

Clinical and legal aspects of hair testing for alcohol

Robert Cohen

Society for the Study of Addiction

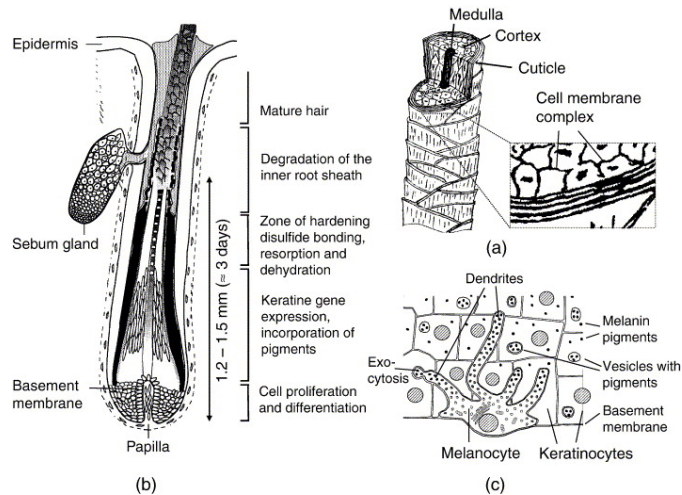
Annual Symposium 2013

That must have been some party!



AHAJOKES.COM

Hair Sampling

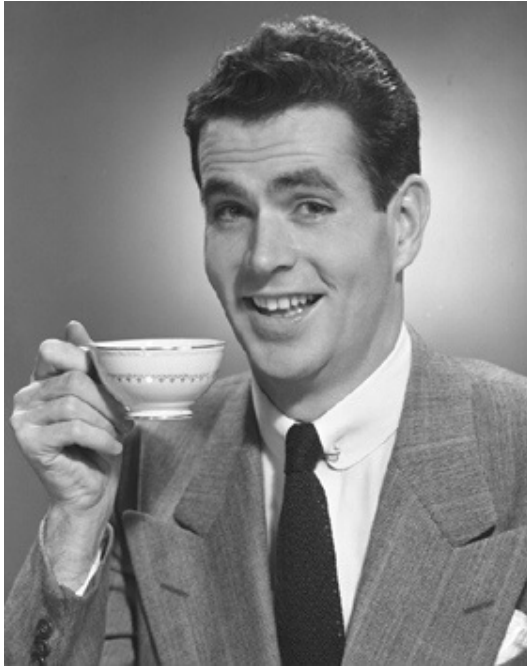


Applications

- Drug-related deaths
- Child custody
- Drug-facilitated crime
- Drug monitoring (*work, rehab*)
- Driving licence assessment

The subject says

- *“I go out once a week and drink 3-4 beers”*



The hair test lab says:

- *“You (are an alcoholic and you) drink 60-120 units/week”*

Benefits of hair testing

- Stable matrix
- Easy to collect sample
- Growth rate of hair permits assessment of use over time (months)
- Scalp, beard, armpit, pubic area



A lock of hair about the width of a thin pencil, or several locks of hair from the back of the head, are twisted and fastened with a string.



The hair is to be cut **just above the skin**, as close to the scalp as possible. The length of remaining segment on the head should be recorded on the request form.



The hair lock(s) are put on separate aluminium foils provided with the root end **exposed at the notched area** of the foil.



Each aluminium foil is to be folded once as shown above.



