

Bob Barrett: This is the podcast from Clinical Chemistry. I am Bob Barrett. In recent years, there have been a number of highly publicized cases involving athletes that have been banned from sports competition for using performance enhancing drugs. It's a little surprising that athletes involved in a doping case often deny any wrongdoing. It's even more surprising that some of the sanctioned athletes later admitted using performance enhancing substances and even assisted anti-doping agencies by revealing the details of their doping programs.

Often the athlete uses performance enhancing drugs not to increase muscle mass or strength, but rather to accelerate recovery for longer and more intense workouts. In the July issue of *Clinical Chemistry*, a Question and Answer article titled '*The Quest for Clean Competition: Are the Testers Catching the Dopers*' summarizes the opinions of four leaders regarding the successes and challenges facing anti-doping programs.

Joining us in this podcast is the lead author Dr. Anthony Butch, Director of the Olympic Analytical Laboratory and the Department of Pathology & Laboratory Medicine at UCLA, and Dr. Larry Bowers, Chief Science Officer for the United States Anti-Doping Agency in Colorado Springs. Dr. Butch, you first, why do you think the topic of doping in sports has attracted so much attention recently?

Dr. Anthony Butch: I think that sports doping has gained widespread attention because over the last several years there have been athletes with highly successful careers that have been caught doping and were banned from competition. This makes sports doping difficult to ignore, especially in light of recent comments by professional athletes suggesting or acknowledging that doping is a problem in sports.

The public used to believe that highly successful athletes were winners because of their God-given talents and solid work ethic. Now, people are unsure if it is talent and hard work that produces winners or the use of performance enhancing drugs that provide the competitive edge to make them winners.

Bob Barrett: Can you walk us through the testing process?

Dr. Anthony Butch: Sure! The testing process begins with the collection of samples which occurs by collection agencies that are separate from the laboratories. Athlete samples, mostly urine samples are shipped to the laboratory and typically arrive one to two days after collection. From the moment the samples arrive in the laboratory, chain of custody procedures are implemented to document the whereabouts

of the samples in the laboratory and who or what has physical custody of the samples at all times.

Chain of custody is established for the original urine containers and all aliquots that are prepared for analysis. The laboratory typically receives two bowls of urine from each athlete which are labeled A and B Bottle. The bottles are checked for leakage and signs of tampering. Since only the A Bottle is used for the initial testing, the B Bottle is not opened and is stored in a locked freezer. The specific gravity and pH of the A Bottle urine is determined to identify signs of sample adulteration and the presence of a liquid other than urine.

Depending upon the testing menu, up to five or more urine aliquots can be prepared for testing. A few hormones such as human chorionic gonadotropin and luteinizing hormone can be measured by simply placing the urine onto an automated instrument without any sample pre-treatment.

However, the majority of testing requires a cleanup step to extract the compounds of interest from the urine matrix and remove interfering substances. For this, either solid phase or liquid phase extraction methods are used for sample cleanup, depending upon the class of compounds in the screen.

After extraction, samples are then dried down to concentrated material and reconstituted with a small amount of solvent. The samples are then placed on the auto-sampler of either a gas chromatography mass spectrometry or liquid chromatography tandem mass spectrometer for detection of specific hormones. Instrument runtimes reach sample range from few minutes up to 30 minutes depending on the class of compounds being monitored.

Standards containing pure reference compounds are analyzed with each batch of unknown urine samples for comparison. The collected data are reduced to dedicated windows containing specific time periods and mass-to-charge ions that correspond to the expected retention times and mass spectrum for each target compound.

Several mass-to-charge ions are typically monitored to identify each drug. Each screen for class of prohibited substances takes approximately two days to complete from sample aliquoting to the reporting of negative results.

If a urine sample screens positive for prohibited substance, then another aliquot of urine is removed from the A Bottle, and is tested a second time by itself with only the appropriate standards and urine controls. The confirmation

test is usually designed to have greater specificity for the drug of interest than the screening test.

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If the confirmation test confirms the presence of the prohibited substance identified during the screen, the A sample is then considered positive. The athlete can request testing of the B Bottle for additional confirmation that prohibited substance is present and also has the right to witness the opening of the B Bottle and observe the entire testing procedure.

Bob Barrett: Okay. Now, how does an anti-doping laboratory know which substances to test for?

