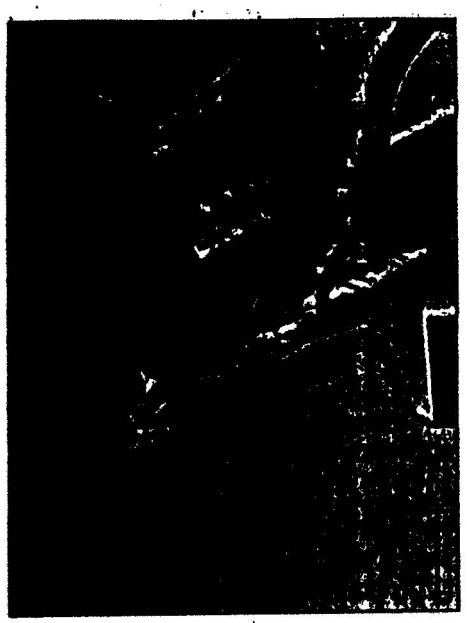
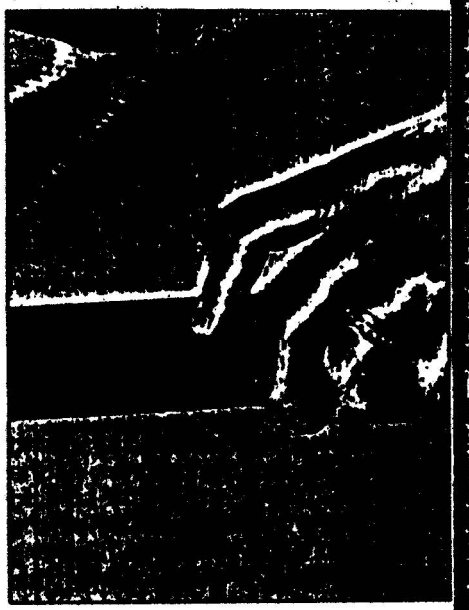
Developed some three years ago, ELISA testing for prohibited medications has grown in scope and application



TEST OF TIME



BY TOM TOBIN

Three years ago, in late 1987, the first ELISA tests developed for use in racing were released for field trial. Within weeks these tests yielded 40 or more positives in the Western United States for the small number of drugs (three/four) for which ELISA tests were then available. This sequence of events was reported in The Blood-Horse *(Jan. 16, 1988, page 294), and about eight months later we reported on the application of this technology to pre-race testing (*The Blood-Horse *of Sept. 24, 1988, page 5476). In the two years since, ELISA testing has matured, and the number of tests available and the number of firms marketing these tests have increased. In this article, we will review the current status of ELISA testing from the perspective of the University of Kentucky team that was involved in the development of these tests.*

Abuse of high-potency drugs in racing was targeted as an area requiring research by the Kentucky State Racing Commission and the Kentucky Equine Drug Council. These groups funded research at the University of Kentucky that by 1989 had led to development of the largest collection in the world of ELISA tests for drugs abused in racing horses. At the same time, differences concerning the commercialization of these tests led to them being unavailable to the Kentucky State Racing Commission laboratory. This, in turn, led to independent distribution of these tests, first for use in Kentucky, and later throughout the world.

Worldwide distribution of these tests led to the setting up of a company called WTT ELISA TESTS from the initials of the UK scientists who developed the tests. Today this company, based in Lexington, markets 30 ELISA tests, the largest panel of ELISA tests available for use in racing horses. These tests are used worldwide.

"ELISA tests are the best thing ever to hit racing in terms of the honest horsemen. ELISA tests have horsemen who wish to use illegal drugs so shook up that they do not dare to use illegal drugs," said Dr. Al Gabel, previously professor of veterinary surgery at Ohio State University and now a Standardbred trainer in Ohio.

Dr. Rick Sams, director of the Equine Drug Testing Laboratory at Ohio State University, said, "All our important positives are developed from ELISA tests. With our TLC screens we get junk medications, but the really important drugs, the potent drugs that have the ability to affect the performance of a horse, are all detected by ELISA testing."

