Institution: King's College London		
Unit of Assessment: 24 Sport and Exercise Sciences, Leisure and Tourism		
Title of case study: Catching those who cheat: Supporting the fight against doping in sport		
Period when the underpinning research was undertaken: 2009 - 2019		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Professor Kim Wolff	Head of Drug Control Centre	From 1997
Professor David Cowan	Emeritus Professor	From 1971
Dr Mark Parkin	Lecturer in Analytical Science, Analytical, Environmental & Forensic Sciences	2005 – 2017
Professor Stephen Harridge	Director, Centre for Human & Applied Physiological Sciences	From 2005
Professor Denise Syndercombe Court	Professor Forensic Genetics	From 2010
Period when the claimed impact occurred: 2014 - 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Doping violates the spirit of sports and is potentially damaging to both the careers and health of athletes, yet it persists. Indeed, the prevalence of the use of performance enhancing drugs in sport is a dynamic challenge that is continuously evolving. The King's College London Drug Control Centre (DCC) is at the forefront of introducing advances in anti-doping science, contributing to both research and providing state-of-the-art laboratory services to both national and international anti-doping agencies. The DCC has developed unique research and analytical improvements to laboratory methods which have led to a strengthening in the UK's anti-doping programme. Research has been integrated into the practice and policy of the World Anti-Doping Agency and has been used successfully to support anti-doping efforts at international competitions and major games around the world. In addition, DCC research has been integral to high profile international anti-doping investigations such as the 2016 McLaren report, which identified numerous anti-doping violations in Russia.

2. Underpinning research

As doping agents evolve and new ways of cheating develop, the requirement to monitor banned substances and maintain analytical competency in sport drug testing means anti-doping is a continuous challenge for scientists. Researchers at the King's College London DCC have undertaken investigations targeted at increasing the understanding and detection of the compounds misused by athletes. The approach has been to detect an ever-greater number of prohibited substances at low concentrations in biological fluids, whilst enhancing testing capability, increasing the quality of analytical data, its sensitivity, and its evidential quality. New knowledge in enhancing the fight against doping in sport as a result of the research from the DCC is outlined in the two examples below.

i. Improving the detection of Human Growth Hormone (hGH)

hGH is a naturally occurring peptide hormone also available for medicinal purposes in a recombinant form (rhGH). Its attraction as a doping agent in sport, includes the reduction of body fat, a potential increase in muscle mass and strength (anabolic effect), and tissue-repairing effects (recovery and increased collagen synthesis). However, distinguishing natural hGH from the recombinant forms and marked inter-individual variability makes detection of doping extremely difficult. Initial work saw King's researchers use acetonitrile protein depletion with a 5

min ultra-high-performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS)-based selected reaction monitoring (SRM) assay to detect both insulin-like growth factor I (IGF-I) and leucine-rich α-2-glycoprotein (LRG) at endogenous concentrations in human serum as a viable means of protein quantification in sports competitors [1]. The DCC scientists, in collaboration with endocrinologists at the University of Southampton, then worked to improve the detection of hGH administration by athletes using a biomarker approach: an indirect method to target biomarkers related to hGH that remain in the body for longer [2,3]. King's researchers successfully advanced the development of methods for the detection of IGF-I and the pro-collagen type III N-terminal peptide (P-III-NP) [2,4]. Since 2015, this collaborative research has improved the test through the introduction of robust and validated decision limits based on the "GH score" (calculated using the IGF-I and P-III-NP concentrations). These limits are now applied to samples from athletes for the detection of hGH administration, offering a more sensitive analytical approach [5] and have been approved for use by the World Anti-Doping Agency (WADA).

