secondary molecules such as metabolites that fluctuate with steroid levels. An athlete's passport is flagged for further evaluation, and possibly more testing, when levels of these biomarkers are outside of the athlete's 99.5th percentile reference range, calculated with a mathematical model that is based on a population of healthy individuals and an individual's own results. As an athlete accumulates more test results, her individual reference range narrows.

“A common misconception is that when an athlete's sample is collected that it’s analyzed with a simple dipstick and that it’s either positive or negative,” says Matt Fedoruk, scientific director at the US Anti-Doping Agency (USADA). “Rather, we are collecting blood and urine, and there is a high level of sophistication that is applied to detect hundreds of prohibited substances. The analyses are quite challenging and complex.”

Anti-doping researcher Jenny Mullen and colleagues at the Karolinska Institute in Sweden have demonstrated that, by establishing a baseline, the ABP could enable detection of a single 100-milligram dose of testosterone gel to the skin—which would be considered a microdose—as long as seven days after application in healthy male volunteers. In another study, Mullen's team found that injection of a 125-milligram microdose of testosterone enanthate, a drug intended to boost levels of testosterone among males with low levels of the steroid, could be detected using the same methods for as long as eight weeks.4

“WADA has rightly set the bar very high for [doping] detection, but that means we don’t always have the sensitivity we would like for our tests,” says Holt. “The ABP is a mechanism by which athletes who travel around the world to compete can be tested by different labs, and those results are comparable among those labs and centrally stored as part of the athlete's history.”

Elusive testosterone

In addition to tracking biomarker levels in athletes over time, anti-doping officials are working to understand what the relative levels of various testosterone precursors and related markers can tell them about an athlete’s normal physiological levels and doping status. Such comparisons are critical to the identification of exogenous testosterone use: numerous precursors of the steroid hormone, as well as epitestosterone, an isomer of testosterone, are suppressed in the presence of exogenous testosterone. Precursor levels relative to the levels of testosterone in the body over time can indicate if something unnatural is going on. In 2007, researchers devised a statistical technique to detect individuals’ abnormal steroid values,5 and WADA added the steroid module to the ABP in 2014.

But there can still be some ambiguity to all these test results. If the variability of steroid biomarkers or their ratios are suspicious to the anti-doping scientist doing the assessment at a WADA-