Acknowledgements: The work reported in this article would not have been possible without the support and cooperation of a large number of people. David Watt and Dan Tai were fellow originators of these tests with the author. Field applications of these tests have been in the very capable hands of Rick Sams and Diane Gerlin in Ohio, Cornelius Ubo in Pennsylvania, Marie Greene at Industrial Laboratories, Shih Ling Chan in Kentucky, and many others. Dr. Al Gabel provided the backstretch perspective, and the work would not have been possible without the support of the University of Kentucky, the Association of Racing Commissioners International, the Kentucky Equine Drug Council, and the Horsemen's Benevolent and Protective Association.

Similar comments came from Dr. Marie Greene of Industrial Laboratories in Denver, Colo.: "All our significant positives now come from ELISA testing. In the last week we have had positives for opiates, Acepromazine, and other drugs, all developed by ELISA testing."

Overall, in the last several months, a large number (60 or more) of potent abused drugs have been detected and called positive using ELISA tests. Most of these incidents of illegal medications that we are reporting have been discovered in the Midwest, especially in one jurisdiction that is running a full panel of WTT ELISA TESTS. The potent medications detected by these new ELISA tests in racing horses include etorphine or "elephant juice," bumetanide or Bumex, ethacrynic acid or Edecrin, propionylpromazine or Tranvet, Metaproterenol and Clenbuterol, dexamethasone, and morphine/codeine.

ETORPHINE (ELEPHANT JUICE, 14 positives)

About three months ago, in response to suggestions that etorphine was being abused, a Midwestern jurisdiction began screening for etorphine using WTT ELISA test for this drug. Almost immediately, a positive ELISA "hit" for etorphine was developed, although the apparent concentration of drug in the sample was very low. However, the sample was "confirmed" as etorphine and further etorphine positives were developed from this same track. Because ELISA testing is only suggestive of the presence of a given drug, it is imperative that all ELISA hits be confirmed by mass spectral analysis.

Investigation as to the source of these etorphines turned up an unusual pattern of distribution. Etorphine, the original source of which is still unknown, was being sold by a practitioner to horsemen in single dose syringes as an "undetectable stimulant." Because the ultimate distribution of these syringes remained unknown, the authorities took the precaution of testing all horses running in the state in question and passed along their information on the availability of etorphine to other testing laboratories. Ultimately, about 13 etorphine positives were called that appeared to be associated with this cluster of events.

The first positive reported with our test for etorphine was from another Midwestern state, where an etorphine positive turned up in a horse which had been heavily treated with the de-worming medication thiabendazole. The laboratory director called to ask whether we knew of any cross-reactivity of our test for etorphine with thiabendazole. We replied that we did not, and she went ahead and confirmed etorphine in the sample. It turned out that the preferred way for some horsemen to use etorphine was with de-wormers, which they incorrectly believed could mask the presence of the etorphine.

Extensive field experience with the etorphine ELISA has shown this test is very sensitive and readily detects clinically effective levels of the drug. This sensitivity is very important since etorphine is the most potent narcotic available for abuse in horses and, as such, is the most difficult to detect. Additionally, the etorphine ELISA is a very clean test with a very low incidence of false positives and, to our knowledge, is virtually specific for etorphine. Since etorphine is a drug that is not readily available and whose use tends to be sporadic, an accurate test is particularly useful.

ILLEGAL DIURETICS: BUMETANIDE (Bumex, 17 positives)

Bumetanide is a high-ceiling diuretic that is marketed for use in humans. It is closely related to furosemide, but bumetanide is 20 times more potent. One can administer much smaller doses of bumetanide and obtain a pharmacological effect that is comparable with that of furosemide. Bumetanide is therefore a drug of choice for individuals who wish to obtain the pharmacological effects of furosemide, but who seek to reduce the likelihood of detection in the horse's urine.

When the ELISA test for bumetanide was put into the screening panel in a Midwestern state, it soon began to show positives for bumetanide. A problem with this ELISA test, however, is that it was much more sensitive than the confirmation methods that were initially available for this drug. The laboratory in question, therefore, developed an improved confirmation method for bumetanide and used it to confirm its ELISA positives. When this confirmation method was put into place, many bumetanide positives were detected and confirmed, abruptly ending a widespread pattern of abuse of this very potent diuretic.

