

THC- 50/15

(Screening/Confirmation)

How Valid is this Still?

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Urine Drug Screening Timeline

Late 1960s
Vietnam war creates need to develop rapid DoA screening techniques

Early 1970s
enzyme immunoassays (EMIT[®]) developed by Syva[®]

1982
President Reagan declared his "War on Drugs"

1988 NIDA published the first mandatory guidelines for workplace drug testing

1995
AS/4308 published

Mid-Late 1990s
PoCT DoA devices became more popular



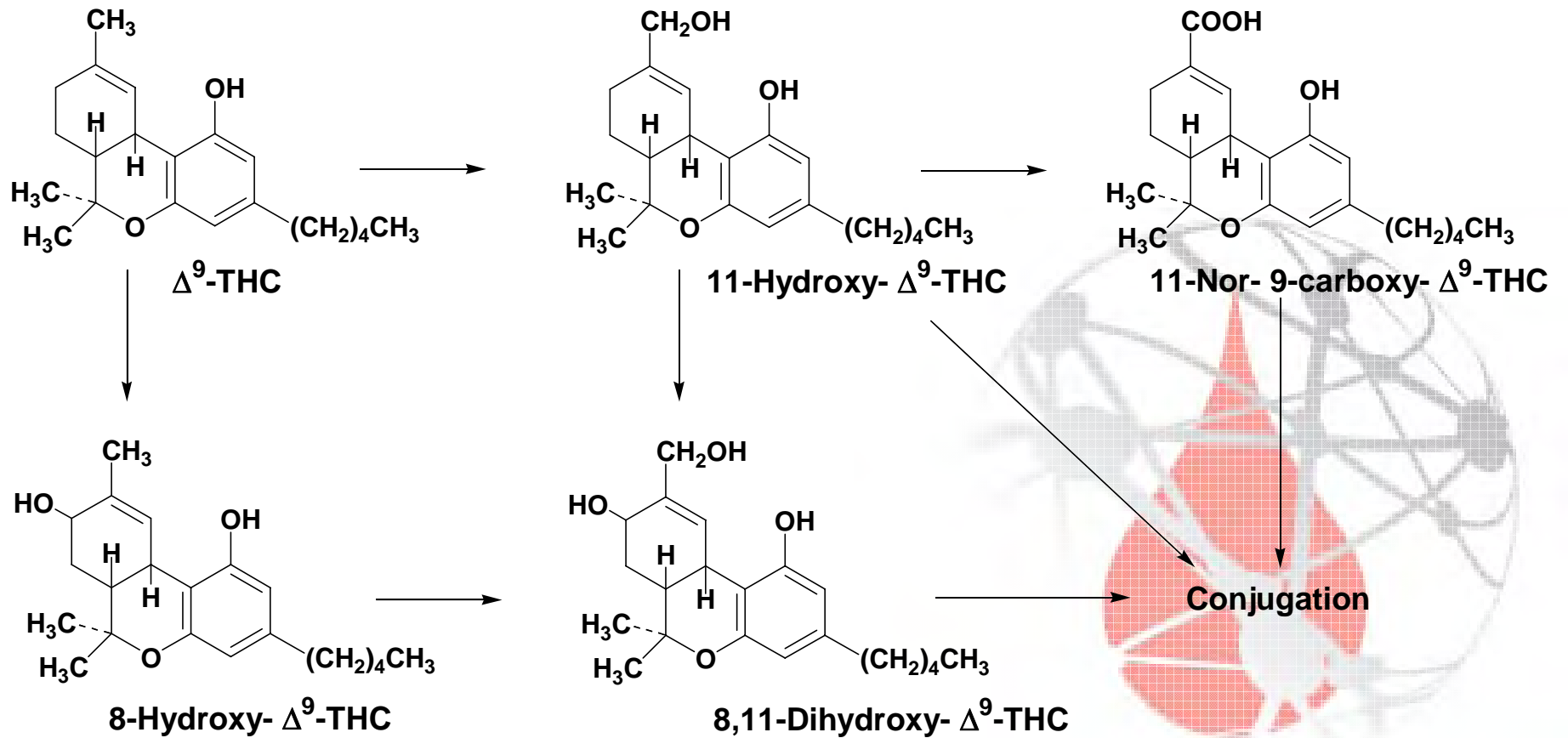
First laboratory assays for drugs of abuse (DoA) and their metabolites in urine were radioimmunoassays in the early **1970s**

1980s both Abbott[®] and Roche[®] develop FPIA[®] DoA assays

Early-1990s Roche introduced KIMS[®] DoA assay

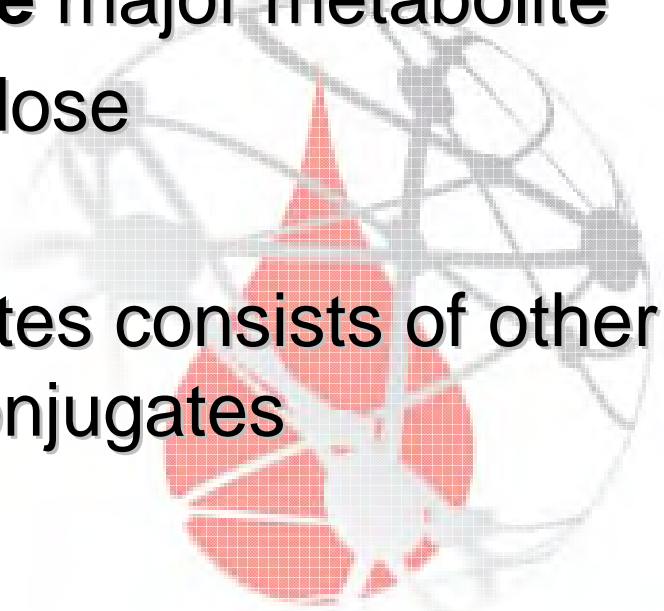
Mid-1990s Microgenics[®] introduces the CEDIA[®] DoA assays

THC Metabolism



Urinary Excretion of THC

- Approx. 30% of a dose of THC is excreted in urine within 72 hours as:
 - **Carboxy-THC-Glucuronide** major metabolite
 - **11-OH-THC** *ca.* 2% of the dose
 - **THC** in low amounts
 - **The remainder** of metabolites consists of other acidic products and their conjugates

