

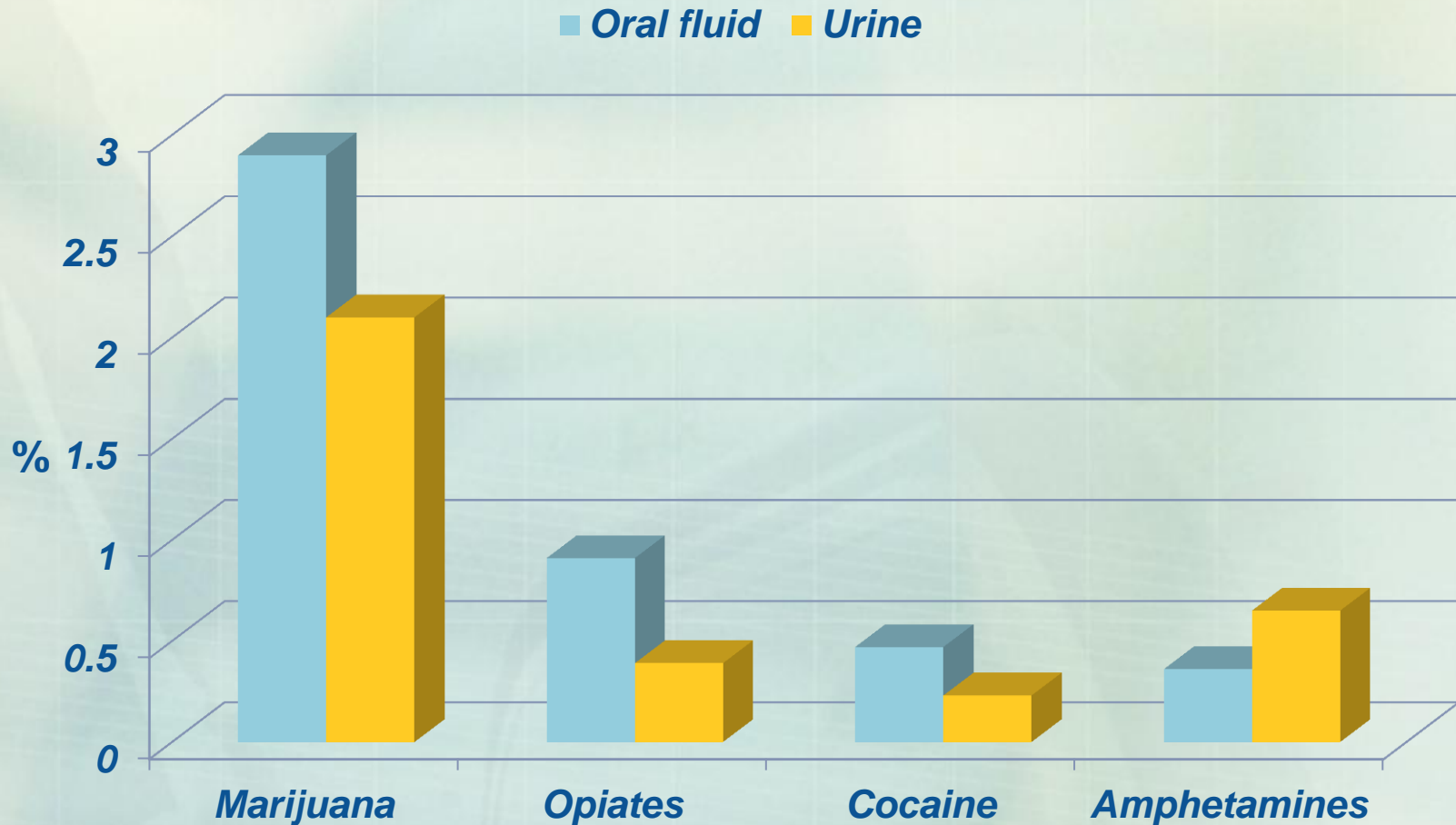
***Oral fluid testing:
Gaining acceptance across the globe***

