APPLICATION NOTE #201507

Extraction of Free Testosterone from Equine Hair on the Bead Ruptor 24

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Introduction

Hair samples are commonly processed for forensic analysis. Hair trace analysis has been a major tool for forensic scientists for decades. Hair samples can be analyzed for the presence of performance enhancing drugs such as hormones and steroids.[1] Testosterone for example, can be abused by athletes due to its ability to enhance muscle development, strength and endurance. The use of performance enhancing drugs, including testosterone, is banned in a majority of sports and scientists are investigating methods in the detection of testosterone from hair. [1]

A key component in creating an experimental workflow that will support the detection of testosterone from hair is the front end sample preparation method. Hair samples are a challenging sample matrix as hair is relatively resistant to disaggregation, hair tends to cling to vessel surfaces and once in powder form is easily aerosolized. An ideal mechanical method for hair disaggregation would have sufficient force to disrupt the target samples, would be compatible with a sealed tube and with the common organic solvent based extraction buffers. Historically, hair disruption methods have included mortar and pestle , cryogrinding and chemical digestion. Although these techniques are adequate, they do have their draw backs. The traditional mortar and pestle is labor and time demanding, cryogrinding is inherently low throughout and only efficient when using small sample sizes and chemical digestion is not only time consuming but uses hazardous acids and bases. Bead milling has recently been demonstrated to be an effective method for disruption of hair and fulfills all the ideal processing requirements. [2] Bead milling is achieved through the high speed shaking of a sample in a sealed tube in the presence of small beads. The high force impacts of the beads against the sample reduces the sample size.

Herein, we evaluate the potential for the extraction of free testosterone from equine hair samples on the Bead Ruptor 24 bead mill homogenizer coupled with sonication using the Sonic Ruptor 400 Ultrasonic Homogenizer.

Materials & Methods

Equipment

- **Bead Ruptor 24** (Cat #19-040)
- **Bead Ruptor 24, 7 mL Tube Carriage Kit** (Cat #19-345-007)
- **7 mL Hard Tissue Grinding Mix** (Cat #19-670)
- **Sonic Ruptor 400 Ultrasonic Homogenizer** (Cat #18-000-115)
- **ALPCO Free Testosterone ELISA Kit** (Cat #11-FTSHU-E01)

Sample Preparation and Analysis

Equine hair was obtained from a local farm and diced into 1 cm fragments. 20 mg of diced hair was washed in DD H20 for 3 minutes, followed by an isopropanol wash for 3 minutes. The hair was thoroughly dried and placed in a 7 ml tube pre-filled with 2.4 mm metal beads. The sample was processed on the Bead Ruptor 24 for two 50 second cycles at 6.8 m/s and chilled in an ice bath for 3 minutes between cycles. The dry ground lysate was resuspended in 100 µl/ mg of methanol and sonicated at amplitude 40 for 30 minutes on the Sonic Ruptor using an 1" diameter horn (OT-T-750). The sample was then incubated overnight at 50°C. Following incubation, the lysate was centrifuged for 15 minutes at 12,000 rpm (Beckman Avanti 30). 100 µl of the supernatant was removed and allowed to dry. The residue was reconstituted in 250 µl of Assay Buffer (ALPCO) and then diluted 1:1 with additional Assay Buffer. The sample and each standard was dispensed into their appropriate polyclonal hormone antiserum coated wells of an ELISA microplate(ALPCO). The remainder of the Free Testosterone Assay kit was carried out per the manufacturer's instructions. 2 µl from each reaction was quantified at 450 nm on a NanoDrop Spectrophotometer (Thermo Fisher) to determine the testosterone assay yields.

Results

Hair is an excellent matrix for the detection of testosterone and other steroid hormones, as it is renewable, non-invasive and non-excretory. [3] Steroid hormones are commonly incorporated into growing hair via the blood vessel that feeds the hair follicle. Testosterone that is administered intravenously can easily be detected in hair over a defined period of time. [4] In this application, we evaluated the Bead Ruptor 24's ability to process equine hair for the purpose of extracting free testosterone as shown in figure 1. As a proof of principle the extraction of testosterone from hair is useful as it demonstrates the potential for the extraction of various other steroid hormones such as cortisol or progesterone.

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20 mg of Equine Hair Before **20 mg of Equine Hair After**

Figure 1: 20 mg of equine hair pre and post processing on the Bead Ruptor 24. Post processing hair was converted to a fine powder that was easily dissolved in extraction buffer.

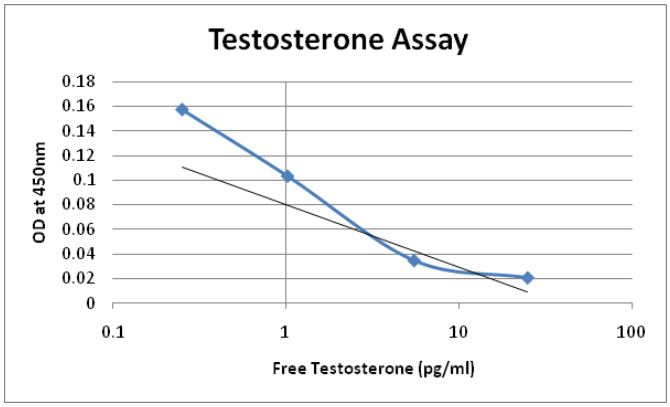

Figure 2 : Testosterone Standard Curve Measured at 450 nm

After dry grinding on the Bead Ruptor 24, a direct quantitative determination of free testosterone was performed by enzyme linked immunoassay using a human serum kit (ALPCO). This procedure is reported to have a free testosterone sensitivity of 0.17 pg/ml. The free testosterone yield of the extracted hair sample was determined to be 0.022 pg/mg of equine hair. According to a study found in the journal of Analytical and Bioanalytical Chemistry, it was found that the maximum values of endogenous testosterone that can be found in horse hair is 1 pg/mg.[5] This shows that the amount of testosterone extracted is slightly less than the maximum value. Extraction efficiency can be increased with additional optimization tests.

Conclusion

The Bead Ruptor 24 is capable of dry grinding equine hair in less than 2 minutes of processing for the extraction of testosterone. The Bead Ruptor 24 was proficient at grinding equine hair into a fine powder so that it may be resuspened in an alcohol solvent. In addition the Sonic Ruptor, is efficient at disrupting the hair powder facilitating the release of testosterone for easy enzymatic immunoassay detection.

References

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Bead Ruptor 24: (Cat #19-040)

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