The Dog Ate My Homework

(Editors' Note: Positive doping tests or "adverse analytical findings" in professional cycling can be highly uncertain and controversial, and can lead to months or years of costly wrangling and disputes, both in the legal system and in the court of public opinion – often without any satisfactory or consensus outcome. While the sport has upped its effort to discover and expose drug cheats, many observers believe that some racers are still getting away with the use of various undetected performance enhancing drugs. What often attracts less attention is the case where it appears that clean riders are being unfairly accused. It is important to realize that while rapidly advancing analytical technology has unquestionably improved the precision and accuracy of drug testing, it's not perfect; there will always be some inherent error in the process. And this means that we may occasionally punish the wrong person. In addition, the ability of modern analytical instruments to detect vanishingly low levels of compounds raises the more fundamental and practical question of relevant threshold levels – at what level should we even be concerned about the presence or absence of a certain chemical? Dr. William Apollo, a cardiologist and former bike racer from Harrisburg, Pennsylvania explores some of these vexing questions and challenges in the article below.)

Professional bicycle racing is a beautiful sport. Every July, both fans and cyclists are transfixed by the French countryside, watching the colorful, rolling pageant of the Tour de France. Over 23 days, an epic drama complete with mountains, sunflowers and castles, is played out on miles of black winding ribbons. Twenty one episodes unfold with panache and romanticism until the peloton reaches the Champs d'Elysees.

But in recent years, this idealized image of professional cycling has been tarnished due to persistent doping scandals. Quixotic results are now viewed with skepticism and are heavily scrutinized by fans and the media alike. Refreshingly, in September 2014, the Cycling Anti-Doping Foundation (CADF) issued a press release indicating that there were no positive doping tests identified at the 2014 Tour de France. There was a collective sigh of relief from the cycling community. According to the UCI, "all the samples collected were systematically analyzed to detect stimulants and erythropoiesis." Additionally, "isotope ratio mass spectrometry (IRMS) was also analyzed in a certain number of samples, in particular to detect testosterone abuse and its precursors." Brian Cookson, President of the UCI, acknowledged the success of anti-doping efforts at this year's Tour de France and cited collaboration among the World Anti-Doping Agency (WADA), CADF, UK Anti-Doping, and French Anti-Doping organizations as the key to this success.

The specific and deliberate wording of the UCI statement indicates that, despite effective collaboration among governing bodies, technological advances cannot be ignored when discussing anti-doping efforts. It must be understood, however, that the science of anti-doping controls is not exact. No laboratory test is completely perfect; any testing procedure is associated with some inherent degree of error. But at the same time, the results of these doping controls have the power to change lives, careers and the history of sport itself. It is therefore important to understand potential sources of inconsistency involved with laboratory testing, as well as reporting of results, from the perspective of determining whether sanctions against an athlete are fair and reasonable. In some cases, the inherent inconsistencies of testing could potentially be used by an athlete to gain an unfair advantage. On the other hand, inherent errors involved in laboratory testing could implicate an entirely innocent athlete in a doping scandal.

It seems that adverse analytical findings are rarely followed by athlete confessions. In fact, alleged doping violations are typically followed by long arduous proceedings in an attempt to determine an athlete's guilt or innocence. Many times, athletes categorically deny any knowledge of doping activity and blame the abnormal test value on a whole range of circumstances beyond their control. Contaminated foods or supplements, mislabeled products, pharmacy mistakes and disappearing twins in utero are only some of the more "creative" excuses. Cycling fans have become weary of this by now predictable sequence of events and have begun to view these excuses as another case of "the dog ate my homework." But what if the dog actually *did* eat your homework? Would anyone believe you? This article, by focusing on two common anti-doping measurements, will illustrate some of the analytical and procedural complexities involved in the effort to ensure clean and fair sport.

Hematocrit – Transporting Oxygen to the Aerobic Furnace: Marco Pantani was at the peak of his cycling career in the late 1990s. In 1998 he became only the 7th rider in history to win the *Giro-Tour* double. His 1999 defense of the *Maglia Rosa* was derailed when a blood test at the Madonna di Campiglio returned an abnormal hematocrit value of 52%. He was issued a two-week ban from competition, effectively removing him from the race. Although the ban was enforced on the basis of "rider safety," there was suspicion of blood manipulation as the cause for his elevated hematocrit. At the time, he was leading the Giro d'Italia by 5:38 over Paolo Savoldelli. Persistent doping allegations plagued Pantani throughout his career, and right up until his untimely death in 2004.

Hematocrit is a simple measure of the percentage of red blood cells within a whole blood sample. Red blood cells can be thought of as the "boxcars" involved in delivering oxygen to an athlete's exercising muscles. Increasing the mass of red blood cells (the number of "boxcars") in an athlete's circulation leads to improved aerobic capacity and a definite advantage in performance. Measuring an athlete's hematocrit is an example of assessing an *endogenous* parameter – a natural biological value that is inherent to a subject's physiology. Blood cells naturally occur in the human body therefore, under normal circumstances, the hematocrit value should fall within a certain acceptable range. In a typical medical laboratory, the measured level of a "normal" hematocrit ranges between 37 – 47%.

