

# Comparative cannabinoid cross-reactivity in THC immunoassays

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## Introduction

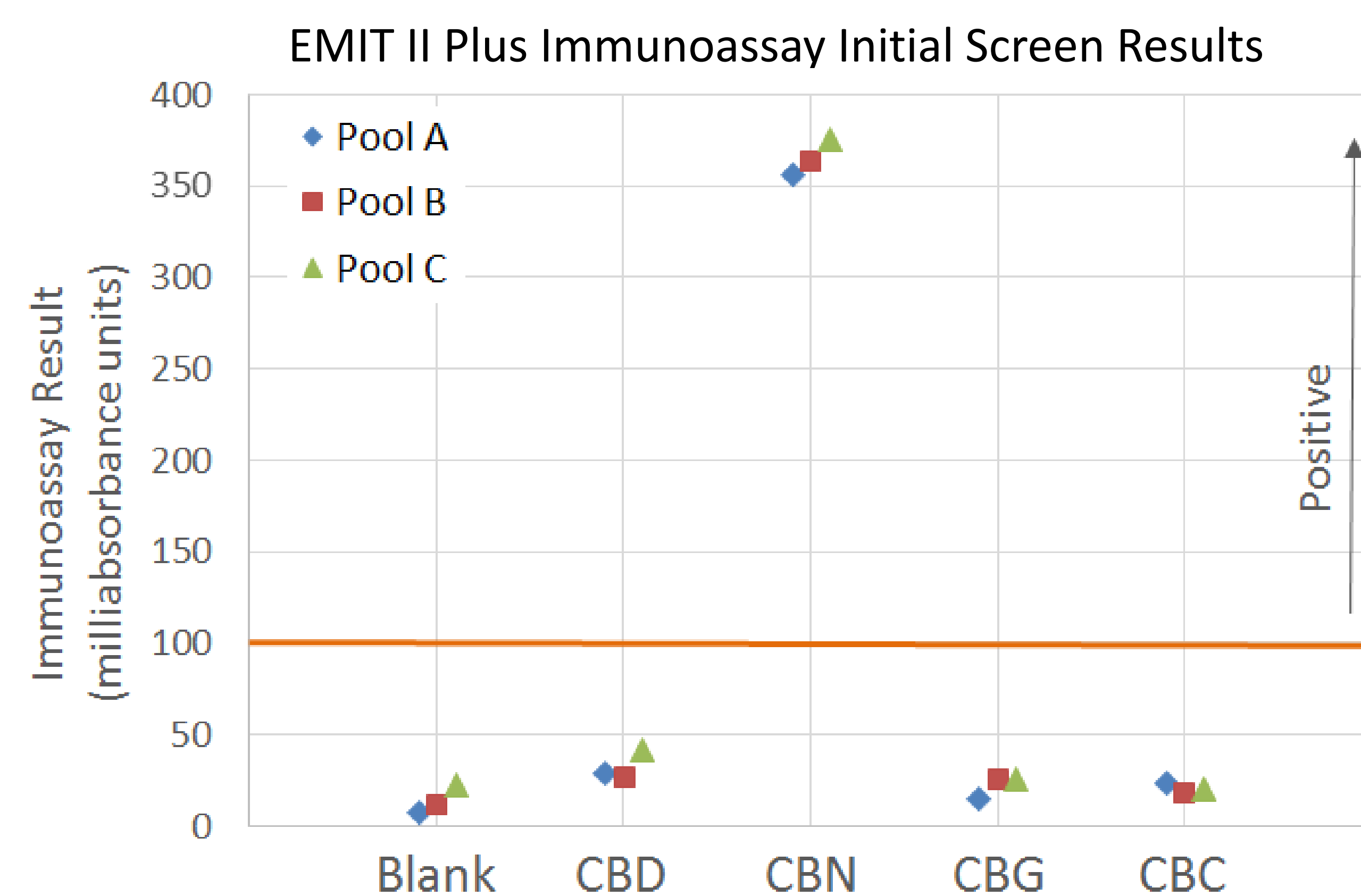
Specificity is a common concern with immunoassays that are used to detect drug use. The active component of marijuana, tetrahydrocannabinol (THC), shares structural similarities with other cannabinoids. The increasing use of cannabinoid products makes it important for labs to be aware of potential cross-reactivity with immunoassays designed to detect THC and related metabolites. The objective of this study was to evaluate the extent of cross-reactivity of two THC immunoassays with four compounds: cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), and cannabigerol (CBG).

