

Understanding Substance Abuse Testing

Michele Gable

Choice Lab Services



Yellow Lab



Black Lab



Chocolate Lab



Meth Lab

funny.com

There are 2 types of Testing

▶ DOT Testing

- ▶ Testing Act of 1991 requires drug and alcohol testing of safety-sensitive employees.
- ▶ DOT rules for drug and alcohol testing procedures. 49 CFR part 40
- ▶ All results are GC/MS confirmed
 - ▶ Drugs tested are limited
 - ▶ Amphetamines, Marijuana, Cocaine, PCP and recently added Opiates

▶ NON-DOT Testing

- ▶ Multiple variations in testing panels
 - ▶ Cut-off levels can be lowered
 - ▶ MRO not required
 - ▶ Screening of sample with immediate results available
- ▶ All non-negative results should be confirmed.

MRO - Medical Review Officer

- ▶ Lab screens the sample and confirms the test result.
Testing lab sends result to MRO
- ▶ The MRO will ask the donor to present a valid prescription or doctor's verification of his medical treatment in support of the positive drug test result to merit a **negative report**.
 - ▶ Quantitative levels do not clearly indicate usage and often the levels will not be on an MRO's report. Report will only indicate positive or negative

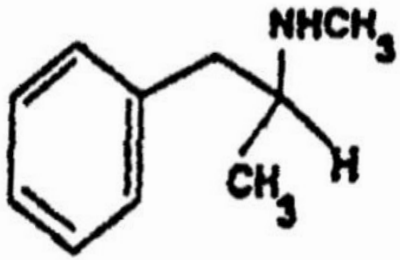
Choosing the correct method of testing

Screen (ELISA)

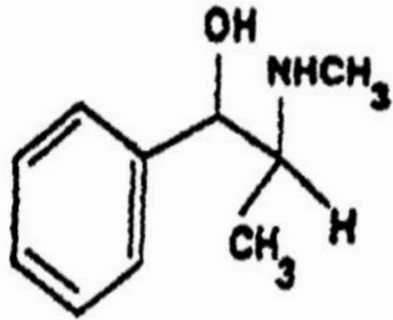


Confirmation (GC/MS)



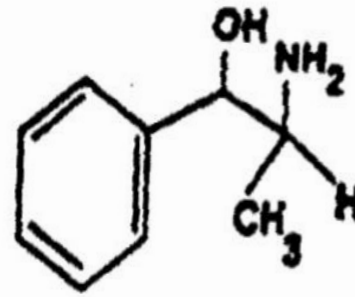


METHAMPHETAMINE

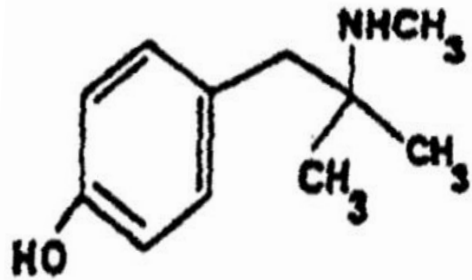


**PSEUDOEPHEDRINE
EPHEDRINE**

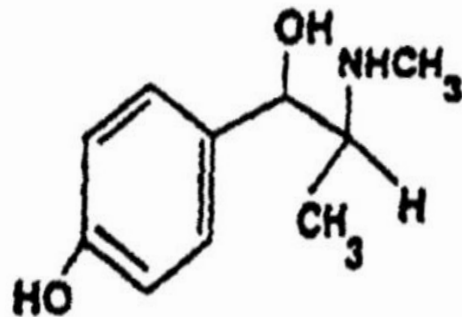
*Actifed, Contac, Sudafed, Drixoral
Bronkaid, Primatene*



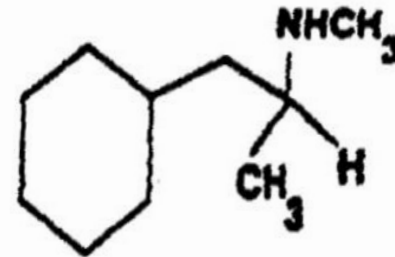
PHENYLPROPANOLAMINE (PPA)
Triaminic, Sinarest, Robitussin



