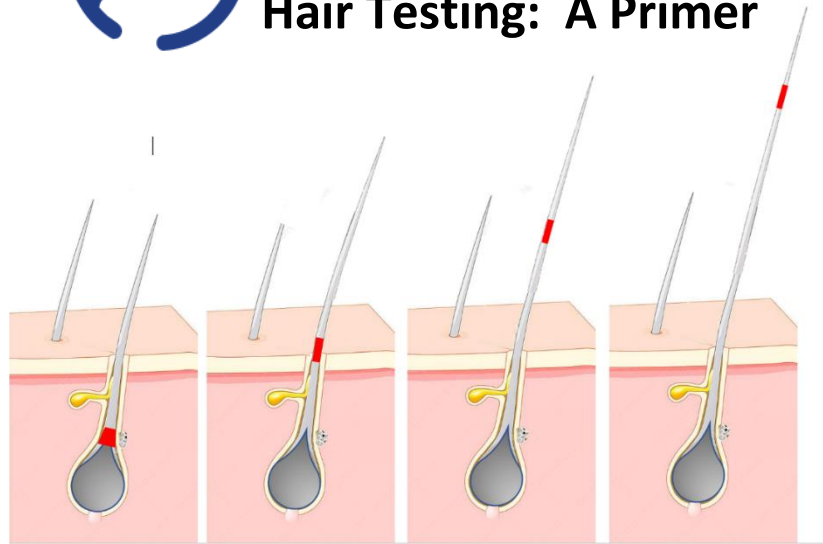




RMITC

Hair Testing: A Primer



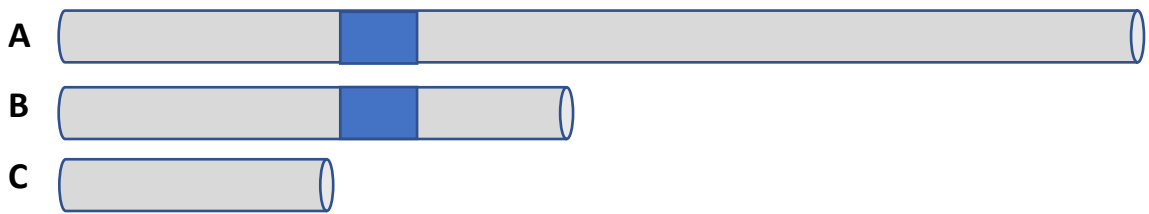
Hair testing is one component of a comprehensive testing strategy. It is a useful complement to programs that are largely based on blood and urine testing and can be useful for both regulatory and intelligence gathering purposes.

Blood and urine testing detect substances administered in the hours, days, or possibly weeks prior to sampling. Hair testing detects substances administered weeks to months prior to sampling. There may be little overlap in the coverage intervals provided by blood/urine and hair testing, and it may be advisable to acquire and test all matrices to ensure complete coverage for a given substance.

An effective hair testing program requires effective communication between regulator and laboratory to establish program goals and expectations.

Will hair testing results will be used for enforcement (and potentially result in administrative action), for intelligence gathering, or both? This determination will establish if screening analysis is sufficient or if confirmatory analysis will be required.

What is the desired interval of testing coverage? Mane hair grows at a rate of approximately 1" per month. The regulator can select the length of hair to be tested that reasonably corresponds to the desired time interval. If the entire length of hair is subjected to testing, coverage can extend well beyond a year. The trade-off for a long coverage interval is the risk that the deposition of drug from a series of treatments may go undetected as its concentration is 'diluted' by the amount of hair from the untreated period.



A, B, and C all represent hair from the same sample collection. A represents the entire length of hair. B represents a trimmed length consistent with a testing coverage interval requested by the regulator. C represents a trimmed length consistent with a testing coverage interval requested by another regulator. Comparing Sample A to Sample B one can appreciate how the concentration of the regulated substance will be lower in A than B and potentially be present in a concentration below the laboratory's lower limit of detection. Despite the same amount of substance in both samples it is possible that Sample A would be reported as passed whereas Sample B would result in the laboratory's issuance of a report of finding. Sample C will be reported as passed as the testing coverage interval did not include the treatment period.

Unlike as with blood and urine where a regulated substance continues to be eliminated over time; once incorporated into the hair matrix the substance remains present, relatively unchanged. It is therefore possible for a horse to be resampled at a later date with minimal risk of drug elimination from, or degradation in, the hair. This allows for a strategy whereby a single initial analysis is performed on a prescribed length of hair; the horse can be resampled if segmental analysis is warranted based on the results of the primary analysis.

In general, segmental analysis—in which a hair sample is portioned, with each segment subjected to labor intensive, pre-analytical processing and instrumental analysis—should be expected to cost more than that analysis of a single sample of a pre-determined length. A testing policy where each hair sample is required to undergo segmental analysis may not represent the most efficient use of limited regulatory funding.

Hair testing cannot detect every substance.

It is important to discuss drug-specific testing capabilities with the laboratory prior to submitting hair samples. The regulator is responsible for selecting the target drugs (or class of drug) and the laboratory can then advise if the substances are amenable to hair testing. Because not all substances are incorporated into hair, the use of hair as a matrix for screening analysis is inadvisable.

