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RACING JUST TO KEEP UP

Anti-doping researchers are looking for new ways to catch cheaters. Can a biological passport help to save the sport?

BY EWEN CALLAWAY

Cyclist Borut Božič drew his hands to his chest with a look of joy, disbelief and exhaustion after defeating some of the world's best sprinters in the Swiss village of Tobel. His stage victory at the week-long Tour de Suisse last month netted the 30-year-old Slovenian a €4,000 (US$5,600) bonus and probably helped to secure his spot in this month's Tour de France, cycling's most prestigious race.

His stage win also automatically earned Božič a trip to a cramped medical trailer. Inside, he and three other riders each filled two small jars with urine. The containers were sealed, anonymized and sent to the Swiss Laboratory for Doping Analyses in Lausanne, where technicians would test them for traces of steroids, stimulants and a potent blood-boosting drug called erythropoietin (EPO).

Such tests have become as much a part of professional cycle racing as carbon-fibre bicycles, but decades of doping scandals show that they are no guarantee of a drug-free race. It is tough to name a Tour de France win in recent years that has gone unmarred by doping accusations. Last year's winner, Alberto Contador, tested positive for the banned drug clenbuterol. He has successfully argued that it came from contaminated meat, but an arbitration hearing could still erase his victory. And last year, it was revealed that seven-time Tour de France winner Lance Armstrong has been the focus of a US Justice Department investigation into doping — although he has never been disciplined and maintains that he never doped. Confronted with increasingly sophisticated dopers, anti-doping scientists face a daunting game of catch-up. "This is an endless whirl," says Martial Saugy, the director of the Lausanne laboratory.

In hopes of slowing the whirl, Saugy's team has pioneered a new kind of anti-doping test: the biological passport. Instead of scouring an athlete's urine for traces of drugs or their breakdown products — as the Lausanne lab would do for Božič's sample — the passport builds up a profile of an individual over time and tries to detect biochemical changes that might indicate doping.

Since 2008, Saugy's laboratory and the International Cycling Union (UCI), cycling's international federation based in Aigle, Switzerland, have created biological passports for hundreds of professional cyclists, some containing data from dozens of blood draws. Other sports are looking to follow suit. Some researchers say that the passport offers the best line of defence against EPO

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use, which has bedevilled inspectors for the past two decades; and biological passports to detect steroid and growth-factor doping are in the works. The technology may see its Olympic debut at the games in London next year. Still, critics — and some athletes — say that it is no match for determined dopers.

"The biological passport is a joke," said Floyd Landis, a former US pro cyclist, to sport-news website ESPN.com in May 2010. After losing a costly four-year battle to overturn his conviction for using steroids during the 2006 Tour de France, Landis admitted to doping for much of his career and said that pro cyclists knew how to defeat the biological passport before it was introduced. But the passport has already led to convictions, and — perhaps briefly — shifted the advantage back to the testers. "I think we are forcing people to decrease their doping habits," says Saugy.

AN ENDLESS CYCLE

Anti-doping efforts started in earnest after the 1960 Olympic Games in Rome. During a team time trial, 23-year-old Danish cyclist Knud Enemark Jensen collapsed, fractured his skull and died. An autopsy reportedly found traces of amphetamine and a blood-vessel dilator in his system. Although the drugs might not have caused his death, the episode forced cycling officials to take a closer look at doping. The UCI banned some performance enhancers, and in 1967 the International Olympic Committee established a commission to ferret out doping in sport.

The task is thankless: anti-doping agencies thwart one cheating strategy, only for another to emerge. The 1972 Olympic Games in Munich, Germany, ushered in testing for stimulants, but athletes had started to take anabolic steroids. A test for steroids arrived at the next summer Olympics, in Montreal, Canada. But four years later, at the Moscow Olympiad, athletes had moved on to undetectable, naturally occurring hormones, such as testosterone. Anti-doping authorities now measure the ratio of testosterone in the blood to a related molecule called epitestosterone. In response, some athletes have reportedly found ways of regulating epitestosterone to keep the ratio in check.

For cycling and other endurance sports, human recombinant EPO fuelled a doping revolution. EPO is a natural hormone that promotes production of oxygen-carrying red blood cells. The first synthetic, or recombinant, version was developed by the biotechnology company Amgen in Thousand Oaks, California, and in 1989 it was approved by the US Food and Drug Administration to treat anaemia. It also offered cyclists an easy endurance boost that helped them to excel in gruelling stage races. The drug is nearly identical to the hormone naturally churned out by the kidneys, so was impossible to detect. It is also easier to administer than blood transfusions, which had been used to the same effect.