PHENTERMINE



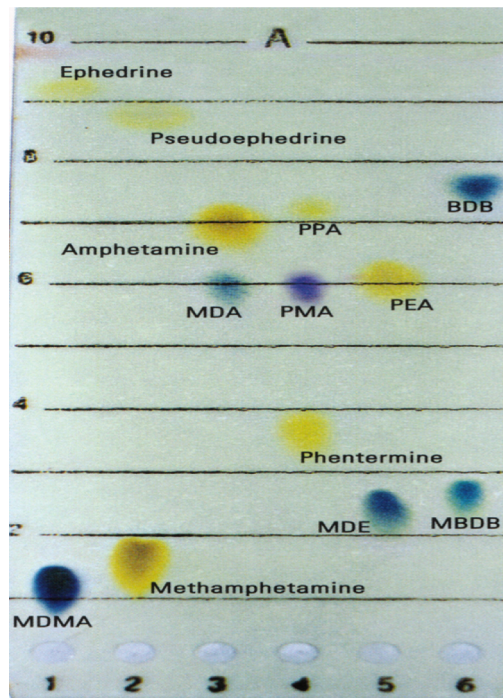
PHENYLEPHRINE
Dristan, Neo-Synphrine



PROPYLHEXEDRINE
Dristan, Benzedrex

Confirmatory Test, GC/MS

Gas Chromatography



Serves as a separation technique to “extract” drug/metabolite for analysis

Specimen injected into a long column at a high temperature

Material converted into gas and separated with a unique “retention” time

Once separated, substance enters MS portion of instrument combination

▶ Questions we answer DAILY

- ▶ 1. Can my work place testing or my screens done at my AME's office count toward my required screens
- ▶ 2. I've DONE my 14 in 12 months you cannot order another test on me
- ▶ 3. What causes a dilute result?
- ▶ 4. Can I eat Poppy Seeds and drink Kombuchi tea?
- ▶ 5. What will make a Peth positive?
- ▶ 6. I just ate _____ am I going to be positive??

Choosing The Right Drug Test:

Direct Alcohol Biomarkers and Other Substances of Abuse

Window of Detection / History of Use

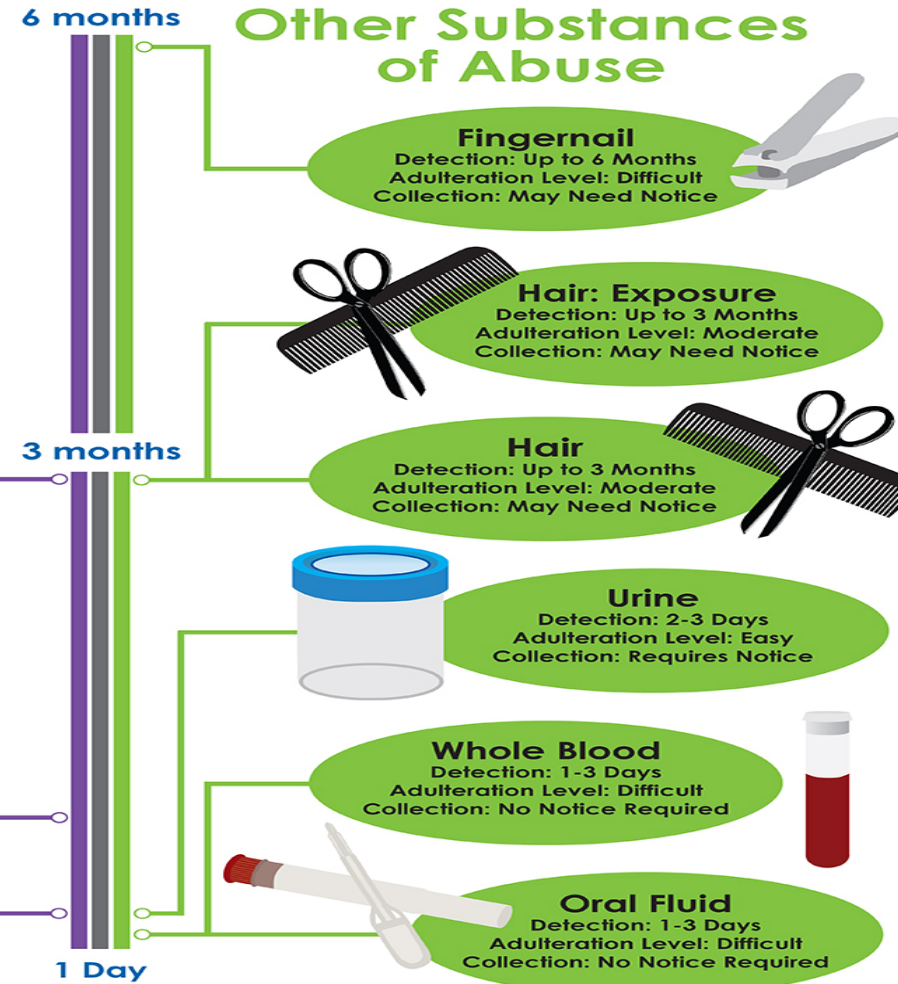
When Choosing a Test That's Right For You, Consider These 5 Factors:

1. Substance being tested
2. Desired window of detection
3. Specimen type
4. Level of adulteration potential
5. Notice required before collection

Alcohol



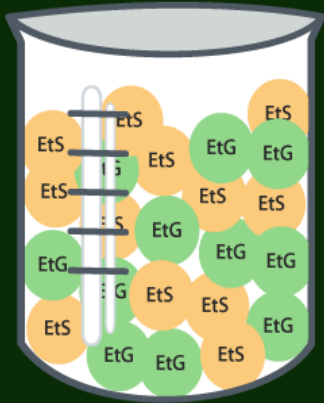
Other Substances of Abuse



Direct Alcohol Biomarker Testing

Urine EtG/EtS

EtG (Ethyl Glucuronide) and EtS (Ethyl Sulfate) are metabolites formed by the body following exposure to ethanol, also called ethyl alcohol, making them direct alcohol biomarkers.



