



## Rapid Detection of Doping with Morphine in Horses

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**ABSTRACT:** In recent years, popularity of horse-riding has increased which has caused many consequences and problems. One of the important issues raised in equestrian sports is the abuse of different drugs for doping. It has been reported that flunixin meglumine has been mainly used for doing of horses in recent years in Iran and also most of doping cases are of morphine type. In this study, urinary morphine detection kit, with the ability to detect at least 300 mg/ml morphine in urine, manufactured by a France company named ABON® was used for early detection of morphine in horse doping. For this purpose, 36 horses of both sexes (26 horses in the experimental group and 10 horses as the control) were obtained. Horses in the experimental group were intramuscularly treated with 0.4 mg/kg morphine sulfate produced by Daroo Pakhsh Company. In addition, 10 control horses received distilled water by the same amount. Then, urine samples were collected 3, 24, and 48 hours after administration and tested using a detection kit. The results showed that morphine test in 100% of horses treated with morphine sulfate was positive in urine samples collected 3 and 24 hours after the administration, while the test results was negative in all horses that received distilled water. Using human morphine test kits, taking of this substance can be detected in racing horses up to 24 hours after consumption.

**Keywords:** Rapid detection of doping; Morphine Sulfate; Horse; Urinary kit

### INTRODUCTION

Providing the appropriate settings is the necessity of healthy development and growth. The veterinary structure provides this situation through dealing with animal diseases and preventive factors. The consequence of public interest in equestrian resulted in new problems. One of the important and preventive problems caused in equestrian and jumping tournaments is doping (Barragry, 1994). Drug tests have been conducted on horses since 1910, and the first positive result was reported in 1912 (King, 1999). Doping control in equestrian started in 1991 in Iran. By definition, every material, except for ordinary nutrition, which influences speed, stamina, and behavior in horses during the match and provides false values for animals of low race, body and stamina is considered doping. Not only is doping a jeopardy for the future of animals, but also it will bring serious physiological dangers such as different strokes, sever damages, and collision with objects during the match (Barragry, 1994; Greydanus & Patel, 2010). In such situations, it is necessary to run anti-doping tests and identify doped animals to provide the race with a clear and fair judgment. Different drugs have been used for doping in horses. Nowadays a broad range of drugs, including brain stimulants and anabolic steroids, are used. A number of the most important group of drugs which are applied in doping are as follows: the drugs influencing the central nervous system; non-steroidal anti-inflammatory drugs; anabolic steroids; diuretics; and pacifying drugs and painkillers. Morphine is among the drugs influencing the central

nervous system. They increase horse's performance by delaying the start of fatigue, increasing the level of awareness, decreasing the perception of match exhaustion, and increasing the cardio-respiratory activity. Although drugs reduce the activities of nerves and muscles in humans, they have effects like amphetamine in horses and increase the threshold of pain. Four liquids can be used for doping tests: sweat, saliva, blood, and urine, among which urine has been completely accepted as the best body liquid for analysis. The majority of drugs are excreted completely or partially through urine. Urine test has many advantages in addition to blood test, some of which can include the separation of drugs or drug metabolites after the match (Barragry, 1994; King, 1999). A greater volume of urine can be taken so that sufficient samples can be available for analytical and verification tests. Another advantage of urine is the fact that drugs and drug metabolites are mostly concentrated in urine (King, 1999; Tobin, 1989). Three types of tests are employed to detect doping in horses: screening tests, determinative tests, and confirmatory tests (Barragry, 1994). There are other separate strip tests which can be used to examine morphine, hashish, benzodiazepines, amphetamine and other drugs. According to the diagnoses of these tests, the presence of monoclonal antibody can be detected against the material being test (Neto, Andraus, & Salvadori, 1996). The morphine test kits are among the most reliable and inexpensive ways of detection in humans.

These tests are based on the detection of materials, drugs or their metabolites in urine (Mikkelsen & Ash, 1988). The most prevalent cause of disorder in the results of strip tests is error in sampling, technical errors, diluents and interveners (Walton, 2013). The aim of this study, conducted for the first time, is to propose a simple, convenient, and field method for the quick diagnosis of doping with the use of morphine in horses before matches and time-consuming complimentary tests like screening tests.

