Don’t be a dope!

_Nina Notman talks to the scientists set to beat the doping cheats at the 2012 London Olympic Games_

“We’ll catch you.” This is the message from the UK government to any athlete planning to dope at this summer’s Olympic Games in London.

Doping means the use of illicit substances or methods to improve athletic performance. It was banned in the 1920s, and is still a growing problem in professional sports. The constant pressure on athletes to win has driven the development of novel and highly sophisticated doping techniques, as wannabe cheats struggle to stay one step ahead of the scientists aiming to expose them.

**The lab**

This year the chances of not being caught are smaller than ever. A laboratory the size of seven tennis courts will be testing athlete’s urine and blood samples 24 hours a day, seven days a week during the Olympic and Paralympic Games. Up to 400 samples will be analysed each day, 6250 in total – more than any previous Games.

The anti-doping lab is based at the GlaxoSmithKline (GSK) site in Harlow, Essex. However, the pharmaceutical company will not be running the testing there. That is the job of David Cowan, a professor at King’s College London’s Drug Control Centre. With the help of anti-doping experts from around the world, Cowan will be overseeing the 150 analysts conducting the tests.

**The process**

Approximately half the athletes competing, including all Olympic medallists, will provide urine and blood samples for anti-doping analysis. These samples will be divided in two, labelled with barcodes (no names) and...
fitted with tamper-proof tops. They will then be couriered from London to the anti-doping lab.

‘The samples arrive in two bottles called the A and the B samples,’ explains Nicola Gray, one of Cowan’s PhD students and an analyst in this summer’s Olympic labs. ‘We freeze the B sample in case we need to do any further tests on it later,’ Sample B will be opened and analysed if sample A tests positive for a doping drug or method. Retesting will also occur up to eight years later if a new doping drug or method is discovered by authorities, to see if it had been by athletes used before scientists knew to look for it.

‘We open the A sample and spit it into smaller portions to undergo different tests,’ Gray explains. More than 240 different known doping drugs and methods will be looked for, with the majority of negative results being reported in less than 24 hours.

Weeeeee Gray herself will be looking for stimulants and diuretics in urine samples during the Games. Stimulants – such as ephedrine and amphetamines – are taken to boost alertness, reduce tiredness and increase competitiveness and aggression. Meanwhile, diuretics increase the amount of urine produced and are banned for two reasons. Firstly, they aid dehydration and therefore weightloss – important to fitting into weight classes for sports such as weightlifting and judo. Secondly, they water down urine making it more difficult for other banned drugs to be detected.

Before analysis, the urine needs to be cleaned up. ‘There are a lot of other compounds in urine that would interfere with the analysis, such as salts, metabolites, proteins and hormones,’ Gray explains. A solid phase extraction method is used to remove these, which takes advantage of the differences in the properties of the compounds. ‘We load the sample into a cartridge, and the analytes we want to look at are trapped inside while we wash it with a range of solvents to get rid of all the rubbish,’ she says. The analytes of interest are then washed off the cartridge into a test tube using a different solvent again. The sample is now ready for testing.

The analytical method Gray uses is liquid chromatography coupled to a mass spectrometer detector, a technique known as LC-MS. The chromatography stage separates the analytes in the sample, while the mass spectrometer detects and records their masses. ‘We look for the masses of the banned compounds at characteristic times in the chromatogram and see if they are there or not,’ Gray explains.

Mimicking nature
Christiaan Bartlett, an analyst at King’s Drug Control Centre, is one of the experts overseeing the Olympic lab’s activities. Bartlett is personally involved in the detection of synthetic – or recombinant – erythropoietin (EPO) and human growth hormone (HGH). EPO and HGH are both naturally present in the body, making detection of recombinant versions more difficult.

EPO is a hormone that regulates red blood cell production. Injecting recombinant EPO will increase an athlete’s red blood cell count, improving their aerobic capacity and delaying fatigue. HGH regulates cell reproduction and regeneration. The ‘benefits’ of HGH doping remain disputed; one claim being that it reduces recovery time between training sessions, allowing athletes to train harder.