Testing for the combination of EtG and EtS in urine eliminates concern for things such as:

- False positive results due to fermentation following collection
- Presence of bacteria that may compromise results
- Potential problems caused by elevated enzymes.

If both biomarkers are present then ethanol has, in some way, been metabolized by the body.

Sensitivity

Window of Detection

Pros: Extremely sensitive, large number of substances detected, moderate advanced notice needed for collection.

Cons: Very short window of detection

For urine testing, it is standard practice in the field of toxicology to include both EtS and EtG, because EtG is subject to bacterial production and degradation if a urine sample is contaminated (e.g. when the donor has a urinary tract infection). EtS is not subject to bacterial production or degradation, and provides a second, more reliable alcohol biomarker in these urine contamination scenarios.

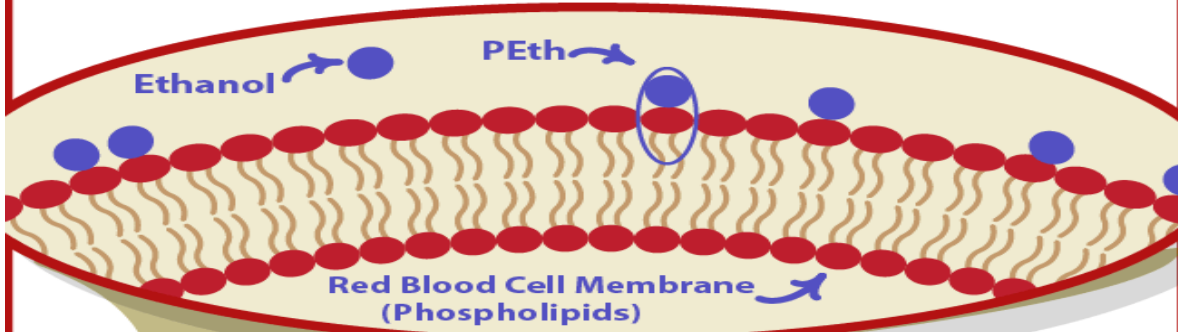
Certain bacteria may interfere with drug detection but will not generate a false positive. Fermenting bacteria in the presence of excess glucose may produce ethanol in the bladder and in the specimen cup.

ETS is only measured and tested via GC/MS

Direct Alcohol Biomarker Testing

Phosphatidylethanol (PEth)

During a series of processes, Phosphatidylethanol (PEth) accumulates in human red blood cells when the body is exposed to ethanol. Since it is formed only when the body is exposed to ethanol it is called a direct alcohol biomarker. The accumulation in red blood cells make it easy to test by collecting blood specimens.



Unlike other markers...
PEth concentrations don't seem to be influenced by¹:

- Age
- Gender
- Certain Diseases
- Other Substances

Half-life
3-5 Days¹

Detectability
28 Days¹

According to a combination of research, analysis demonstrates good efficiency of PEth for detecting chronic heavy drinking¹



Benefits:
Highly sensitive, collection can be done anywhere*, no notice needed for collection*, mid-term window of detection

The literature suggests that it requires multiple servings of ethanol on a single occasion to produce a positive PEth result. PEth has a half-life of approximately 4.5 days.

There are zero instances in the scientific literature (over 25,000 articles) of anything other than consumption of ethanol creating Peth results.

1. Guido Viel, (et al.) (2012) *International Journal of Molecular Sciences*, 13,14788-14812, doi: 10.3390/ijms131114788

* When collected via USDTL BloodSpot® collection



Binge Drinking Defined

- A “binge” is a pattern of drinking alcohol that brings blood alcohol concentration (BAC) to 0.08 gram percent or above.
- For a typical adult, this pattern corresponds to consuming 5 or more drinks for a male, or 4 or more drinks for a female, in about 2 hours.

National Institute on Alcohol Abuse and Alcoholism. NIAAA Council approves definition of binge drinking. NIAAA Newsletter. 2004 Winter;:3.

How Drugs Are Incorporated In Hair

Hair is a reservoir matrix, which provides a long-term window of detection for drug and alcohol (ab)use.

Hair can trap drug and alcohol biomarkers through multiple routes:



Blood

Biomarkers traveling through blood vessels enter the root of the hair and become trapped in the core as the hair grows.



Sebum

Sebaceous glands in the hair follicles deposit an oily substance, known as sebum, that lubricates the growing hair shaft. Drug and alcohol biomarkers in the sebum can be deposited and absorbed by hair.



Sweat

Hair becomes saturated with sweat as we perspire. Biomarkers can be secreted through the sweat glands and are absorbed by the hair shaft.