#### MATERIAL AND METHOD

The quick human-urine morphine test strips, made by ABON® in France, were used to detect the existence of morphine in urine. They are capable of detecting at least 300 mg/ml morphine in urine. To conduct this study, 36 horses were selected. In other words, 26 horses were considered to be the samples, and 10 more were used as controls. An amount of 0.4 mg/kg sulfate morphine, made by Darupakhsh, was injected into the

neck muscle in the 26 sample horses, and the 10 control horses received the same amount of distilled water through injection. The urine samples were collected before the injection, 3, 24, and 48 hours after the injection. Using the strip kits of quick diagnosis, the morphine in urine was investigated according to the instruction of the manufacturing company. In the morphine detection strip kits, the monoclonal antibody of morphine taken from mouse serum was used (Neto *et al.*, 1996). If there is morphine or its metabolites in the urine sample, they move up with the capillary migration method or get bonded with the existing antibody. In this case, the color line will not be observed on the strip, and the result of the test will be positive. There is another line as control which should be observed all the time. If urine is kept at a temperature between 2 and 8 degrees, this test can be conducted in 48 hours (Mikkelsen & Ash, 1988) (Fig. 1).



**Fig. 1.** Morphine detection strip test.

#### RESULTS AND DISCUSSION

The research results indicated that there was no intervening factors in horse urine causing the test result to be falsely positive or falsely negative. In other words, no positive reactions were observed in horse urine samples before the injection, and all the horses receiving morphine injections had positive results in their urine samples after 3 and 24 hours. After passing 24 hours, the samples were negative in terms of the existence of morphine. All the urine samples taken from horses, being injected distilled water, were negative.

According to the results, morphine quick detection strip test can be used for the initial detection of doping in horses before matches. The problem of doping in horses is known as an unacceptable and unfair action which can cause horses permanent damages in terms of natural and physiological performance and intervene the selection of animals with respect to various aspects. Usually, some groups are sampled for testing drugs in matches; these samples are taken from the winners,

losers and also taken from the injured animals and the losers with the highest winning possibilities. In most countries currently, samples are usually taken from winners (Barragry, 1994; King, 1999). In Iran, samples are also taken from winners, and the collected samples are usually sent abroad for doping tests. This is very important in sophisticated cases. Many drugs and substances are used for doping in horses. In Iran, opioid drugs are most commonly used as one of the most applied traditional doping substances which exists in blood usually in an almost intact form; however, it is changed or metabolized in urine (Wood *et al.*, 1990). Regarding acidic drugs such as phenylbutazone and furoseme which are highly connected to protein and are highly concentrated in blood, they are exceptions because they can be easily tracked in blood (Tobin, 1989; Wood *et al.*, 1990). The cleanse time for drugs in race horses is the main problem of controlling drugs in horses (King, 1999). It has been well identified that the difference in urine pH influences the concentration of drug and the speed of excretion for some drugs.

Horse urine pH is usually alkaline influenced by the food portion. It normally ranges from 7.5 to 8.5, which might become acidic under metabolic circumstances and severe physical trainings so that it can have different substances such as calcium carbonate and pigments probably tested as an intervening factor causing disturbances in the test results (Jones, 1988). Horse urine sample pH is almost constant in 48 hours; however, it increases after 48 hours (Warner, 1989). The abovementioned conditions may cause the test result to be falsely negative. In this study, a simple and inexpensive method was used in the field form. Given the existence of different intervening factors, more accurate tests should be conducted to confirm the diagnosis. However, before carrying out time-consuming methods, screening tests can be used to investigate suspicious doping cases in which traditional common drugs containing morphine in equestrian matches at the minimum cost. On the other hand, the impact of intervening drugs should be taken into account in doping test. The drugs intervening tests can be detected as covering substances, diuretics, and acidic substances causing test results to be falsely negative. They are easily identified with tests conducted to detect these kinds of cheats such as measuring keratins and the special weight of urine and measuring the acidity of urine. Drugs containing codeine cause the test results to be falsely positive in the morphine test, a fact which can be detected in thin layer chromatography (TLC) test. However, it is less used in horse medicine. Moreover, the consumption of 40 mg furosemide dilute the urine and does not have any effects in the results of morphine test. Amitriptyline, diazepam, and antibiotics do not usually cause any interventions in the detection of morphine (Walton, 2013). In this study, it was found out that although heather metabolism is different in human and horse, human morphine test kits can be used as a screening test for equestrian matches, and there are

no intervening factors in horse urine influencing the test results. Therefore, this kit can be used for test until 24 hours after taking morphine.

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