An Evaluation of Pre- and Postrace Testing and Blood and Urine Drug Testing in Horses

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1. **Blood and urine are the major biological fluids used in drug testing in North America. Blood is used in both prerace and postrace testing, urine in postrace testing only.**
2. **Most drugs and drug metabolites are detectable in blood and urine, although certain drugs may be more readily detected in one than the other.**
3. **Although no testing program can analyze for all possible drugs, best coverage is obtained by testing both blood and urine. The addition of blood to all testing programs is recommended.**
4. **In prerace testing, blood samples are taken from all horses in every race within two hours of racing. The samples are analyzed at trackside laboratories. Horses with illegal medication are scratched.**
5. **In postrace testing, urine and/or blood are taken from selected horses after the race.**
6. **The combination of prerace and postrace testing of blood and urine offers the best drug coverage and prevents the racing of many illegally medicated horses. An affordable alternative is postrace blood and urine testing.**
7. **In the last analysis, each racing jurisdiction must evaluate the operational and scientific merits of prerace and postrace testing and blood and urine testing. The procedures which best support their rules of racing and are operable within their financial structure should be selected.**

Definitions

Positive Test

For the purposes of this report, a positive is a drug finding which violates a medication rule. An analyst reports a positive when there is sufficient data to substantiate the presence of a specific drug or foreign chemical substance. Partial data, which suggests, but does not satisfy the analyst that a specific drug is present constitutes a "suspicious." A "suspicious" may be upgraded to a positive by the acquisition of more data, depending on the medication rules of the particular jurisdiction.

Prerace Testing

Prerace testing involves the testing of all horses in every race for illegal[a] drugs before racing. In the United States, horses are detained in a secure paddock, and an initial 20-ml blood sample is drawn within two hours of post time.[18,27,32,40,41] These samples are analyzed[b] immediately for the presence of drugs at a trackside laboratory. All drug findings are confirmed by resampling the horse(s) involved, and results are reported before opening of the pari-mutuel windows. The judges or stewards may withdraw any horse(s) from the race.[c] A subsequent hearing may be held to investigate a drug positive in accordance with the rules of racing. Prerace testing is currently in operation at harness tracks in New York, New Jersey, Pennsylvania, and Ohio, and is always performed in conjunction with postrace blood and urine testing at trackside laboratories. In New Jersey, New York, and Ohio, all drug findings are reconfirmed at a central research facility.

Postrace Testing

Postrace testing involves the testing of selected horses for the presence of illegal drugs after racing.[23] Urine and/or blood from these horses are analyzed for drugs in a central laboratory, usually remote from the track. Drug positives are reported to the designated official who may take disciplinary action.

Selection of Biological Fluids

The biological fluids available for drug testing are sweat, saliva, blood, and urine. The selection of a fluid depends on the disposition of drugs, the analytical techniques available, the medication rules involved, and the degree of regulation required by the racing jurisdiction. In general, drugs are distributed to body tissues by the blood and are then eliminated or excreted from the body in urine, feces, and sweat. In certain instances drugs are detectable in saliva. Drugs and, in particular, drug metabolites, can be present in higher concentrations in urine which is essentially an ultra-filtrate of blood. If biological fluids which have lower concentrations of drugs are selected, more expensive and sensitive analytical techniques are required. Depending on the available resources, the rules of racing, and the amount of regulation required, a single fluid or combination of fluids may be analyzed.

Sweat

Limited data are available concerning the detection and excretion of drugs in sweat.[13,30] Another objection to the use of sweat is that it is difficult to refute a defense that drugs reported in sweat were surface contaminants and were never actually present in the horse. Sweat is usually not available prerace, which precludes its use in prerace testing.

Saliva

Collection of the sample. Saliva is collected by swabbing the horse's mouth with a gauze pad held in a forceps. The pad may have been moistened with a dilute solution of acetic acid. This procedure yields between 3 and 15 ml of undiluted saliva. Saliva is rapidly and easily obtained in this way, and collection of a single sample takes about four minutes.[14] Unfortunately, however, the concentrations of many drugs in saliva are low.

Properties of the sample. Acidic, highly protein-bound drugs do not appear to enter saliva in sufficient concentrations to be detectable.[8,10,14]

Because saliva is alkaline (basic) relative to plasma, the entry of basic (alkaline) drugs into saliva is restricted.[1,15,42] Thus, concentrations of certain drugs in equine saliva are only about 25% of the concentrations found in plasma.[14] Many basic drugs are pharmacologically active at very low plasma concentrations, so this reduction in concentration in saliva is important. Neutral drugs are found in about the same concentrations in plasma and saliva.