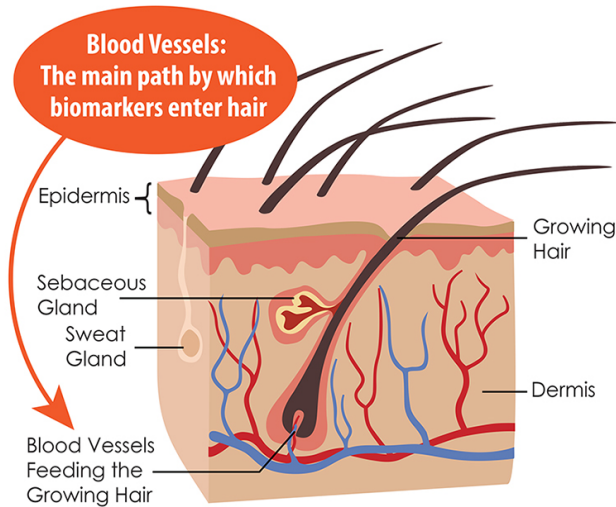


Environmental Exposure

When drugs such as marijuana, methamphetamine, or crack cocaine are smoked, drug residue in the air can be deposited on, and incorporated into, the hair shaft.

Bleaching, perming, dyeing and straightening can affect the outcome of a hair test. Cosmetically treated hair should not be collected.

The only interpretation that can come from a positive hair test is that the individual used or was exposed to drug during the three months prior to collection.



10-14 Days

It takes 10-14 days for drug and alcohol biomarkers to be deposited in the hair root and then emerge past the scalp line.

3 Months

Optimal samples will provide a 3 month window of detection for drug and alcohol (ab)use.



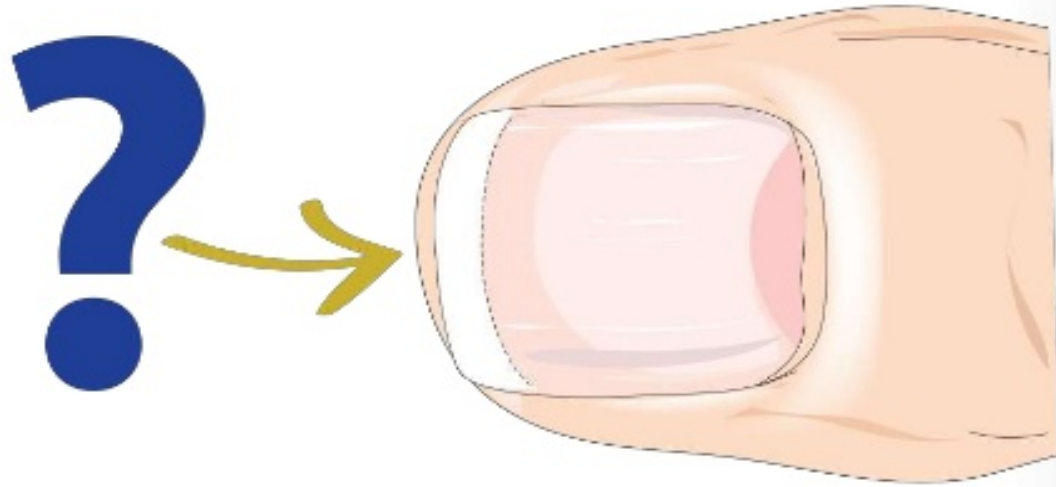
The average rate of growth for hair is a half inch per month.



An optimal sample is a 1.5 inch hair sample of about 200 strands, cut close to the scalp (the width of a #2 pencil).

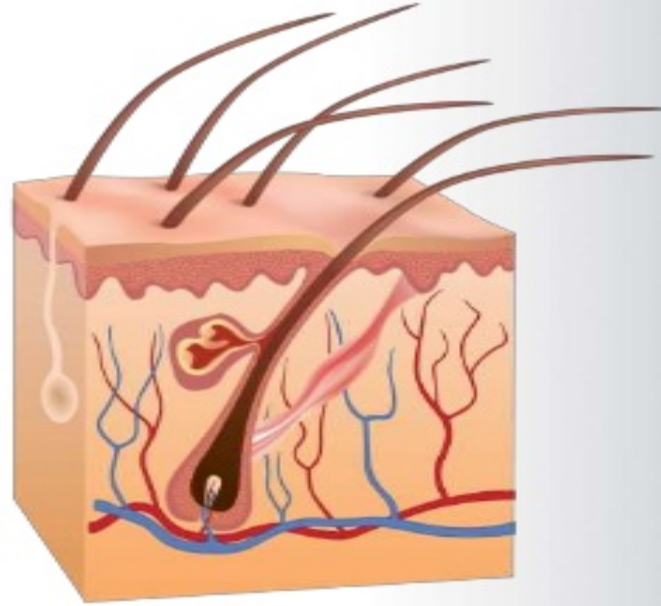
Kintz, P., Villain, M., and V. Crimele. (2006). Hair analysis of drug detection. *The Drug Monitor*, 28(3), 442-446.