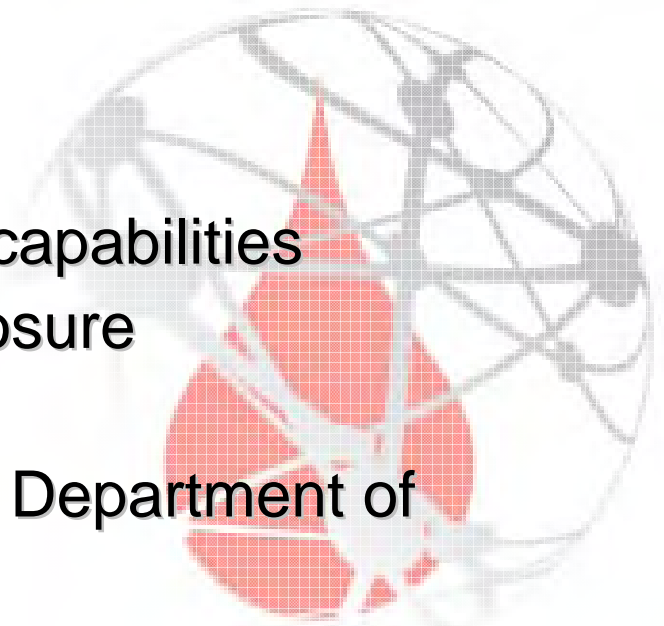


How Were Cannabis Cut-Off Levels Set?

- In the early days of screening cut-offs were manufacturer specific
- Confirmation levels were determined by the sensitivity of the equipment and methodology used by the reference lab
- Everything changed with the release of Mandatory Guidelines for Workplace Drug Testing by NIDA in the US

Mandatory Guidelines for Workplace Drug Testing (1988)

- Cut-off concentrations were based on evaluations of several factors including:
 - Drug metabolism
 - Excretion studies
 - Drug detection time windows
 - Instrument and methodological capabilities
 - Risk of environmental drug exposure
 - Cost of analyses
 - Urine drug testing data from the Department of Defence

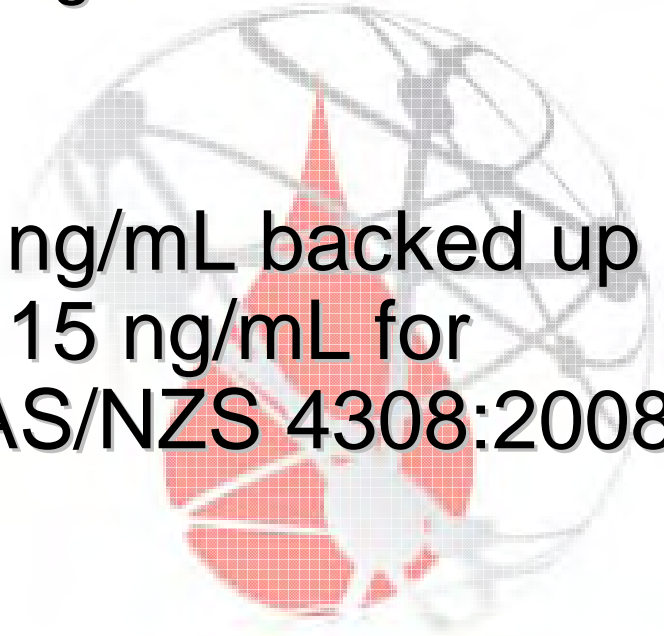


AS/NZS 4308:2008

- Australian standard for the analysis of drugs of abuse urine was first published in 1995 as AS 4308-1995.
- Two iterations later we have AS/NZS 4308:2008.
“Procedures for specimen collection and the detection and quantitation of drugs of abuse in urine”.
- Inclusion of use of point-of-care screening devices
- Confirmatory test cut-off concentrations for compounds in the amphetamine type substances and benzodiazepines classes of drugs

The Question

- Given that:
 - THC immunoassays have improved since initial guidelines were set
 - Use of PoCT devices play a significant role in drug testing
- Is a screening cut-off of 50 ng/mL backed up by a confirmation cut-off of 15 ng/mL for carboxy-THC as stated in AS/NZS 4308:2008 still valid today?



Important Considerations

- Drug testing is conducted as a deterrent to drug use
- Critical that a person is not falsely accused of drug use.
- To ensure safety of individuals, the converse is also true
- Drug test results are often stand-alone results often unaccompanied by the additional information ordinarily found in medical testing/ diagnosis
- It is therefore imperative that the accuracy and validity of analysis is considered so we can make the call “Positive” or “Negative”

Experimental

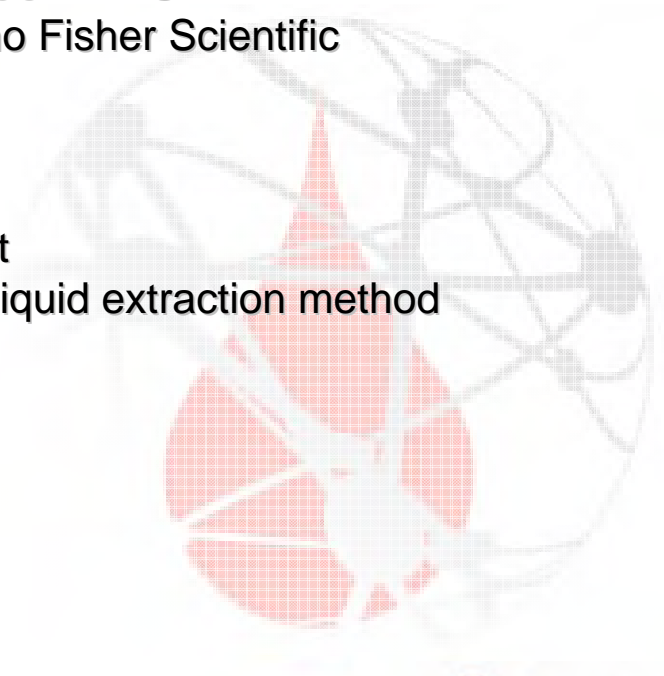


Specimen Selection

- Specimens which returned DRI THC semi-quantitative results near the screening cut-off of 50 ng/mL (ca. 100)
- 6 month collection period
- Random donor specimens were used
- Specimens were only used once they were ready for disposal
- Aliquots were made for each analysis methodology (min. freeze thaw cycles)
- Where possible analysis was carried out on one day.