Insert the aluminium foil into the envelope

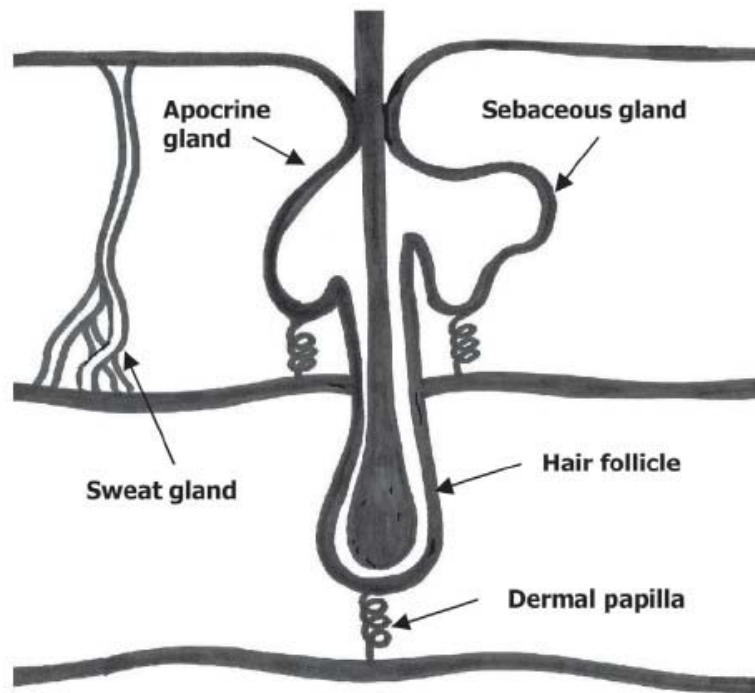


Fill in the donor's particulars and the required

Agius R, Kintz P. Drug test analysis
Drug Test Analysis 2010;2;367

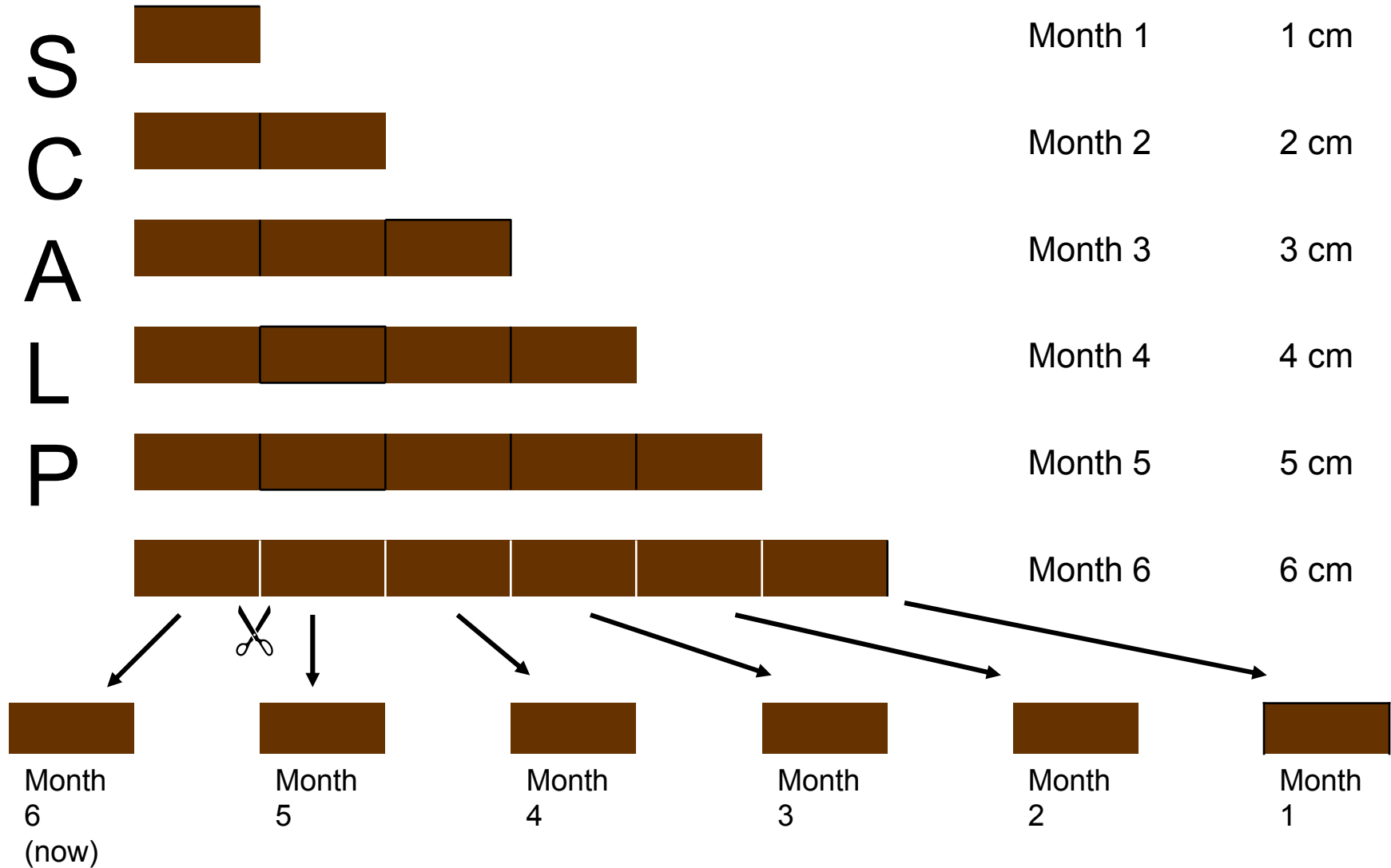
Routes of incorporation of drugs into hair

Cooper G. *Ann Clin Biochem* 2011; **48**:516



- Blood capillaries at the hair root bulb
- Sebum
- Sweat
- External contamination

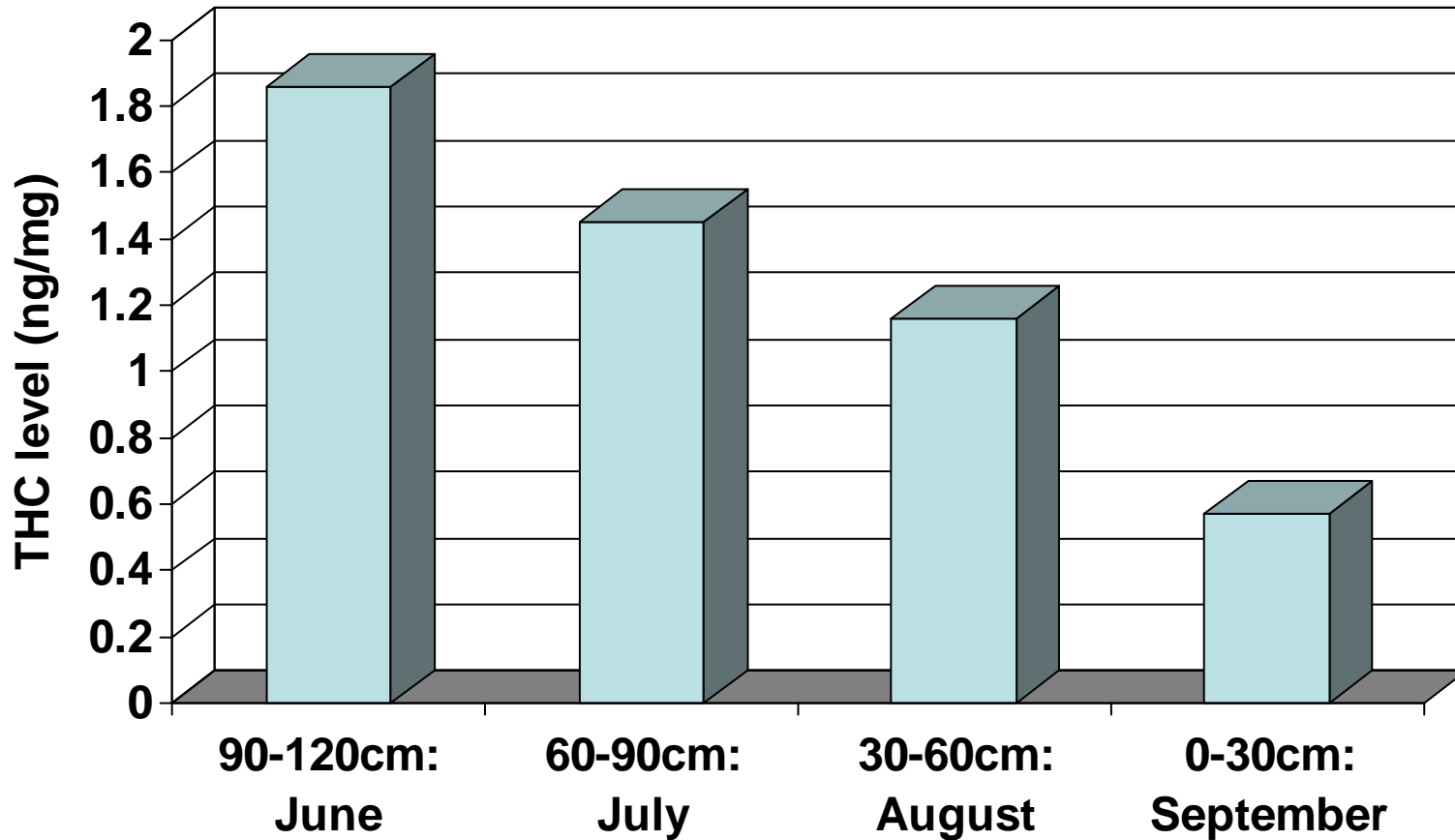
Diagrammatic representation of the growth of hair



Hair test in a patient who gave a history that he had reduced his cannabis use over the last 6 months

Segment	Period (days prior to sample)	Dates	Cannabis level (ng/mg)
1	0-30	September	0.57
2	30-60	August	1.16
3	60-90	July	1.45
4	90-120	June	1.86

Hair test in a patient who gave a history that he had reduced his cannabis use over the last 6 months
(same data as previous slide)



BARBITURATE CONCENTRATIONS IN THE SKIN AND HAIR OF GUINEA PIGS*

1ST LT. RAYMOND W. GOLDBLUM, M.C., LEO R. GOLDBAUM, Ph.D.
AND LT. COLONEL WILLIAM N. PIPER, M.C.

