QUO VADIS?

A History and Projected Future for Equine Drug Testing

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QUO VADIS?

Latin; Where are you going?

UBI TE

Latin; Where have you been?

100 BC- Honey and water were supposedly used to stimulate chariot horses racing in ancient Rome; crucifixion awaited those that were caught in the act of doping.

1899- The word "doping" enters the English lexicon.

Early 1900's- Drug testing in racing begins but testing methods are primitive. Cocaine was an early "winner".

1910- In France, the Russian chemist Bukowski proves it is possible to detect drugs in equine saliva; method detects strychnine, morphine, cocaine and caffeine.

1932- Bukowski's methods are adopted in Florida and "modern drug testing" begins in the US.

Late 1930's- Pre-Race testing involved identification of crystals or injecting rats with urine or saliva to examine the effects on behavior.

1944- Paper chromatography separation and detection is introduced. By extracting drugs from body fluids they could be separated, detected and identified.

Late 50's- Thin-layer chromatography becomes the most used form of chromatography for drug detection.

1950's- Radio-immunoassay (RIA) was developed. In the 1960s, immunoassay technology was enhanced by replacing radio-isotopes with enzymes for color generation.

1950's- Gas chromatography-Mass spectrometry is introduced.

1960's- High performance liquid chromatography (HPLC) instruments become commercially available. Specific gravity, ultraviolet and fluoresence detectors are added over next decade which permits improved detection of some drugs.

1960's- Introduction of gas chromatography with various detectors to examine body fluid extracts introduced in Ohio racing laboratory.

1968- Malcolm Dole develops electrospray ionization.

1979- First commercial LC-MS offered for sale.

2010- Exact mass/high resolution MS coupled to UHPLC detects and confirms almost any drug at picogram levels (depending on method of sample preparation).

UBI TE!

Over the years, labs serving one or more jurisdictions have instituted these different technologies and applied them to equine testing in saliva, blood and/or urine, often without notice. How up-to-date such testing was and is today has largely depended on the willingness of the jurisdiction served to pay the price of what truly excellent testing costs.

Despite the advances, there remains no single methodology that detects all drugs all the time and the more coverage requested or required, the greater the expense.

Newer LC-Exact Mass methods may solve this problem.

Advanced sample preparation methods and UHPLC-high resolution mass spectrometers make it possible to detect almost any compound (peptide, acid, base, neutral, amphoteric compound, etc.) in blood and/or urine in a highly efficient, very rapid analytical screen. This may allow such technology to eventually be placed at the racetrack and pre-race sampling may again become useful and popular. However, these advances will continue to lower the thresholds for detectability of many substances.

At some point, this instrumental sensitivity and "background" contamination will merge and no one will be able to produce a truly negative result on any sample.

QUO VADIS?

A regular paper clip weighs about 1.0 g.

Divided into 1,000 pieces, each piece weighs 1.0 mg.

(1/1000$^{th;}$ 10^{-3})

Divide one piece into 1,000 pieces and each weighs 1.0 ug.

(1/1,000,000$^{th;}$ 10^{-6})

Divide one ug piece into 1,000 pieces and each weighs 1.0 ng.

(1/1,000,000,000$^{th;}$ 10^{-9})

Divide a 1 ng piece into 1,000 pieces and each weighs 1.0 pg

(1/1,000,000,000,000$^{th;}$ 10^{-12})

Divide a 1 pg piece into 1,000 pieces and each weighs 1.0 fg

(1/1,000,000,000,000,000$^{th;}$ 10^{-15})

THIS IS THE LEVEL NEW MASS SPECTROMETERS CAN DETECT

WHERE DOES THIS END?

Ubi est finis ?

When is a low drug level **too low** to call a positive?
Micrograms, Nanograms, Picograms, Femtograms, Attograms, Zeptograms, Yoctograms?

Have we already exceeded the limit?

In too many cases the answer is Yes. In fact, many current thresholds could just as easily be caused by or exceeded by environmental contamination.