**So, why does fingernail work so well
for testing long-term biomarkers
compared to other specimen types?**

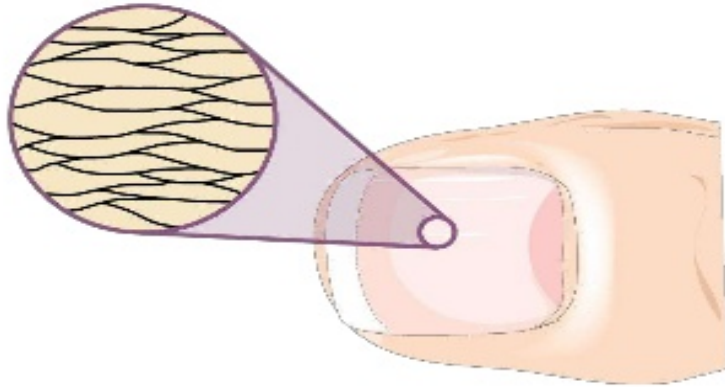


Fingernail Testing vs. Hair Testing

- Hair is not always available
- Hair disadvantages
 - Pigmentation (color) has a large influence on biomarker absorption in hair: dark hair vs. blonde & red hair
 - Chemical treatments can washout biomarkers more rapidly: bleaching, perming, straightening, dying, etc.



Fingernails Are Made of Keratin

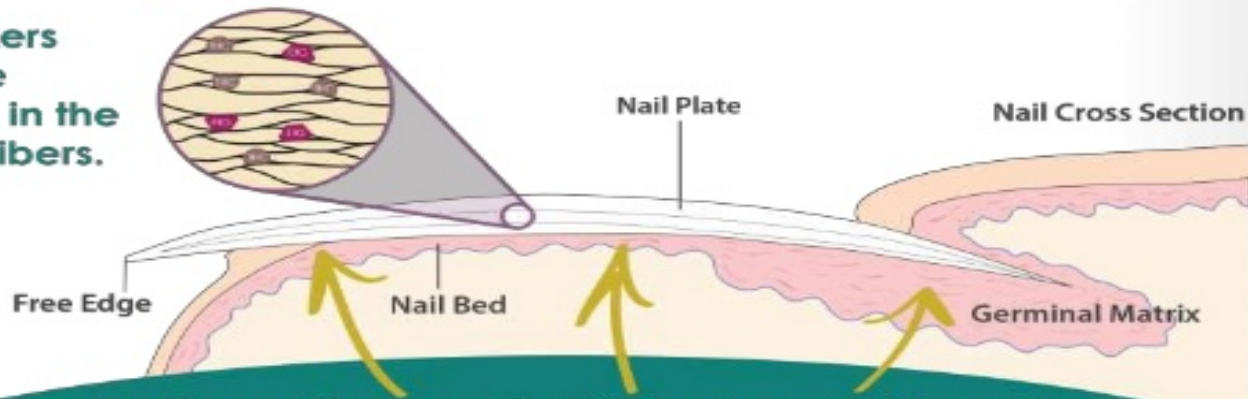


- **Keratin:** fibrous structural protein of hair, nails, horn, hoofs, wool, feathers, and of the epithelial cells in the outermost layers of the skin. The polypeptide chains of keratin are arranged in parallel sheets held together by hydrogen bonding.

~Encyclopædia Britannica

Incorporation of Alcohol & SOA

Biomarkers become trapped in the keratin fibers.



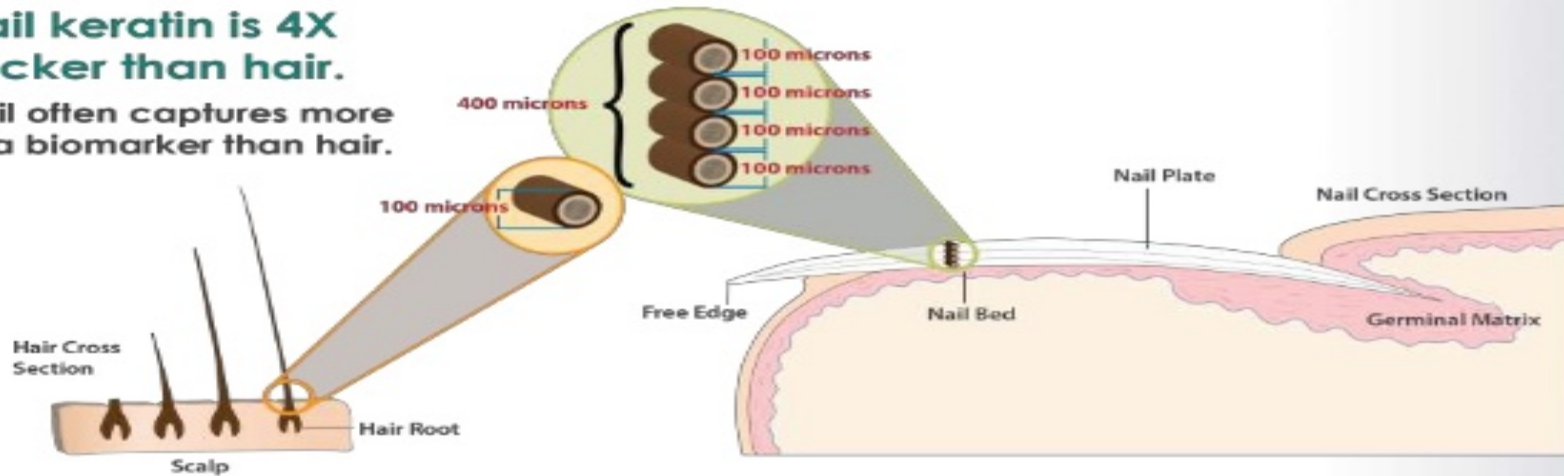
As the nail grows in thickness and length, biomarkers from alcohol and other substances of abuse build up in the nail at the germinal matrix and along the nail bed.

