Phencyclidine False Positive Immunoassay Induced by the Bath Salt α-PVP

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Immunnoassay screening procedures are susceptible to producing false positive results from cross reacting compounds (1). Our facility recently experienced false positive phencyclidine (PCP) urine samples tested with the Thermo Fisher DRI Immunoassay on a Biolis 50i Automated Clinical Analyzer. The Thermo Fisher DRI assay is a homogeneous competitive monoclonal enzyme immunoassay with activity measured spectrophotometrically at 340 nm. The Biolis was calibrated using a 25 ng/ml cutoff with two quality control levels to verify calibration. Each of the patient samples confirmed negative for PCP at 5 ng/ml by tandem mass spectrometry on an Agilent Infinity 1290 liquid chromatographic system coupled to an Agilent triple quadrupole 6490 MS/MS using a seven point calibration curve, r² > 0.99, and three quality control concentrations using deuterated internal standardization. Chemically, PCP is described as 1-(1-phenyl cyclohexyl)piperidine.

A urine specimen from a 32-year old male monitored in a pain clinic was submitted for analysis. His sample screened positive by the DRI Immunoassay for PCP and confirmed negative by LC/MS/MS. The sample was positive by LC/MS/MS for > 200 ng/ml α-PVP and > 1,600 ng/ml cotinine. His urine creatinine measured > 300 mg/dl on the Biolis 50i, which is outside the expected range of 20 - 300 mg/dl. All other results for muscle relaxants, opiates, tricyclic antidepressants, stimulants, benzodiazepines, synthetic opioids, anticonvulsants, and other illicit drugs included in the panel, were negative by LC/MS/MS. The patient screened negative
for PCP on two subsequent urine collection days. However, a close review of the two subsequent immunoassay screen data indicated negative at the 25 ng/ml cutoff, but showed semi-quantitative cross reactivity results of approximately 1 and 7 ng/ml. Each sample confirmed negative at 5 ng/ml for PCP by LC/MS/MS and confirmed positive for α-PVP at 25 ng/ml and 110 ng/ml, respectively. An approximate 4 to 6% cross reactivity relationship was indicated at this low range.

The synthetic cathinone drug, alpha-pyrrolidinopentiophenone (α-PVP), is chemically related to the norepinephrine dopamine reuptake inhibitor (NDRI), Centroton. Both are central nervous system stimulants. Alpha-PVP, listed by the Drug Enforcement Administration as Schedule 1, is used recreationally (2). It is sold in retail products offered by convenience stores and over the internet as an ingredient of bath salts, labeled "Not for Human Consumption" in attempts to avert the Synthetic Drug Abuse Prevention Act of 2012. On the streets α-PVP is referred to as flakka.

Thermo Scientific reports in the Drugs of Abuse, DRI® Phencyclidine Cross Reactivity Guide that the bath salt (MDPV) methylenedioxypyrovalerone causes a false positive PCP screen at 7,000 ng/ml (3). A protocol was developed to determine if the structurally related α-PVP also cross reacts. Blank urine was spiked with α-PVP and analyzed using the DRI PCP Immunoassay on a Biolis 50i. A preliminary test revealed that a concentration of 1,000 ng/ml α-PVP produced a false positive result of 37 ng/ml for PCP. Additional immunoassay screening in triplicate at 300, 400, 500, 600, and 1,000 ng/ml α-PVP resulted in linear average apparent PCP semi-quantitations of 17, 19, 21, 23, and 29 ng/ml, $r^2 > 0.99$, (Fig 1). The nicotine metabolite, cotinine, was also tested by immunoassay at concentrations ranging to 4,000 ng/ml and found to be negative.

In conclusion, α-PVP was demonstrated to be a strong cross reacting compound when analyzed with the Thermo Fisher DRI Immunoassay for Phencyclidine. Multiple alpha-PVP urine samples at 1,000 ng/ml produced measured false positives PCP results. The curve indicates that an α-PVP of 747 ng/ml cross reacts equivalently to 25 ng/ml PCP representing about a 3% apparent relationship. Our findings have been submitted to the manufacturer. While the significantly increasing availability of α-PVP and other illicit sympathomimetic amines warrants growing public health surveillance, point of care clinics and laboratories utilizing only DRI immunoassay screening procedures should be aware of the cross reactivity and submit all presumptive positives for PCP to a reference laboratory for confirmatory testing using a higher level of specificity and sensitivity (4).
Fig 1. False Positive Phencyclidine Induced by the Bath Salt α-PVP

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References


2. Lists of Scheduling Actions, Controlled Substances, Regulated Chemicals (PDF) (February 2015), US Department of Justice, Drug Enforcement Administration, Office of Diversion Control.


Editor’s Corner

Our Division Events at AACC National Meeting (2015) in Atlanta

Our Division events at the AACC National Meeting were well attended and very successful.

The Annual Division meeting and lunch was held on Monday, July 27. After Jim (Chairman), Loralie (Treasurer), and Valerie (Secretary) presented their reports, Leland (Chair of Award Committee) presented the Division Awards. Jill Wolken of Univ of Wisconsin got the first place with the topic: “Inappropriate Use of Suboxone Film to Pass Drug Testing with Cross-Talk to 6-MAM”. Kelly Doyle from Univ of Utah and Katrangi Waddah from Mayo Clinic had two Honorable Mentions. Mark Marzinke from Johns Hopkins (2014 Award Recipient) presented: “Antiretroviral Testing: Development and Validation of LC-MS/MS Assays in Unique Specimen Sources to Support Clinical Trials”. There are 24 antiretroviral drugs available now. Mark developed LC-MS/MS methods for two of these new drugs in clinical trial: FTC (emtricitabine) and TFV (tenofovir):

He presented the assay performances: imprecision (intra- and inter-assay CV were <15%) and LoQ (defined as less than 20% CV).

The division conducted a poster walk at 2 PM on Wednesday with Patrick Kyle. 46 posters were presented in TDM/Toxicology/DAU.

Other topics of interest to our Division were:

- July 28, a morning symposium on ‘how to manage patients on controlled substances via risk stratification’
- July 30, symposium of ‘Survey of Personalized Medicine: The PGx, PK, PD of it all’

In summary, the division was well represented at this year’s AACC National Meeting.

Pradip Datta
Upcoming Conferences

- **G2: Lab Institute** Oct 14-16 Washington D.C
- **2015 Northeast Lab Conference** Oct 20-22 Portland, ME
- **ASCP** Oct 28-30 Long Beach, CA
- **LAB QUALITY CONFAB** Nov 3-4 New Orleans, LA
- **KnowledgeLab 2016** March 20-23 Orlando, FL

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