Specimen Analysis

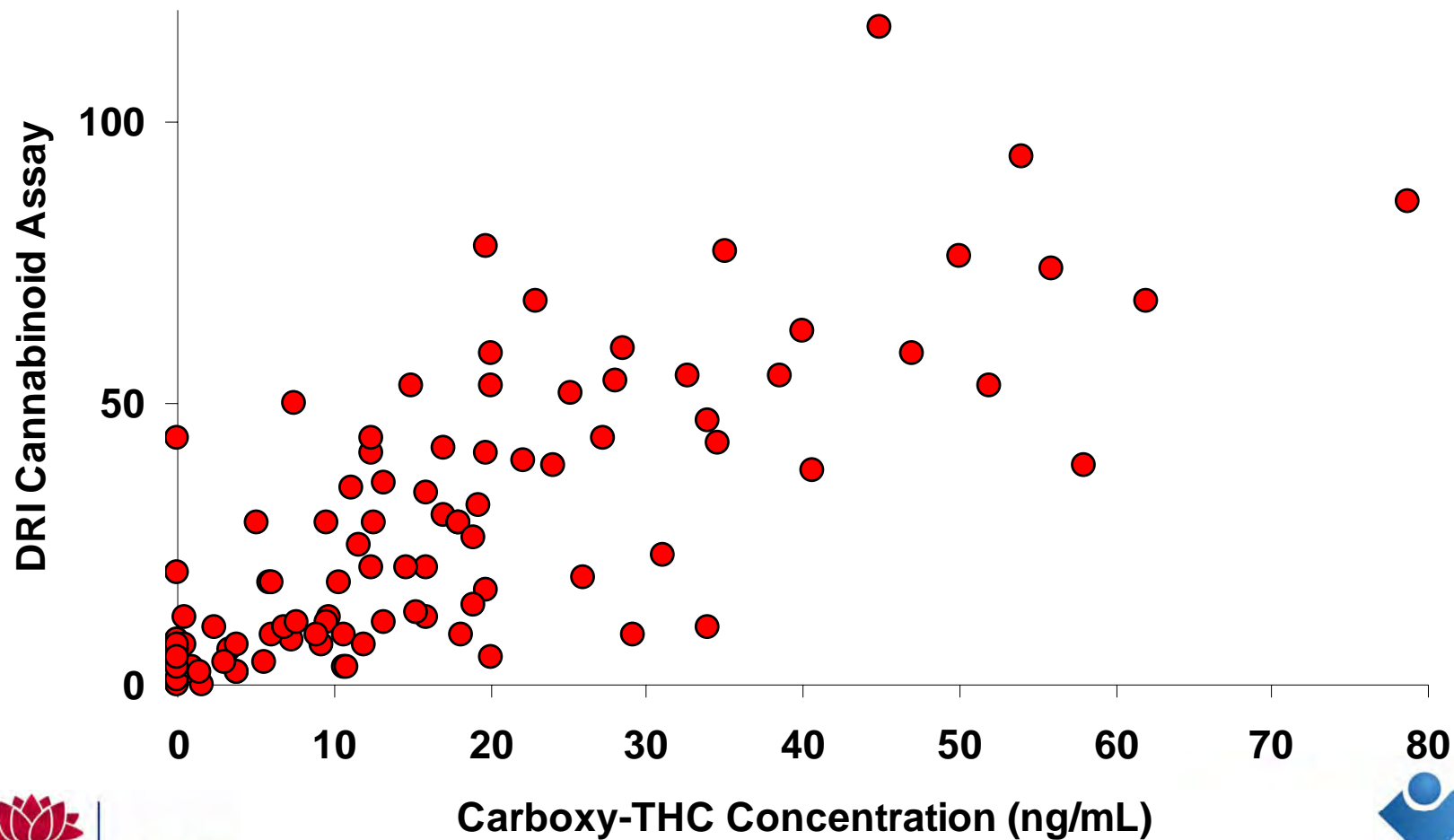
- **DRI Cannabinoid assay**
 - Thermo Fisher Scientific
 - MGC 240
 - HAPS
- **CEDIA THC Plus**
 - Thermo Fisher Scientific
 - Olympus AU400
 - Racing Analytical Services
- **KIMS Cannabinoids II**
 - Cobas Integra 800.
 - Perth Medical Laboratories
- **EMIT THC**
 - Siemens
 - Dimension RxL Max
 - HAPS
- **MicroCheck THC**
 - Thermo Fisher Scientific
 - HAPS
- **GC/MS**
 - Agilent
 - liquid/liquid extraction method
 - HAPS



