The Scientific Reliability of the Processes

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Introduction

Sports Drug Testing (SDT) and Workplace Drug Testing (WDT) both fall into the medicolegal type of drug testing in that the results must be able to withstand legal challenge whether it be via the sporting organizations' hearings or employment courts. The results must therefore be absolute accurate, provide irrefutable evidence of proof of the presence of a drug or its metabolite and accurately quantitate that substance if such data is relevant. The consequences of a positive test can be very serious.

- Sport: banned from competition from three months to two years for the first offence and for life after a second offence for some drugs.
- Workplace: an individual's likelihood of obtaining or retaining employment may be determined by the result of a drug test. Most employers would refer their employees to an EAP drug treatment programme after the initial positive but the employee would be required to undergo regular tests to make sure he/she is staying clean".

Consequently SDT and WDT must be conducted by the best possible processes available.

Steps in reliable process

There are a number of very important steps all of which must be strictly adhered to, in order to ensure the outcome is accurate. These are:

1 sample collection
2 transportation and laboratory receipt
3 screening
4 confirmation
5 quantitation
6 interpretation
7 reporting.

If any of these steps is not conducted to the protocols and standards which have been established, then the results can justifiably be challenged.

Standards

1 *Sports drug testing*

- IOC Medical Commission (IOC-MC) (1994)
- College of American Pharmacologist (CAP)

The IOC-MC lists the selected classes of "Pharmacological Agents" and doping methods that are banned or subject to certain restrictions. All possibilities are covered by listing a few examples of drugs in each class and then adding "related substances", for example:

Class C. Anabolic agents. 1 Anabolic androgenic steroids

clostebol	fluoxymesterone
metandienone	metenolone
nandrolone	oxandrolone
stanozolol	testosterone

... and related substances.

Also with the exception of caffeine and testosterone, any amount of any of the banned substances present in the urine constitutes a positive result. For caffeine the concentration may not exceed 12 micrograms per millilitre and for testosterone, the presence of a testosterone (T) to Epitestosterone (E) ratio greater than six to one constitutes an offence unless there is evidence that this ratio is due to a physiological or pathological condition.

Therefore, for SDT a positive is not only determined by the laboratory's ability to accurately identify a substance but also the sensitivity of the instruments and its ability to detect a substance at minute levels. As instrument become more sophisticated obviously the limits of detection are lowered. Also, different laboratories will be able to detect drugs to different levels depending on their ability to keep abreast of the "state of the art" technology.

The IOC-MC accreditation programme is very strict and laboratories become suspended or lose their accreditation unless they demonstrate 100% accuracy in the regular quality control programmes.

2 *Workplace drug testing*

- USA: "Mandatory Guidelines for Federal Workplace Drug testing Programmes" Department of Health and Human Services (DHHS) (June 1994). These were originally known as the NIDA guidelines.
- Australian Standards for testing for Drugs of Abuse in Urine (April, 1995).

These standards have been established to combat the many "cheap" inferior quality and worrying inaccurate testing services which are offered by entrepreneurs seeking to make a quick buck by offering inadequate screening services or "do-it-yourself" kits.

They require the use of the best available technology for ensuring the full reliability and accuracy of drug tests and strict procedures for governing the chain of custody of specimens collected.

They also list the "performance impairing" substances which are recommended for testing and the "cut-off" levels for both the screening and confirmatory analytical phases. Below these cut-offs a test is deemed to be negative so passive inhalation of cannabis, for example, would not give a positive response at these testing cut-offs.

In Australia and New Zealand the recommended suite of drugs is

- Cannabinoids
- Opiates
- Cocaine
- Amphetamines
- Benzodiazepines.
- Alcohol (optional)

Sample collection

The collection of the sample is a very important part of the process since if the sample is not collected properly the integrity of the sample is in question. Trained personnel are required to supervise this process.

The donor must be able to be identified in some way.

The procedure requires the collection of a urine sample which is non-invasive and the simplest sample to collect. The options for providing the sample are "witnessed" or "unwitnessed". Collection of samples for SDT is always witnessed whereas most employers opt for the unwitnessed option for obvious reasons and the collector is required to carry out the following practices to ensure the urine sample is not diluted or a blank sample is not smuggled in as a substitute.

- Colouring agent is added to the toilet cistern.
- Faucets are taped up.
- The donor washes hands prior to sampling.
- Bags, coats, etc, are removed from donor.
- The urine sample is examined visually for possible contamination or dilution.

The collection kit is packaged in a heat sealed plastic bag and consists of the following components:

- sealed container with a temperature strip which ensures the sample is at body temperature;
- specimen label with a unique identification number;
- sample security seal with same unique number;
- tamper-proof bag with same unique number;
- mailing box with tamper-proof seal.