The volume of saliva which can be obtained is small, being not more than 20 ml and often as low as 2 to 3 ml.[14] Because of the reduced concentration of many drugs in equine saliva, this small volume can be a serious problem. Saliva is not, to our knowledge, routinely used as a biological fluid in most drug testing programs in the United States.

Blood

Collection of the sample. Blood sampling using prepackaged, sterile vials and needles is rapid and inexpensive. For sampling alone, minimal facilities are required. Since a professional veterinarian does the blood sampling, ancillary information about the clinical condition of the horse is obtained. Blood is, *per se*, the simplest, most economical, and rapidly obtainable biological sample to take from the horse. It is the only sample suitable for prerace testing under North American racing conditions. There is no evidence that venipuncture as utilized in pre- and postrace testing harms horses.

[a] For the purposes of this report, an illegal medication is a medication whose use is prohibited in the jurisdiction in question.

[b] Throughout this report analytical work is reported as the state-of-the-art in March, 1978.

[c] Withdrawing a horse from a race is commonly referred to in racing circles as "scratching."

Advantages of blood testing. The prinicpal advantage of blood testing is that almost all drugs detectable in blood are present in an unchanged form. Unlike urine, in general, only very small concentrations of metabolites are present, provided large doses of drugs are not administered.[21] For this reason, drugs which are excreted totally in urine as unidentified metabolites are detectable only in blood.[3,16,19,21,22,27,40] Blood samples are readily and easily obtained almost without exception. Normal endogenous biochemical material or "background" is significantly lower in blood than urine, so that very low concentrations of drugs can be detected without extensive sample cleanup and preparation. Because drugs are actually found in blood at or about the time of racing, the significance of the finding is usually relatively clear-cut.[39] Blood levels of drugs are essentially not affected by other drugs such as diuretics,[39] in contrast with the marked dilution effects observed on some urinary drug concentrations.[33] Because the disappearance of most drugs from the blood occurs relatively rapidly in the horse, the possibility of an analyst finding traces of a drug in blood days after administration is in most cases remote.[1]

Disadvantages of blood testing. An apparent problem with blood testing is the small size of the sample, which is usually not greater than 20 ml. Compounding this problem is the fact that some drug or drug metabolite concentrations in blood are a good deal lower than in urine.[4,6,28,29] The upshot of this is that the analytical techniques required in blood testing are somewhat more exacting than those required for urine.[2,4-6,12,18,22,40] Some classical analytical techniques used in urine testing are not readily applicable to blood testing.[4,18] Given sensitive analytical techniques, the small size of blood samples is not a problem. Another disadvantage is that some drugs and drug metabolites detected in urine may not be detected in blood.[2,3,17,21,38,40] Use of a blood sample also limits the time during which drug administration can be detected.

Relationship Between Blood and Urinary Concentrations of Drugs

The principal difference between blood and urinary levels of drugs is that urinary concentrations of drugs or their metabolites tend to be higher than plasma concentrations of drugs. In the kidney, all drugs and drug metabolites pass freely into a freshly formed plasma filtrate which is the first step in urine formation.[39] During urine formation any drug which is predominantly water soluble, and especially drug metabolites, can be concentrated several-fold.[39] For this reason, some drugs which are marginally detectable in plasma, are readily detectable in urine. On the other hand, drugs which are excreted as metabolites in urine may be detectable as the parent drug in plasma.

Urine

Collection of the sample. Urine collection is slow, difficult, and expensive compared to blood and saliva. While up to 90% of horses will urinate within one hour, some horses must be held for up to three hours to produce a urine sample, and occasionally no sample is obtained for testing. Routine use of diuretics to facilitate urine collection is not recommended, as these drugs interfere with urinary concentrating mechanisms and result in reduced urinary concentrations of many drugs.[21,33] If a large number of horses is being tested, this means that a considerable holding facility must be available. Further, urine collection is apparently an acquired skill, so a staff of trustworthy and skilled urine collectors must be maintained. Due to these limitations, usually only several horses from a given race are sampled. The time constraint virtually eliminates the use of urine as a prerace testing medium in North American racing, although urine is used in Europe and Asia.[7,31,41]

Advantages of urine testing. The major advantage of urine testing is that relatively large quantities (200 to 500 ml) are usually available compared to blood or saliva. Many drugs and drug metabolites are found in urine in concentrations greater than those found in plasma.[6,23,35-37] These concentrations render the analyst's job with urine easier if classical analytical techniques are used. A sufficient sample is also available for a number of confirmatory tests.