Biomarker: a measurable substance whose presence is indicative of ingestion or exposure to substances of abuse (SOA).

Fingernail Testing vs. Hair Testing

Nail keratin is 4X thicker than hair.

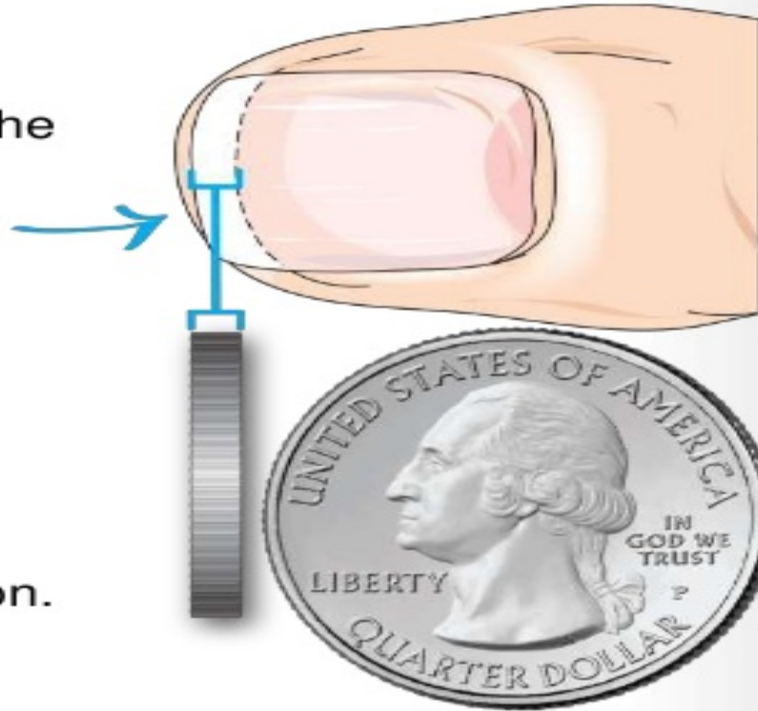
Nail often captures more of a biomarker than hair.



- Additionally, hair growth is relatively slow compared to fingernails, which can be grown in as little as 1-2 weeks. Some notice may be needed for the donor to refrain from clipping their nails in preparation for a test.

Fingernail Collection

- Fingernail samples are clipped and collected by the donor in front of a trained collection staff member.
- A clipping of 2-3 mm long (about the width of a quarter) from all ten fingernails will provide about 100 mg of sample, the ideal amount for screening and confirmation.



Multiple Binges And Keratin Testing

- **Multiple** binges raises alcohol biomarker levels.
- These levels are what we are measuring in keratin based tests (fingernail/hair).
- **Negative Test** = Few to no binges during 3 months
- **Positive Test** = Multiple binges during 3 months
- This is a test for dangerous drinking
- This is **NOT** a test for social drinking