***Christine Moore, PhD, DSc
Immunoanalysis Corporation, CA***

Introduction

- *Oral fluid as an alternative to urine has been increasing in popularity internationally*
- *Europe and the USA are taking steps to establish analytes, cut-offs and to manage expectations in workplace testing*
- *EWDTS: has submitted guidelines for oral fluid testing to regulatory body*
- *SAMHSA/DTAB: In July 2011, oral fluid was accepted as a potential matrix for workplace testing*

Workplace positivity rate January – June 2010



Quest Diagnostics Drug Testing Index 2010

Oral fluid options

- *Easier implementation of random testing*
- *Easier matrix to collect than urine*
- *Fewer complications with collection in the workplace*
- *Similar or better positivity rates*
- *Seamless implementation into laboratory testing facilities already testing urine*
- *Devices associated with laboratory tests widely available and validated over many years*

Beating the saliva test

- *Ultra Saliva Cleansing Kit; Aqua-Clean pills; Ultra-Wash Toxin*
- *Quick Fizz Detoxifying Tablets: contains powerful ingredients such as vitamin B2, Creatine, Uva, Ursi, Dandelion, Cranberry, Gingko Biloba and Stevia. Because toxins are stored in your fat cells and are released when your body burns fat, **Quick Fizz** prevents you from burning fat cells so the toxins aren't released during the drug test.*
- **Cleanse:**
 - *5 day: light marijuana user (1-2 times/week for a month) \$79.95*
 - *30 day: chronic marijuana user (1-4 times a day for a year) \$249.95*

All:

- *Drink a lot of water a few days in advance*
- *Pass urine as often as you can since it helps the medicine to take action*
- *Do some exercise to accelerate the metabolism in your body*
- *Take the medicine exactly as prescribed; Take it at the very time as specified*
- ***Don't take any drugs or alcohol a couple of days before your test***

EWDTs: Guidelines for European workplace drug testing in oral fluid
Gail Cooper, Christine Moore, Claire George, and Simona Pichini
Drug Testing and Analysis 2011

Sections from Abstract:

- *Over the past decade, oral fluid has established itself as a robust testing matrix for monitoring drug use or misuse.*
- *One of the main advantages of oral fluid relates to the collection of the matrix which is non-invasive, simple, and can be carried out under direct observation making it ideal for workplace drug testing*
- *Laboratories offering legally defensible oral fluid workplace drug testing must adhere to national and international quality standards (ISO/IEC 17025); however, these standards do not address issues specific to oral fluid testing.*
- *EWDTs recognizes the importance of providing best practice guidelines to organizations offering testing and those choosing to use oral fluid drug testing to test their employees.*

European Workplace Guidelines

- *From: Bednarova Martina [mailto:BednarovaM@cai.cz]*
Sent: 12 September 2011 15:03
To: Gail Cooper; ragius@laborkrone.de
Subject: RE: EA Approval for EWDTS and UKIAFT Guidelines

- *Dear Colleagues,*
- *Coming back from the EA Laboratory committee meeting I have a pleasure to inform you that EWDTS document Drug and Alcohol Testing in Hair has been recognised as the EA advisory document and it was agreed to send out the document Guidelines for European workplace drug testing in oral fluid for comment.*
- *We will keep you informed on the progress.*
- *Best regards,*
- *Martina Bednarova*

Comparison: Collection devices

Both EWDTS and SAMHSA acknowledge that the collection device can affect the result: Specimen volume, drug recovery from a pad, split samples and drug stability in a device all part of the discussion

EWDTS

- *Must not contain stimulants*
- *Collects known volume, through in-built indicator or laboratory testing*



- *Known volume +/-10%*
- *Either split samples, or collect two samples simultaneously*
- *Storage: Short-term: Refrigerated; Long-term: Frozen (-15°C or less)*