The detection and quantification of proteins, in particular of peptide hormones in urine, has continued to pose analytical challenges in sports testing. Historically, testing has relied on the measurement of relevant biomarkers using immunoassay tests, which have been associated with varied performance, poor correlation among methods and differential cross-reactivity. King's researchers developed a superior approach for the analysis of IGF-I and P-III-NP peptides with improved sensitivity and evidential quality [6]. They were able to differentiate between exogenous (illicit) peptides and those found endogenously in the body by pioneering the use of tryptic digestion and subsequent micro/nano-liquid chromatography-mass spectrometry (LC-MS), with two surrogate tryptic peptides (hT1 and T5) for the mass spectrometric confirmation of P-III-NP. This facilitated the detection of P-III-NP in a human serum sample by LC-MS for the first time [6]. This research has been recognised by awards such as the Barber prize for Mass Spectrometry (British Mass Spectrometry Society, 2017) and best article (Partnership for Clean Competition Funded Research, 2018).

ii. Developing novel anti-doping methods to identify illegal sample manipulation

Ensuring the integrity of the biological samples provided by athletes is critical to the anti-doping system. Athletes seeking to subvert the system may try to manipulate their sample in such a way that the subsequent analytical results are less likely to demonstrate that they have been taking a prohibited substance. An example of this behaviour is providing an alternative "clean urine" sample from themselves or a colleague which can negatively affect the analytical data. To solve this problem the DCC developed sensitive and rapid methods to detect and profile DNA within an athlete's urine [7]. This meant that it was now possible to verify that the correct athlete provided the sample.

3. References to the research

[1] Kay RG, Barton C, Velloso CP, Brown PR, Bartlett C, Blazevich AJ, Godfrey RJ, Goldspink G, Rees R, Ball GR, **Cowan DA, Harridge SDR**, Roberts J, Teale P, Creaser CS (2009). High-throughput ultra-high-performance liquid chromatography/ tandem mass spectrometry quantitation of insulin-like growth factor-I and leucine-rich a-2-glycoprotein in serum as biomarkers of recombinant human growth hormone administration. *Rapid Communications in Mass Spectrometry*; 23: 3173–3182. DOI: 10.1002/rcm.4237.

[2] Velloso C, Godfrey R, Blazevich A, Bartlett C, **Cowan DA,** Bouloux PM, **Harridge SDR,** Goldspink G (2013). The effects of two weeks recombinant growth hormone administration on the response of IGF-I and PIIIP to a single bout of high resistance exercise in resistance trained young men. *Growth Hormone & IGF-I Research* 23:76-80. DOI: 10.1016/j.ghir.2013.01.002

[3] Lopes F, **Cowan DA**, Thevis M, Thomas A, **Parkin MC** (2014). Quantification of intact human insulin-like growth factor-I in serum by nano-ultrahigh-performance liquid chromatography/ tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*. 15;28(13):1426-32. DOI: 10.1002/rcm.6908.

[4] Guha N, Erotokritou-Mulligan I, Bartlett C, Nevitt SP, Francis M, Bassett EE, **Cowan DA**, Sönksen PH, Holt RI (2014). Biochemical markers of insulin-like growth factor-I misuse in

athletes: the response of serum IGF-I, procollagen type III amino-terminal propeptide, and the GH-2000 score to the administration of rhIGF-I/rhIGF binding protein-3 complex. *The Journal of Clinical Endocrinology and Metabolism*. 99(6):2259-68. DOI: 10.1210/jc.2013-3897.

[5] Holt RI, Böhning W, Guha N, Bartlett C, **Cowan DA**, Giraud S, Bassett EE, Sönksen PH, Böhning D (2015). The development of decision limits for the GH-2000 detection methodology using additional insulin-like growth factor-I and amino-terminal pro-peptide of type III collagen assays. *Drug Testing Analysis*. 7(9):745-55. DOI: 10.1002/dta.1772.

[6] Moncrieffe D, **Parkin MC**, **Cowan DA** (2018). Peptide selection for the quantification of P-III-NP in human serum by mass spectrometry. *Rapid Communications in Mass Spectrometry* 15;32(7):535-542. DOI:10.1002/rcm.8066.

[7] Devesse L, **Syndercombe Court D, Cowan DA** (2015). Determining the authenticity of athlete urine in doping control by DNA analysis. *Drug Testing Analysis*. 7(10):912-918. DOI: 10.1002/dta.1785.

4. Details of the impact

The King's College London DCC seeks to protect both the integrity of sport and the health of athletes and is the only WADA accredited laboratory in the UK. The DCC aims to increase awareness of the danger of doping for the health of those participating in sport at any level and keep up with the multifaceted technical nature of cheating in sport to ensure that the UK remains at the forefront of the fight against doping. The DCC has collaborated with key actors in the anti-doping world, ensuring that cutting edge science remains effectively employed in practice and policy.

