



HAIR TESTING FOR DRUGS OF ABUSE **Commonly Asked Questions and Answers**

1) What methodology is used?

- Hair samples are first screened using ELISA (Enzyme Linked Immunosorbent Assay), a proven reliable methodology for routine drug testing. Confirmation of ELISA presumptive positives are performed by gas chromatography /mass spectrometry (GC/MS) or gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS).

2) What is ELISA?

- ELISA is an acronym for enzyme-linked immunosorbent assay. ELISA assays are heterogeneous nonisotopic assays that usually have an antibody immobilized onto a solid support. The ELISA assay uses a microtiter plate that has the antibody to the drug, drug metabolite or drug class coated to each well of the microtiter plate.

3) What is the difference between EIA and ELISA?

- EIA is the more traditional enzyme immunoassay. The technology has been widely used for the analysis of drugs of abuse. It is homogenous in nature meaning that the analysis is performed without any physical separation during the analysis. ELISA is heterogeneous — the microtiter plate is washed before the reaction is allowed to go to completion. In general, ELISA assays may offer greater sensitivity than most EIA procedures.

4) Is ELISA forensically defensible?

- Yes, the technology is well established and has been used in many formats for the analysis of both drugs of abuse, Therapeutic Drug Monitoring and Serology (antibody tests) and Blood Banking procedures. Furthermore, ELISA technology is used extensively in the Pharmaceutical industry for new drug screening.

5) What is the turnaround time?

- The laboratory receives the samples via overnight courier. Preparation of the sample for testing is performed the day the samples arrive and the initial test is completed overnight. Negative results are reported within 24 hours of receipt. Positive results are confirmed, reviewed, and reported within 48-72 hours of receipt.

6) What drugs are tested with Hair testing?

- The drugs tested are the following: Amphetamines (Amphetamine, Methamphetamine, MDMA and MDA), Opiates (Codeine, Morphine and 6-monoacetylmorphine), Cocaine (and cocaine metabolites), Marijuana (THC and THC Carboxylic Acid metabolite), and PCP.

7) Can Hair testing detect Ecstasy?

- Yes, Ecstasy (MDMA) and MDA are screened utilizing the Amphetamines group ELISA and confirmed by GC/MS and/or GC/MS/MS.

8) Can Hair testing detect oxycodone?

- No, currently our Hair testing procedures do not target oxycodone.

9) How long are positive (non-negative) specimens retained by the laboratory?

- Non-Negative specimens are retained for a minimum of 12 months (the same as non-negative urine specimen).

10) Will the test results really reflect 90 days use of drugs?

- Hair follicles underneath the scalp are surrounded by a dense network of capillary blood vessels. Drugs in the bloodstream are able to incorporate and bind to the hair follicles underneath the scalp. It takes approximately 3-5 days for hair containing drug to reach the outer environment on top of the scalp to be collected based on the average rate of head hair growth. Head hair grows approximately 1.3 cm or ~1/2-inch per month. The standard length of hair tested by the laboratory is the first 3.9 cm or ~1 1/2 inches from the root end. Therefore, a hair analysis of 3.9 cm covers a time span of approximately 90 days, 3-5 days after drug use. Many employers find it useful to test both hair and urine for pre-employment purposes. Urine is useful for a current picture (last 3-5 days except Marijuana which is longer) of possible drug use and hair for an approximate three-month drug history.

11) Is Hair analysis appropriate for other workplace drug testing?

- Because Hair testing detects drug use over a long period of time, usually around 90 days, it is not an appropriate method for post-accident or reasonable suspicion testing. In both of these situations, the result should detect the drug use of an individual as close as possible to the time of the incident.

12) How does the lab distinguish between ingestion and contamination with PCP, which has no metabolites?

- An initial wash of all specimens is performed prior to the screening process as well as on a separate portion of hair prior to the confirmation. To date there have been no claims of external contamination with PCP in hair on any positive PCP results performed by lab. Positive hair specimens are stored by lab for one year and the hair can be retested for potential contamination, by analyzing the final wash and final extract by GC/MS and comparing these results to user hair and hair artificially spiked with PCP.

13) Does lab report out as "positive" the presence of cocaine alone and/or cocaine and/or cocaine and benzoylecgonine (BE) without other metabolites?

- The lab does not report out cocaine alone without the presence of cocaine metabolite on employment testing specimens. However, when cocaine exposure of children by adults is suspected, lab does report out cocaine alone due to exposure in the hair of children.
- The lab does report out positive hair specimens containing cocaine and benzoylecgonine only, which have a sufficient amount of benzoylecgonine or ratio of benzoylecgonine to cocaine to rule out external non-enzymatic hydrolysis of external cocaine to benzoylecgonine. Spiked hair samples with cocaine or exposure samples have significantly less benzoylecgonine to cocaine than user hair samples. In addition, upon request, the laboratory can analyze additional unique "in vivo" metabolites such as cocaethylene and norcocaine at very low limits of detection using tandem mass spectrometry (1). Upon request the final confirmation wash solution can be tested by tandem mass spectrometry and compared to the final extract by tandem mass spectrometry. User hair samples have significantly less drug in the wash than artificially spiked samples mimicking external contamination.

14) Since cocaine and BE are found in the environment, how does the lab ensure the results are from ingestion and not contamination ?