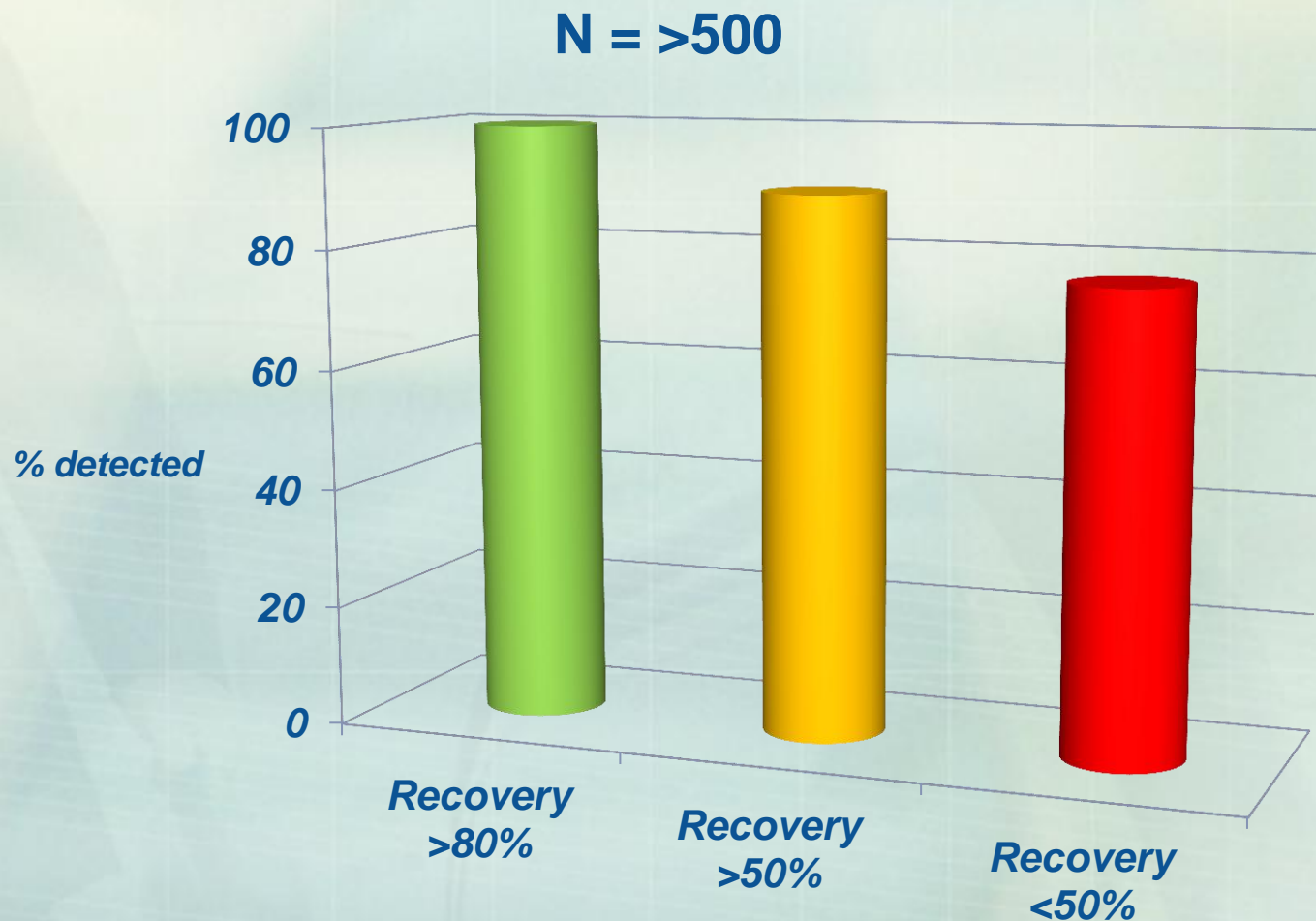
SAMHSA (proposed)

- *Must not contain stimulants*
- *Collects at least 1mL +/-10%*

- *Discussion on minimum percentage (%) drug recovery from device*
- *Discussion on drug stability (short and long term)*

Device recovery of THC

4ng/mL cut-off concentration



Proposed screening cut-off concentrations (ng/mL)