The UCI, based on statistical analysis, has settled on a hematocrit value of below 50% as being an acceptable *threshold* for rider safety. But it should be noted that establishment of a threshold value for hematocrit is somewhat arbitrary and highly dependent upon the sample population itself. For example, Vasquez & Villena published a study in *High Altitude Medicine and Biology (2001)* measuring hematocrit levels in a group of young men residing in Bolivia. When living at an altitude of 4,000 meters, the average hematocrit value was 52.7% (with a range 45 – 61%). This clearly illustrates both the impact of altitude on red blood cell mass as well as the importance of considering a sample population when assigning a normal threshold value for a given test population, or assay. The current UCI threshold was chosen because it was felt to include, with reasonable certainty, most normal professional cyclists, while at the same time reducing the number of false positive tests – inaccurate test results indicating doping in an innocent rider – leading to unnecessary sanctions.

However, it is important to understand that a reported hematocrit value may be affected by many factors. Varying laboratory techniques for measuring hematocrit carry an inherent degree of error which may alter the obtained value by as much as five percentage points. Potential sources of laboratory error can be related to operator handling, storage conditions and shipping times of the sample to the lab, machine calibration and dilution of the blood sample during analytical preparation. Assuming an accurate laboratory process and test result, a higher hematocrit could indicate dehydration, altitude training, manipulation by blood transfusions, administration of erythropoietin (EPO) or Xenon gas treatments – some of which might represent an attempt to increase the number of "boxcars," and thereby gain a performance advantage.

In this example, hematocrit values can be adjusted and manipulated to a cyclist's advantage as long as external testing at the time of an anti-doping control shows that the hematocrit is less than 50%. Conversely, a rider could also take advantage of dilution errors associated with hematocrit measurement. For example, if a doped cyclist suspected an upcoming anti-doping control, use of intravenous hydration immediately prior to testing could facilitate lowering of the hematocrit below the acceptable 50% threshold level, thus avoiding an adverse analytical finding. Longitudinal monitoring of a rider's hematocrit levels (a series of similar tests over a longer period of time), using the so-called Biological Passport Program, has been instituted to place these types of fluctuations into proper perspective. Additional investigation, such as testing for the presence of recombinant EPO, can assist in determining the medical reason for, or "etiology" of, an elevated hematocrit.

Clenbuterol – Where's The Beef (From)? In the 2010 Tour de France, Alberto Contador and Andy Schleck were engaged in a tense struggle for the Maillot Jaune, culminating in a showdown during the final time trial on the penultimate stage of the race. Contador prevailed on the road as the two riders battled across the vineyards of Bordeaux. But he was eventually stripped of his title on the basis of an adverse analytical finding indicating trace amounts of the chemical clenbuterol in his system. His defense, although ultimately unsuccessful, was predicated on the ingestion of contaminated meat (clenbuterol is sometimes used in certain countries to promote livestock growth). More recently, Michael Rogers returned an adverse analytical finding for clenbuterol in 2013. He had participated in the Tour of Beijing and was tested three days later at the Japan Cup Cycle Road Race. In this case, Rogers was able to successfully defend himself on the basis of accidental ingestion of clenbuterol from contaminated meat while racing in China. Some have suggested that the handling of these two cases represents a double standard regarding the sanctions delivered. Be that as it may, these two cases also raise several other important questions which are pivotal when assessing the significance of an adverse analytical finding for certain performance enhancing substances: (a) should there *ever* be an acceptable level of clenbuterol in a doping control test? (b) do trace amounts of this drug really enhance performance? and (c) what are the potential causes or sources of clenbuterol in the positive specimen?

Clenbuterol is an example of an *exogenous* substance – a chemical that never occurs naturally inside the subject's physiology – which may sometimes be administered to improve athletic performance. This drug is not approved by the U.S. Food and Drug Administration, but in some areas of the world it is used as a long-acting bronchodilator for treatment of asthma. In addition to its use as a pulmonary medication, clenbuterol has also been shown to increase lean muscle mass and reduce body fat. From the perspective of a professional cyclist, this could enhance performance by improving the power/mass ratio, and for this reason, clenbuterol is specifically banned by WADA, both in and out of competition. It is considered to be a *non-threshold* drug – any detectable level is considered to be an adverse analytical finding because it does not naturally occur in the human body. Furthermore, there is no Therapeutic Use Exemption (prearranged allowance, upon request, of drug usage for certain athletes under certain conditions) for the drug. If even a miniscule amount of clenbuterol is identified, the worst-case scenario must be assumed – the rider is guilty of recent doping and was tested prior to the drug being completely metabolized and excreted from the system.