After voiding the specimen must remain within the sight of both the donor and collector at all times until properly sealed.

The documentation covers details and signatures as listed:

- date

- donor and company/sporting event
- collecting supervisor
- witness (SDT)
- testing options
- medication: last two weeks
- chain of custody
- specimen receipt at laboratory.

Analysis: screening

1 *Workplace*

The analysis at the laboratory has a two-tiered approach. Initially screening for the menu of drugs of interest at and above the predetermined cut-off levels is carried out by trained science technicians. Typically immunoassay methods are used and we use the SYVA EMIT systems since SYVA have specifically focused on urine substance abuse when designing their products. Standards and control samples are analysed with each batch of samples.

The screening tests will either give a negative response which results in the sample being reported negative, or a positive response. However a positive response after screening is in no way sufficient evidence of proof of the presence of that drug since there are a number of legitimate substances which can interfere with the screening tests and result in positive responses.

A positive response after screening must therefore be subjected to confirmation and quantitation. It is also important to mention that the "do-it-yourself" or "on-the-spot" screening practices are not acceptable by these standards.

2 *Sports*

Chromatography

Because of the much bigger range of drugs and metabolites being tested for and also because of the requirement for the screening to be as sensitive as possible, SDT mainly uses automated chromatographic instruments interfaced to a variety of detectors selected for their sensitivity to the substances of interest. These instruments are:

- Gas Chromatograph (GC)
- High Performance Liquid Chromatograph (HPLC).

Some drugs, ie, Anabolic Steroids, require even more sophisticated instrumentation for this screening phase and Gas Chromatography/Mass Spectrometry (GCMS) is used.

The primary function of a GC or HPLC is to separate the components of a mixture. Hence the extract from a urine sample is injected onto the top of a chromatographic column. With the assistance of gas or liquid the various components of the extract will pass along that column at different rates depending on the packing inside the column, the programmed external environment the column is subjected to and the chemical make-up of the

components. The time at which the individual components are detected at the end of the column is reproducible for a set of conditions but it is possible for two or more different substances to pass through the column at indistinguishable rates. Therefore whilst this Retention Time (RT) can provide a very good indication of what substances are present, it is not an absolute identification.

Analysis: confirmation/mass spectrometry

The samples giving a positive screen result are referred to a qualified scientist for confirmation and quantitation. These samples are first extracted by liquid/liquid or solid phase methods. Controls and accurately measured amounts of a range of standards are simultaneously extracted. Wherever possible we use deuterated analogues of the drug in question as internal standards for very accurate quantitation.

The concentrated extract is subjected to derivatization to enhance the sensitivity, specificity and chromatographability. Gas Chromatography/Mass Spectrometry (GCMS) is then carried out and this sophisticated method provides a chemical fingerprint of the drug or metabolite of interest. A positive result from MS is irrefutable evidence of the presence of a substance in the sample and this is the only result which will withstand legal challenge.

Quantitation

In SDT accurate quantitation is critical for determining whether caffeine is greater than 12 microgrammes per millilitre or whether the T/E ratio is greater than six. It can also be important for interpretation of results, for example, whether a positive morphine is due to codeine or morphine ingestion since morphine is a metabolite of codeine and codeine is not a banned substance for either SDT or WDT.

For WDT since a positive can only be reported if the substance is present above the predetermined level it is essential that the methodology is designed so that the accuracy around that cut-off level is beyond question.

The most accurate quantitation methods use GCMS and utilize deuterated analogues of the drugs as internal standards.

Interpretation and reporting

It is the scientist's role to ensure that the most accurate and precise results are delivered to the sporting body or workplace and to make sure that procedures have been enforced to protect the integrity of the sample. The responsibility then lies in the hands of trained medical personnel to determine the meaning of these results when related back to the individual.

In WDT this responsibility lies in the hands of a Medical Review Officer (MRO) who will interview the donor to determine whether there is any legitimate reason for the drug to be present before the result is reported to the employer.

The sporting bodies similarly have medical experts skilled in playing this interpretative and advisory role.

Conclusion

Both SDT and WDT must only be carried out by laboratories who are able to perform to the international standards dictated by the recognized authorities. SDT laboratories must be accredited by the IOC or CAP. For WDT, North America laboratories should be either NIDA or CAP accredited. In Australia and New Zealand such a specialist accreditation system is not available at this stage but the members of Australian/New Zealand Standards committee will be addressing this gap over the next 12 months. In the meantime there is one laboratory in New Zealand (ESR) and a few in Australia observing the international standards.

If results of a test can determine the future of an athlete's sporting career or an individual's working career, they must be absolutely accurate.