Drug class	EWDTs	SAMHSA	Comments
<i>Amphetamines</i>	40	50	(120)
<i>Cannabinoids</i>	10	4	(3)
<i>Cocaine</i>	30	20	(15)
<i>Opiates (morphine)</i>	40	40*	(30) <i>*Includes synthetic opioids: oxycodone and hydrocodone</i>
<i>6-acetylmorphine</i>	4	4	
<i>Phencyclidine</i>		10	(3)
<i>Methadone</i>	50		
<i>Buprenorphine</i>	5		
<i>Propoxyphene</i>	40		
<i>Benzodiazepines</i>	10		

Proposed confirmation cut-off concentrations (ng/mL)

Drug class	EWDTs	SAMHSA	Comments
<i>Amphetamines</i>	30	50	(120) AMP, METH, MDMA, MDA, MDEA
<i>Cannabinoids</i>	2	2 (0.02*)	(1.5) THC *THC-COOH for SAMHSA ?
<i>Cocaine</i>	8	8	(6)
<i>Opiates (morphine)</i>	40**	40*	(30) ** Includes dihydrocodeine *Includes synthetic opioids: Oxycodone, oxymorphone, hydromorphone, hydrocodone
<i>6-acetylmorphine</i>	4	4	
<i>Phencyclidine</i>		10	(3)
<i>Methadone</i>	20		
<i>Buprenorphine</i>	5		
<i>Propoxyphene</i>	40		
<i>Benzodiazepines</i>	10		Temazepam, Oxazepam, Nordiazepam

Perceived drawbacks

- *Window of detection is too short*
 - *Parent drug presence may give good information on recent use*
 - *2011: Clin Chem, Lee et al. Chronic daily marijuana smokers: THC detectable for 48 hrs; THC-COOH for 29 days (median = 13 days)*
- *Insufficient specimen for multiple or repeat testing*
 - *Device specific*
 - *2011: JAT, Heltsley et al – up to eight drug classes screened and confirmed from one Quantisal™ device*
- *Drug concentrations too low to measure reliably*
 - *2011 proficiency reports from RTI: laboratory reliability improving*
- *Marijuana detection is difficult*
 - *True for **some** point-of-care devices; pad recovery is low on **some** collection devices, but an informed choice of collection device and analytical laboratory negates this disadvantage*

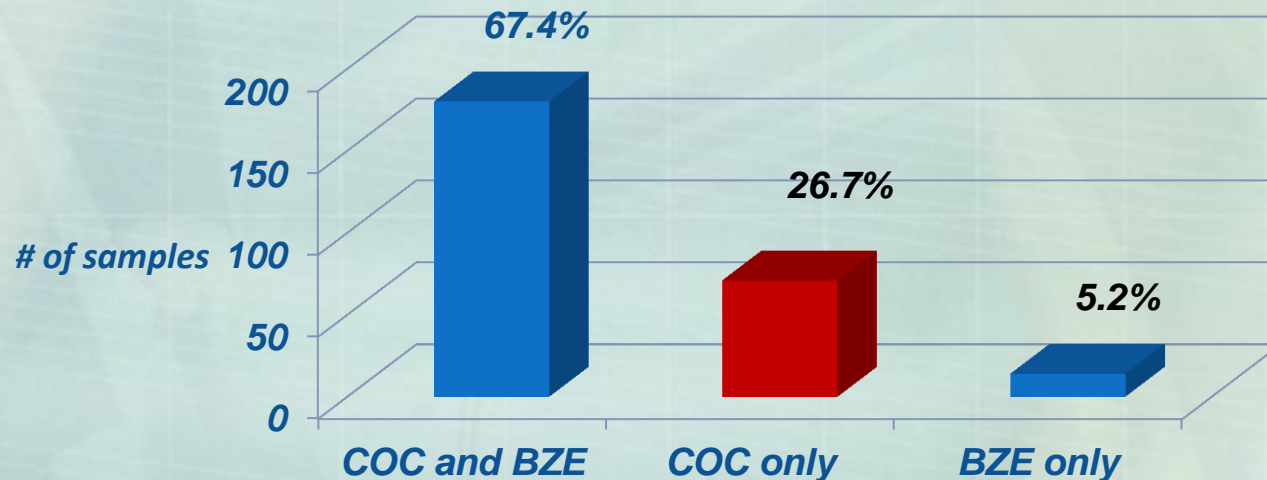
Actual drawbacks

- 1. *Insufficient specimen for **split** samples (A and B)*
 - *Not enough data (yet) regarding sequential collections; simultaneous collections may be required*
- 2. *Immunoassay screens may not be targeted at correct drug*
 - *Specifically cocaine*
- 3. *Passive exposure to marijuana may cause positive results*
 - *Potential acceptance of oral fluid as a specimen in the Federal workplace testing program*
 - *THC positive: you smoked marijuana*
 - *Well, things are not always what they seem*