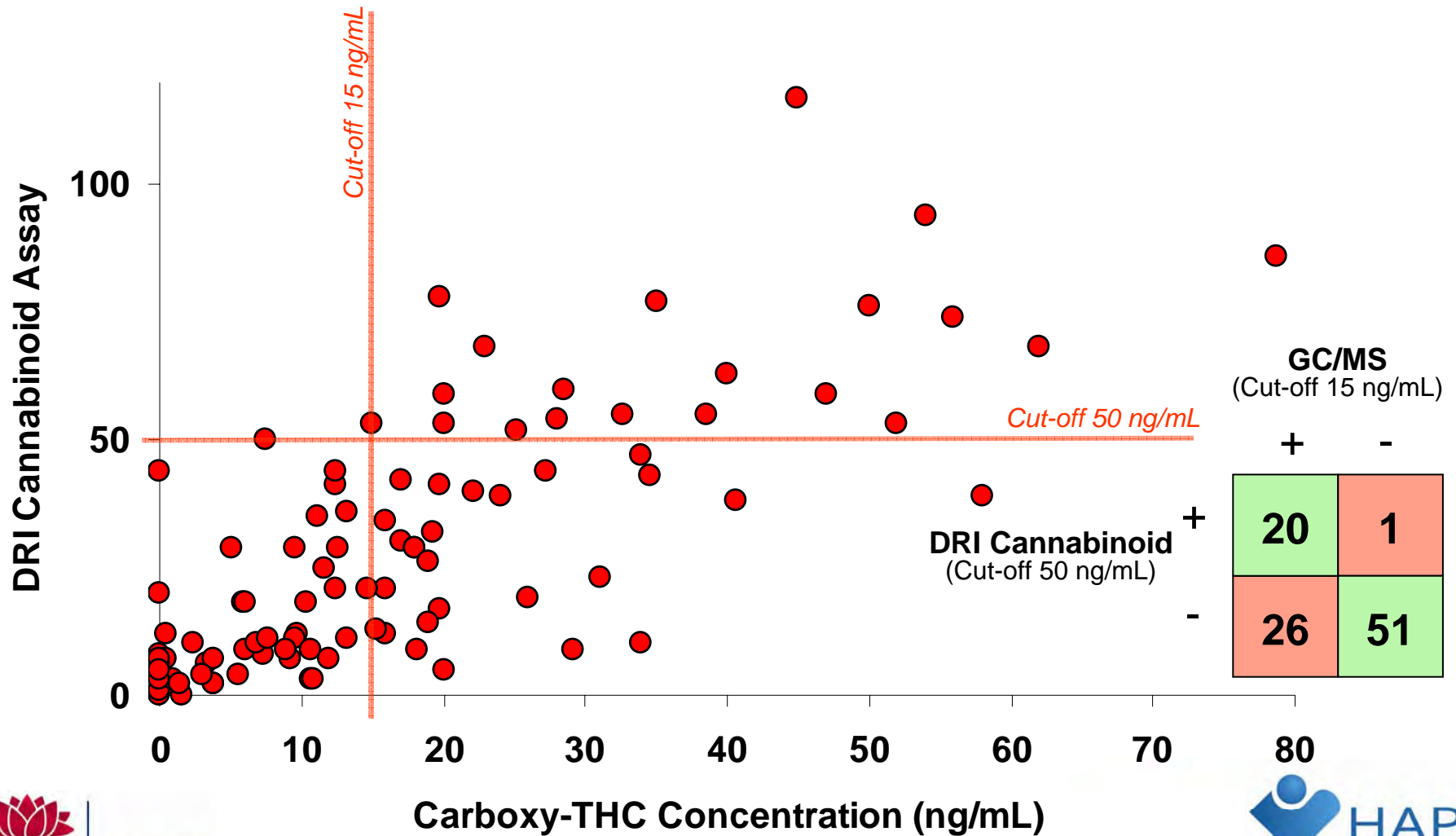
Results



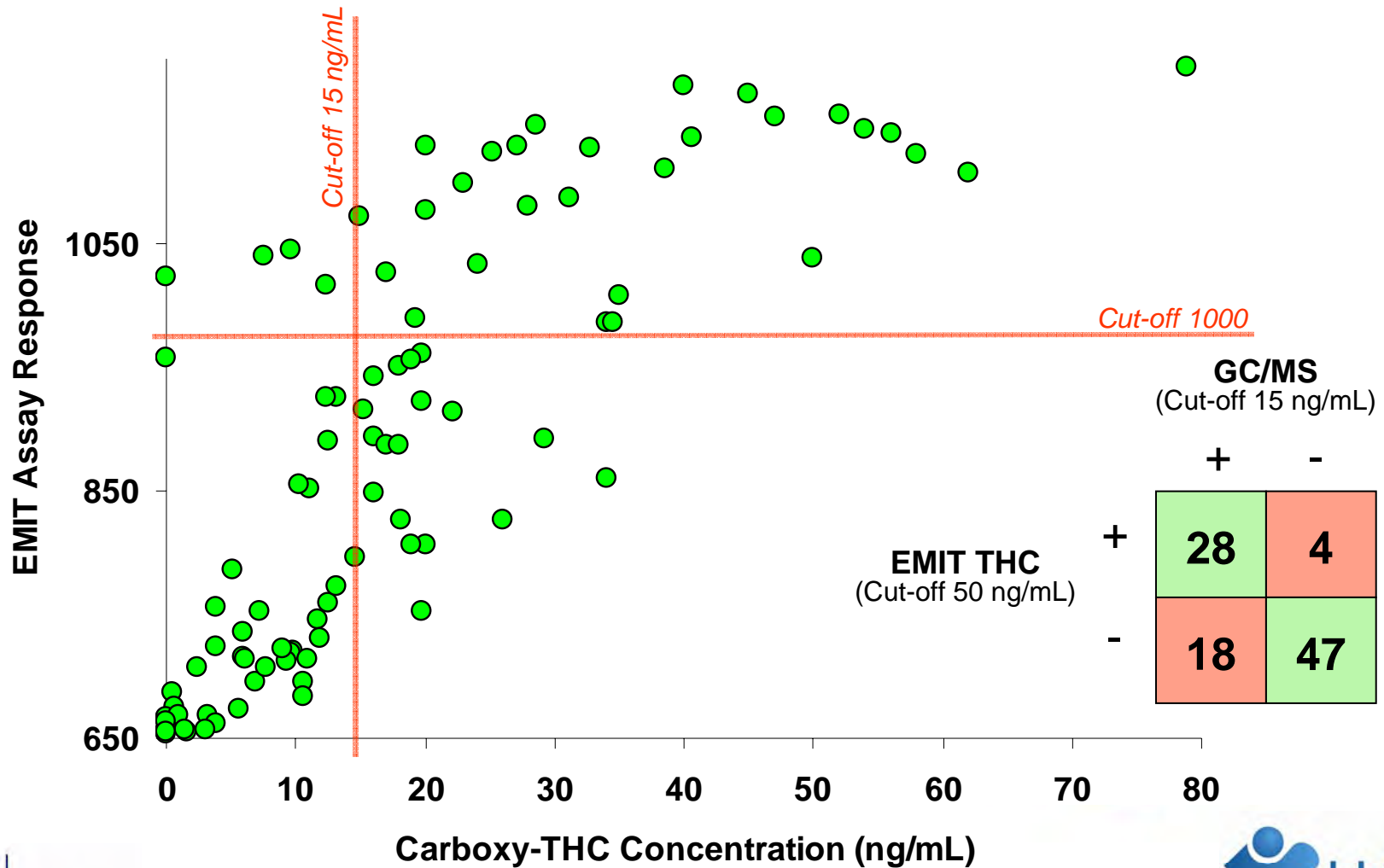
DRI Cannabinoid V_s Carboxy-THC Concentration