TIMING is the most important factor

When asking for a screen know what you want to test for and the source

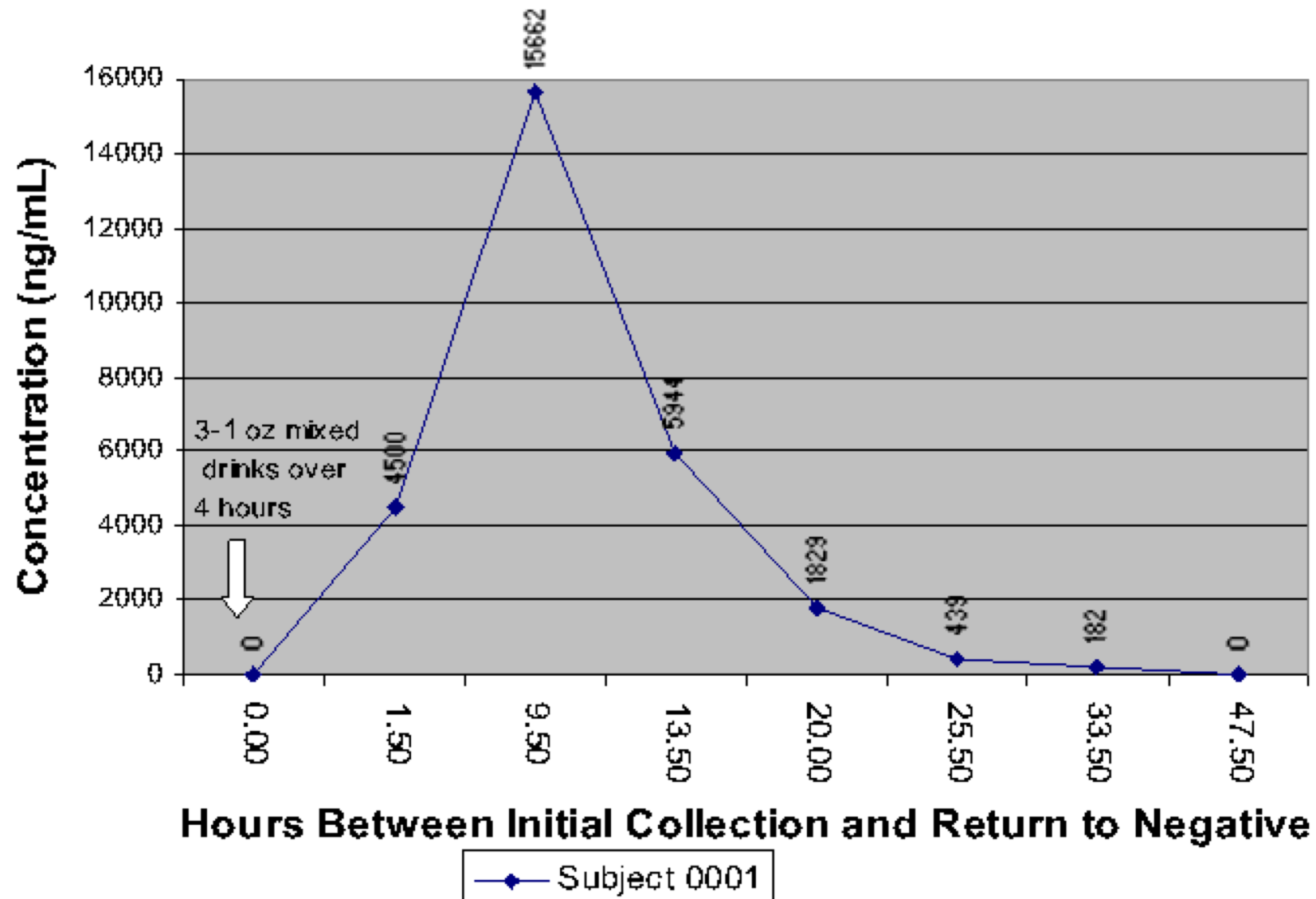
Detection time varies

Urine -2-3 days

Hair and Nail - 3 months

Blood - Peth, alcohol approx. 2 weeks

- ▶ When a person uses drugs or alcohol, there is an ongoing process of biomarker absorption and loss.
- ▶ Build up and break down of drug and alcohol biomarkers happens at the same time, making it impossible to accurately determine the amount of substance ingested.
- ▶ Factors that can affect how much substance may get trapped in a reservoir matrix
- ▶ Age, Body Mass, Overall Health, Metabolism, Timing, Frequency and Amount



Dilute Urine result --- Peth ordered

Date	Creat Level	Sp grav	Date of PETH	Peth result	Day	2 nd Peth	Result	3 rd Peth	
8/4	18.1	1.002	8/14	76	10	8/23	Negative		
9/22	11.1	1.002	10/3	Negative	11				
9/17	15	1.002	10/3	Negative	13				
10/22	8.8	1.002	10/29	102	9				
1/7	13.9	1.002	1/11	403	4	1/21	134.3	2/6	Negative
2/4	10.57	1.002	2//12	23	8				
2/20	14.8	1.002	3/1	45	9				

Low level ETG/NEGATIVE ETS... resulting in Positive Peths

Urine collected	ETG Level	ETS Level	Date of PETH	Peth Result	Day between
7/17	170	Negative	8/1	29	15
10/17	110	Negative	11/2	136	19
11/22	264	Negative	12/11	140	77
5/4	164 and DILUTE	Negative	5/14	141	10
9/10	193	Negative	9/19	50	9
6/7	169	Negative	6/22	376	15
1/28	127	Negative	2/6	22	9

LB

Tests Requested

HAIR14ETG	HairStat-14 + ETG	Sample POSITIVE		
Test		Result	Quantitation	Screen Cutoff Confirm Cutoff
AMPHETAMINES		negative		500 pg/mg
BARBITURATES		negative		200 pg/mg
BENZODIAZEPINES		negative		200 pg/mg
COCAINES		negative		500 pg/mg
METHADONES		negative		200 pg/mg
MEPERIDINE		negative		500 pg/mg
OPIATES		negative		200 pg/mg
PCP		negative		300 pg/mg
OXYCODONE		negative		200 pg/mg
PROPOXYPHENE		negative		200 pg/mg
CANNABINOIDS		negative		1 pg/mg
TRAMADOL		negative		500 pg/mg
FENTANYL		negative		25 pg/mg
SUFENTANIL		negative		10 pg/mg
ETHYL GLUCURONIDE		POSITIVE		20 pg/mg
Ethyl Glucuronide LCMSMS		POSITIVE	124 pg/mg	20 pg/mg

Additional Sample Information

Body Hair

LB

Tests Requested

NAIL12ETG	NailStat-12 + ETG	Sample POSITIVE		
Test		Result	Quantitation	Screen Cutoff Confirm Cutoff
AMPHETAMINES		negative		500 pg/mg
BARBITURATES		negative		200 pg/mg
BENZODIAZEPINES		negative		200 pg/mg
COCAINES		negative		500 pg/mg
METHADONES		negative		200 pg/mg
MEPERIDINE		negative		500 pg/mg
OPIATES		negative		200 pg/mg
PCP		negative		300 pg/mg
OXYCODONE		negative		200 pg/mg
PROPOXYPHENE		negative		200 pg/mg
CANNABINOIDS		negative		1 pg/mg
TRAMADOL		negative		500 pg/mg
ETHYL GLUCURONIDE		POSITIVE		20 pg/mg
Ethyl Glucuronide LCMSMS		POSITIVE	>200 pg/mg	20 pg/mg