## Methods

Two immunoassays were tested in this study: the EMIT II Plus immunoassay run on a Beckman AU5810 instrument and the Microgenics MultiGent Cannabinoids assay run on an Abbott Architect instrument. To screen for cross-reactivity initially, we tested three different urine pools fortified with 1000 ng/mL of each compound. Pools were prepared by mixing at least three deidentified patient samples that were negative for THC via the EMIT II Plus immunoassay. We next tested a range of fortified CBN concentrations to determine what concentration of CBN was required to trigger a positive immunoassay result. The immunoassay is qualitative: an absorbance unit response greater than the established cut-off value of 100 indicates that the sample contains more than 20 ng/mL THC metabolite. We tested three different urine pools fortified with either 1000, 250, 100, 50, 20 or 10 ng/mL CBN. Finally, we sought to establish whether CBN has an additive effect with THC in the immunoassay. We fortified 21 individual samples that were weakly positive for THC via a mass-spectrometry based method (between 5 and 10 ng/mL), but negative by the EMIT II Plus immunoassay, with a concentration of CBN not itself sufficient to yield a positive immunoassay response (50 ng/mL).

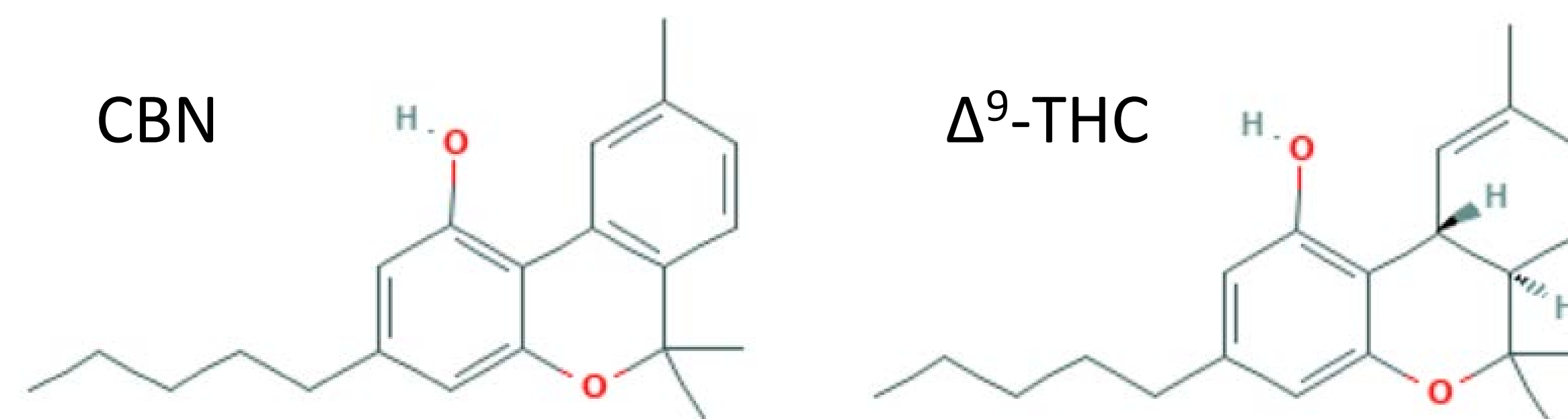
## Screening Results

- Microgenics MultiGent Cannabinoids assay showed no cross-reactivity with CBD, CBN, CBG, or CBC
- EMIT II Plus assay only showed cross-reactivity with CBN