Barbiturates are known to frequently cause dermatitis medicamentosa. Soon after the introduction of barbital in 1903 and phenobarbital in 1911, cases of dermatitis were reported from the use of these drugs. The etiology of barbiturate dermatitis remains unknown. Potter and Whitacre expressed the opinion that long acting barbiturates cause skin eruptions more frequently than short acting

Barbiturate Concentrations in the Skin and Hair of Guinea Pigs FREE

Raymond W Goldblum MC, Leo R Goldbaum PhD and William N Piper MC

J Invest Dermatol 22: 121-128; doi:10.1038/jid.1954.16

[Abstract](#) | [PDF](#)

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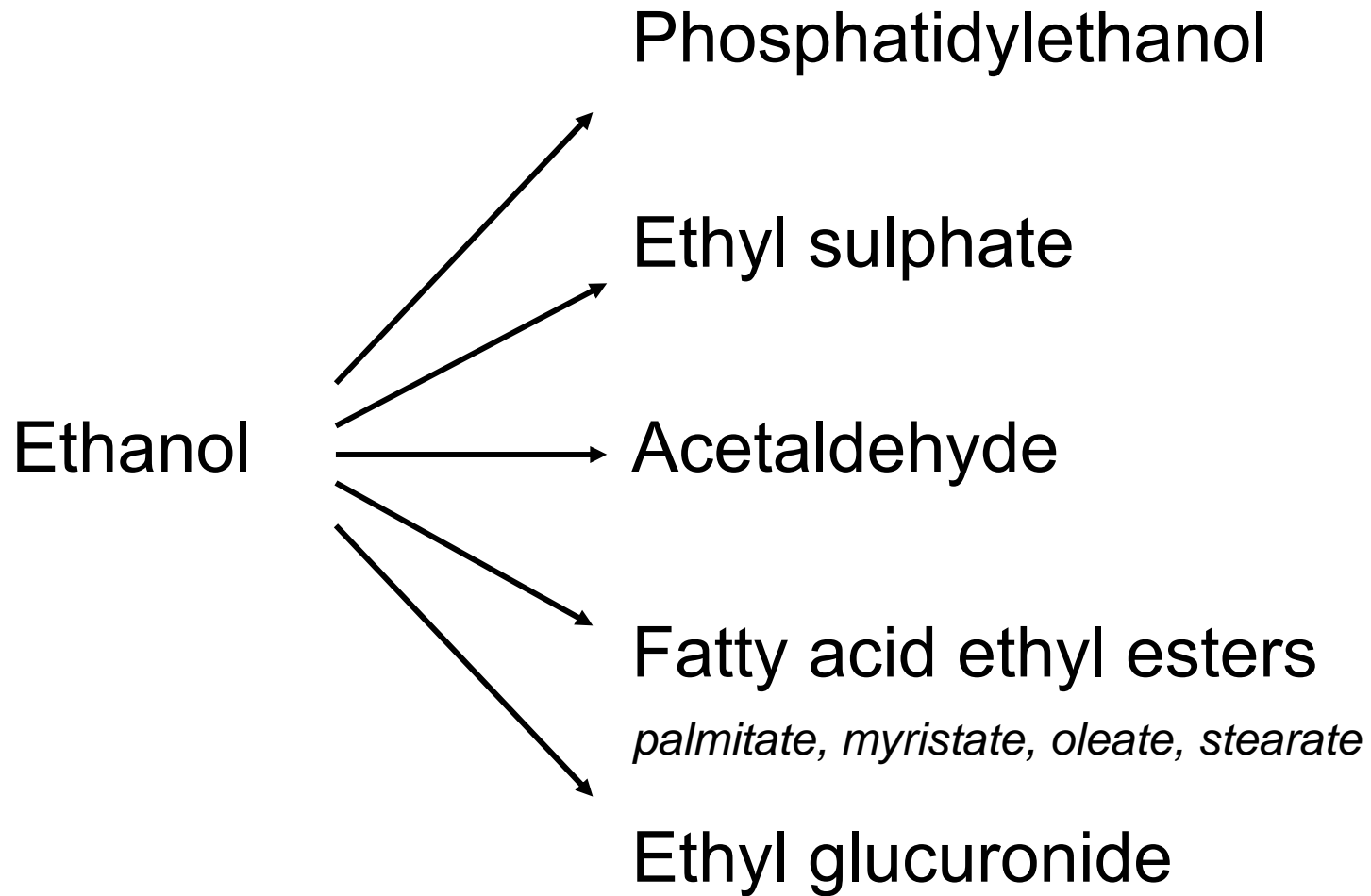
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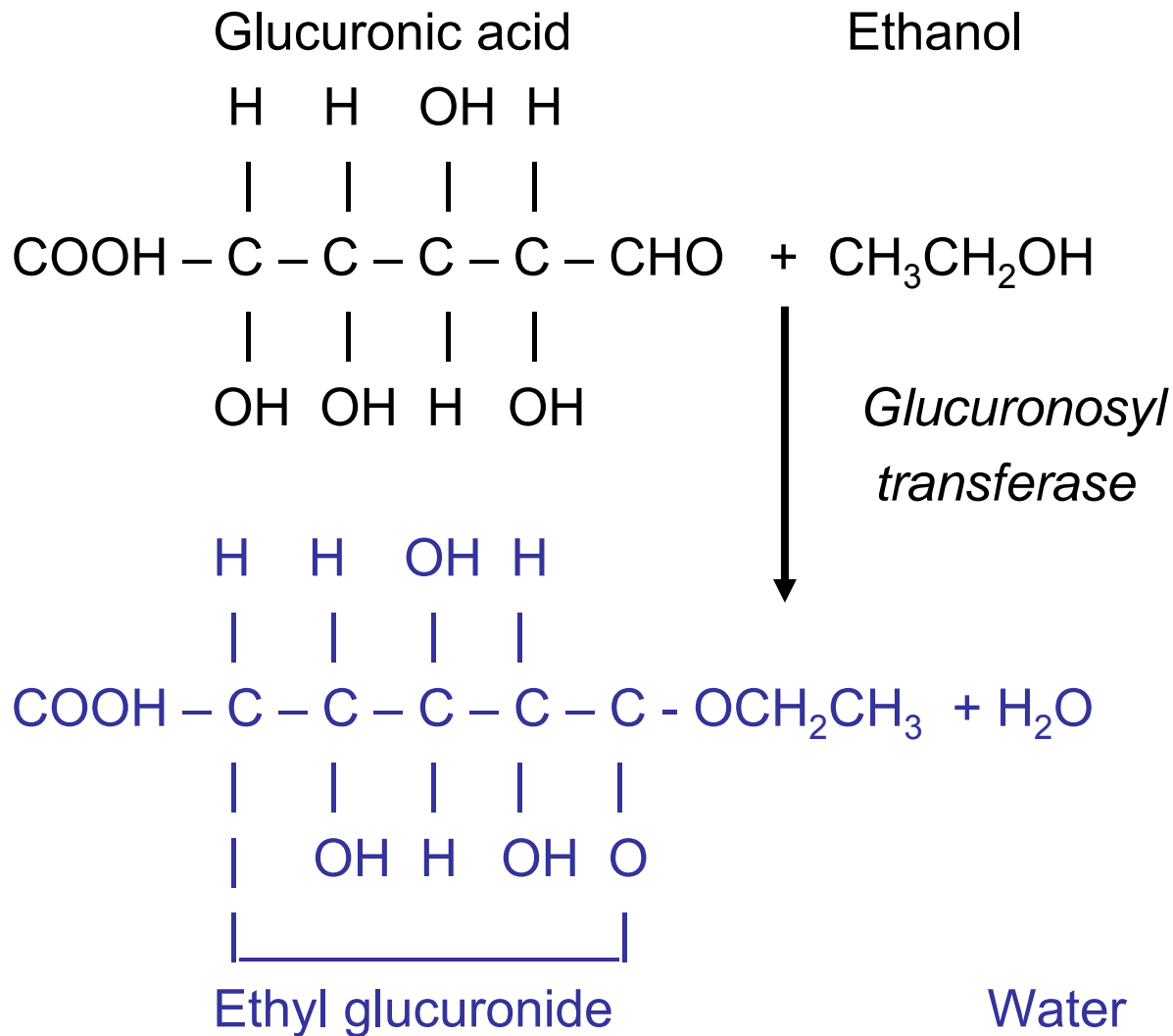
Drugs of abuse which are commonly sought in hair tests