- See above response to previous question. Although it is possible to produce BE non-enzymatically from cocaine in the environment, typically the proportion of BE formed is relatively small and concentrations of cocaine are significantly greater than BE originating from external sources. In addition, BE does not incorporate into the hair as readily as cocaine and therefore very little incorporation of BE would occur from exogenous sources. In a 1995 study, BE was not detected in hair samples exposed to "crack" cocaine smoke, the most likely mechanism of external exposure to cocaine (2).

15) For marijuana, the lab reports out as "positive" the presence of THC alone without the presence of the metabolite carboxy- THC. Since THC is present in smoke, how does a THC positive distinguish between ingestion and contamination?

- The lab does not report out THC positives without the presence of carboxy- THC metabolite for employment specimens. We do offer a marijuana "exposure" test where we do report out THC only at the limit of detection. This test is primarily used to test children exposed to marijuana smoke from adult marijuana users.

16) In the CBS News blind sample comparison, why did the lab incorrectly report the contaminated negative hair sample as "positive"?

- First of all, the so-called negative hair sample was artificially prepared by an individual who either purposely or inadvertently spiked a sample with cocaine and cocaethylene. Cocaethylene, is a unique metabolite, produced *in vivo* from concomitant cocaine and ethanol use. The CBS sample that our lab and another lab reported as cocaine positive contained measurable amounts of cocaethylene.
- In addition, the CBS study of three hair testing laboratories was a totally unscientific comparison of the laboratories' abilities to accurately detect drug(s) in hair samples. The CBS study was publicly denounced as being a totally unscientific study by the Drug Testing Advisory Board and all three laboratories involved.

Literature References:

1. Bourland, J. A., Hayes, E. F., Kelly, R.C., Sweeney, S.A., and Hatab, M.M., Quantitation of cocaine, benzoylecgonine, cocaethylene, methylecgonine, and norcocaine in human hair by positive ion chemical ionization (PCI) gas chromatography -tandem mass spectrometry. *Journal of Analytical Toxicology* Vo124, No.7: 489-495 (2000).
2. Wang, W.L. and Cone, E.J., Testing human hair for drugs of abuse. IV. Environmental cocaine contamination and washing effects. *Forensic Science International* 70: 39-51, (1995).

REPORTING

17) How are the results reported?

- As with all laboratory-based testing, results are logged in the laboratory information system and reported to the client by confidential fax, or internet reporting via secure email notification.

ADULTERATION

18) Can Hair testing be beaten?

- We have not found any adulterants that can beat the test at this time.

TRANSPORTATION

19) How do I get the specimen to the laboratory?

- The specimen should be sent via overnight courier to our Las Vegas laboratory.

COLLECTION/SUPPLIES

20) Can I use my urine Custody and Control Form?

- There is an alternative specimen Custody and Control Form that should be used. It is intended for lab based testing of both hair and oral fluid specimens.

21) Can the test be run if I use the regular Custody and Control Form by mistake or I have run out of the alternate Custody and Control Form?

- Yes, but the tamper-evident tape on the urine Custody and Control Form is designed for the urine bottle and is too long for the hair envelope.

22) How do I order the Hair Collection Kits?

- You can order the Hair Collection Kits from ASC. You can order these supplies by the same procedure as you would order the routine Custody and Control Forms and the urine collection kits. Either call 1-866-526-2873 or you may use our e-mail or fax line.

23) What do I receive when I order Hair Collection Kits?

- A specimen transportation bag, Hair Specimen Collection Envelope, alcohol pad and aluminum foil for each kit ordered.
- Airbills for shipping the specimens to the Las Vegas laboratory are available as a separate orderable item.
- Custody and Control Forms must be ordered separately.

24) How are the hair samples collected?

- In order to be fair to the donor, we must have enough hair to repeat assays if necessary. The test requirements are 120 mg of hair by weight. Hair weight varies

among individuals. Since collectors do not have access to sensitive scales, it is easier to visualize in numbers of hairs or sample size.

If the hair is over 4 inches long, then we require approximately 120 strands. If placed in a bundle this quantity of hair would resemble the circumference of a pencil. It is critical that the root ends of the cut hair are aligned and placed with the root ends extending about 1/4-inch beyond the pointed portion of the arrow formed by the foil in the collection kit. In order to approximate time of use (prior 90 days), the laboratory will cut and use about 1-inch from the root end.

If the hair is shorter than 4 inches, but longer than 1 inch, more hair is required. If the hair is curly, root ends do not have to be kept aligned and the sample size should resemble the size of a standard cotton ball.

If the donor has no head hair or hair shorter than 1-inch long, the collector may use chest, underarm, leg, or face hair — in that order of preference. Please note the source of the sample on the hair collection envelope. This will aid in a more accurate interpretation of the results. If body hair is collected, make sure to collect as much hair as possible. This hair is usually lighter in weight and therefore more hair is needed for testing. This specimen type will be reported with a disclaimer indicating that interpretation of results may not be consistent with expected results from head hair.

The issues of sample tampering, adulteration and substitution are not an issue for the collector since he/she is cutting the hair directly from the donor. As stated previously, the donor and the sample are never out of the view of the collector.

25) What acceptance/rejection criteria does the laboratory use for proceeding with the analysis of a hair specimen?

- The following would be reasons for rejecting a hair specimen:
 - a. No Chain of Custody.
 - b. No way to link specimen with the donor.
 - c. Quantity of hair insufficient for analysis.
 - d. Length of hair less than one centimeter (1 cm).

REGULATIONS

26) Can Hair testing be used for Department of Transportation (DOT) testing?

- No, the Department of Transportation (DOT) has not approved Hair testing at this time.