When requesting analysis for substances not routinely included in the scope of hair testing, it may also be advisable for the regulator to consult other laboratories to verify they have commensurate testing capabilities should split sample analysis be a consideration.

Substances currently amenable to detection through hair analysis include: beta agonists (e.g. clenbuterol, albuterol, zilpaterol, and ractopamine); Selective Androgen Receptor Modulators; anabolic steroids and their esters, sedatives, analgesics, and Selective Serotonin Reuptake Inhibitors (SSRIs) (e.g. sertraline [Zoloft] and fluoxetine [Prozac]). Detection can be further

dependent on dose, route of administration, duration of exposure, and characteristics of the individual substance.

Performing hair analysis to detect a substance that is not known to be incorporated into (or onto) hair can result in erroneous conclusions about a horse's exposure to that substance.

Analysis of hair samples detects substances introduced in two ways: 1) Incorporated into the hair matrix as it is formed in the follicle or 2) secreted onto the hair through sebaceous (oil) or sweat glands in the skin.

Hair testing may not detect administrations performed close to the time of sampling (days to weeks).

The method of sample collection—pulled or cut—may affect the ability to detect recent administrations.

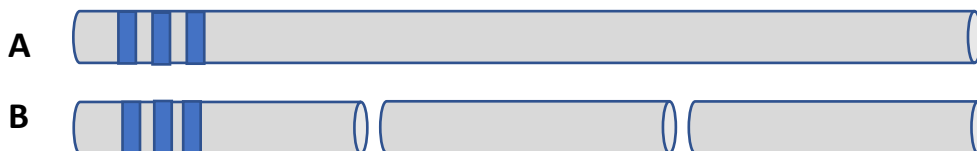
Again, if the overall amount of 'untreated' hair far outweighs the amount of 'treated' hair, the relative concentration of the substance is decreased and thus may not be detected through hair analysis.

A testing strategy that combines blood/urine and hair analysis represents the most comprehensive coverage.

Hair testing may not detect a single dose or even repeated administration of very low doses.

This is particularly true when the entire length of a hair submitted is tested as a single sample.

As with blood and urine testing, the sensitivity of each laboratory's analytical method establishes what is the lowest measurable concentration of a given substance that the laboratory is able to detect, and thus report.



The use of segmental analysis (Sample B) not only increases the probability that the regulated substance will be detected, it can also provide insight into the time period of the treatment interval.

Hair testing may be useful for regulators in reconciling treatment reports with specific restricted administration times or stand down intervals to determine compliance.

Note that the lab can only report substances detected and their estimated concentrations per unit weight (e.g. ng of drug/gram of hair). It is the regulator's responsibility to interpret laboratory findings as they relate to reported treatments.

At best, a laboratory can only provide a rough estimate of the administration time of a substance. It is not possible to declare that a substance was administered at 50 days rather than 60 days prior to sampling.

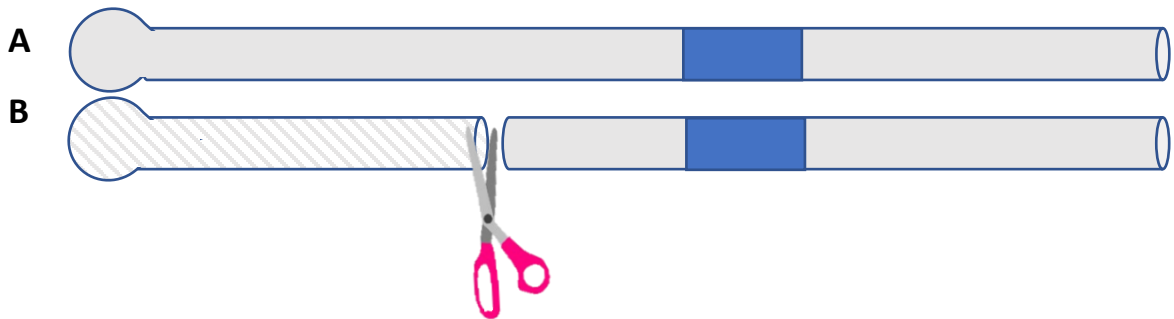
Estimating a time interval during which an administration likely occurred is problematic when cut (rather than pulled) hair samples are submitted. The laboratory was not involved in sample collection and cannot determine from the sample submitted how close to the skin line the hair was cut. Thus, the laboratory cannot form any conclusions about an estimated interval from treatment to sampling.

Photo 1 below shows a sample of mane being cut close to the hair/skin interface whereas Photo 2 shows the cut being made well-removed from the hair line. Inconsistency in the acquisition of cut hair samples can confound the interpretation of testing results. Ideally, hair that is not pulled should be cut as close to the body as possible.