Screening by immunoassay

- Many immunoassays are diluted versions of urine assays (target BZE), therefore do not detect cocaine
- Based on a cohort of 265 positive oral fluid samples, 26% of true positives will be missed if the wrong screening assay is used by the laboratory
- Importance: **Presence of cocaine indicates recent drug use**



Passive exposure to marijuana

■ Objective:

- To determine the presence of cannabinoids in oral fluid after realistic passive exposure to marijuana smoke

- Collaborative project with Dr. Donald Uges
University Medical Center, Groningen

The Netherlands



Physical characteristics of volunteers (n = 10)

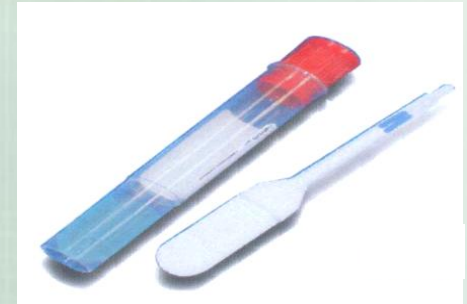
<i>Subject</i>	<i>Age</i>	<i>Gender</i>	<i>Weight (kg)</i>	<i>Height (m)</i>	<i>BMI (kg/m²)</i>
S1	23	M	77	1.9	21.33
S2	22	M	88	1.92	23.87
S3	22	F	75	1.82	22.64
S4	23	F	57	1.67	20.44
S5	24	F	65	1.74	21.47
S6	23	M	83	1.86	23.99
S7	23	M	90	1.95	23.67
S8	23	M	84	1.87	24.02
S9	25	F	55	1.58	22.03
S10	25	F	60	1.75	19.59

"Coffee shop" menu in Holland

HASH		WEED		Pre Rolled JOINTS	
maroccan Hash	€10,-	*Indoor*		* WEED *	€7,-
Sachlang		Silver [redacted] Cup Winner	€12,-	Silver Haze	€5,-
4 very strong stoned	€8,-	The Best indica Haze	€11,-	Bubble Gum	€4,50
TBIZla		Black Widow Haze		Blue Berry	€4,-
astmore Blond hash		Real Powerfull sativa	€11,-	White Widow	€4,-
Super Palm	€6,-	Bubble Gum		Fishermans Friend	€4,-
Our House Special		Strong indica Great taste	€9,-	Prix Special	€3,50
Black Hash		Soma Diesel	€9,-	Super Skunk	€3,50
Melana Cream	€15,-	Sweet strong stoned	€9,-	Purple Haze	€3,50
The best black Hash		Kali Mist		Jamaica	€3,50
Manali pasvati	€11,-	Very Smooth High	€8,-	Thai	€3,-
Spicy but strong High		Orange Bud		* Hash *	
Nepal tempel ball	€11,-	A real nice Red Hair	€8,-	Isolator	€7,50
Great taste powerfull High		Blue Berry	€8,-	Royal Cream	€4,50
Pakistani Palm	€8,-	Pro smokers Only		Nepal	€4,-
Straight from the Desert		Fisherman's Friend	€7,50	Super Palm	€3,50
Tempel Afgaan	€7,-	This is very strong shit		* Pure Joint *	
High Quality afgaan		White Widow	€7,-	No tobacco	
Afgaan		A nice strong Bio weed		Just weed	€5,-
Soft and Sticky	€5,-	Prix d'ami Special skunk	€6,-		
Exclusive Hash		This is our house special			
Sticky Fingers	€35	Super Skunk	€6,-		
Moonshine Isolator	€15,-	This is a special offer for you!			
Isolator	€20,-	*Outdoor*			
*KILLER HASH!		thai	€5,-		
		thai stick	€6,-		
		Jamaica	€5,-		

