Cocaine Confirmation Problems Solved

Thomas J. Farrell
Elmhurst Memorial Hospital
Department of Pathology
Elmhurst, IL 60126

Value of CAP Survey Specimen

- Regulatory - CAP Accredited labs must run external Quality Control specimens
- Peer evaluation of method, technologists, and instrumentation.
- Test the accuracy of method compared to the weighed-in amount in specimen.
- Discover and correct inherent, unknown problems with our method and/or analysis

CAP Survey UDC-C 2005 for Benzoylecgonine Directed Challenge Samples

I.D.	CEDIA Screen 300 ng/mL	GC/MS	CAP TARGET +/- 1 SD
UDC-21	Negative	134 ng/mL	87 +/- 8.4
UDC-28	Negative	151 ng/mL	87 +/- 7.5
UDC-29	Negative	224 ng/mL	87 +/- 8.9

TYPICAL GC/MS SAMPLE RUN ORDER

1. 150 ng/ml QC
2. 500 ng/ml QC
3. 2000 ng/ml QC
4. Negative QC
5. Commercial QC
6. Patient #1 undiluted
7. Patient #1 x50
8. Patient #2 undiluted
9. Patient #2 x50
10. Etc

1. 150 ng/ml QC
2. 500 ng/ml QC
3. 2000 ng/ml QC
4. Negative QC
5. Commercial QC
6. CAP Survey (target=87) (SBP)
7. Patient #1 undiluted
8. Patient #1 x50
9. Patient #2 undiluted
10. Patient #2 x50
11. CAP Survey(target=87) (SAP)

Investigation and Observations

- On first 4-5 runs, both SBP and SAP were within 1 S.D. of group mean (87 ng/ml)
- Then a run with SBP=87, but SAP=195 ng/ml
- After instrument maintenance, SAP=224 ng/ml
- At this point, we considered three possibilities:
  - "extended" carryover
  - contaminated (by injector) sample vial
  - technician sloppiness in setting up extractions

Investigation and Observations

- We added a Negative QC to each run before SAP
- A subsequent run had SBP=87, Neg. QC=129, SAP=324
- The last patient from the run was repeated undiluted and x50, followed by Neg QC and SAP. Both Neg QC and SAP were OK.
- Vial contamination and tech sloppiness seem ruled out, but we are unable to reproduce apparent carryover.
- Future runs showing elevated SAP were completely re-extracted and repeated on GC/MS, and we were again unable to reproduce the elevated SAP.
- No logic to these observations, but we notice that SBP is never elevated.

Investigation and Observations

- Our current thoughts:
  - still not sure about technician sloppiness
  - Only certain high samples would cause carryover
  - Many samples > 100,000 DON'T cause carryover
- Discovered that techs were using the same pipette tip to add Internal Standard to all samples.
- This technique was corrected but problem reappeared 5 runs later.
- Entire run was re-extracted and run on GC/MS with SAP run in triplicate. (128, 182, 147)
- Instrument maintenance done, 5 blank samples run, SAP run = 87.

Investigation and Observation

- Our current thoughts:
  - this is real carryover
  - carryover lasts > 1 vial and could be many vials
  - need to quantitate and determine extent of carryover

Extent of Carryover

- After a run of many high patient samples:
  - Neg QC 55 ng/ml
  - Survey 232 (target = 87)
  - 150 Std 226
  - Com QC 172 (target = 162)
  - Neg QC 22
  - Neg QC 476
  - Survey 128
  - Survey 182
- Decided that the best way to avoid this extended and inconsistent carryover was to avoid running UNDILUTED patient samples.

Documentation of No Carryover

- Patient #1=55,000 Patient #2=177,000 and Patient #3=1,000,000 were assayed as follows:

  - 1) Pt.#1 x100
  - 2) Pt.#1 x200
  - 3) Pt.#2 x100
  - 4) Pt.#2 x200
  - 5) Pt.#3 x100
  - 6)Pt.#3 x200
  - 7)Neg QC
  - 8)Survey
  - 9) Pt.#1 x50
  - 10)Pt.#2 x50
  - 11)Pt.#3 x50
  - 12)Neg QC
  - 13)Survey

- Negative QC's (#7 &12) and Surveys (# 8 &13) showed no carryover

CONCLUSIONS

- Although SAMSHA cutoff level for Benzoylecgonine confirmation is 150 ng/ml, actual patient samples range between zero and greater than one million ng/ml
- This presents extraordinary challenges to an analytical system expected to produce an interlab SD of 8.0 @ 87 ng/ml
- Under these extremes, a patient sample of 1 million ng/ml which carries over only 0.016% (160 ng/ml), will cause a following negative sample to be reported positive by GC/MS confirmation

Conclusions

- Demonstrated method has carryover large enough to cause Neg (<150 ng/ml) to be positive
- Cause of carryover - unknown.
  - One theory = random, high specimens contain unknown substance(s) that change column dynamics and binding.
- The best way of avoiding these circumstances is to never inject high patient samples.
- Our current policy:
  - run only x50 patient samples
  - if Benzoylecgonine < 400 ng/ml, an undiluted sample is then
  - run. The GC/MS will never be challenged with a sample >20,000 ng/ml, a level which has caused no carryover