Dr. Anthony Butch: For anti-doping organizations that are signatories to the World Anti-Doping Code, the testing menu is defined by the prohibited list that is part of the code. The prohibited list is updated on a yearly basis, and new compounds are continually being added to the list. The most current prohibited list can be found on the World Anti-Doping Agency website.

For out-of-competition testing, a prohibited list includes anabolic agents, peptide hormones such as erythropoietin-growth hormone, beta2-agonist, hormone antagonist and modulators, diuretics and masking agents. To give you an idea about the number of drugs being tested, the exogenous anabolic steroid list alone contains 49 specific compounds.

For in-competition testing, more classes of compounds are prohibited besides those on the out-of-competition prohibited list; stimulants, narcotics, cannabinoids and glucocorticosteroids are also prohibited in-competition, beta blockers could also be prohibited depending upon the sport. This involves a lot of additional drug testing. As an example, there are currently 64 stimulants on the prohibited list that World Anti-Doping Agency accredited laboratories are expected to routinely screen for during in-competition testing.

Bob Barrett: Dr. Butch, are all doping control programs the same or are there differences?

Dr. Anthony Butch: Doping control programs are not all the same when it comes to drug testing. If the Doping Control Organization follows the World Anti-Doping Code, then the compounds monitored are all the drugs on the World Anti-Doping Prohibited List, and would be the same for all doping organizations following the code.

This means that the list of banned substances for Olympic athletes in the United States would be identical to the list used for Olympic athletes in other countries. This ensures a leveled playing field.

However, there are many sports organizations that have not signed onto the World Anti-Doping Code for many different reasons. This includes professional, college, and high-school sports organizations. They typically create their own list of banned substances that often include some but not all of the compounds on the World Anti-Doping Prohibited List.

Anabolic steroids are usually prohibited and mirror the World Anti-Doping Prohibited List, but other classes of compounds such as beta2-agonist and glucocorticosteroids are usually not prohibited.

Stimulants are usually prohibited, but the number of stimulants on the list is considerably smaller than the World Anti-Doping Prohibited List of Stimulants.

Another example is diuretics, which may not be routinely tested for unless the urine has a very low specific gravity and is extremely dilute.

Bob Barrett: We now turn to the United States Anti-Doping Agency's Chief Science Officer Dr. Larry Bowers. Now doctor, how does a National Anti-Doping Organization like USADA deter athletes from using prohibited substances?

Dr. Larry Bowers: Well, as you pointed out, the goal of any anti-doping program is deterrence for those athletes that are struggling with the decision as to whether to use performance enhancing drugs or not. It's really important that they see the risks and accountability for their behaviors.

So we use a model that was developed in Social Science called the Perceptual Deterrence Model or Rational Choice Model. And according to that model, there has to be a perceived risk of being tested, a concern that the substance used is going to be detected and an understanding that there will be a significant consequence for breaking the rules.

So just to give an everyday example, if we know there is no law enforcement for speed limits, driver's speed at will pretty much. If there is an indication of a radar trap, all the traffic slows down, even if the police car has a manikin in it and no radar. But if we were to leave that fake trap at the same location for too long, people would notice and speeds would again increase because perceived risk would be low.

Again, just sort of finish the whole concept, if the fine for speeding was \$5, no one won't mind paying it even if you were caught. So there is really no deterrence. So the key to having an effective deterrence program is that we use intelligence information that we can gather to guide our collections. Our laboratory testing must continue to develop through research so that we can detect all of the substances the athletes are using, and our sanctions have to be such that we maintain perceived risk.

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And as athletes change their behaviors in order to avoid detection we have to also change our strategies in order to continue to have an effective risk of detection.

Bob Barrett: Some athletes have claimed that inadvertently ingested prohibited substances contained in dietary supplements. Now how can an athlete avoid this possibility?

Dr. Larry Bowers: Well, first and foremost, athletes need to be informed consumers. They need to carefully review the products that they plan to take, to contact authorities and product manufacturers to get detailed information about substances that they are taking, and perhaps even have the product tested themselves.

We usually advice athletes to exercise common sense and avoid products with suspicious names and exaggerated claims. For example, athletes should be cautious when they encounter marketing term such as stacked or muscle, mass, tren, bol, legal steroid, stimulant and terms like that.

In general, supplements that promise sexual performance, more muscle, spectacular weight loss, and instant recovery should be suspect to really put it most simply if for supposedly safe supplement benefits sound too good to be true, they probably are.

