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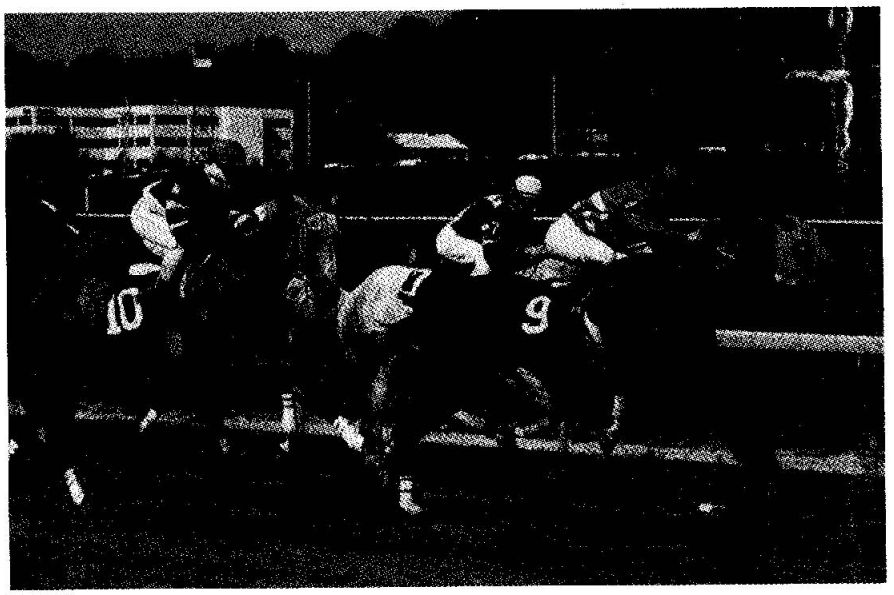
# Kentucky Laboratory Takes Lead Against Horse Dopers

by Thomas Tobin

Within the last three years, a research group at the University of Kentucky has developed more than 50 high sensitivity enzyme-linked immunosorbent assay (ELISA) tests of drugs abused in racing horses. These include tests for narcotic analgesics, stimulants, depressants, tranquilizers, local anesthetics, anabolic steroids, diuretics, and other drugs abused in horses. These inexpensive tests are up to 1000 times more sensitive than comparable thin-layer chromatographic (TLC) tests, and can be performed in an hour. Their development has given equine drug-testing laboratories the tools required to control drug abuse in racing horses.



**Researchers hedge their bets by developing tests that detect low levels of high-potency drugs in racing horses. Expanded tests will make racing a winner.**

Introduction of these tests in 1988 revolutionized equine drug testing. Prior to their use, unscrupulous horsemen abused high-potency drugs, which are effective at extremely low dose, with impunity. The sensitivity of the ELISA tests led to a sudden increase in the number of "positives" detected at the racetrack. When the tests were introduced in one Southwestern state, the new tests took more than 40 trainers by surprise over a very short period, and all of them were suspended.

The improper use of drugs to stimulate performance ("horse doping") was pioneered by American trainers more than 100 years ago, and when these trainers went to Europe at the turn of the century, they became known as the "Yankee alchemists." The problem started with the purification of cocaine and morphine by chemists in the 19th century. These purified drugs, along with the invention of the hypodermic syringe, made acute stimulant medication of racing horses a practical proposition. The racing successes of the American trainers led to rules prohibiting medication in racing horses, and to the introduction of routine drug testing for the track.

The first recorded drug tests in racing took place in Russia in 1903, where an American trainer named James Keene was having a very good season. One afternoon, however, Keene was approached in the paddock by a Russian racing official with a chemist by the name of Bukowski in tow, Bukowski had a basket of frogs in hand, and he took saliva samples from Keene's horses and injected them into the frogs. The frogs behaved in a most un-froglike way, "croaking piteously." These croaks constituted the first drug positive in racing, and James Keene was ruled "off the turf" in Russia.