"In the 1990s and 2000s, it was quite easy for the cheaters to use huge amounts of EPO," says Saugy. Don Catlin, a pharmacologist who used to run an anti-doping laboratory at the University of California, Los Angeles, has a grimmer view. "Everyone in cycling was doping," he says.

Without a test for EPO, cycling regulators turned to an indirect measurement called the haematocrit — the percentage of blood volume made up of red blood cells. Typically, red blood cells account for 40–45% of the blood, but in the heyday of EPO doping, some riders were showing up at starting lines with haematocrits of more than 60%. Their blood was so viscous that they would collapse before races, says Neil Robinson, who led the development of the passport at the Lausanne laboratory. The UCI instituted a 'no-start' rule, disqualifying riders if their haematocrits on the morning of a race were above 50% for men and 47% for women. So cyclists began diluting their EPO-boosted blood with saline solution to keep their haematocrits below the threshold, says Robinson.

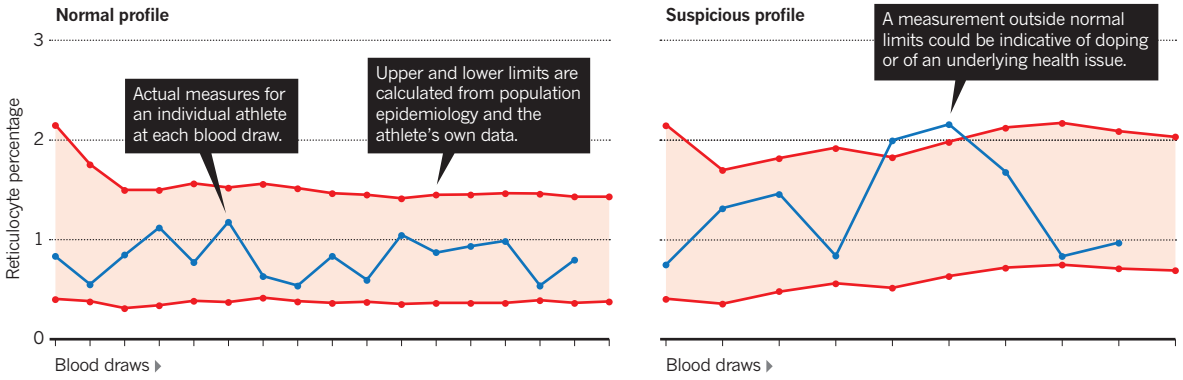
The drug companies that produce EPO have helped anti-doping laboratories to develop direct tests based on subtle biochemical differences between the recombinant molecules and the natural form. The first of these was approved for use in 2000. But athletes increasingly obtain knock-off forms produced in China and India, and researchers have struggled to keep up, says Robinson: "The solution is the passport."

The passport started taking shape in 1999, when Robinson and Saugy began clinical studies of EPO doping in volunteers. "We immediately realized that there were major differences between subjects," says Robinson. For example, in one volunteer, levels of immature red blood cells called reticulocytes might rocket up in response to the hormone, whereas in another, they might barely rise. The researchers realized that instead of comparing such blood metrics against a wide range of values based on the general population, it made more sense to use an athlete as his or her own control and look for unusual fluctuations.

Today, the passport is an electronic record of several different characteristics of red blood cells — haematocrit, the concentration of the blood protein haemoglobin, the percentage of reticulocytes and other metrics — collected

GOOD BLOOD, BAD BLOOD

The biological passport tracks nine blood characteristics for an athlete over time. Below are normal-looking (left) and suspicious-looking (right) measurements for one of these: the percentage of reticulocytes, or immature red blood cells, in the blood. Although an abnormal result for one characteristic doesn't necessarily raise suspicion, abnormal readings for more than one could indicate that the athlete is doping.



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periodically in and out of competition for an individual athlete (see 'Good blood, bad blood'). A statistical model that accounts for factors such as an athlete's sex or the altitude at which a sample was collected (thinner air boosts red-blood-cell production) estimates the probability that a rider's profile is anomalous. "The model will not tell you whether they've doped or not — it tells you the degree of abnormality," says Robinson.