- Amphetamine
- Antidepressants
- Barbiturates
- Benzodiazepines
- Buprenorphine
- Cannabis
- Cathinone
- Cocaine
- Fentanyl
- Flunitrazepam (Rohypnol)
- GHB (complicated by natural occurrence)
- Ketamine
- LSD
- Mephedrone
- Methadone
- MDMA (ecstasy)
- Methamphetamine
- Opiates
 - Heroin
 - Morphine
 - Codeine
 - Dihydrocodeine
 - Tramadol
- PCP

Metabolites of ethanol



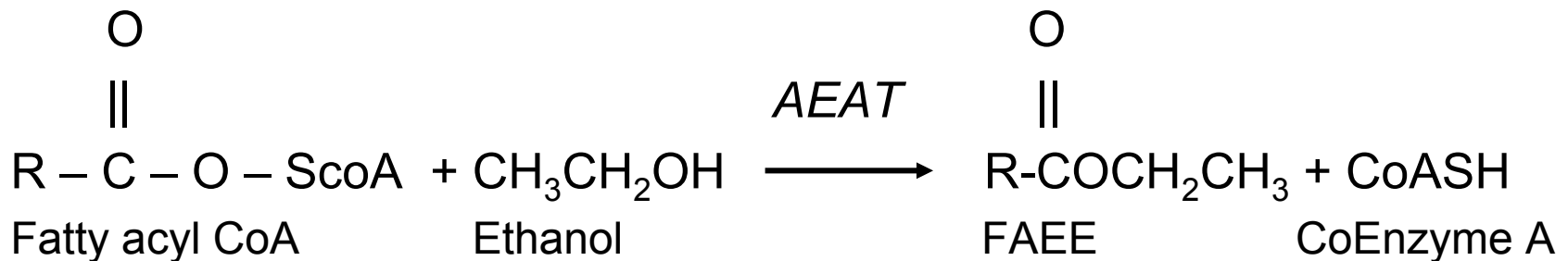
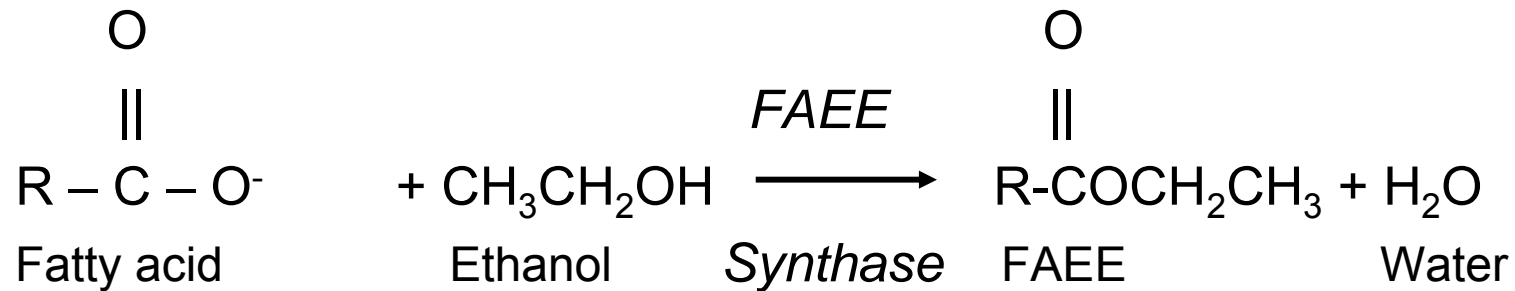
Ethyl glucuronide (EtG)



Ethyl glucuronide (EtG)

