to undertake the 24/7 anti-doping operation based on its cutting-edge bio-analytical research in drug control. Although a number of athletes were disqualified in the pre-Games testing, the deterrent effect of the KCL work was evidenced by the few doping cases during the Games itself. Using the new biomarker test developed by the DCC at KCL in collaboration with colleagues at the University of Southampton, the team identified for the first time the administration of recombinant human growth hormone (hGH) in two athletes. The findings of the KCL-led operation are already being used to develop similar testing facilities for the 2016 Olympic Games in Rio de Janeiro and have opened up the science of drug-testing to schools through the "Scientists in Sport" initiative.

2. Underpinning research

The Drug Control Centre (DCC) at King's College London (KCL) is at the forefront of research into the detection of drug abuse in sport, taking the lead in changing the way in which anti-doping analysis is conducted both in the UK and internationally. The underpinning research has been led by Professor David Cowan, Director of the DCC since 1990, and has resulted in the development of new and improved analytical approaches for the detection of a number of prohibited substances in sports. This research has also involved Dr Andrew Kicman (1990-present, Head of Research & Development Group), Dr Norman Smith (2002-present, Senior Research Fellow), Dr Alan Brailsford (2004-present, Post-Doctoral Analyst), Dr Ivana Gavrilovic (2007-present, Post-Doctoral Research Analyst), Dr Alessandro Musenga (2010-present, Post-Doctoral Analyst), Dr Mark Parkin (2005-present, Lecturer in Analytical Science) and Dr Christopher Walker (1986-present, Senior Analyst).

Key examples of cutting-edge bioanalytical research undertaken at the DCC to deter drug misuse have included the development and application of the first tests for stimulants (such as amphetamines), anabolic steroids and the protein hormone human chorionic gonadotrophin, as well as the development of more complex tests to confirm administration of the naturally produced anabolic steroid 5α-dihydrotestosterone.

Human Growth Hormone (hGH): In collaboration with the University of Southampton, work on hGH detection has been pursued to develop a reliable biomarker approach for detection of misuse with a longer window of detection than was provided by existing approaches (Erotokritou-Mulligan I, *et al.* 2009). This includes characterising physiological variability of two markers of exogenously administered hGH: insulin-like growth factor-1 (IGF-1) and pro-collagen type III N-terminal peptide. For this work, DCC scientists were essential in helping plan the research from the anti-doping perspective and performing all of the analytical measurements. World Anti-Doping Agency's (WADA) accreditation of the DCC was an essential part of making the approach legally defensible for anti-doping work. The development of ultra-high-performance liquid chromatography/tandem mass spectrometry for quantifying IGF-1 is helping further to enhance detection methodology.

Nandrolone: In a study funded by the United States Anti-Doping Agency, scientists at the DCC refined the detection of misuse of this anabolic steroid by using state of the art gas chromatography-tandem mass spectrometry to define the upper limits for urinary 19-norandrosterone (19-NA), the principal urinary metabolite of nandrolone. This research was undertaken on samples taken from a large cohort of healthy women not taking anabolic steroids

but including those using permitted contraceptives containing norethisterone (Walker CJ, *et al.* 2009). These important results confirmed that the WADA revised threshold for 19-NA for females was acceptable, negating the need for a more complex approach for 19-NA analysis. New techniques have also been developed to enable the administration of nandrolone to be distinguished from the oral contraceptive norethisterone.

In additional work, KCL researchers have improved the ability to detect the administration of naturally produced substances, especially the male hormone testosterone, by using chemically synthesised molecularly imprinted polymeric substances rather than using antibodies produced from animals (Gavrilović I, *et al.* 2011). They have also furthered the development of combustion isotope ratio mass spectrometry, including sophisticated two-dimensional gas chromatography to simplify and improve sample purification (Brailsford AD, *et al.* 2012).

Improvements achieved in both performance and efficiency of doping control analysis include the development and validation of a simple, but sensitive and rapid, analytical procedure based on flow cytometry to detect human haemoglobin and haemoglobin-based oxygen carriers potentially misused in endurance sports. KCL researchers have also developed methods to deal with the quantification of difficult basic drugs such as ephedrines (Gray N, *et al.* 2011) and a fast multi-analyte screening method specifically developed for the detection of prohibited xenobiotics in urine that allows the screening of several classes of substance in a single chromatographic method with a very rapid run-time (Musenga A, *et al.* 2013). These novel techniques proved invaluable in the anti-doping analysis at the London 2012 Olympic and Paralympic Games. Post Games, and of particular note, is the ability to review archived data to be able to check for new 'designer' drugs to see whether athletes may have misused them under the WADA 8 year rule to be able to re-investigate samples (Musenga A, *et al.* 2013).