Bumetanide is a classic drug for which there is a high potential for abuse. In the first place, bumetanide is readily available, an important requirement for widespread abuse of a drug. Secondly, it is thought to be a treatment for the very common equine problem, "bleeding" or exercise induced pulmonary hemorrhage (EIPH), and there is a strong demand for treatment of this condition by horsemen. Thirdly, while treatment of EIPH with furosemide is legal in most jurisdictions, its use is controlled by rule, and it is illegal in a few jurisdictions, creating further pressure for illicit treatment of this condition. Fourthly, and most importantly, bumetanide is a drug that is 20 times more potent than furosemide and is therefore 20 times more difficult to detect than furosemide. All of these factors contribute to a higher than average potential for abuse of this medication, especially in jurisdictions that do not permit use of furosemide.

ILLEGAL DIURETICS: ETHACRYNIC ACID, (five positives)

Ethacrynic acid (Edecrin) is another diuretic that is a highly effective substitute for furosemide and that also is difficult to detect. Ethacrynic acid is given to horses at doses of about five mg/horse, about 1/20 of the dose of furosemide. As well as being given in small doses, it is very difficult to detect by standard TLC screening techniques. For these reasons, ethacrynic acid has often been used in jurisdictions that ban the use of furosemide, or those in which the requirements for furosemide use, such as use of a detention barn, are considered bothersome.

When our ethacrynic acid ELISA test was first introduced, it immediately uncovered a pattern of ethacrynic acid abuse. For the first week or so, ethacrynic acid was being discovered in harness horses at a rate of one to two positives/day, suggesting relatively free use of this drug. However, once the first positive was called, abuse of this drug stopped, and ethacrynic acid has no longer been a problem in harness racing in jurisdictions using the ELISA test for ethacrynic acid.

ILLEGAL TRANQUILIZERS: PROPIONYLPROMAZINE (TRANVET, three positives)

Propionylpromazine, or Tranvet, is an analog of Acepromazine used in small animal medicine as a phenothiazine tranquilizer. Tranvet was at one time marketed for use in horses, but it turned out to produce prolonged prolapse of the penis in stallions. Tranvet was therefore withdrawn from the equine market, but it has remained available to small animal practitioners. As a potent tranquilizer readily available to veterinarians, Tranvet has a considerable abuse potential in racing and show horses.

Tranquilizers such as Acepromazine and Tranvet are used in racing and show horses to "take the edge off" nervous or "washy" horses. A "washy" horse is one which runs his race in the paddock and cannot put in a good performance on the track. Acepromazine can also be used to calm nervous horses and to enable jockeys to rate horses more effectively during a race. All of these theories are based on empirical observations by horsemen, and there is no hard scientific evidence to suggest that tranquilizers actually produce these effects. Nevertheless, there is probably a continual search by the unscrupulous for undetectable tranquilizers that can be used to "take the edge off" racing horses and thereby help them win races.

Acepromazine is a phenothiazine tranquilizer that has been widely used in equine medicine and in racing for 20 years. While its actions in the horse are well understood, Acepromazine also is an exceptionally potent drug, in that doses of as little as one mg/horse have clear-cut pharmacological effects. With the development of improved TLC detection techniques for this drug, some horsemen switched to propionylpromazine, a structural analog of Acepromazine, when a tranquilizer was required close to post time.

Because of the suspected abuse of phenothiazine tranquilizers in horses, Acepromazine was one of the first drugs for which we developed an ELISA

(Continued on next page)

ELISA TESTING

(Continued from page 6189)

test. This test turned out to be a very sensitive and useful one in that it detected a large number of Acepromazine analogs. It cross reacts with Acepromazine, chlorpromazine, propriopromazine, trifluprornazine, and a number of other phenothiazine analogs. Like our etorphine test, it has very low background values in horse urine so "hits" on the ELISA test have a very high probability of being confirmable positives.

This test has developed a large number of positive hits, initially for Acepromazine and, more recently, for propriopromazine. Working with this assay, a Midwestern laboratory reports detecting about three propionylpromazine positives and confirming them. As with the better ELISA tests, the test is highly sensitive for a number of structurally related drugs and has very low background values allowing highly effective screening for this group of drugs. Screening by group for highly potent drugs is the preferred mode of use of ELISA testing for drugs such as Acepromazine and terbutaline.

ILLEGAL BRONCHODILATOR: METAPROTERENOL (10 positives)

Terbutaline is a member of a very large and potent group of drugs structurally related to adrenaline and amphetamine and in which actions on the respiratory system are accentuated. Terbutaline dilates small airways and as such is a potent anti-asthmatic. It is a member of a very large family of drugs that includes Clenbuterol, Salbutamol, Albuterol, Isoproterenol, Metaproterenol, all of which are exceptionally potent drugs and many of which are undetectable or very difficult to detect by thin layer chromatography. Members of this group of drugs reportedly have been widely abused in racing horses.