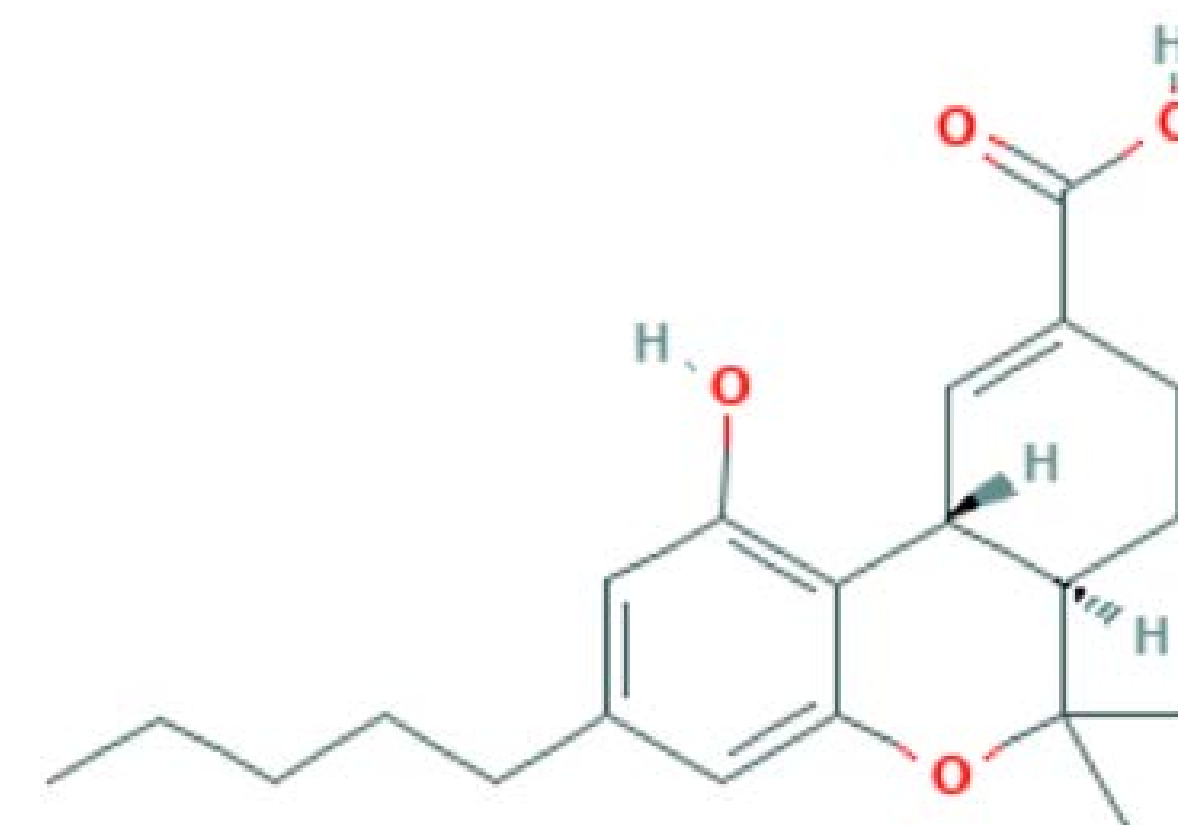
## What is CBN?

- CBN is an aromatized derivative of THC



Structures of CBN (CID=2543) and  $\Delta^9$ -THC (CID=16078) from PubChem Database, National Center for Biotechnology Information, accessed 4/2019.