As well as ruling Keene "off the turf," these events started a tradition of effective and aggressive drug testing that has since become a hallmark of racing. In the 1930s, when few sensitive drug testing techniques were available, the racing industry developed crystal tests, highly sensitive for their time, that used silver, gold, copper, and other metals that could complex with plant alkaloids to form specific crystals. In the 1950s and '60s, racing adopted gas/liquid chromatography as the preferred technique for drug detection, and by the mid-1980s, all racing jurisdictions had drug-testing laboratories in place. ELISA testing is fast replacing TLC and radioimmunoassay methods. Based on the effectiveness of ELISA testing and to ensure medication-free racing, the industry is committed to extending its panel of high-sensitivity ELISA tests.

In the mid 1980s, equine drug testing typically consisted of broad-based TLC screening followed by mass spectral confirmation of suspected positives. While this method worked reasonably well, its Achilles heel was the low sensitivity of TLC screening for very-high-potency drugs. Over the years horsemen had discovered that high-potency drugs were undetectable by TLC, and they started using them without check. Foremost among these drugs were the narcotic analgesics or opiates.

As a group, opiates act somewhat differently in horses than in man, and have three actions that improve the performance of horses in a race. Opiates suppress pain, as *in* man, and can enable slightly unsound horses to run good races. Beyond this, this class of drugs stimulates locomotor activity or running in horses, whereas in man it produces narcosis or sleep. The third and most recently discovered action of the narcotic analgesics in horses is the delay of fatique. Opiates apparently suppress the inhibiting effects of blood lactate on performance, allowing racing horses to run stronger and finish stronger, especially in longer races.

Historically, these drugs have also been used in combination with stimulants, which potentiate the locomotor stimulant effects and likely also the analgesic actions of the opiates. Such drug combinations are commonly the case in illegal medication.

In selecting an opiate, it makes sense for a horseman to select a high-potency drug. The low doses necessary for doping with these drugs makes them hard to detect, and unfortunately for racing, the pharmaceutical industry has developed opiate analogs that are among

the most potent drugs available. For example, a large number of fentanyl drugs have been developed, some of which are 1000 times more potent than morphine. The most potent opiate known, etorphine or "elephant juice," is 10,000 times more potent than morphine. A dose of 50 ug of etorphine to a horse produces a very good pharmacological effect, and this dose is impossible to detect by TLC. In addition, effective drug doses for horses may be lower than for humans because the surface area-to-mass ratio, which the dose depends on in part, is lower in the horse than in man.

By 1985, a large number of potent drugs were used because they were undetectable by TLC. Among the drugs that were being abused by horsemen were stimulants such as cocaine, mazindol and amphetamines, bronchodilators such as terbutaline, clenbuterol, and glycopyrrolate. Tranquilizers such as acepromazine and the benzodiazepines were used to calm excitable horses so that they would not "run their race" in the paddock before the race. Local anesthetics were being used to block painful joints to enable sore horses to run. Anabolic steroids were being used to improve performance in the same way that these drugs are used in human athletics. Finally, and somewhat unusually, diuretics were being used to eliminate fluid from horses to improve their breathing. The problem had become critical and the racing industry had no ready solution.

In 1985, the Kentucky State Racing and Harness Racing commissions approached the University of Kentucky (UK) for help. UK's researchers proposed the development of a panel of highly sensitive immunoassay tests for these drugs. The project was ambitious, since the number of drugs abused in racing is large and the specific drugs abused changes constantly. The outcome of this project, after five years of intensive development, is a unique and expanding panel of 50 ELISA tests that can detect the presence of about 100 high-potency drugs in racing horses.

To be useful for horse racing, a test must be cheap, quick to perform, and exquisitely sensitive to subtherapeutic doses of the drug it detects. ELISA tests cost about eighty cents a piece, can be performed in an hour, and are capable of detecting drug concentrations on the order of parts per billion. Occasionally blood samples are taken pre-race, in which case all the horses are screened, but because only 10 ml can be drawn, there isn't enough drug in the blood for mass spectrometry confirmation. Drug concentrations in urine are generally about 50-fold higher than in blood, and larger sample volumes are possible, so ELISA test results can be confirmed. However, urine confirmation is generally possible post-race only, given that collection is time-consuming and races are run every 30 minutes, so most testing at the racetrack is administered post-race and post-race testing, not every horse is screened with every test. Instead, rotating panels of about 10 tests may be used in horses on a random basis. Currently, about 10 percent of runners are routinely tested post-race.