Experimental design

- IRB approved
- Two locations: “Coffee shops” in Groningen, Holland
- Oral fluid specimens collected **outside the shop** where marijuana use was on-going
- Collected two samples with Quantisal™ devices at each time point
 - Prior to exposure; 20 min; 40 min; 60 min
 - 2 hrs; 3 hrs
 - Subjects left shop
 - Final sample: 12 -22 hrs after entering shop
 - In addition:
 - one collection pad at location #1; two pads at location #2 were opened, left on table in the shop throughout the experiment



Passive marijuana exposure

■ *Location #1*

- *16 x 23 x 11 ft*
- *4 – 16 active smokers over 3hrs (x = 8)*
- *2 male, 3 female drug free volunteers*

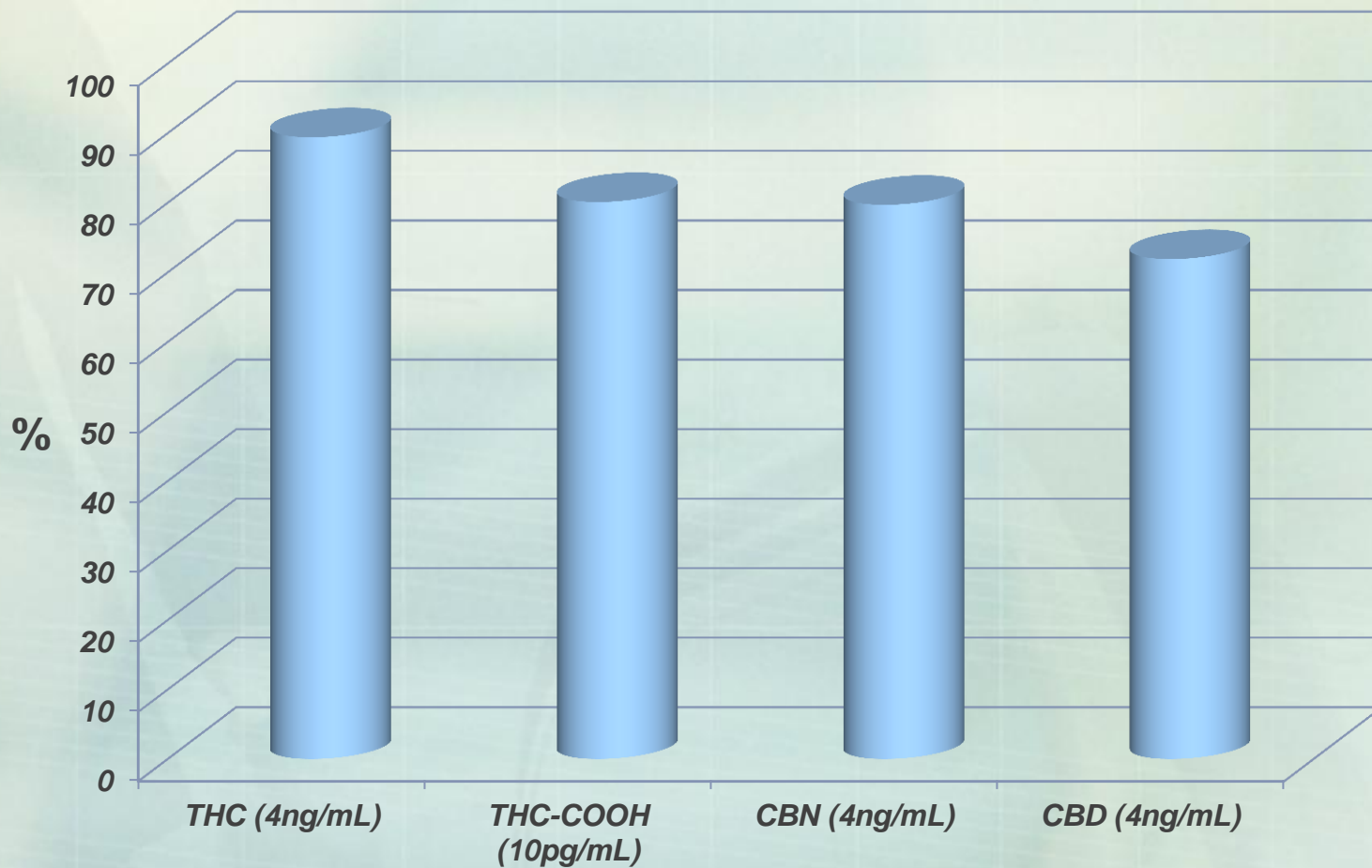
■ *Location #2*

- *6.5 x 23 x 10 ft*
- *0 – 6 active smokers over 3 hrs (x = 2.5)*
- *3 male, 2 female drug free volunteers*

Analytical procedures

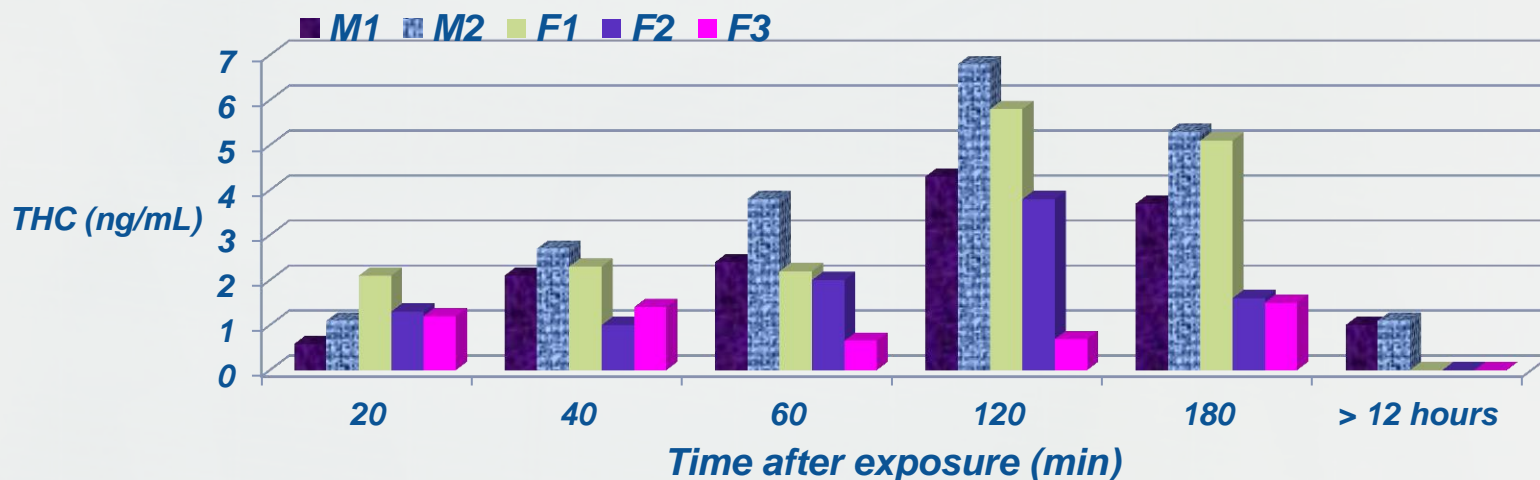
- *After collection, specimens shipped to test facility*
- *All specimens analyzed using ELISA: 4ng/mL*
- *All specimens analyzed using GC/MS:*
 - *THC (0.5ng/mL)*
 - *Cannabidiol (CBD; 1ng/mL)*
 - *Cannabinol (CBN; 0.5ng/mL)*
- *All specimens analyzed using GC-GC/MS*
 - *THC-COOH (5pg/mL)*