3. References to the research

Brailsford AD, Gavrilović I, Ansell RJ, Cowan DA, Kicman AT. Two-dimensional gas chromatography with heart-cutting for isotope ratio mass spectrometry analysis of steroids in doping control. Drug Test Analysis 2012;4:962-69. Doi: 10.1002/dta.1379 (1 Scopus citation)

Erotokritou-Mulligan I, Bassett EE, Cowan DA, Bartlett C, Milward P, Sartorio A, Sönksen PH, Holt RIG. The use of growth hormone (GH)-dependent markers in the detection of GH abuse in sport: Physiological intra-individual variation of IGF-I, type 3 pro-collagen (P-III-P) and GH-2000 detection score. Clin Endocrinol 2009;72:520-26. Doi: 10.1111/j.1365-2265.2009.03668.x (15 Scopus citations)

Gavrilović I, Mitchell K, Brailsford AD, Cowan DA, Kicman AT, Ansell RJ. A molecularly imprinted receptor for separation of testosterone and epitestosterone, based on a steroidal cross-linker. Steroids 2011;76:478-83. Doi: 10.1016/j.steroids.2011.01.004 (7 Scopus citations)

Gray N, Musenga A, Cowan DA, Plumb R, Smith NW. A simple high pH liquid chromatography-tandem mass spectrometry method for basic compounds: Application to ephedrines in doping control analysis. J Chromatogr A 2011;1218:2098-105. Doi: 10.1016/j.chroma.2010.10.104 (6 Scopus citations)

Musenga A, Cowan DA. Use of ultra-high pressure liquid chromatography coupled to high resolution mass spectrometry for fast screening in high throughput doping control. J Chromatography A 2013;1288:82-95. Doi: 10.1016/j.chroma.2013.03.006 (Recent paper, no citations)

Walker CJ, Cowan DA, James VH, Lau JC, Kicman AT. Doping in Sport - 1. Excretion of 19-norandrosterone by healthy women, including those using contraceptives containing norethisterone. Steroids 2009;74:329-34. Doi: 10.1016/j.steroids.2008.10.008 (8 Scopus citations)

Research Funding (PI Professor David Cowan):

- 2005-7. Norethisterone. US Anti-Doping Agency, £120,000
- 2005-8. Development of novel high sensitivity and specific methods to provide reliable forensic evidence of drug administration in vulnerable groups. Engineering and Physical Science Research Council, £300,297

- 2007-9. Confirmation of Doping with Natural Androgens by Isotope Ratio Mass Spectrometry; simplifying the analytical Procedure and Increasing the Evidential Power. World Anti-Doping Agency, £150,250
- 2010-11. Can genomic analysis be the answer to autologous blood transfusion detection? Partnership for Clean Competition, £81,704
- 2011-13. RNA based testing for the detection of autologous blood transfusion. Partnership for Clean Competition, £144,920
- 2012-13. Subject-based profiling for the detection of testosterone administration in sport-investigating the value of serum analysis. World Anti-Doping Agency, £64,254

4. Details of the impact

The bio-analytical research in forensic drug analysis by King's College London (KCL) researchers into the testing of sports competitors for prohibited substances has been recognised as world-leading. In support of the work of the Drug Control Centre (DCC), the Chief Science Officer of the US Anti-Doping Agency (USADA) confirms that "*the underpinning research at the internationally renowned Drug Control Centre has developed new and improved analytical approaches for prohibited substances in human sports drug testing*." He goes on to detail, referring to the specific studies discussed above, how "*the research knowledge and expertise of the Centre continue to have significant impact in the field of anti-doping*" (1).

In 2002, due to their expertise and extensive research on methods for detecting prohibited substances, the DCC at KCL (2) delivered anti-doping testing for the Commonwealth Games in Manchester. Following this, the World Anti-Doping Agency (WADA) produced an independent observer's report that, in reference to the DCC, said that "future Games Laboratories [should] adopt the procedures for analysis as carried out in this highly professional and effective laboratory" (3). This expertise led the DCC to become a key advisor to testing laboratories for the 2008 Summer Olympic Games in Beijing and the 2010 Commonwealth Games in Delhi. Most significantly, the DCC was then chosen to run the anti-doping facility at the London 2012 Olympic and Paralympic Games. As such, the DCC played a pivotal role in protecting the health of the athletes and ensuring these Games were conducted to the highest ethical standard, as reflected in a number of worldwide media articles at the time (4a-d). Due to his role as Director of the DCC, Professor Cowan was also a member of the London 2012 Summer Olympic Games Bid team, the London Organising Committee of the Olympic and Paralympic Games and was the Laboratory Representative on the International Olympic Committee's Medical Commission (posing no conflict of interest in respect to winning the anti-doping contract as decisions were made independently).

The testing of competitors for prohibited substances was successfully delivered from a dedicated state-of-the-art laboratory set up at the research and development site of the pharmaceutical company GlaxoSmithKline (GSK) at Harlow in Essex. This Anti-Doping Science Centre (ADSC) carried out analysis of more than 6,250 samples (up to 400 per day) throughout the Games, 24 hours a day, 7 days a week, using instruments in accordance with the highest possible standard of accreditation for analytical work and accredited by WADA. The ADSC employed nearly 400 staff in total, including 37 DCC core staff and over 175 temporary analysts and overseas scientists.