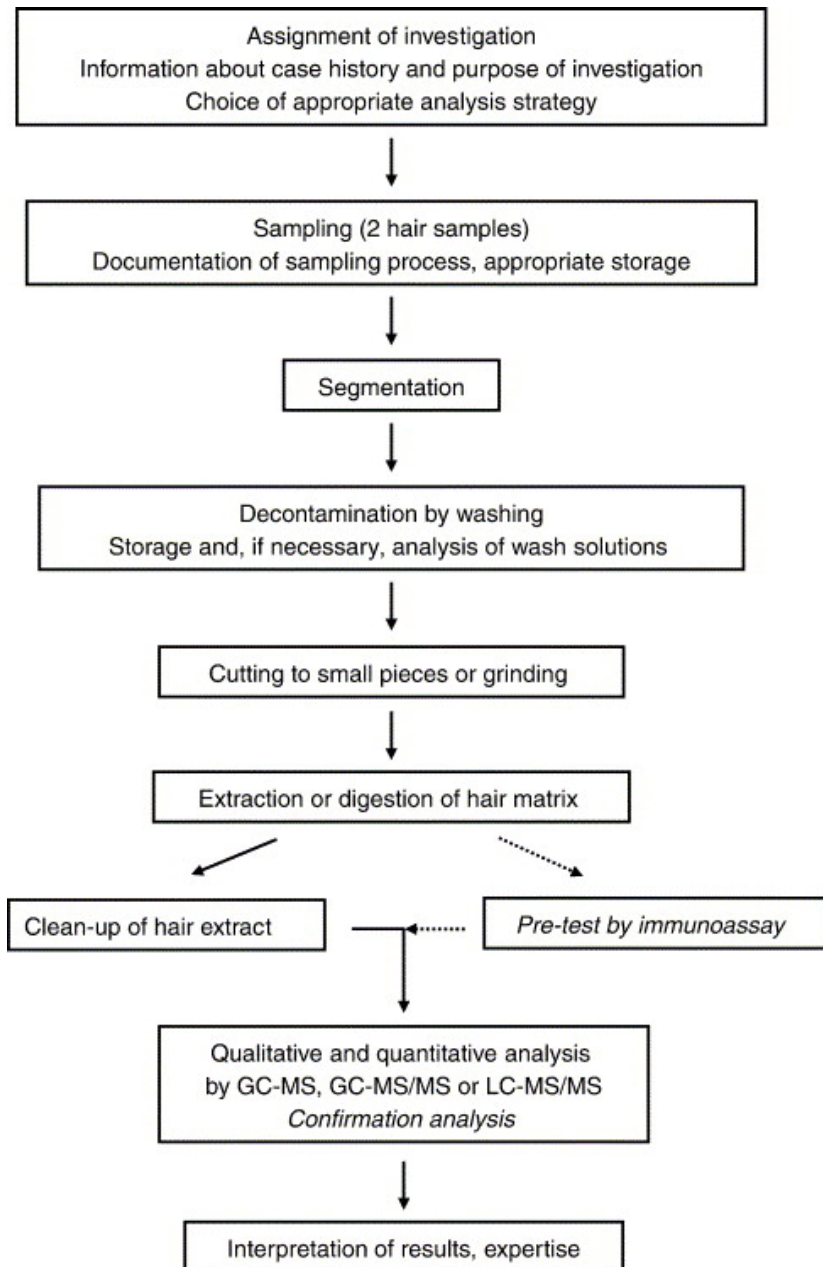
glucuronosyl transferase Glucuronic acid + ethanol \longrightarrow Ethyl glucuronide	
Location of synthesis	Hepatic endoplasmic reticulum
EtOH \rightarrow EtG	0.04%
Solubility	Hydrophilic
Peak time	2-5 hours after EtOH peak
Breakdown	WBC, liver, pancreas
Excretion	Sweat Urine
$t_{1/2}$	2-3 hours
Detection time	Serum 36 hours Urine 5 days

Fatty Acid Ethyl Ester (FAEE) synthesis



Fatty Acid Ethyl Esters (FAEEs)

<p style="text-align: center;">FAEE synthase</p> <p>Fatty acid + ethanol $\xrightarrow{\hspace{2cm}}$ FAEE + water</p>	
<p style="text-align: center;">AEAT</p> <p>Fatty acid CoA + ethanol $\xrightarrow{\hspace{2cm}}$ FAEE + Coenzyme A</p>	
Location of synthesis	Erythrocyte, pancreas, heart, liver, duodenal mucosa, lung, adipose tissue, gall bladder, brain
EtOH \rightarrow FAEEs	1%
Solubility	Hydrophobic, serum bound to albumin and lipoprotein
Peak time	2 hours
Breakdown	In cells to EtOH and FFA; in blood to TG
Excretion	Sebum
$t_{1/2}$	58 sec (initial turnover); 16-24 hours (adipose tissue)
Detection time	Serum 24 hours



Hair Test
Procedure
Pragst & Balikova
Clin Chim Acta
2006; **370**:17

Chain of custody procedure

- Chain of custody is a procedure to ensure that the sample received by the lab is the sample given by the subject and that the sample has not been tampered with

It involves

- identifying the subject carefully
- taking and storing the sample openly
- putting the sample into a sealed bag before the sample leaves the subject.
 - The lab will reject the sample if the sealed bag is not intact

Chain of custody procedure

Cooper G. *Ann Clin Biochem* 2011; **48**:516

Standard hair collection kit

- Chain of custody form
- Foil and collection envelope
- Security seals
- Evidence bag
- Transportation envelope (optional)
- Instructions for collection of a hair sample

Information recorded in a standard hair collection kit

Cooper G. *Ann Clin Biochem* 2011; **48**:516

- Unique sample identification number
- Name and contact details of instructing authority / employer
- Name and donor identification (dob, passport, driving licence)
- Collector details (print name, signature and date)
- Date and time of collection
- Description of hair sample (length, colour, condition)
- Donor declared cosmetic treatments and medication
- Donor declared period and frequency of substances abused
- Analysis required
- Name and contact details of testing laboratory
- Record of individuals who receive / handle sample

In the lab

- The sample is weighed
- Cut into segments
- Decontaminated
- Cut into small pieces or ground up
- Extraction or digestion of the hair matrix
- Tested with immunoassay
- Confirmatory tested with GC/MS

Our Laboratory



Weighing hair samples prior to analysis.



Preparation of samples for analysis.



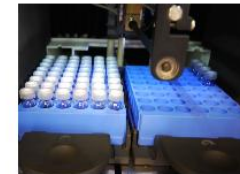
Sample preparation station.



Analysing the chromatographs.



Gas Chromatography sample analysis.



Samples being analysed for drugs.