Given the current regulatory framework and the fact that it can't ensure that a supplement is free of contaminants or that what is listed on the label is what the supplement actually contains, USADA continues to advice athletes to use supplements at their own risk.

Bob Barrett: Doctor, what are some strategies that are used to optimize the drug testing programs?

Dr. Larry Bowers: Well, a well-developed and effective anti-doping program is not based on testing alone, we must also focus on deterrence, education, a thorough and fair results management process, and timely insignificant consequences.

We also know that an effective drug testing program really has to contain year around no notice testing in order to be effective. Research is a critical aspect of our work. We continually evaluate our collection strategies. We've learned over the years the cheaters change their behaviors in response to our improvements in testing.

For example, when a urine test for recombinant erythropoietin was developed with a detection window of seven days, the cheaters switched from subcutaneous injection to intravenous administration, specifically to reduce the detection window.

Another important element is monitoring whether or not an athlete is using prohibited substances, we use the monitoring of intra-individual reference ranges, which is sometimes called the Athlete Biological Passport in sports.

To identify non-physiological changes in blood or urine analytes. For example, each urine we collect is tested for a number of steroid concentrations and those concentrations are analyzed for deviations from that person's previous tests. And by doing that we can narrow the range of variability before we can detect clear case of doping.

The same is true for collection of hematological parameters like hematocrit, reticulocytes and so on. Again, the intra-individual changes are much smaller than population changes, which is well-known in Clinical Chemistry, and we use that same principle to follow-up on individuals within the anti-doping testing pool.

Usually but not always abnormal results would be used to perform additional strategic testing rather than evidence in a particular doping case.

Bob Barrett: And doctor, other than drug testing what have doping programs done to enhance their ability to catch and deter athletes from using performance enhancing substances?

Dr. Larry Bowers: Our testing program is highly effective, but we do understand that in order to be truly successful we must continue to advance our intelligence gathering and our research efforts. It's clear that a single approach cannot be successful.

In the fight against doping it's critical that we remain vigilant. Any pattern that we establish such as, for example, collecting no notice out-of-competition samples at a particular time of day, or any rule that we right can be used cheaters to undermine the process.

We have to continue to use intelligence to understand what the athletes are doing, and modify our test distribution plan and specific test allocation to catch cheaters and maintain deterrence.

Research is really also a very important point. We have to be able to detect doping agents and methods and their unique effects on the body in order to achieve any level of deterrence.

Any new doping substances and methods or changes in behavior due to new tests must continue to be researched.

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In this regard, the partnership for clean competition, which is a Research Consortium formed by the U.S. Anti-Doping Agency, the U.S. Olympic Committee, the National Football League and Major League Baseball, is also a critical factor for the future.

By the way, any clinical chemist or toxicologist is eligible to apply for a PCC Research Grant. The website is [www.cleancompetition.org](http://www.cleancompetition.org).

And finally, it's important that we enhance the true value of sport and break the cycle of win at all cost, that is the root of so many problems in our society.

USADA has developed curricula that are in use in our elementary and high schools. USADA has conducted a survey on the value of sport, and we're working to develop educational programs based on the results of that survey.

We need to create a culture of excellence and values rather than a culture of winning at all costs.

Bob Barrett: And finally, back to you Dr. Butch, based on your interviews, do you think athletes who are tested for performance-enhancing substances support anti-doping programs?

Dr. Anthony Butch: Yes, I do. I think that the majority of athletes truly believe that drug testing programs are an effective tool for preventing doping to gain a competitive edge. When an athlete is caught doping, I think that other athletes feel that this damage is the integrity of the sport.

Doping scandals make it harder for the public to believe that athletes are successful because of his or her natural abilities, and not because they cheated by taking a prohibited compound.

Many athletes believe that along with good educational programs that emphasize the health risk associated with doping, more announced random testing of athletes is needed to prevent the use of performance-enhancing substances.

Bob Barrett:

Dr. Anthony Butch is the Director of the Olympic Analytical Laboratory in the Department of Pathology and Laboratory Medicine at UCLA; and Dr. Larry Bowers is the Chief Science Officer for the United States Anti-Doping Agency in Colorado Springs. They have been our guests in this podcast from *'Clinical Chemistry'*.

I'm Bob Barrett. Thanks for listening!

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