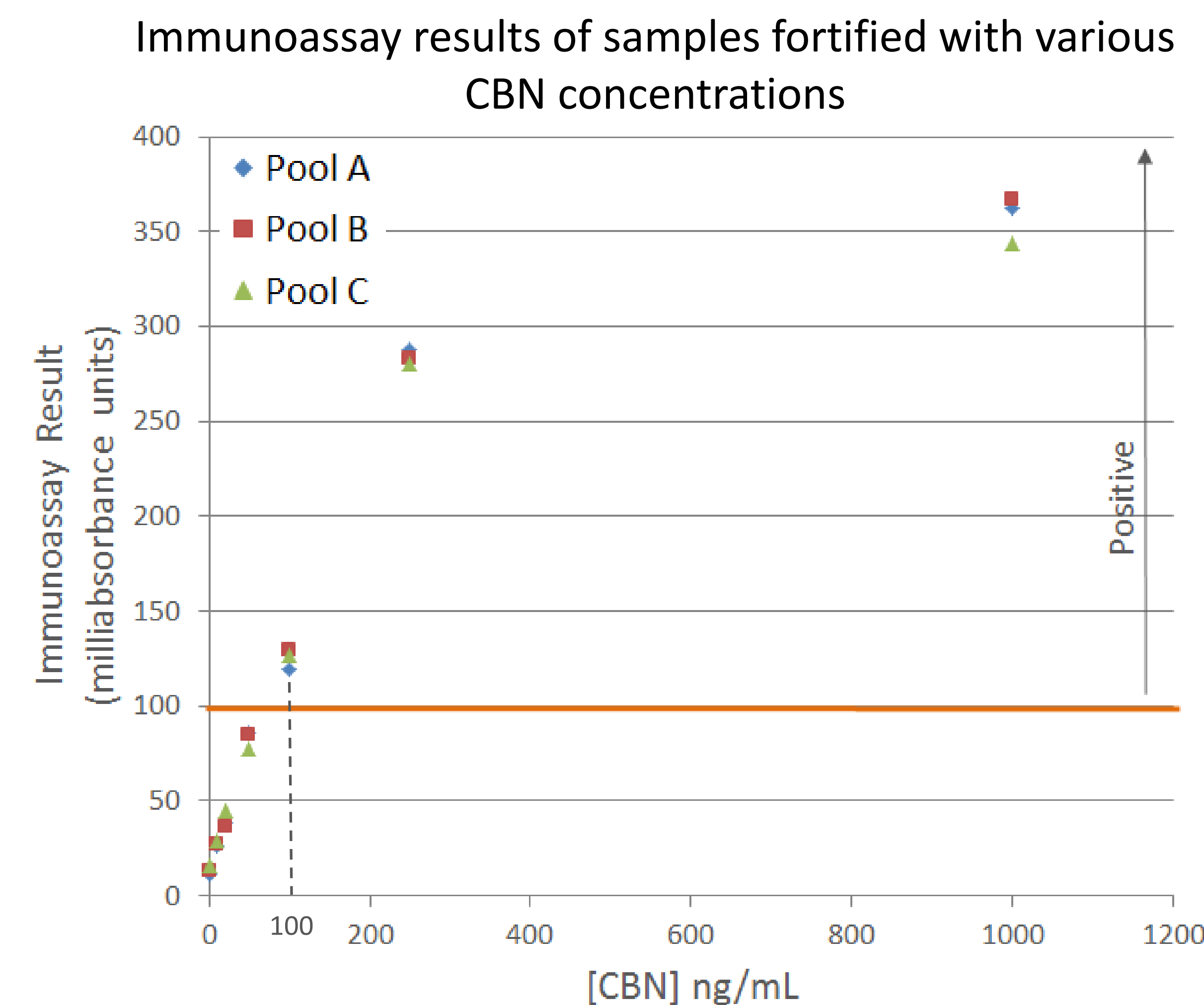
- CBN likely exists in small quantities in cannabis plants
- CBN mostly produced during aging and storage of cannabis products
- CBN products are directly marketed as sleep aids
- Immunoassay designed to detect major  $\Delta^9$ -THC metabolite, 11-nor- $\Delta^9$ -THC-9-carboxylic acid



Structure of 11-Nor-9-carboxy-delta-9-tetrahydrocannabinol (CID=108207) from PubChem Database, National Center for Biotechnology Information, accessed 4/2019.