MODEL OF HONESTY

A panel of anti-doping experts reviews profiles identified as suspicious and determines which cases merit a full-blown investigation. Although it is generally used to target riders for direct testing, cyclists have been successfully prosecuted on the basis of their biological passports alone. And in March, the Court of Arbitration for Sport, an international supreme court of sport based in Lausanne, upheld two of these prosecutions, further legitimizing the approach. More cases may be on the way. A report leaked to the French sports newspaper *L'Équipe* revealed a list compiled by the UCI, rating last year's Tour de France riders on a scale of 0 to 10 on the basis of their biological passports. From a total of 198 riders, 42 were rated at 6 or higher, meaning that they showed "overwhelming" evidence of doping, according to *L'Équipe*. Although it isn't proof of doping, the list may be used in deciding which riders to scrutinize in the future.

"There are still chinks in the armour," says Catlin. A team led by Michael Ashenden, an anti-doping researcher who heads the Science and Industry Against Blood Doping consortium in Gold Coast, Australia, simulated EPO 'microdosing' in ten volunteers[1]. They received small intravenous injections twice weekly for 12 weeks. The treatment boosted the subjects' haemoglobin mass by 10%, equal to two bags of transfused blood, but the biological passport didn't flag a single profile as suspect.

In another study[2], Carsten Lundby, a cardiac physiologist at the University of Zurich in Switzerland, and his team subjected three groups of volunteers to different EPO regimens for ten weeks. A testing approach similar to the biological passport caught only 58% of the doped volunteers. "I'm happy I'm not working in anti-doping, because it must be frustrating," says Lundby.

Some researchers say that the statistical model underpinning the passport might produce an unacceptably high number of false positives — clean riders who look dirty on a test. Clifford Spiegelman, a statistician at Texas A&M University in College Station, complains that the model wrongly assumes that biological variations follow what statisticians call a normal distribution. Normal distributions resemble bell-shaped curves, with few outliers. The problem, says Speigelman, is that biological measurements are chock full of outliers — far more than would be predicted by a normal distribution. Proponents of the passports are "presenting themselves as more accurate than they really are", he says, and he estimates that the false-positive rate of the passport could be off by a factor of 10 or even 100.

> **"WE HAVE TO USE THE SAME APPROACH AS A CRIME SCENE."**

Pierre-Edouard Sottas, a Lausanne-based scientist with the World Anti-Doping Agency who developed the statistical model underpinning the passports, says that tests on thousands of clean athletes show that the blood characteristics used do follow a normal distribution. Moreover, he notes that a panel of experts, not his statistical model, makes the final decisions about an abnormal profile.

NO SIGN OF THE FINISH LINE

Robinson acknowledges that the passport cannot catch everyone, but it could deter dopers. The UCI points to a study[3] from its scientists indicating that the incidence of blood metrics that suggest doping has declined since the introduction of the passport.

Anti-doping scientists think that they can improve the tests using tactics such as monitoring sudden spikes in performance, which could indicate something other than intensive training. Robinson and his team want to incorporate information garnered through police investigations of telephone and customs records into the biological passport's predictive model, so that suspicious behaviour and blood chemistry could both be used to flag a rider for closer follow-up. "We have to use the same approach as a crime scene," says Robinson.

His team is also developing versions of the passport to detect steroid and growth-hormone abuse by charting changes in the urine or blood levels of compounds such as testosterone and insulin-like growth factor-1. These researchers and others are also looking to improve the biological passport by searching for new molecular indicators of blood doping. For example, according to unpublished research by the Lausanne lab, circulating levels of a microRNA called miR-144, which is involved in regulating red-blood-cell production, spike after volunteers take EPO. Yorck Olaf Schumacher, an anti-doping scientist at the University of Freiburg in Germany, says that his lab has found changes in gene expression in response to transfusions of a patient's own blood, which can't be detected using conventional markers. Robinson says that it will be several years before these new markers make their way into the biological passports. "We need to validate all these approaches, and that gets tricky."

As the three-week, 3,400-kilometre trek of the Tour de France nears its finish on 24 July on the Champs-Élysées in Paris, Božič has yet to duplicate his Tour de Suisse stage victory. But his biological passport has gained another data point. Before setting off on this year's Tour de France, Božič and the other 197 riders gave blood samples for their passports, says Robinson, whose team plans to use these data anonymously to estimate the prevalence of blood doping in this year's race.

The team hopes that the passport will keep more riders honest. But after running an anti-doping laboratory for a quarter of a century, Catlin is convinced that tests, no matter how sophisticated, will never keep up with the most determined dopers. "For every move to the right, the other guys are moving to the left and it balances out again." ■

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