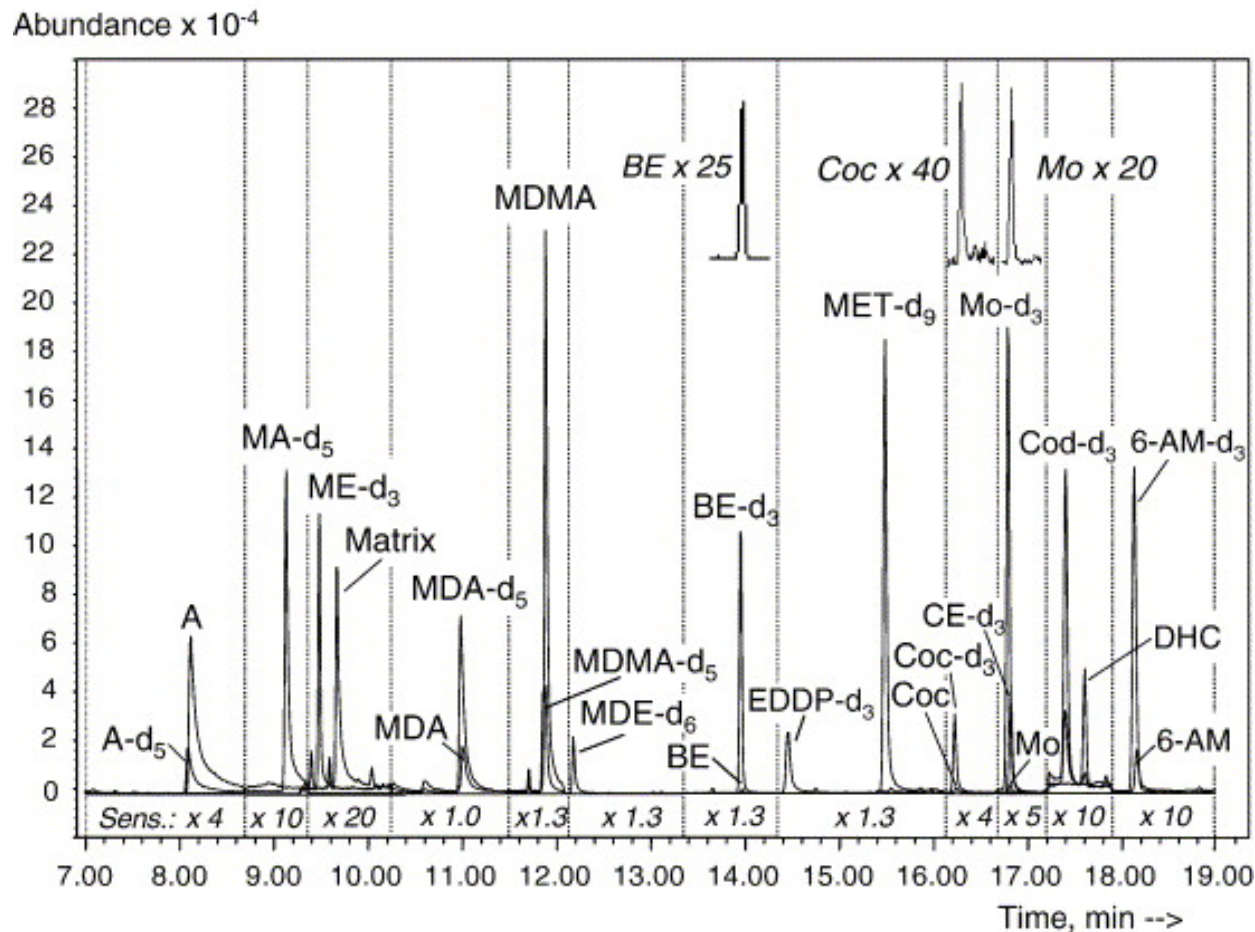


Examining the sample analysis results.



Analysing the results and writing the report.

Readout from GC-MS testing for different substances



Is the cardholder pregnant?

1. Buys pregnancy test



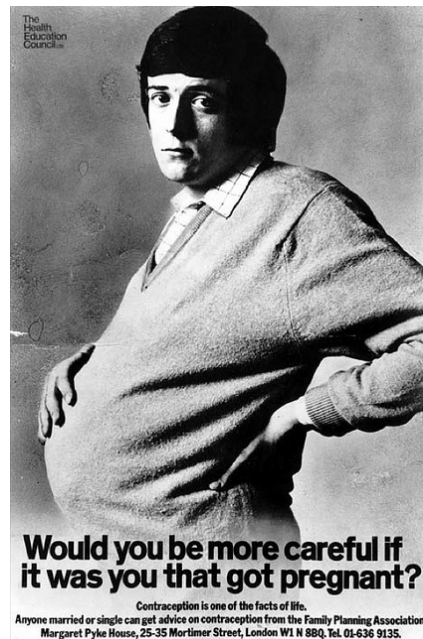
Is the cardholder pregnant?

1. Buys pregnancy test
2. Buys baby stuff



Is the cardholder pregnant?

1. Buys pregnancy test
2. Buys baby stuff
3. Is male



Would you be more careful if it was you that got pregnant?

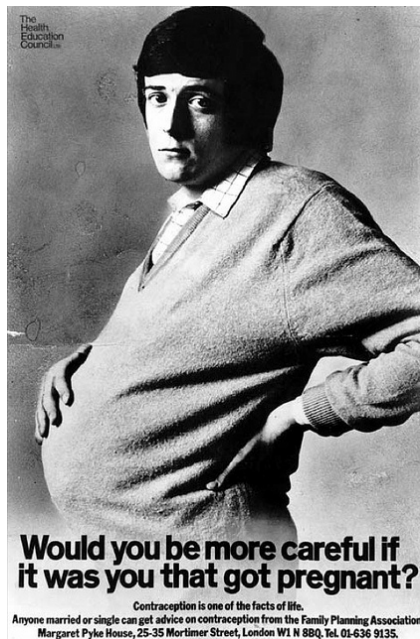
Contraception is one of the facts of life.
Anyone married or single can get advice on contraception from the Family Planning Association.
Margaret Pyke House, 25-35 Mortimer Street, London W1 N 8BQ, Tel. 01-636 9135.



Is the cardholder pregnant?

(see eg. Duhigg C. How companies learn your secrets.
www.nytimes.com/2012/02/19/magazine/shopping-habits.html)

1. Buys pregnancy test
2. Buys baby stuff
3. Is male







- All you have is a sample of hair
- The lab does – or does not – detect the presence of EtG / FAEEs in this hair sample
- If EtG / FAEEs are detected, it is an assumption when saying the person is 'positive' for alcohol use
 - You do not have enough information for a diagnosis of an alcohol use disorder or even a full picture of recent use

Areas for misinterpretation

- Technical aspects of taking the sample
- Contamination of the sample
- What is the origin of the alcohol?
- Relation of history of alcohol intake to the quantity of EtG/FAEE in the hair sample
- Interpretation of the sample result

Technical Issues

Problem	Solution
Have you got the right patient?	ID
Was the sample properly collected?	Trained Collector, standard kit
Was the sample tampered with in transit?	Chain of custody
Contamination	Complex washing procedure (not fully effective)
Quality Assurance	Lab accreditation

Contamination

- Hair is washed to removed dirt
- Contaminants
 - Hair products (shampoo, styling gel, hairspray)
 - Dandruff, head lice
 - Body fluids
 - Drugs
- Washed with an organic solvent then an aqueous solution
 - Positive wash solutions indicate external contamination (but also extract drugs from the hair)
- Standard washes that remove contamination without removing active drug are not available



Origin of the alcohol

- Alcoholic drinks
- Fermented foods
- Endogenous alcohol production
- Other sources of alcohol

Fermented foods

- Fermentation of foods to alcohol is of ancient origin
- Alcoholic beverages are free from pathogens



Endogenous alcohol production

- Low level ethanol generation by microbial fermentation in the gastrointestinal tract (see Rosano & Lin, JAT 2008, 32: 594)
- Autobrewery syndrome
 - Term that has been used to describe patients who become repeatedly inebriated after ingestion of food of high carbohydrate nature in the presence of abnormal yeast proliferation, particularly of *Candida* species (J Pediatric Gastroenterology and Nutrition 2001;33:214), e.g., in short gut syndrome, surgical jejunoileal bypass (ibid)
 - *Klebsiella pneumoniae*, *Saccaromyces cerevisiae* and *Torulopsis glabrata* utilising infant feed (Paediatric Research 1984; 18:195a), *Lactococcus garvieae*
- End stage renal disease (see Rap Comm Mass Spect 2006;20:61)
- Possible Intracellular synthesis of alcohol (Antoshechkin Alc Alc 2001; 36:608)
- Studies suggest that the amount of alcohol produced in this way is small (ed Dasgupta Pharmacogenomics of Alcohol and Drugs of Abuse, CRC Press, 2012: 22)
 - Medicolegally not effective as a defence to drink driving (Med Sci Law 2000;40:206)