Shaping world class testing

As the only testing centre accredited by WADA in the UK, the DCC has, since 1978, provided robust and trusted analytical services. Due to the high quality of work and their impact on standing in the anti-doping community, the total number of samples collected and analysed by the DCC continues to rise year on year. In 2015, the DCC analysed 8,800 samples, whilst in 2018 this number had increased to 12,587 samples, demonstrating that professional sports bodies are increasingly acknowledging the importance of testing athletes as a deterrent to the use of performance enhancing drugs. Using the accredited laboratories at King's as an advanced and trusted testing site, DCC research has impacted the shape and trajectory of testing in the UK.

King's researchers have a proven track record in hGH research [2,3,4]. Working closely with WADA the DCC team developed a new methodological approach to test for hGH, the Human Growth Hormone (hGH) Biomarkers Test. This test was incorporated into guidelines published by WADA in 2015 for all anti-doping laboratories through across the world [A]. These guidelines provide the regulatory documents, implementation processes and monitoring procedures to ensure that tests are carried out systematically and that a harmonised approach is taken across all test centres. As the Senior Deputy Director of the Science and Medicine department at WADA acknowledges, *"the research output of the DCC was pivotal in the establishment of legally defensible biomarker scores and the implementation of the WADA Guidelines - Human Growth Hormone (hGH) Biomarkers Test in 2015"* [B]. [text removed for publication]

Appropriate long-term storage and further analysis of archived samples collected from athletes has emerged as an important factor in the protection of clean sport. Under the World Anti-Doping Code, samples can be stored for up to 10 years after their initial analysis and still retain the same legal impact if analysed further retrospectively. Taking advantage of DCC research to improve techniques and detect novel compounds, King's researchers have been able to conduct enhanced retrospective analysis on over 100 samples, with more planned.

Shaping practice and policy in UK anti-doping (UKAD)

UKAD, as the organisation responsible for protecting sport in the UK from doping, works towards 'protecting the right to participate in clean sport'. The DCC is the predominant laboratory used by UKAD for the analysis of athlete samples with the aim of catching those in sport who cheat.

In 2018, the Department for Digital, Culture, Media and Sport published a 'Tailored Review of UKAD' [D] which contained an assessment of current services along with 45 recommendations

for the future. The report highlighted the close partnership between the UKAD and the DCC, stating the King's centre as the *"most impressive example and contributes to the UK's international standing in sport and anti-doping"* [D]. The report recommended a 50% increase in testing across sport, and in January 2018, the UK Government gave UK anti-doping a £6 million funding boost to educate athletes, share intelligence and conduct testing in the fight against drug cheats to keep sport clean [E]. As a result, the number of samples analysed by the DCC has increased to nearly 13,000 in 2019. [text removed for publication].

Shaping antidoping testing at key events

The DCC has provided the analytical science to ensure that high class anti-doping is provided at the world's premier sporting events to maintain fairness and safety. After successfully supporting testing at the London 2012 Olympic Games the DCC team have gone on to support the development of policy, practice and process in a variety of settings.

For example, King's research has ensured that major games now use techniques from mass spectrometry, detecting peptide and growth hormones more effectively. Former director of the Brazilian Doping Control Laboratory noted that *"the breakthrough of growth hormone exogenous administration detection for the first time in 2012, showed the athletic community that the era of protein doping was due to be over"* [G]. As a result, more compounds were tested at the 2016 Rio Games than any other previous games ensuring that the health of the athletes and tests conducted are of the highest ethical standard, giving confidence to individual athletes that major competitions are as fair as possible.

By providing communication skills, laboratory organisation, procurement processes, training of personnel and volunteers, researcher exchanges and advanced training the DCC significantly impacted on the preparations and processes during the Games. As the former director of the Brazilian Doping Control Laboratory stated *"this sizeable contribution of Professor Cowan, the DCC and King´s College London surely had an impact on the quality of the doping control analysis performed at the Rio 2016 games"* [G]. The Independent observer for the Rio 2016 Olympic Laboratory report stated *"the laboratory was superbly equipped, operated very securely and generally very efficiently, and now represents an outstanding legacy from the Games for the anti-doping movement in South America"* [M].