Disadvantages of urine testing. The principal disadvantage of urine testing is that many drugs are excreted in urine as water-soluble metabolites which are difficult to extract and in some cases exist as unidentified compounds.[3,9,11,17,20,25,26,28,34] Drug metabolites of adequate quality for analytical standards are very difficult to obtain commercially. Another problem with urine testing is that the volume and pH of urine can vary considerably in response to changes in the animal's environment and to drugs which may be administered. Thus, diuretics can greatly increase the volume of urine and dilute much of the renal concentrating effect.[39]

Prerace Testing

Sampling Procedures

The only biological sample used in prerace testing in the United States is blood. An initial blood sample is drawn from all horses within two hours of racing and analyzed at the adjacent laboratory. Once sampled, the animals must be held under close supervision until race time to insure that no drugs are administered between sampling and racing. All drug findings are confirmed by resampling the horse(s) involved. In the event the horse is

scratched, urine samples are also collected for additional confirmatory analysis.

Analytical Procedures

The general analytical approach used in prerace blood testing involves a preliminary screening procedure to test for a wide variety of drugs and use of confirmatory techniques to indentify positive samples. Solvent extraction is used to isolate drugs from blood according to functional groups present. The extracts are analyzed using a combination of ultraviolet spectroscopy, thin layer chromatography, gas chromatography and, in Pennsylvania, high pressure liquid chromatography.[40] Based on these analytical techniques, a drug is identified to the satisfaction of the analyst and a report is made to the designated official prior to post time.

Benefits of Prerace Testing

Prerace testing is the best mechanism by which the actual running of illegally medicated horses can be prevented. Its presence on a track acts as a highly visible and immediate deterrent to illegal medication, and as such, creates an atmosphere of confidence in racing. The integrity of the testing system is increased because the chain of evidence is shortened. Because the results of the test are available while the animal is still in the secure area, the animal may be isolated and resampled for confirmatory or independent testing if necessary. Since all horses entered are tested prerace, testing coverage is more equitable than postrace testing of selected horses. Because prerace testing is completed before the race, it simplifies regulatory problems for both horsemen and track management. Because the horse is disqualified before racing rather than after winning a purse, both the incentive to and incidence of legal challenges are reportedly reduced.

Blood is the only biological fluid presently used in prerace testing in North America. The benefits of blood from a technical point of view are discussed above under advantages of blood testing.

Limitations of Prerace Testing

The major limitations associated with prerace testing are the technical disadvantages associated with the use of blood as a biological fluid. These are discussed above under disadvantages of blood testing. In addition, the time constraints imposed on prerace testing are more demanding than in postrace analysis. It is imperative that strict security be maintained between sampling time and post time.

Costs of Prerace Testing

Prerace testing and postrace urine testing are used in combination in New Jersey, New York, Ohio, and Pennsylvania. Each operating racetrack requires a separate laboratory located at trackside. In some instances, two tracks may share the same facility if racing dates are compatible.

Each laboratory requires approximately 600 to 700 sq ft of fully equipped space. Total cost is $80,000—approximately—$30,000 for the facility and $50,000 for analytical instrumentation. Instrumentation costs are usually amortized over a five-year period. Salaries, benefits and supplies for operation of each laboratory are approximately $350/day, thus, the average cost of testing 80 bloods prerace and 20 urines postrace for a 200-day racing season is $70,000.

Postrace Testing

Sampling Procedures

Postrace testing is performed on either urine and/or blood. Usually a few selected horses such as winners, beaten favorites, or others in accordance with the rules of racing are tested in each race. After the race, the horse is taken to a detention barn and cooled-out. At this point a blood sample is taken, if required, and a urine collector left in the stall to collect the urine sample. Once collected, the samples are cooled, frozen, or otherwise preserved and prepared for shipment to the testing laboratory.

Analytical Procedures

The general analytical approach used in postrace blood and/or urine testing involves a preliminary screening procedure to detect a wide variety of drugs and/or metabolites followed by confirmatory techniques to identify the exact chemical involved. A combination of solvent extractions and resin extractions are used to separate the drugs from endogenous biochemical material. Most analytical protocols involve the use of ultraviolet spectroscopy, thin layer chromatography and gas chromatography. An increasing number of laboratories are now using high-pressure liquid chromatography, infrared spectroscopy and mass spectrometry in addition to the above techniques.

Benefits of Postrace Testing

Postrace testing has been and remains the backbone of drug testing systems in most jurisdictions. The major benefit of postrace testing is the technical advantage of testing urine as a biological fluid. These are outlined above under advantages of urine testing. In addition, the time constraints imposed on prerace testing are more demanding than postrace analysis. Thus, a greater number of tests and analytical techniques could be used for screening and identification purposes.