## CBN Titration Results

- CBN showed a non-linear relationship in the EMIT II Plus immunoassay

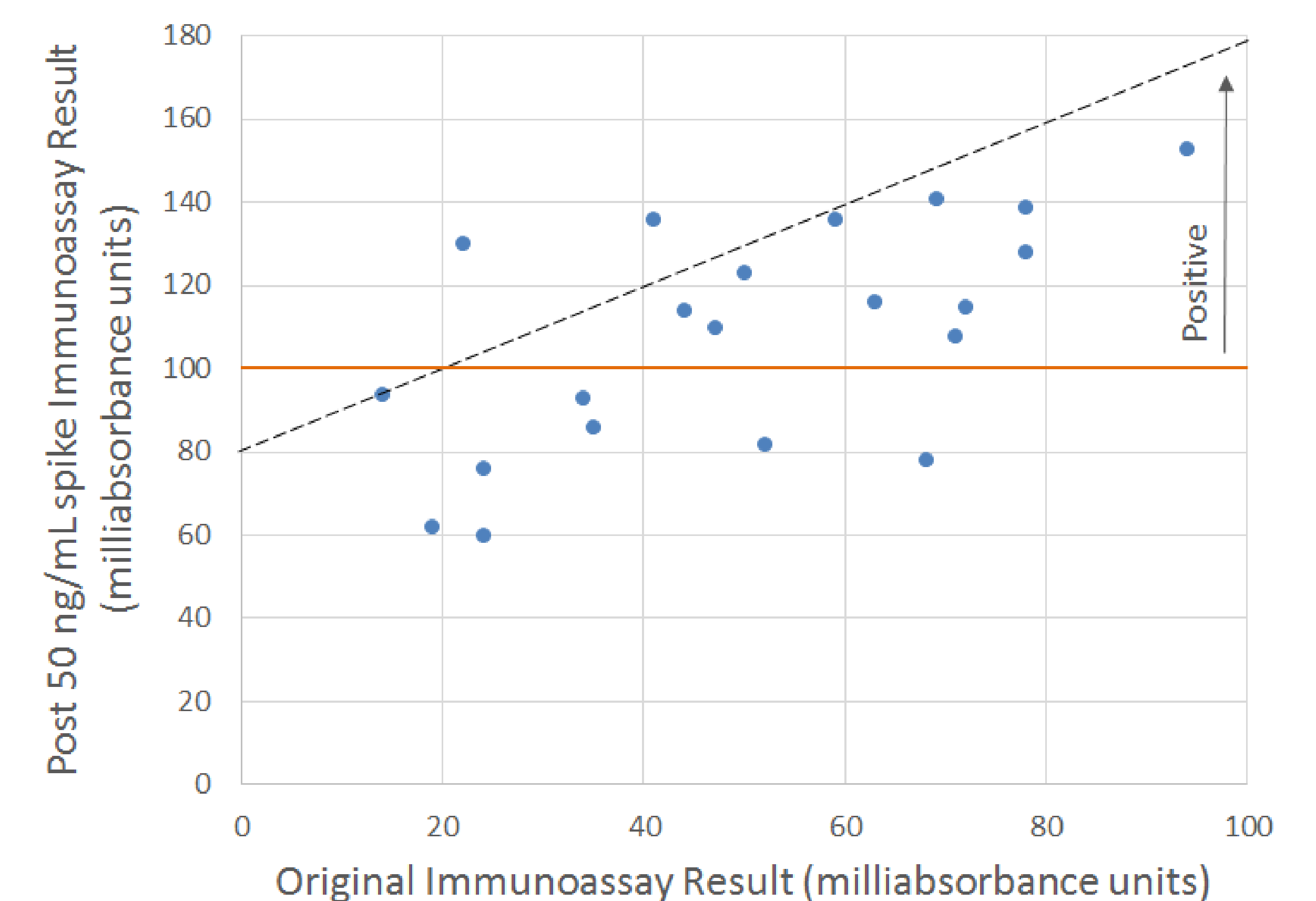


- Samples fortified with at least 100 ng/mL CBN produced a positive result
- Since the assay is calibrated to be positive at 20 ng/mL THC metabolite, this suggests about five-fold lower reactivity with CBN

## Additivity Results

- Immunoassay results for all 21 samples weakly positive for THC vis mass spectrometry were higher following 50 ng/mL CBN addition
- Thirteen samples became positive by immunoassay following the addition
- Samples did not respond consistently to CBN addition
- Differential response was not correlated to the creatinine concentration of the urine

Immunoassay results pre- and post- 50 ng/mL CBN addition. The dashed line represents the relationship expected based on 50 ng/mL CBN in negative urine.



## Conclusions

- Variability in cross-reactivity profiles exists between different immunoassay manufacturers
- Since the purity of cannabinoid products is not regulated, there may be some THC present that produces reactivity on the drug screen
- False-positive drug screen results can cause serious consequences for patients, so it is critical to consider cross-reactivity when interpreting such results

## Acknowledgements & References

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