In addition, the King's team impacted on the preparations for the PyeongChang Winter Olympic Games 2018. As stated by the former head of the South Korean WADA laboratory at the Korea Institute of Science and Technology in Seoul, the DCC were *"key in helping us to fulfil our work to the international standard required… for the preparation of 2018 Games, I have made the contract with [DCC] for solution of WADA ISL requirements in staff, facility, security and scientific methods. They were involved in training our staffs, designing document systems for traceability of overall process of sample analysis, preparing WADA site visits, and defending WADA reports requested after the result of the site visit… [the DCC] was the best supporter to us in advising and suggesting the technical solutions of the sample analysis for its successful achievement"* [H].

Investigating doping violations - Russia

Following a 2014 German TV documentary which contained allegations of widespread doping in Russia, WADA established an Independent Commission (IC) to conduct an investigation into the allegedly corrupt practices around sample collection and results management that implicated athletes, coaches, trainers, and doctors, as well as the accredited laboratory based in Moscow and the Russian Anti-Doping Agency (RUSADA) in doping violations. As one of the most important events in the world-wide anti-doping community for many years, the WADA President appointed Professor Richard McLaren to lead the IC, with findings reported in 2015 and 2016 [I].

King's researchers contributed significantly to the IC, utilising new research and techniques to provide validated methodologies which could detect sample manipulation with salt [J], and urine swapping through DNA analysis [7]. Prof McLaren turned to the DCC since they either *"had in their repertoire of analytical methods or rapidly developed methods that provided me with legally defensible evidence that samples had been manipulated as claimed by Dr Rodchenkov. This greatly assisted me in proving the involvement of individuals who could then be identified in my formal and public reports"* [K].

The IC ultimately proved systematic cheating and a universal ban was placed on Russian athletes participating in worldwide sporting events such as Rio 2016 Olympics and Paralympics [L] as well as the WADA accreditation of the Moscow Laboratory being revoked. As noted by Prof McLaren *"Without the assistance of the Drug Control Centre, my investigation would at best have been delayed and possibly may not have been able to reach the clear conclusions as published… I am very indebted to the DCC for the very significant contributions they made to my work"* [K].

These events had a huge impact on international sport, demonstrating to the world that the fight against doping is being taken incredibly seriously, and those who seek to subvert the system, no matter how powerful, will be heavily sanctioned. As a result, large organisations such as WADA and the International Olympic Committee have reiterated their critical commitment to the maintenance of fairness in competition, where King's researchers continue to play a key role.

5. Sources to corroborate the impact

[A] WADA World Anti-Doping Program (April 2016) Guidelines: Human Growth Hormone (hGH) Biomarkers Test for Doping Control Analyses Version 2.0

[B] Testimonial from Senior Deputy Director Science, Laboratories, World Anti-Doping Agency

[C] [text removed for publication]

[D] Department for Digital, Culture, Media & Sport (Jan 2018) Tailored Review of UK Anti-Doping

[E] Gov.uk (Jan 2018) UK Anti-Doping receives £6 million funding boost [press release]

[F] [text removed for publication]

[G] Testimonial from Director of the Laboratory for the Support of Technological Development Chemistry Institute Federal University of Rio de Janeiro

[H] Testimonial from former head of the South Korean WADA laboratory, Korea University Science & Technology, Korea Institute of Science and Technology

[I] World Anti-Doping Independent Commission Investigation (Nov 2015) The Independent Commission Report #1: Final Report and (Jan 2016) The Independent Commission Report #2

[J] Angeli Mehta (2016) Anti-doping scientists expose cheating Russian athletes, ChemistryWorld.com

[K] Testimonial from Professor Richard McLaren, O.C., DD, Faculty of Law, Western University, London, Canada, CEO, McLaren Global Sport Solutions Inc.

[L] Olympic.org (July 2016) Decision of the IOC Executive Board Concerning the Participation of Russian Athletes in the Olympic Games Rio 2016 [news article]

[M] World Anti-Doping Agency (2016) Report of the Independent Observers Games of the XXXI Olympiad, Rio de Janeiro 2016