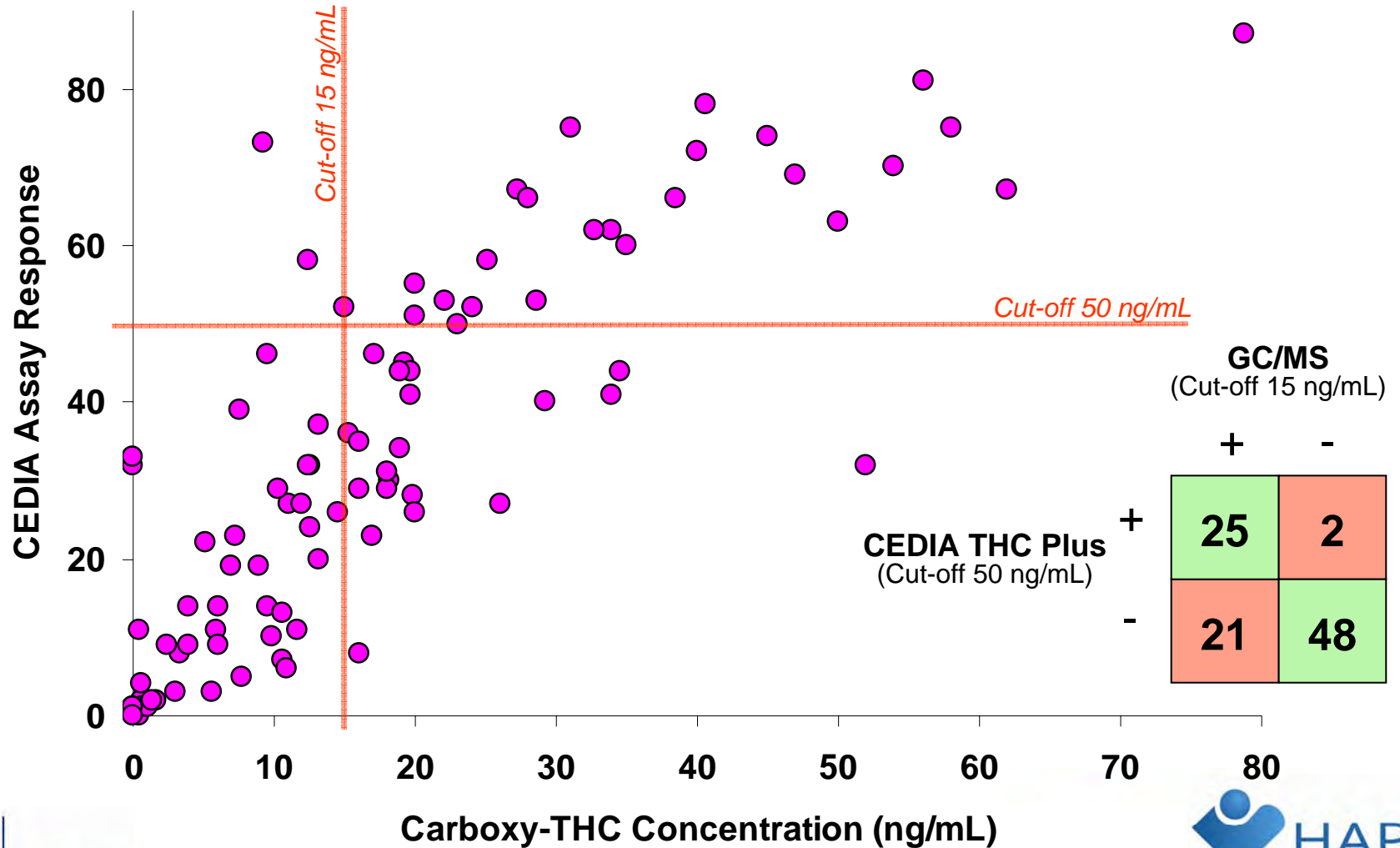
DRI Cannabinoid V_s Carboxy-THC Concentration