Because horses are herbivores, there is a high matrix effect in urine due to plant phenols, but clean-up steps are being developed, and the ELISAs in current use at the track have been shown effective. Currently, there are 22 or 23 legally available opiates on the market for human consumption, and 19 of these have been identified so far in horse-doping cases. The number of illegal "designer drugs" is unknown, and can only be guessed.

When these tests were introduced into post-race testing, they immediately exposed substantial patterns of abuse. They exposed Sufentanil abuse in one Southwestern state, and when the results of the test were announced, the leading trainer at one meet lost his first place standing. In other states, large numbers of positives for buprenorphine, etorphine, mazindol, terbutaline, clenbuterol, and acepromazine were uncovered and long-standing patterns of illegal medication were brought to an end. More recently, patterns of butorphanol, corticosteroid, and diuretic abuse have been exposed and halted by recently developed tests. Table 1 presents a comparison of the effectiveness of some of these ELISA tests with the older TLC-based methodology. The success of these tests is such that they are now in use in over 50 laboratories worldwide, and to have credible equine drug testing a jurisdiction must have an ELISA-based program.

TABLE 1

**Efficacy of ELISA Based Tests Compared With TLC**

| DRUG | TLC Status | ELISA Positives |
|---|---|---|
| Buprenorphine | No test | Multiple, >40 |
| Oxymorphone | Marginal | Multiple, >40 |
| Sufentanil | No test | 10 in 300 tested |
| Mazindol | Marginal | Multiple, >30 |
| Cocaine | Marginal | Multiple |
| Acepromazine | Marginal | Multiple, >25 |
| Etorphine | No test | Multiple, >10 |
| Bumetanide | Marginal | Multiple, >17 |
| Ethacrynic Acid | No test | 5 |
| Metaproterenol | No test | Multiple, >10 |
| Dexamethasone | Marginal test | 10 |

The left-hand column lists drugs detected in racing horses after the introduction of ELISA testing. The second column lists the status of the TLC tests available for each drug, while the third column lists the approximate number of positives developed on introduction of an ELISA test for this drug. The current list of ELISA tests from ELISA Technologies, A Division of Neogen Corporation consists of 50 individual tests that detect the presence of 100 or more high-potency drugs.

While these events served to establish the utility of ELISA testing, they also created a major challenge for the racing industry. If ELISA testing was so effective, would the racing industry be able to develop new ELISA tests for all the drugs abused in racing? ELISA tests are quite difficult to develop, requiring the talents of a number of researchers with different expertise. To date, the research group at UK, consisting of a synthetic chemist, Dr. David Watt, an immunochemist, Dr. Daniel Tai and a veterinarian, Dr. Thomas Tobin, has met this challenge and produces about one new test a month for application in equine drug testing.

In addition to their sensitivity, these ELISA tests have the advantage that they can be automated. We foresee most equine drug testing being done with automated ELISA tests, which will improve both the scope and the efficiency of testing. It also appears likely that we will be able to develop quantitative tests for drugs such as phenylbutazone, or "horse aspirin," that are legal below a specified level. ELISA testing is likely to replace TLC and radioimmunoassay for most equine testing applications.

These tests are also useful for drug screening in other areas. They are clearly applicable to human urine testing for minor drugs of abuse, and also for life insurance and employee testing. Agricultural uses include screening of livestock in stock shows and screening of meat, poultry, and dairy products for drug residues. They should also be equally applicable to screening human athletes in competition, in the same way they are used for racing horses.

These ELISA tests are available to racing jurisdictions worldwide and are being commercially produced by ELISA Technologies, A Division of Neogen Corporation under license from the University of Kentucky. Similar tests for other drugs abused in racing horses and other markets are currently under development.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