Levels of mepivacaine metabolite following IV injection of mepivacaine

DOSE	Time post-dose (hrs)	Level (ng/ml urine)
1 ug (2.5 ng/kg)	1	0.15
	2	0.15
	3	0.17
	4	0.17
10 ug (25 ng/kg)	1	0.54
	2	1.26
	3	0.56
	4	0.65
100 ug (250 ng/kg)	1	6.55
	2	7.47
	3	6.73
	4	3.06
	8	3.40

Soon to be required protective clothing for trainers, veterinarians, grooms, hot walkers, jockeys…

SOLUTION: When in Doubt, Apply Common Sense:

Regulation of drug use in the racing industry:

1. Categorize the drugs

2. Establish penalty scheme that reflects the seriousness of the violation

3. Apply the science of pharmacology to the use of drugs and to the interpretation of data obtained from detection/confirmation of drugs in equine samples

1. Categorize the drugs

A Scheme for Categorization of Drugs of Abuse

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Abstract

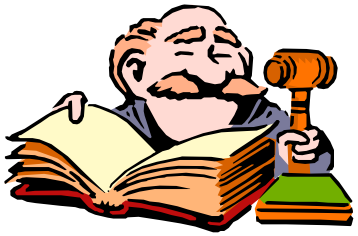
Members of racing commissions are often uninformed regarding the pharmacology of drugs detected in racehorses. The commission for the State of Louisiana requested a list of drugs categorized according to the surveillance for the Louisiana State Racing Commission in July of 1987. A newly appointed Commission took office in January of 1988 and asked whether or not the "Official Chemists" laboratory would provide a list of drugs categorized according to severity of infraction of the Louisiana Rules of Racing. As a veterinary pharmacologist, I felt that this could be accomplished, but I was also certain that no such list would be unarguable. Nevertheless, the Commission now has such a categorization, which is applied to every drug violation case at every hearing, and which has the input and sanction of the equine veterinarians in this State.

The first step was to determine whether any other U.S.

2. Penalty scheme that reflects the seriousness of the violation

Louisiana Penalty Guidelines by RCI Category (LAC 35:I.1797)

- Class 1- License suspension for > 1yr ≤ 5yr
  - ➔$5000 fine, purse redistribution
  - ➔Class 2- License suspension > 6 mo < 1 yr
  - ➔Fine > $1500 < $2500, purse redistribution
  - ➔Class 3- License suspension > 60 d < 6 mo
  - ➔And/or Fine <$1500, purse redistribution
- Class 4 & 5
  - ➔1st violation in 12 months, $200 fine
  - ➔2nd violation in 12 months, $500 fine
  - ➔3rd violation in 12 months, same as Class 3



3. Apply the science of pharmacology to the use of drugs and the interpretation of data obtained from detection/confirmation of drugs in equine samples. This approach mandates that thresholds for “trace levels” of equine therapeutics and drugs used by humans be established.

But that’s not what we’ve got.

In fact…

Racing jurisdictions have bought “A Pig in a Poke”, been sold “A Bill of Goods”, etc.

More and more withdrawal times and thresholds in the “ARCI Controlled Therapeutic Medications Guidelines” are being proven to be “built on a pillar of sand.”

Pletcher overage that wasn’t has major implications for racing

Posted by The Biz on Jul 24, 2015

Rocky Transition for New Drug Rules in West Virginia

by Ray Paulick | July 29, 2014

Regulation of medications should be based on good science, not just technology (LOD, LOQ of instruments).

It should permit adequate therapeutic treatment of horses, not limit the use of effective drugs based on "public opinion", emotion, hyperbole or uninformed or unproven speculation, mythology, or financial and political agendas.

Studies to establish thresholds/withdrawal times should be based on scientific, pharmacological principles, using relevant dosing, routes, regimens and formulations to obtain needed data. Environmental contamination must be taken into account.

Good science requires that such studies address the effects of breed, weight, age and sex of horses and use statistically relevant numbers of animals for each to obtain an accurate assessment , including variability. Inherent variability must be taken into account.

Data should be interpreted by independent experts, not stewards, Commissioners or Equine Medical Directors.

QUO VADIS?