Other sources of alcohol

- Alcohol based mouthwashes
- Alcohol containing sanitizer gels (Rosano & Lin, JAT 2008, 32: 594)
- Hair lotions
- Alcohol-free beers
- Communion wine
- Medications, such as chloral hydrate (Arndt *et al* 2009)
- Alcohol in everyday products, such as vanilla extract; hygiene products, cosmetics, foods (SAMHSA 2006)
- Sauerkraut
- Matured Bananas
- Baker's Yeast
- Alcohol in the environment (e.g., wine bar) (De Giovanni et al JAT 2008;32:156) / ambient air (Rap Comm Mass Spect 2006;20:61)
- Also, FAEEs have been consumed as nutritional supplements (Saghir et al, Am J Physiol 1997; 273 Gastrointest Liver Physiol 36: G184-G190)

Quantitative estimation from hair samples of alcohol consumed

- Various studies have sought to relate the history of the amount that a person drinks and the amount of alcohol metabolites in hair
- The Society of Hair Testing has produced guidelines
 - 2009 (www.soht.org, accessed 31 March 2010):
 - Chronic alcohol consumption defined as $\geq 60\text{g EtOH/day}$
 - Cutoffs for levels in the scalp hair in the 0-3cm proximal segment indicative of chronic alcohol consumption:
 - EtG 30pg/mg
 - FAEEs 0.5ng/mg
 - 2012 (www.soht.org, accessed 17 October 2013): levels suggesting repeated alcohol consumption
 - Chronic alcohol consumption not defined
 - EtG $\geq 7\text{pg/mg}$ in the 0-3 up to 0-6cm proximal scalp hair
 - FAEEs $\geq 200\text{pg/mg}$ in the 0-3 proximal hair segment or $\geq 400\text{pg/mg}$ in the 0-6 proximal hair segment

Evidence for a relationship between amount of alcohol stated and hair test levels

- Note that Auwärter's paper shows that even in hair of teetotal subjects FAEEs can be detected
- Other findings of a positive correlation between history of alcohol intake and EtG / FAEEs

	N	Range (ng/mg)	Mean (ng/mg)
Alcohol fatalities	10	2.50-13.5	6.80
Alcoholic in treatme	19	0.92-11.6	4.00
Mod soc drinkers	13	0.20-0.85	0.41
Teetotal	5	0.06-0.37	0.16
Conc of FAEEs in proximal 0-6cm (Auwärter <i>et al. Clin Chem</i> ;2001; 47 :2114)			

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Sensitivity of EtG

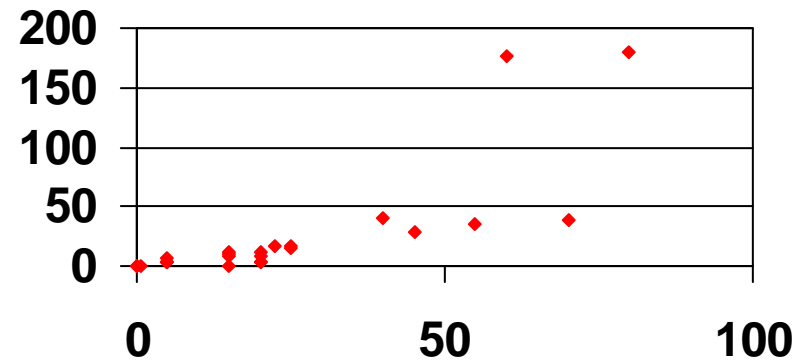
Lees *et al Alc Alc* 2012; 47:267

- 100 participants
- 4 groups, giving alcohol history via AUDIT-C
 - Teetotal (0 units/week) 5.3% above SOHT
 - Low risk drinkers (1-21 units/week) 9.8%
 - Increasing risk drinkers (22-50 units/week) 45.5%
 - High risk drinkers (50+ units/week) 57.9%
- 1-3cm hair sample tested
- 29 samples positive
- Correlation between alcohol history and EtG concentration 0.42
- Comparison with SOHT guideline (2009) of 30µg/pg to indicate high drinking (60 units/week)
- Sensitivity 0.52
- Positive result highly likely to indicate drinking (PPV 1.00)
- Negative result not good evidence for abstinence (NPV 0.23)

Problem with relationship between amount of alcohol stated and hair test levels

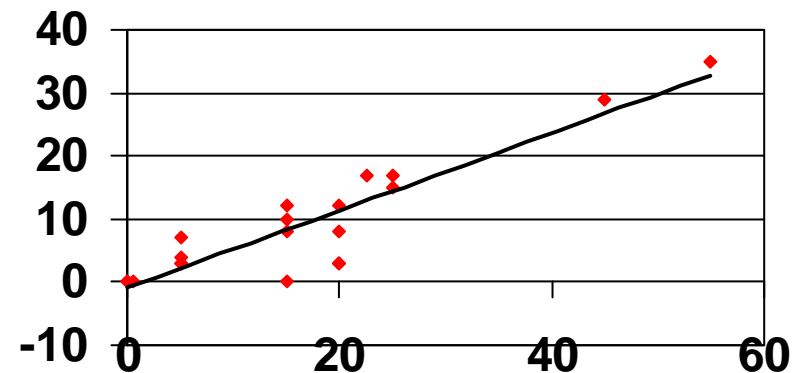
Kerekes *et al.* *Alc Alc* 2009;44:62

- Correlation is not proportionality
 - *You cannot work back from the hair test result to work out how much a person is drinking (even if the histories given by subjects were accurate)*