Methodologies were utilized that had been developed by the DCC. These include novel high resolution mass spectrometric screening method for all of the urine samples (Musenga 2013) and the improved isotope ratio mass spectrometric method for confirmatory testing of all samples where there was suspicion of endogenous steroid administration (Brailsford 2012). In a letter of support, the GSK Director for this operation writes that: "*As the Drug Control Centre has been in the forefront of introducing advances in anti-doping science for many years, we were particularly impressed by their analytical chemistry ability which is evidenced by their publication record, especially in such challenging areas as quantitative handling of basic compounds, working with minor contaminants such as those found in contraceptive steroids and the application of biomarkers at an evidential forensic level*" (5).

The unprecedented scale of the anti-doping operation at London 2012 is demonstrated by statistics revealing that over 5,000 athletes' samples were tested during the Olympics and a further 1,400 during the Paralympic Games, equating to almost 50% of the competitors and including all medal

winners. Working in a highly regulated environment, where confidentiality and security were paramount at all times, DCC staff demonstrated their track record of quality and delivery. Both these attributes came to the fore in an environment that mandated not only a rapid turnaround of results, but also accurate identification of prohibited substances and interpretation of the findings. Indeed, unequivocal identification is essential, since laboratory findings may be the subject of prolonged and intense legal scrutiny. Negative analytical findings were securely reported no later than 24 hours of receipt at the laboratory and even adverse analytical findings were reported within very short time-frames. The latest research on the interpretation and application of the human growth hormone (hGH) biomarker projects described above were accepted by WADA and resulted in the undisputed disqualification of two Paralympic athletes for hGH misuse, as described in the letter from USADA (1).

The procedures set up at the ADSC for the London Olympic Games created a base of knowledge around operations and processes. As such, the DCC is currently working with scientists who will set up a similar laboratory in Rio de Janeiro for their 2016 Olympic and Paralympic Games (6). The DCC also continuously works with the UK Anti-Doping Agency (UKAD), providing assistance through their research and expertise. In a letter of support, the Chief Executive of UKAD testifies that "*the Drug Control Centre is the current provider of comprehensive analytical services to UK-Anti-Doping and has demonstrated not only its ability to provide consistent tests but also its commitment to lead the science behind anti-doping testing*." He goes on to describe how UKAD has "*valued the advice and experience of the DCC*" and how they are *"experienced in presenting its analytical results at athlete hearings, appeals and courts of law both nationally and internationally*" (7).

In partnership with GSK, the DCC has delivered further impact based on its drug monitoring programme role through public outreach programmes such as "Scientists in Sport" (8). These encompass the promotion of anti-doping messages as well as chemistry and science within schools and the broader community through visits to/presentations at both the Harlow Olympic testing facility and the DCC laboratories at KCL. Professor Cowan has also served on the WADA Laboratory Accreditation sub-committee and the Council of Europe Working Party Investigating Drug Abuse in Sport that led to the first World Anti-Doping Convention.

5. Sources to corroborate the impact

1. Letter of professional support from the Chief Scientific Officer of the US Anti-Doping Agency, Colorado Springs, CO, USA (on request)
2. Drug Control Centre Website: www.kcl.ac.uk/biohealth/research/divisions/aes/research/drugcontrol/index.aspx
3. Observer's Report XVII Commonwealth Games, Manchester, 25th July To 4th August: http://www.wada-ama.org/rtecontent/document/manchester.pdf
4. Media coverage:
   a. Olympics-London 2012 to be most tested Games, says WADA expert. Reuters (US) June 16 2011: http://www.reuters.com/article/2011/06/16/olympics-london-doping-idUSLDE75F24220110616
   b. ANALYSIS-Olympics-Testers gear up for sophisticated dopers.Reuters (US) 19 July 2011: http://www.reuters.com/article/2011/07/19/olympics-london-doping-idUSL6E7HU05Y20110719
   c. Doping: Journey of a sample at London 2012 Olympics. Reuters (Canada) 19 Jan 2012: http://ca.reuters.com/article/sportsNews/idCATRE80I1Z520120119
   d. London Olympic lab declared ready by WADA.ESPN 23 April 2012: http://sports.espn.go.com/espn/wire?section=oly&id=7844695
5. Letter of professional support from the Director of London 2012 operations at GlaxoSmithKline, Brentford, UK (on request)
6. Letter of professional support from the Laboratory Director of the 2016 Rio Games, Rio-de-Janeiro, Brazil (on request)
7. UKAD website: http://www.ukad.org.uk/ and letter of professional support from the Chief Executive of UKAD, London, UK (on request)
8. Scientists in Sport: http://www.scientistsinsport.com/resources/london-2012-resources.aspx