Additional Sample Information

Toe Nails

Sample Comments

ACTUAL VALUE: EthylGlucuronid - 447 pg/mg

LB

Tests Requested

20-PET-BLD	20-Phosphatidyl Ethanol (Bld)	Sample POSITIVE		
Test		Result	Quantitation	Screen Cutoff Confirm Cutoff
PHOSPHATIDYL ETHANOL		POSITIVE		20 ng/mL
Phosphatidyl Ethanol LCMSMS		POSITIVE	>200 ng/mL	20 ng/mL

Sample Comments

ACTUAL VALUE: 20-PhsphtdylEth - 478 ng/ml

Tests Requested

20-PET-BLD	20-Phosphatidyl Ethanol (Bld)	Sample POSITIVE		
Test		Result	Quantitation	Screen Cutoff Confirm Cutoff
PHOSPHATIDYL ETHANOL		POSITIVE		20 ng/mL
Phosphatidyl Ethanol	LCMSMS	POSITIVE	151 ng/mL	20 ng/mL

Sample Comments

Test developed and characteristics determined by United States Drug Testing Laboratories, Inc. See Compliance Statement on our website http://www.usdtl.com/compliance_statement

Tests Requested

ETG-HAIR	Ethyl Glucuronide (hair)	Sample POSITIVE		
Test		Result	Quantitation	Screen Cutoff Confirm Cutoff
ETHYL GLUCURONIDE		POSITIVE		20 pg/mg
Ethyl Glucuronide	LCMSMS	POSITIVE	149 pg/mg	20 pg/mg

Additional Sample Information

Head Hair

Tests Requested

NAILETG	NailStat-ETG	Sample POSITIVE		
Test		Result	Quantitation	Screen Cutoff Confirm Cutoff
ETHYL GLUCURONIDE		POSITIVE		20 pg/mg
Ethyl Glucuronide	LCMSMS	POSITIVE	>200 pg/mg	20 pg/mg

Additional Sample Information

Finger Nails

Sample Comments

ACTUAL VALUE: EthylGlucuronid 334 pg/mg

PT

Tests Requested

HAIR14ETG	HairStat-14 + ETG	Sample POSITIVE		
Test		Result	Quantitation	Screen Cutoff Confirm Cutoff
AMPHETAMINES		negative		500 pg/mg
BARBITURATES		negative		200 pg/mg
BENZODIAZEPINES		negative		200 pg/mg
COCAINES		negative		500 pg/mg
METHADONES		negative		200 pg/mg
MEPERIDINE		negative		500 pg/mg
OPIATES		negative		200 pg/mg
PCP		negative		300 pg/mg
OXYCODONE		negative		200 pg/mg
PROPOXYPHENE		negative		200 pg/mg
CANNABINOIDS		negative		1 pg/mg
TRAMADOL		negative		500 pg/mg
FENTANYL		negative		25 pg/mg
SUFENTANIL		negative		10 pg/mg
ETHYL GLUCURONIDE		POSITIVE		20 pg/mg
Ethyl Glucuronide	LCMSMS	POSITIVE	87 pg/mg	20 pg/mg

Additional Sample Information

Head Hair

AT

Tests Requested

20-PET-BLD	20-Phosphatidyl Ethanol (Bld)	Sample POSITIVE		
Test		Result	Quantitation	Screen Cutoff Confirm Cutoff
PHOSPHATIDYL ETHANOL		POSITIVE		20 ng/mL
Phosphatidyl Ethanol	LCMSMS	POSITIVE	>200 ng/mL	20 ng/mL

Sample Comments

ACTUAL VALUE: 20-PhosphatidylEth - 1516 ng/mL

AT

Tests Requested

UEM-URINE	Urine Ethanol Metabolites	Sample POSITIVE		
Test		Result	Quantitation	Screen Cutoff Confirm Cutoff
ETG/ETS METABOLITES		POSITIVE		100 ng/mL
Ethyl Glucuronide	LCMSMS	POSITIVE	>10000 ng/mL	100 ng/mL
Ethyl Sulfate	LCMSMS	POSITIVE	>10000 ng/mL	25 ng/mL
VALIDITY CREATININE/SPGR		Normal		
Creatinine	Urine	Normal	34 mg/dL	

Sample Comments

ACTUAL VALUE: EthylGluc-0100 - 220562 ng/mL

ACTUAL VALUE: EthylSul-100 - 28288 ng/mL

The background features abstract, overlapping green geometric shapes in various shades of green, ranging from light to dark, creating a modern and dynamic look. The shapes are primarily triangles and polygons, some with thin white outlines, set against a white background.

Michele Gable

678-491-9404

Michele@choicelab.org

michelegable@gmail.com