‘There is a subtle difference between the naturally occurring form of EPO that everyone produces and the recombinant EPO that you would inject yourself with if you were trying to cheat,’ Bartlett says. ‘We utilise that very slight difference in our method for differentiating in one athlete whether the EPO in them is natural or recombinant.’

Recombinant EPO is made by injecting the human gene that codes for EPO into hamster cells, he explains. ‘The hamster cells then produce their version of human recombinant EPO.’

Natural and recombinant EPO protein molecules have the same amino acid backbone. However, due to the different cells in which they are made, different carbohydrates are attached to this backbone. These different attachments give the two molecules a slightly different charge. ‘It is that difference in charge that allows us to separate the two forms,’ Bartlett explains.

‘The test is the most complex test that we run.’ As with Gray’s LC-MS procedure, the first step in EPO analysis is sample clean up – removing the other more abundant proteins from the urine. Again it is passed through a cartridge, this time containing antibodies selective for EPO. The EPO molecules (both natural and recombinant)
in the urine stick to the column, while everything else is washed through. A buffer is then used to release the EPO from the column, and it is collected for analysis.

To determine if there is any recombinant EPO present in the sample, a technique called isoelectric focusing is used. The EPO is placed on a gel with a pH gradient, and an electrical current is passed through it. ‘All of the proteins will move through the gel based on their charge,’ Bartlett says, ‘recombinant EPO moves closer to the cathode compared to natural EPO.’ The gel is then visualised and assessed to see if recombinant EPO is present.

The HGH test also takes advantage of a slight difference between its recombinant and natural versions. ‘Natural HGH is made up of a variety of different molecular weight proteins, known as isoforms,’ Bartlett says. To make recombinant HGH, ‘the gene that codes for just one of these isoforms is injected into animal DNA, making the recombinant HGH that the animal produces a pure form of one isoform only,’ he explains.

‘If you consider a tube of Smarties you have lots of different colours, always in the same sort of proportions. Imagine recombinant HGH is the blue Smartie. If you poured 20 blue Smarties into a tube you would have a change in the ratio of blues compared to all the other colours,’ he explains. The HGH ratios are measured in blood serum using a technique called an immunoassay, and any change in the expected ratios of the isoforms indicates HGH doping has occurred.

**Excitement among the analysts**

Speaking at the unveiling of the anti-doping lab in January, the UK Minister for Sport and the Olympics, Hugh Robertson, said: ‘Our message to any athlete thinking about doping is simple – we’ll catch you.’ With all this state-of-the-art chemistry on his side, one can see why he is so confident that any cheats will be ousted.

But away from the headlines lies another happy tale – for Cowan and his team this is a chance of a lifetime to see their work in the spotlight. ‘It is immensely exciting,’ says Gray. ‘I’ve spent three years researching in this field and now I’m going to be able to apply it to real life.’

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**Staying ahead of the game**

Doping at the Olympics is as old as the Games themselves, the ancient Greeks are known to have ingested certain foods and substances to improve athletic performance. Hallucinogenic mushrooms, for example, were eaten to aid physiological preparation – reduce fear, one assumes, as the sports of the time were pretty brutal.

Sport authorities started to ban doping in the late 1920s. But drug testing of competitors was not introduced at the Olympics until 1968. Throughout the 1970s and 1980s, stories of athletes being caught out regularly hit the headlines.

One of the most famous doping cases occurred at the 1988 Seoul Olympics. Canadian Ben Johnson won the 100 m gold medal, beating arch rival Carl Lewis and setting a new world record. A couple of days later he was stripped of both his medal and record – and banned from athletics for two years – when he tested positive for stanozolol (an anabolic steroid).

This incident further heightened the media attention on the problem, and in 1999 the World Anti-Doping Agency (Wada) was set up. Wada is an independent body that coordinates global efforts to eliminate doping in sports. However, illicit methods for enhancing athletic performance are still evolving and the race to keep up with new testing methods is a constant struggle.

At the last summer Olympics, in 2008 in Beijing, Rashid Ramzi was one of over a dozen athletes to fail anti-doping tests. Ramzi won Bahrain’s first ever gold medal at an Olympics – in the 1500 m race. In 2009, after a decision to retest some of the samples from the Games, he tested positive for CERA (a newly developed type of EPO) and was stripped of his medal that November.

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