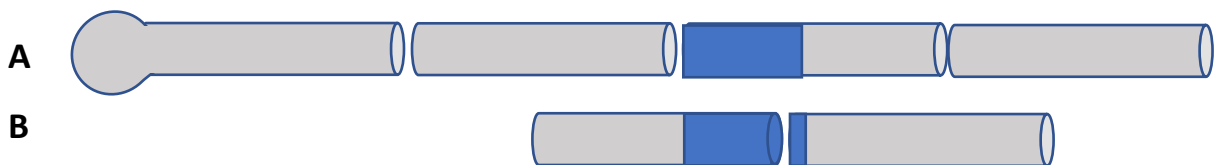
Photo 1



Photo 2



Sample A represents a pulled hair sample, with the root bulb attached. Sample B represents a cut sample with the cut made an unknown distance from the hair-skin junction.



If segmental analysis is performed on both samples, one could draw erroneous conclusions about Horse B's interval from treatment to sampling.

However, if treatment reports are submitted, the dosing interval can be reconciled with the results of segmental analysis. It is important to remember that the interval during which a substance is incorporated into the hair will extend beyond the treatment interval. However long a substance is present in the blood following cessation of treatment it will continue to be incorporated into the hair.

A negative hair test does not nullify the results of a positive blood or urine test. Conversely, a negative blood or urine test does not nullify the results of a positive hair test.

Negative hair testing results in conjunction with positive blood and/or urine results can indicate a relatively recent exposure.

Negative blood and/or urine results in conjunction with a positive hair testing result are likely indicative of a longer exposure-to-sampling interval.

Sample procurement is key. Refer to the RMTc/AQHA Hair Sampling Procedure Brochure: (<https://rmtcnet.com/wp-content/uploads/2018-07-Hair-Sampling-Procedure.pdf>) for sampling guidance.

A pulled sample is preferable both for estimating treatment timelines and to allow for DNA analysis should the identity of the horse from which the sample originated be questioned. (DNA can be extracted from the cellular material in the root bulb. The hair shaft lacks cells; it is not possible to extract DNA from a cut hair sample.) It may be advisable to acquire a blood sample at the same time cut hair samples are collected should DNA analysis be subsequently requested

Note: Some labs prefer cut hair to ensure that adequate sample amounts are submitted. It is recommended that the 'cut vs. pulled' discussion occur between regulator and laboratory in advance of sampling. Even where pulling is the designated method, it is advisable to provide individuals performing sampling with instructions and supplies to acquire cut hair should pulling not be an option for a given horse.

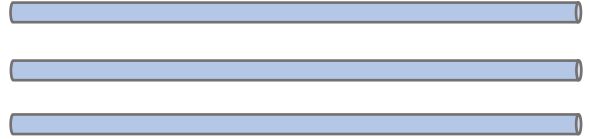
It is recommended that gloves be worn when hair samples are collected to avoid transfer of substances from the collector's skin to the hair sample.

Hair should be clean and dry. The sample bundle should be cinched with an elastic band or zip tie to maintain hair alignment. Loose or tangled hair should not be submitted.

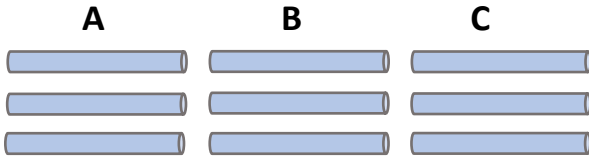
Before conducting any sampling consult the laboratory for minimum sample requirements to ensure that analysis can be performed. The laboratory must be provided with a sample of sufficient size, weighing approximately one gram. If segmental analysis to be performed, each segment must weigh a minimum of 1 gram—thus requiring a larger total sample.

To illustrate:

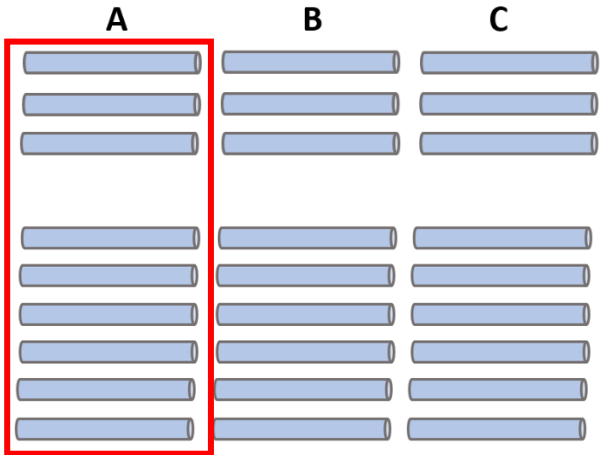
Three cylinders, each 3' in length,
weigh a combined total of 1 lb.



The cylinders are each cut into equal thirds.
The three A sections weigh a total of 1/3 lb.
Likewise for the B and C segments.



If there is a requirement for 1 lb. of each
of segments A, B, and C, it is necessary to
section 6 more cylinders.



Summary

Hair analysis is a useful regulatory and investigative tool. Its implementation in a testing program requires thoughtful planning, effective communication, and oversight by individuals sufficiently versed in matters related to sample collection, capabilities and limitations of the analytical methods in use at drug testing laboratories, and interpretation of testing results.

Reference

Analytical and Practical Aspects of Drug Testing in Hair, Forensic Science Series, Kintz, P, ed., CRC Press, Taylor and Francis Group, Boca Raton, FL, USA; 2007.