Cannabinoid recovery from Quantisal™



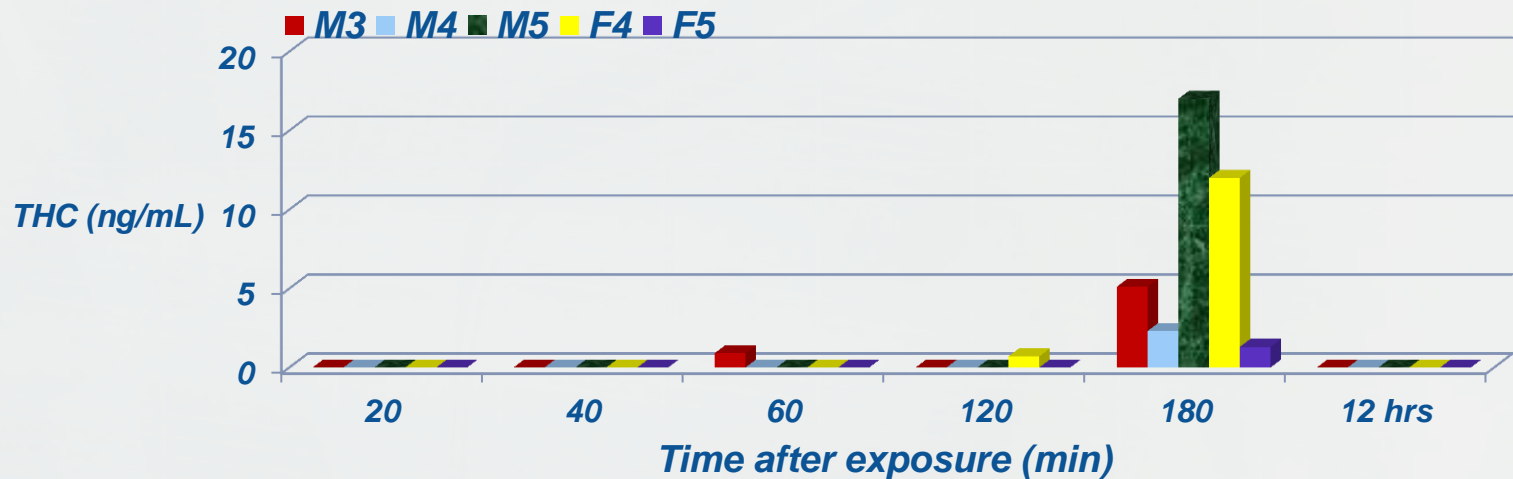
Results

- Location #1 (5 “passive” subjects)
- Active smokers 4 – 16; mean 8
- Results:
 - All subjects negative before entering shop
 - THC detected in all subjects from 20 min – 3 hrs



Results

- Location #2 (5 “passive” subjects)
- Active smokers 0 – 6; mean 2.5
- Results:
 - All subjects negative before entering shop
 - Relatively high THC in 3 subjects at 3hr time-point



Open pads

	Location #1	Location #2	
		Pad 1	Pad 2
THC (ng/mL)	290	212	216
CBN (ng/mL)	48	40	42
CBD (ng/mL)	16	28	38
THC-COOH (pg/mL)	0	0	0



Passive exposure

- *THC is absorbed by drug-free individuals under realistic conditions when exposed to marijuana smoke*
- *After only 2 hrs of exposure some individuals showed concentrations above proposed screening and confirmatory cut-offs*
- *The metabolite, THC-COOH, not detected in any of the specimens*
- *THC-COOH: appears to be a bio-marker for active marijuana use as opposed to THC which may be present due to passive exposure*

Limitations and further research

- *Not all active smokers have THC-COOH in oral fluid*
- *Subjects sampled after leaving shop, then >12 hrs later*
 - *Add time points in between to determine when exposure drops below proposed screening cut-off*
- *If this study is acute exposure (one time, limited timeframe) then an assessment of chronic THC exposure is needed*
 - *More research with individuals chronically exposed to legal medical marijuana*

Summary

- *Oral fluid testing for drugs and alcohol is being implemented in many market segments*
- *Oral fluid gives as good as, if not better information, than urine for workplace testing*
- *Europe and the USA are embracing the potential for oral fluid testing in the workplace*
- *Europe has submitted guidelines for oral fluid testing to regulatory body*
- *SAMHSA is continuing to discuss and produce guidelines to introduce testing*

Thank you for your attention..