Oral dosing with slowly absorbed drugs or "time release" capsules immediately before prerace sampling may not give rise to detectable blood levels of drugs. However, if dosing in this way gives rise to blood levels of

drugs effective at the time of racing, detectable drugs will show up in postrace blood and/or urine testing.

Limitations of Postrace Testing

For reasons previously indicated, postrace testing strongly deters, but does not totally prevent, the running of illegally medicated horses. It has no effect on betting payoff. Because the principal disciplinary action is confiscation of purses, fines and suspensions, there can be substantial financial inducement for legal challenge of the postrace testing process.

Postrace testing, as presently employed, samples 25% or less of the horses in a race on a selective basis. In the event a sample is accidentally lost in shipment or in the laboratory, no replacement can be taken.

Costs of Postrace Testing

The cost of contract postrace testing is variable, ranging from $10 to $20/urine or combined blood and urine analysis.

Relationships of Blood and Urine and Prerace and Postrace Testing

Combined prerace and postrace testing offers the best and most comprehensive medication control system available today. In the absence of prerace testing, selective postrace testing of blood and urine offers an affordable alternative. What is lost, of course, is the ability to scratch any of the postitive horses before they race.

Postrace urine testing offers good drug coverage. The inclusion of a blood sample helps under some circumstances and must be considered well worth the extra cost. The advantages of postrace blood samples include the ability to detect many drugs in blood which may be diluted in urine by diuretics, whose use is permitted in some jurisdictions. It also helps to detect a parent drug which may be found in urine only as metabolites. Finally, under some circumstances it may not be possible to obtain a urine sample, in which case the postrace blood sample may be the only sample available.

An ideal testing situation would be one where blood or urine levels of drugs would be detected in all horses before post time, with any offender of the rules of racing scratched. Prerace blood testing approaches this ideal and should be encouraged and regarded as the method which could ultimately be the best approach to racing's medication control problems.

Future Developments

Applied research in analytical chemistry and equine pharmacology is needed in order to improve the state of the art of drug testing. The chemist must be capable of detecting a wide variety of potent new drugs and the veterinarian must have knowledge of drug action in the horse, so that the use of illegal medication can be controlled. This is no small task.

New analytical methods are constantly being developed for use in drug analysis. These must be evaluated for use in drug testing and applied whenever possible.

There is a dearth of reliable information concerning the effects that drugs have on racing performance or speed of a horse.[18] Drugs must be administered to horses in doses comparable to racing situations under controlled, experimental conditions. Studies in drug metabolism, pharmacokinetics (clearance times) and exercise physiology must be expanded if we are to improve our knowledge of equine pharmacology.[18] A number of research programs are now conducting these studies using swimming pools, treadmills and simulated race track conditions, but much work remains to be done.

In the last analysis, each racing jurisdiction must evaluate the operational and scientific merits of prerace and postrace testing, and blood and urine testing. The procedures which best support their rules of racing and are operable within their financial structure should be selected.

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May 11 and 12, *Mastitis in Depth II*. Sponsored by the New York State College of Veterinary Medicine, Cornell University. Further details are available from the Office of Continuing Education, N.Y.S. College of Veterinary Medicine, Ithaca, NY 14853, (607)256-7700.

May 14–19, *Veterinary Mini-Course Series*. Sponsored by the Tuskegee Institute, School of Veterinary Medicine. *Orthopedic Surgery*. Instructor: Dr. C. D. Newton. *Clinical Dermatology*. Instructor: Dr. A. A. Stannard. For further information contact: Mrs. C. T. Lyman, School of Veterinary Medicine, Office of Associate Dean, Tuskegee Institute, AL 36088, (205)727-8176.

May 17, *Mid-Atlantic States Veterinary Clinic*. Sponsored by the Maryland and Pennsylvania Veterinary Medical Associations. For more information contact: Mr. Ray Thompson, 330 N. Charles St., Baltimore, MD 21201, (301)539-1700.

May 17–19, *Sixth Equine Nutrition and Physiology Symposium*. Sponsored by the Equine Nutrition and Physiology Society. Various papers will be given. For further information contact: Gary D. Potter, Secretary-Treasurer, Animal Science Department, Texas A&M University, College Station, TX 77843.

May 19–20, *Diagnostic Dermatology (A Team Concept)*. Sponsored by the Auburn University of Continuing Education School of Veterinary Medicine. A Team presentation. For further information contact: Mrs. Marion Moore, School of Veterinary Medicine, Auburn University, Auburn AL 36803, (205)826-4546.

May 28–31, *Veterinary Acupuncture Workshop*. Sponsored by the Tuskegee Institute, School of Veterinary Medicine. For further information contact: Dr. E. W. Adams, School of Veterinary Medicine, Tuskegee Institute, AL 36088.