False-Positive Carbamazepine Results by Gas Chromatography–Mass Spectrometry and VITROS 5600 Following a Massive Oxcarbazepine Ingestion

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Guest: Dr. Uttam Garg is Professor of Pediatric Pathology and Director of Clinical Chemistry, Toxicology, and Biochemical Genetics at Children's Mercy Hospital in Kansas City, Missouri.

Randye Kaye: Hello, and welcome to this edition of "JALM Talk" from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

Conventional approaches to drug testing rely on a primary screening step followed by a secondary confirmation. Best practice dictates that the secondary confirmation step utilize a separate methodology than the initial screening step and have improved sensitivity and specificity. This approach is widely utilized in clinical, pre-employment, and forensic settings. Despite the standard adoption of this approach, issues can occur that impact the accuracy of results.

"False Positive Carbamazepine Results by Gas-Chromatography Mass Spectrometry and VITROS 5600 Following a Massive Oxcarbazepine Ingestion" was published in the July 2018 issue of *The Journal of Applied Laboratory Medicine*. The case study highlights unique circumstances that affected both screening and confirmatory methods for urine drug testing.

The first author is Dr. Uttam Garg. Dr. Garg is Professor of Pediatric Pathology and Director of Clinical Chemistry, Toxicology, and Biochemical Genetics at Children's Mercy Hospital in Kansas City, Missouri.

Welcome Dr. Garg. This Case Report of a false positive carbamazepine result is very interesting. Can you briefly describe the Case Report for us?

Dr. Uttam Garg: Randye, first of all thanks for the nice introduction. This Case Report is on a two-year-old patient who presented to our emergency department with symptoms of depression and seizure-like movements, and the patient was being treated for epilepsy. It was known that the patient ingested his anti-seizure medication, however the name of the medication was not known. But, it was suspected that the patient might have ingested carbamazepine. It was not known how much drug the patient had ingested. Also, it

was not known if the patient co-ingested another medication.

So, based on this history, carbamazepine levels and urine comprehensive drug screen was ordered. In our lab, we perform carbamazepine by amino acid on VITROS 5600 Chemistry Analyzer. And we perform comprehensive drug screen by gas chromatography/mass spectrometry. The drug screen includes screening for more than 200 drugs and toxins. Since carbamazepine is analyzed on automated analyzer, the results became available fairly quickly. Carbamazepine result was 8 microgram per ml which is within therapeutic range of 4 to 12 microgram per ml. This level of carbamazepine of 8 microgram per ml did not explain the patient's symptom. And suspicion of co-ingestion of another drug remained.

In the meantime, the results of comprehensive drug screen were reported. Carbamazepine and oxcarbazepine were detected on GC Mass Spec. So, it was thought that the patient ingested both carbamazepine and oxcarbazepine and the patient's symptoms are due to co-ingestion of these drugs. By that time, the history became more clear and it was noted that the patient ingested his medication oxcarbazepine, and the patient had no access to carbamazepine. This raise the question of false positive carbamazepine results.

Randye Kaye: And just can you confirm, did you say the patient was two years old? How old was the patient?

Dr. Uttam Garg: Yeah, a two-year old, 23-month old. Yeah, two-year old.

Randye Kaye: Wow. So, how did you discover and confirm that the carbamazepine results were incorrect?

Dr. Uttam Garg: First of all, we reanalyzed the samples to make sure that the results are reproducible and they were. Then to resolve this issue, we used HPLC with UV detection. The HPLC method we use, it measures both carbamazepine and oxcarbazepine and their metabolites. On HPLC, oxcarbazepine and its metabolite were seen, and they were present in fairly large concentrations. Interestingly, carbamazepine or its metabolite were not detected on HPLC. So, these findings indicated that carbamazepine result by immunoassay and GC Mass Spec was false positive.

Then the next question was, what caused the false positive results? Was it oxcarbazepine or its metabolite? So, to resolve this issue, we prepared samples with different concentrations of oxcarbazepine and its metabolite. These spiked samples were then analyzed by immunoassay and the GC Mass Spec. On immunoassay, increasing

concentrations of oxcarbazepine exhibited increased measured concentration of carbamazepine. On the other hand, carbamazepine was not detected in the samples spiked with oxcarbazepine metabolites. So, these studies showed that the parent drug oxcarbazepine, but not oxcarbazepine metabolites, caused false positive carbamazepine amino acid results.