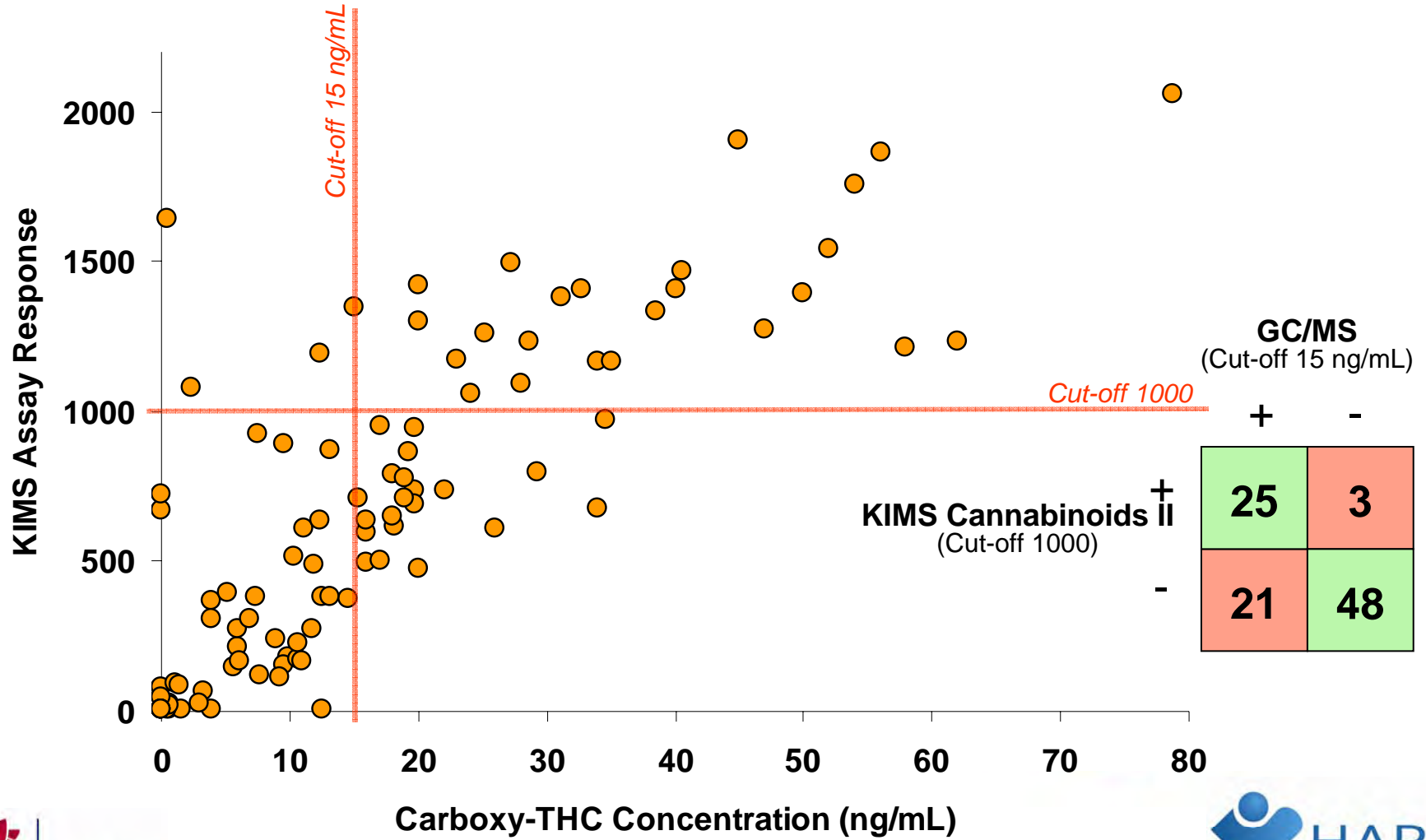
EMIT THC V_s Carboxy-THC Concentration



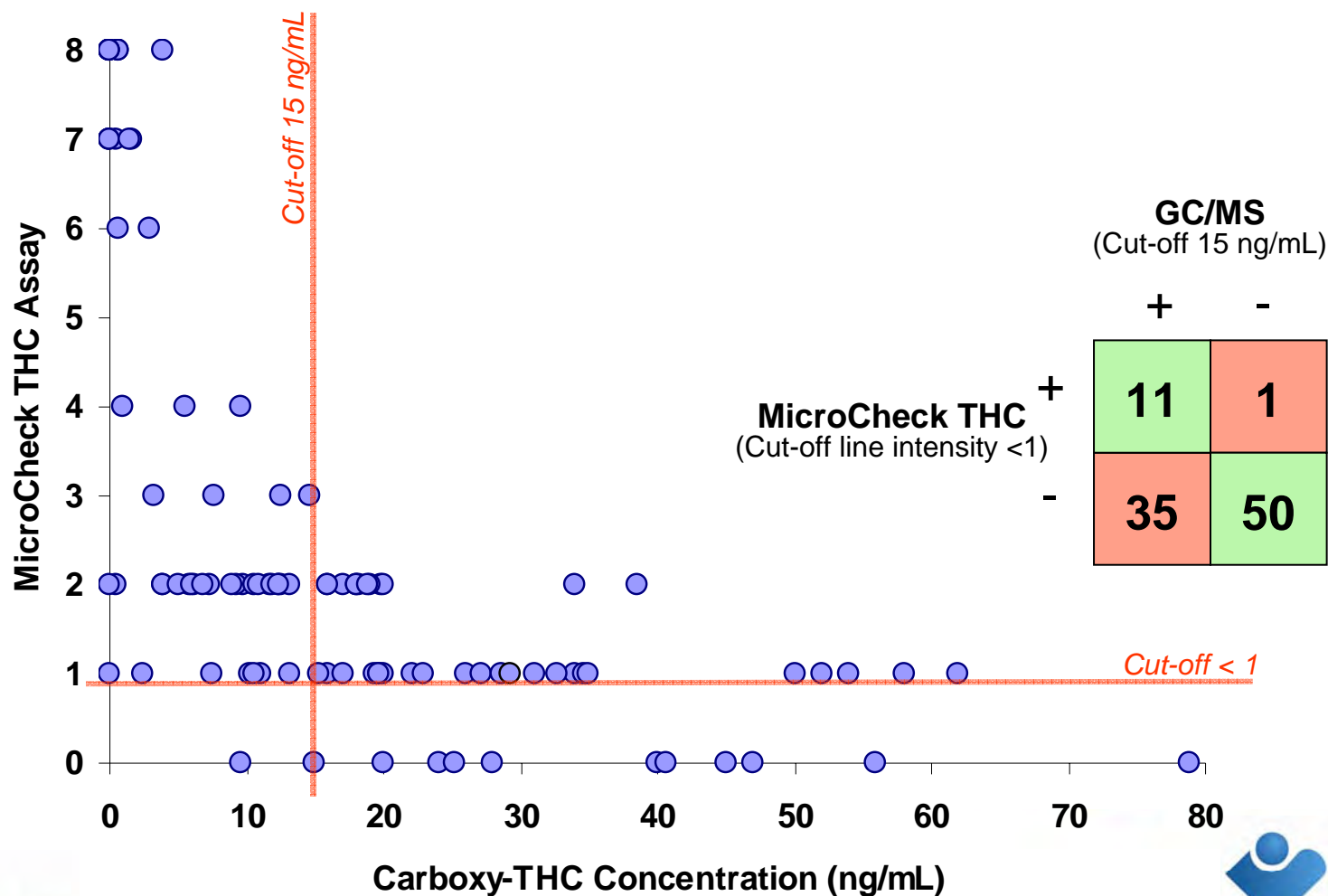
CEDIA THC Plus V_s Carboxy-THC Concentration