Clenbuterol has also been used therapeutically in veterinary medicine as a bronchodilator and a tocolytic (a medication used to suppress premature labor). When administered in supra-therapeutic doses it also has the capability to improve beef yield in cattle, thereby improving profits. Although now illegal in most industrialized countries, this practice continues with regularity in some areas of the world, particularly China and Mexico. Administration of clenbuterol in this dosage range (10 – 100 times the typical therapeutic dose) may result in detectable levels of the drug in humans following inadvertent consumption of contaminated meat products. In 2012, Guddat, et.al. from the German Sports University in Cologne explored this issue. They studied 28 athletes returning from China and were able to identify a 79% positive clenbuterol rate. Based on their findings, they cited regional food contamination as a possible source for inadvertent positive doping tests in sport.

Clenbuterol can be administered as a pill or liquid reaching peak concentration in about 2.5 hours. The half-life of the drug is approximately 35 hours, meaning that it would be metabolized from the system within several days. Most of the drug is excreted unchanged in the urine, allowing for clinical detection. Identification of clenbuterol in urine samples is accomplished by highly sophisticated equipment with tremendous sensitivity and specificity. WADA accepts two specific analytical procedures for this particular assay: Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS). These methods provide specific structural information about the clenbuterol molecule itself, making identification very accurate. When individual laboratories are certified by WADA, they must be able to prove a *Minimum Required Performance Level (MRPL)* – the ability to meet certain minimum standards of laboratory technical performance. It does not represent an acceptable drug threshold, a limit of detection or limit of quantification. Although WADA requires an MRPL of 2ng/mL (two parts per billion) for detection of clenbuterol, some labs may be able to detect levels much lower than this, depending on the sensitivity of their equipment.

The rapidly advancing ability of contemporary laboratory equipment to detect minute traces of various compounds – including clenbuterol in urine samples – has prompted extensive recent discussion about how to place these findings into proper context. The unexplained presence of even tiny trace amounts of clenbuterol in a urine sample allows legal accusation by doping authorities, while the rider must prove beyond the shadow of a doubt the source of the clenbuterol. Because of this, some observers have argued that clenbuterol should be reclassified as a *threshold* drug whereby relatively small amounts would not be considered a positive test. This would significantly reduce the possibility of false positive results from inadvertent exposure. Current technology, however, is incapable of distinguishing intentional from accidental exposure. Therefore, according to WADA, a positive finding for clenbuterol is an adverse analytical finding, no matter how minute the concentration or the performance enhancing potential.

These competing opinions are nicely illustrated in the case of Alberto Contador. His adverse analytical findings were a function of the sensitivity of the equipment used in a specific anti-doping lab. Contador's abnormal samples contained 0.05 ng/mL and 0.02 ng/mL (*nanogram= one billionth of a gram; one gram = 1/30 ounce*) respectively. This is less than 10% of the statutory MRPL levels for clenbuterol. If he had been tested in a different laboratory, with less sensitive equipment, clenbuterol might never have been found in his system at all. Given this information, defense consultant Dr. Douwe de Boer introduced the concept of "*fair and reasonable concentration.*" This defines a certain threshold level above which sanctions against a rider are justified. This value is typically 10% of the MRPL, where a concentration below this level would typically not be reported. But although this threshold level may be accurate for some therapeutic medications, it is not true of clenbuterol. Clenbuterol can never be of endogenous origin and hence, according to WADA regulations, is forbidden both in and out of competition. Unlike Michael Rogers, Contador was unable to provide sufficient evidence linking himself to an area where regional food contamination occurs with regularity.

WADA recognizes the potential for food contamination resulting in inadvertent exposure to clenbuterol. Because there is no test available today to determine whether clenbuterol was purposely or accidentally ingested, adverse analytical findings are evaluated on a case by case basis. WADA is conducting research in this regard. Director General David Howman, in an interview with *Cycling Weekly*, stated, "We've got that under the radar and we are conducting several research projects to see where we can get to a situation where clenbuterol, if detected in a sample can be shown to be taken through food or not."

In summary, technological advances in laboratory testing have had an unquestionable impact on the quest for clean and fair sport. At times, however, these highly sensitive assays are capable of producing results which are confusing and open to interpretation. In some cases, based on a simple test result alone, significant doubt can be cast regarding a rider's guilt or innocence – and hence, an appeals process must place the adverse analytical finding into proper perspective. What must be remembered – as the sport strives to correct and minimize the pervasive doping culture of the past – is that the current system can yield up false positives as well as false negative tests. Just as the current anti-doping system may fail to detect certain guilty dopers, it may also sometimes unfairly accuse certain clean athletes. In other words, sometimes it really *is* possible that the "the dog ate your homework" – we must work to minimize the potential for that situation. What remains clear is that the most foolproof anti-doping control is a confession.

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