This group of drugs proved difficult to develop antibodies to, but more recently we have raised an excellent antibody to Terbutaline. This antibody cross reacts with Salbutamol, Clenbuterol, Metaproterenol, and likely with other members of this group. We therefore brought up an ELISA test based on this antibody and released it for evaluation and also for general use. Preliminary work showed that this test was sensitive and would detect administration of Clenbuterol, Salbutamol, and, of course, Terbutaline.

We released this test even though no generally accepted mass spectral confirmation method for Terbutaline existed. This was not a problem, since the test was an excellent one and we thought it likely that positive ELISA "hits" would develop and stimulate chemists to develop confirmation methods. This is exactly what happened, and at this time a number of positives for drugs related to Terbutaline have been called based on this test.

One of the first groups to try this Terbutaline ELISA was an East Coast laboratory, and this laboratory soon began to report ELISA positives. They were not able immediately to identify the source of these positives, however, other than Terbutaline. The laboratory then simply kept examining possibilities, and soon they had eliminated Clenbuterol. Finally, after several days of work, the lab reported the first Metaproterenol positives ever reported in Terbutaline. These positives are now being confirmed, and a positive for a very low level of Clenbuterol developed from this test has been reported in a Western state.

ILLEGAL CORTICOSTEROIDS: DEXAMETHASONE (10 positives)

Another group of drugs to which we have developed ELISA tests are the corticosteroids. The corticosteroids are potent anti-inflammatory drugs that act to suppress tissue responses to inflammatory mediators. They can be administered systemically for their anti-inflammatory actions or directly into inflamed bursae or joints to supress lameness. They are very potent drugs and can be very difficult to detect. For example, dexamethasone can be given intravenously shortly before a race and cannot be detected by the old testing methods for more than six hours after its administration. Similarly, some of the long-acting corticosteroids can be administered directly into joints and produce effects that last for days or weeks after administration of the drug.

We have developed and brought to market tests for dexamethasone, a corticosterold commonly administered within hours of racing, and methylprednisolone, a corticosteroid that can be given directly into the joint to reduce lameness. Each of these drugs is active for long after the old detection methods can detect their presence, and new analytical methods were badly needed. With the introduction of ELISA tests for these agents, control of abuse has been advanced substantially.

Working with the dexamethasone test, an East Coast jurisdiction developed several positives for dexamethasone, but initially had difficulty confirming them. However, careful TLC/immunoassay analysis of the samples strongly suggested the presence of dexamethasone, so an improved mass spectral confirmation method was developed. When this method was applied to the samples, they turned out to have confirmable levels of dexamethasone, adding a further 10 positives to the positive score for ELISA tests.

ELISA TESTING: IMPACT AND FUTURE DIRECTIONS

Overall, the availability of ELISA tests has made an enormous difference in equine medication control. It is now possible to custom-make highly sensitive tests for drugs for which no tests previously were available. These tests are rapid, sensitive, and economical and require a minimum of equipment and training. Once a useful ELISA test is developed for a drug, it is relatively easy to ensure that abuse of a particular drug does not occur, and the pace at which ELISA tests can be developed is increasing.

In addition to developing new tests, we are making a particular effort to develop what we call generic ELISA tests. A generic ELISA test is one that detects several members of a family of drugs, thereby allowing the chemist to screen for a number of related drugs at once.

Other work concentrates on the development of a new immunoassay-based technology that will dramatically increase the speed and sensitivity of ELISA testing. When this technology becomes available, ability to use this technology in pre-race testing will be dramatically improved, and its efficacy in post-race testing also will be markedly increased.

In summary, therefore, ELISA testing is a highly effective technology that can be targeted to detect specific illegal drugs abused in racing horses. The targeted drugs are the highly potent drugs that have a reputation for improving performance in racing horses. These drugs have long been abused by horsemen, confident that these drugs were undetectable by thin layer chromatography.

The advent of ELISA testing means that any illegal medication that is abused is, in principle, detectable. Horsemen who abuse illegal medications therefore never know when the next ELISA trap will be sprung, a circumstance that has had a very beneficial effect on the rate of illegal drug use in jurisdictions that employ vigorous ELISA testing programs. ■