KIMS Cannabinoid II V_s Carboxy-THC Concentration

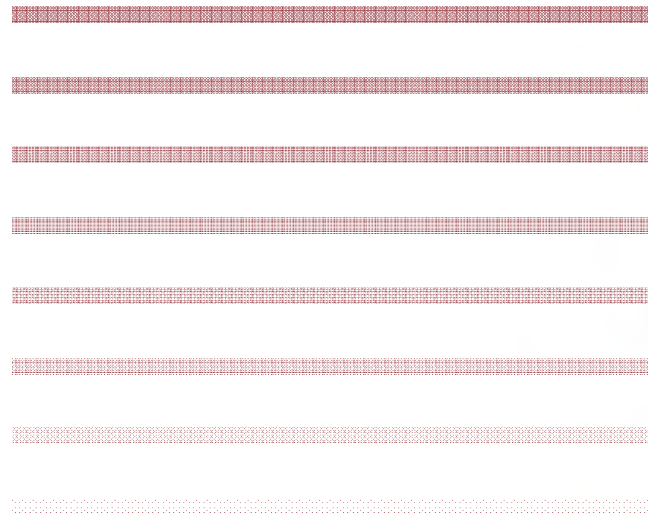


MicroCheck THC V_s Carboxy-THC Concentration

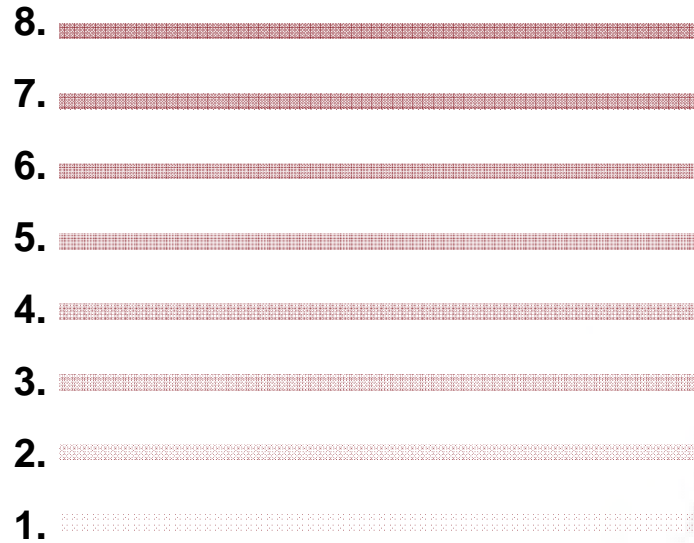


PoCT Device Result Reading

- How many lines can you count?



PoCT Device Result Reading

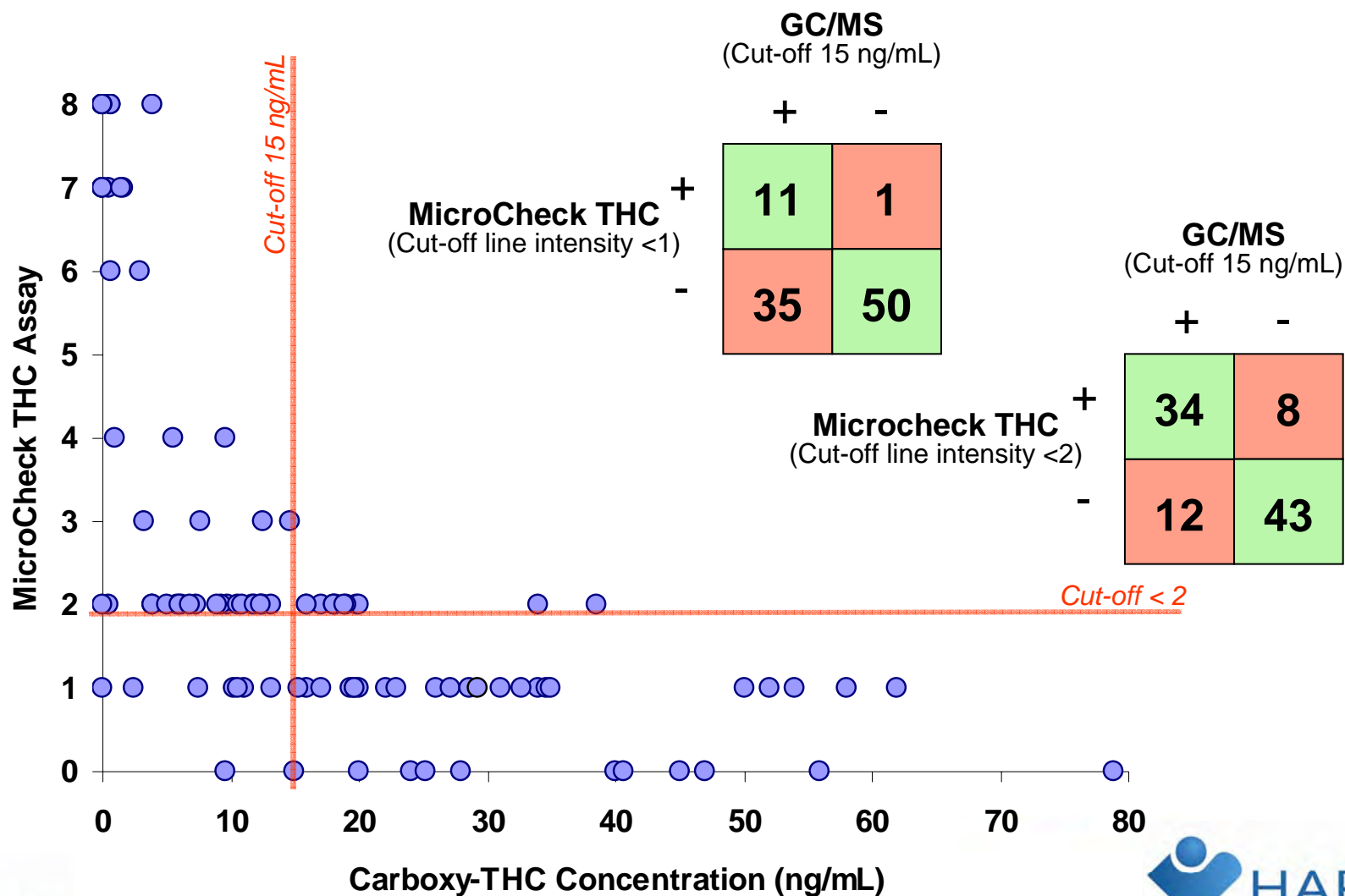


- Near-cut-off result reading can be challenging
- Inherent variations in reading certain colours and colour intensities between individuals.