So the next question was, what caused false positive results on GC/MS? For that, we prepared urine samples spiked with oxcarbazepine and its metabolite, and analyzed these samples by the GC Mass Spec. Interestingly, in contrary to immunoassay, the samples supplemented with oxcarbazepine metabolite showed carbamazepine peak. And the samples supplemented with oxcarbazepine did not show carbamazepine peak. So, it is postulated that at high temperature of the injection port, oxcarbazepine metabolite got converted to carbamazepine. So in summary, false positive result in immunoassay was due to oxcarbazepine, and false positive results in GC Mass Spec was due to oxcarbazepine metabolite.

Randye Kaye: So, it's really interesting that you got false positive results from two different methods. Just tell me, is this common?

Dr. Uttam Garg: Yes, Randye. It is very interesting that two different methods give false positive results. It is very rare to have false positive results by two different methods particularly, when they are based on two different principles. In fact, detection of a drug by two different methods, which are based on different principles, is considered confirmatory for the presence of a drug. You mentioned earlier in drug screening, it is very common to use two methods. Generally, the first method is immunoassay. Immunoassay positive results are confirmed by mass spectrometry, such as GC Mass Spec or LC-Tandem Mass Spec. It is not uncommon to have false positive result by immunoassay, but it is very rare to have false positive result by mass spectrometry.

Randye Kaye: Isn't mass spectrometry considered the "gold standard technique" in drug analysis?

Dr. Uttam Garg: In most part, yes. Mass spectrometry is considered the gold standard. However, it is important to keep in mind that although rare, false positive results do happen and can happen in mass spectrometry. There are several reasons for false positive results in mass spectrometry. One of the common reasons for false positive results is the presence of structurally similar compounds in a patient's sample. The interfering compound produces similar ions to that of drug of interest and it is difficult to distinguish mass spectrum of the interfering compound from the compound of interest.

This problem is commonly seen for drugs which produce only few ions. For example, this problem can be seen with sympathomimetic amines. Also, this problem can be seen in what's called selective ion monitoring, in that only few ions are monitored rather than looking at the whole mass spectrum of a drug. One way to eliminate this interference is to separate the interfering compound using chromatography. Then, there is another issue, although it's not a real interference, but can be a problem. Mass spectrometry cannot distinguish stereoisomers as they produce similar ions. For example, D and L amphetamines cannot be distinguished by a routine mass spectrometric analysis. Special techniques are needed to distinguished stereoisomers.

Then there is another very unique problem, which can cause false positive results. This problem is mostly seen in GC Mass Spec. At high temperature in GC Mass Spec injection port, a certain compound can get converted to another compound. This phenomenon was seen in 1990 by false positive methamphetamine results. It was discovered that a certain derivatives of ephedrine are converted to methamphetamine derivative at a high temperature of injection port. So, that is what happened in our case. Oxcarbazepine metabolite got converted to carbamazepine in the injection port. This type of false positive results may be eliminated by lowering the injection port temperature or using some other techniques.

Randye Kaye: Okay, thank you. So that explains a lot. Are there any other takeaways from this study that you'd like to mention?

Dr. Uttam Garg: Yeah, there are several takeaways. First, although rare, false positive results can be seen in mass spectrometry and it is generally accepted that a particular drug is present if the drug has been detected by two different methods based on different principles. Despite the use of two independent methods, false positive results can still happen.

The other takeaway is, when managing a patient, clinical correlation with the lab data is very important, and the communication between the healthcare team and the laboratorians is extremely valuable. For example, in our case, the medical toxicologist who was managing this patient was very savvy and knowledgeable. He realized that the measured level of carbamazepine did not correlate with the patient's clinical symptoms. He asked questions and work with the lab. This led to further investigation and uncovering of false positive results.

Randye Kaye: Okay, thank you very much. That was a very enlightening. Thank you so much for joining us today Dr. Garg.

Dr. Uttam Garg: Thank you Randye.

Randye Kaye: That was Dr. Uttam Garg, Professor of Pediatric Pathology at Children's Mercy Hospital talking about “False-Positive Carbamazepine Results by Gas Chromatography–Mass Spectrometry and VITROS 5600 Following a Massive Oxcarbazepine Ingestion” from July 2018 issue of JALM. Thanks for tuning in for “JALM Talk.” See you next time and don't forget to submit something for us to talk about.