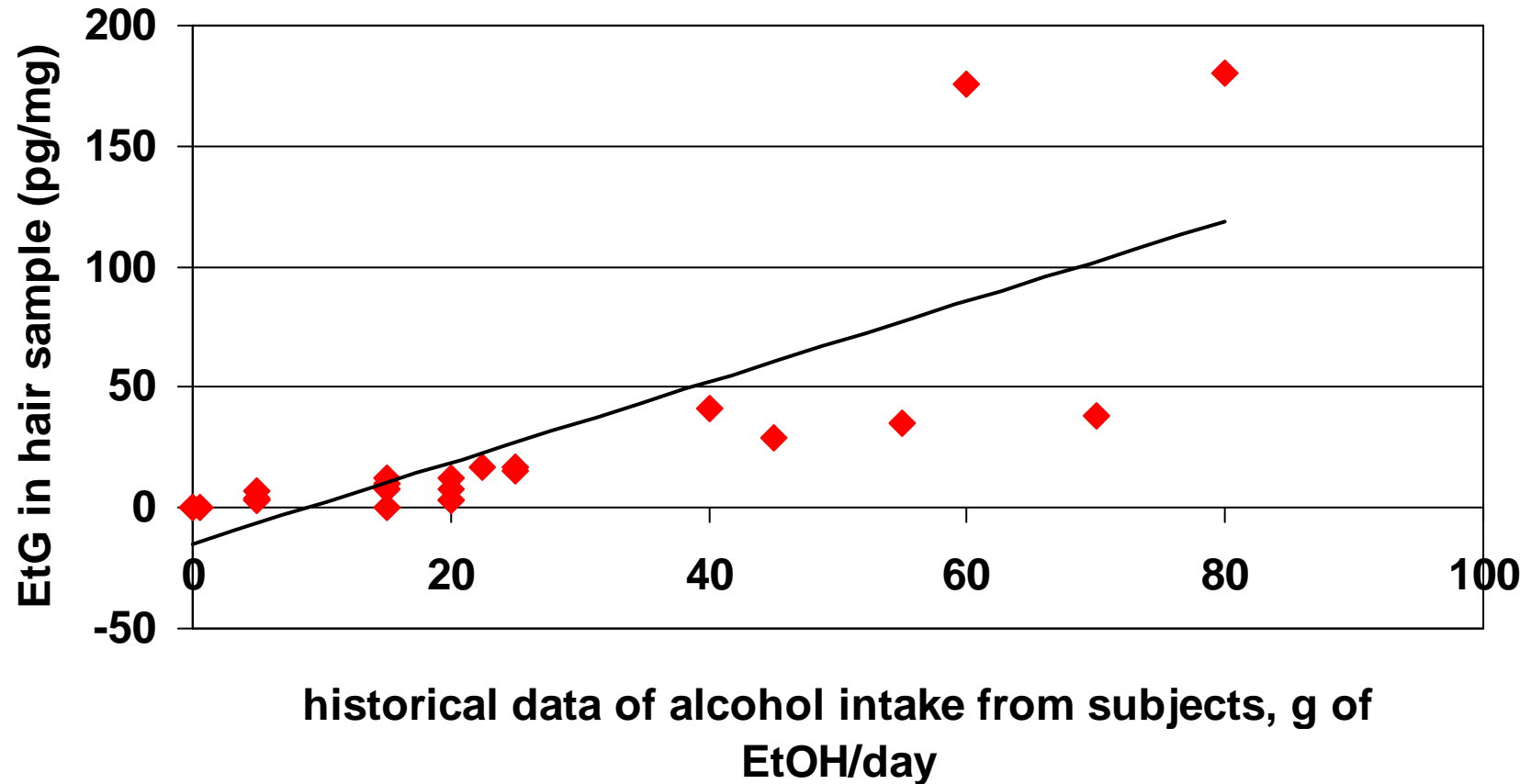
These are two graphs of selected data that allow the point to be made.

- X-axis: historical data of alcohol intake from subjects, g of EtOH/day
- Y-axis: EtG in hair sample (pg/mg)



Problem with relationship between amount of alcohol stated and hair test levels

Kerekes *et al.* *Alc Alc* 2009;44:62



Mother in childcare proceedings

FAEEs	ng/mg
Jan-Jul	0.12
Mar-Sep	0.54

- Had not drunk for over a year
- Seen 12 times in clinic in 5 months
- On each occasion
 - History of not having taken alcohol since last seen
 - Appearance consistent with not having drunk recently
 - Breathalyser reading 0.00mg/l

Problem interpreting hair test result

Pragst F. *Forens Sci Int* 2012; **217**:e4-7

- Mother required to show 1 year abstinence
- Entered residential rehab
- After 2 months, hair sample showed
- Conclusion that she had drunk 20-50 units/week in the previous month
- Mother disputed this interpretation and denied alcohol intake
- Further sample 7 weeks later showed concentration of 0.03ng/mg FAEEs in the 0-6cm segment, interpreted as her not having used in the previous 6 months
- (there are further details in this case that illustrate the discussion of the interpretation of later test samples later in the paper)

0-1cm (most recent)	1-2cm	2-3cm
22pg/mg EtG	0	0

43 year old widow

- Children 13 and 10
- 4 episodes of heavy drinking for 1 week over a 2 year period
- At assessment
 - No physical signs of chronic drinking or liver disease
 - Breathalyser 0.00mg/l
 - MCV, LFTs, CDT normal, GGT 70 IU/l
 - Hair test negative for alcohol
- Attended alcohol treatment service
- Seen in Court 6 months after assessment. Court advised she needs to demonstrate 3 months of abstinence
- Hair test result at month 9 unexpectedly positive. Clinical appearance of abstinence between months 6 and 9
- Further test at month 11

43 year old widow

Month	0	1	2	3	4	5	6	7	8	9	10	11
	Inter view						Hair Test 1			Hair Test 2		Hair Test 3
Hair Test 1					EtG negative FAEEs neg							
Hair Test 2							EtG 0.254 FAEEs neg					
Hair Test 3					EtG negative FAEEs negative							

Other types of assessment information

- Clinical assessment
 - History from the patient about their alcohol intake
 - History of alcohol related problems
 - Observations of the clinicians in the interview
 - Mental state examination – intoxication, stigmata of chronic liver disease
 - Motivational state
- Other investigations
 - Breathalyser, platelets, WBC/CRP, MCV, GGT, LFTs, CDT, urine / blood testing for alcohol
 - Rating scales (AUDIT, SADQ)
- History from others
 - Family, friends, professionals

LB Richmond v B & W & B & CB [2010] EWHC 2903 (Fam)

- Care proceedings in which a mother had a history of alcohol abuse
- Assessment of a hair test sample had been reported as being positive for alcohol abuse, disputed by the mother
- The Judge (Moynan J) reviews the evidence about hair testing and its interpretation from Professor Pragst and Mr O'Sullivan (Trichotech)
- The Judge concludes:
 - “22 (i) When used, hair tests should be used only as part of the evidential picture”

Concluding remarks

- Hair testing for alcohol is a valuable addition to the repertoire of biological tests that assist with the assessment of how much alcohol a person is drinking.
- It gives useful information about the presence of metabolites of ethanol in hair, which raises the possibility that alcohol has been consumed over a prolonged period
- The results must not be taken uncritically
 - A 'positive' hair test result does not “trump” a negative history
 - It does not prove the patient is lying
 - But it does need to be explained
- Sometimes, you cannot resolve the ambiguity between the clinical presentation, other forms of data and the hair test result, and you must say so.

Concluding remarks

- Proper clinical practice is that the clinical picture should take priority over any laboratory test result (unless the test result is the basis of the disease diagnosis), but you should leave a cautionary note
- Diagnosis is a working hypothesis and is always subject to revision in the light of further information
- Be careful not to let your own prejudices interfere with the interpretation of the data
 - “she’s an alcoholic. She must be lying.”
- The Courts do not like such uncertainty. They want you to tell them the answer. Sometimes you have to disappoint them
- Remember that, in Court, the erroneous judgement of an incompetent judge trumps your perfect medical assessment. Live with it. Life is not fair.

Thank you for listening!

Robert Cohen

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