PoCT Performance

- Using calibration materials 40 50 and 60 ng/mL the line intensity was 1.
- We found the performance of the device was insensitive to a difference of $\pm 20\%$ of the cut-off
- Insert quoted performance is:
20% false negative rate (n=5) for the device from the cut-off to +25% of the cut-off

Microcheck THC V_s Carboxy-THC Concentration



Performance Characteristics @ a Confirmation Cut-off of 15ng/mL

	True Positive	True Negative	False Positive	False Negative
EMIT	28.9%	48.5%	4.1%	18.6%
CEDIA	26%	50%	2.1%	21.9%
DRI	20.4%	52%	1%	26.5%
KIMMS	25.8%	49.5%	3.1%	21.6%
Microcheck < 1	11.3%	51.5%	1%	36.1%
Microcheck < 2	35.1%	44.3%	8.2%	12.4%

Specificities to THC Metabolites

Compound	% Cross-Reactivity				
	EMIT	CEDIA	DRI	KIMS	MicroCheck
11-nor- Δ^9 -THC-9-Carboxylic acid	100	100	100	100	100
11-nor- Δ^8 -THC-9-Carboxylic acid		62.5	50	56.2	100
8- β -11-Dihydroxy- Δ^9 -THC	86.2	38.5		86.2	
8- β -11-Hydroxy- Δ^9 -THC	73.5	38.5	100		
8- β -Hydroxy- Δ^9 -THC			50.0		
8- α -Hydroxy- Δ^9 -THC				32.9	
11-Hydroxy- Δ^8 -THC	74.6				
11-Hydroxy- Δ^9 -THC	64.9	62.5	50.0	43.5	2.0
9-Carboxy-11-nor- Δ^9 -THC-glucuronide	52.6	58.8		80.6	
Δ^9 -THC		25.0	100	1.9	0.5
Δ^8 -THC					0.7
Cannabinol		10.0	50.0	2.5	0.1
Cannabidiol		1.3			0.05

Performance Characteristics @ a Confirmation Cut-off of 20 ng/mL

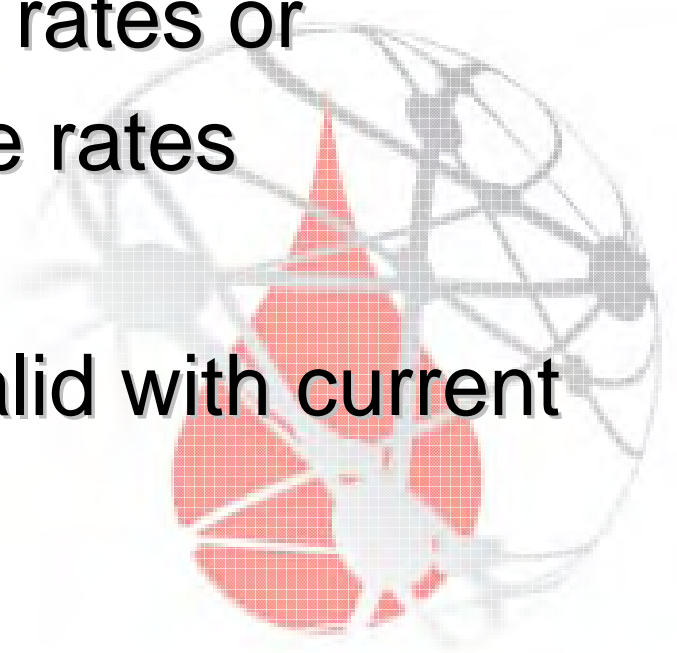
	True Positive	True Negative	False Positive	False Negative
EMIT	25.8% ↓	61.9% ↑	7.2% ↑	5.2% ↓
CEDIA	25% ↓	65.6% ↑	3.1% ↑	6.3% ↓
DRI	18.4% ↓	66.3% ↑	3.1% ↑	12.2% ↓
KIMMS	24.7% ↓	64.9% ↑	4.1% ↑	6.2% ↓
Microcheck	10.3% ↓	67% ↑	2.1% ↑	20.6% ↓

Performance Characteristics @ a Confirmation Cut-off of 10ng/mL

	True Positive	True Negative	False Positive	False Negative
EMIT	29.9% ↑	35.1% ↓	3.1% ↓	32% ↑
CEDIA	27.1% ↑	36.5% ↓	1% ↓	35.4% ↑
DRI	20.4%	37.8% ↓	1%	40.8% ↑
KIMMS	26.8% ↑	36.1% ↓	2.1% ↓	35.1% ↑
Microcheck	11.3%	37.1% ↓	1% ↓	50.5% ↑

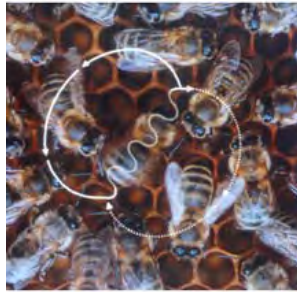
Conclusion

- While marginal gains could be made by moving the confirmation cut-off by 5 ng/mL up or down this comes at the cost of either:
 - Increased false positive rates or
 - Increased false negative rates
- Current cut-offs are still valid with current assays





Bees + Cocaine = High Flyers



- Neuroscientists at Macquarie University, have found that after giving cocaine to bees they threw themselves into unusually energetic waggle dances and they even went "cold turkey"

"They overvalue the quality of the nectar. They think it is the best nectar they've ever had."

"People have the same response. Everything you experience when you are on cocaine feels fantastic. You feel amazing."

- They believe that bees can provide a tool for looking at addiction, such as which genes are activated when the bee's brain goes cold turkey.
- So what would happen if the bees were dosed with Cannabis?

Acknowledgements

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