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INTERPRETATION OF BUPRENORPHINE AND NALOXONE RESULTS: CONSIDERATIONS FOR DOSAGE FORM AND SPECIMEN TYPE

Buprenorphine prescribing is extremely common across multiple clinical specialties, primarily opioid use disorder (OUD) and pain management treatment providers. Legislative changes have allowed for more prescribers to offer buprenorphine treatment for OUD, including nurse practitioners and physician assistants. This comes at a time when America is suffering from the opioid epidemic, and overdose deaths are surging. Testing for buprenorphine is ordered by a large percentage of Aegis clients, and with so many dosage forms and potential sources of the drug available, proper result interpretation is essential for optimal clinical care.

Period of Detection & Specimen Type Considerations

The possible period of detection of buprenorphine varies according to the matrix – or specimen type – used for testing. In general, buprenorphine may be detectable up to 7 days in urine, up to 48 hours in saliva, and up to 24 hours in blood.[1] The dosage form of buprenorphine can affect the detection, however. Transdermal buprenorphine (Butrans®) delivers smaller doses of buprenorphine (5 to 20 mcg/hr) compared to the sublingual formulations (2 to 8 mg).

Markman, et al. assessed buprenorphine urine concentrations following use of transdermal and sublingual formulations in reportedly compliant patients.[2] Median concentrations of analytes were higher post-sublingual use (see table below). Clinicians assessed adherence to transdermal therapy by checking to see if patients were wearing their patches, but this does not offer definitive proof of patient compliance. Inconsistent or inappropriate use of the transdermal patch could also lead to lower concentrations in urine.

	Transdermal Median Concentration (IQR*)	**Sublingual Median Concentration (IQR*)**
Buprenorphine	n/a	5 (4-9)
Norbuprenorphine	6.5 (3.8-23)	88 (47-196)
Buprenorphine-glucuronide	18 (8-32)	284 (119-501)
Norbuprenorphine-glucuronide	14 (9-29)	492 (155-1011)

**IQR = Interquartile Range*

Based on the reported median concentrations it is reasonable to conclude some patients may not excrete detectable concentrations of buprenorphine and/or norbuprenorphine in urine, even with compliant use of transdermal buprenorphine. This is also supported by an internal analysis of 42,000 urine specimens submitted to Aegis for buprenorphine compliance testing. For samples with reported sublingual prescriptions, 95.9% demonstrated evidence of compliance to the prescribed medication. In contrast, only 73.4% of samples with reported transdermal buprenorphine demonstrated evidence of compliance. Urine is still considered the preferred specimen to collect for transdermal buprenorphine, but negatives may occur for some patients, even when patients are adherent. Oral fluid and blood are not viable options for assessing compliance with transdermal buprenorphine. [3-4]

Injectable buprenorphine options such as Sublocade® are also available, and may present challenges to toxicology result interpretation. Sublocade® is prepared in 100 or 300 mg doses and administered as a monthly subcutaneous injection.[5] The manufacturer labeled half-life ranges between 43-60 days because of the slow release of buprenorphine from the subcutaneous depot. In patients with moderate to severe hepatic impairment the half-life was found to be longer but not in patients with mild hepatic impairment. Based on the pharmacokinetics of this

dosage form, patients may continue to experience positive buprenorphine toxicology results for an unknown period of time following drug discontinuation, although a downward trend in detected buprenorphine concentrations is likely, while also accounting for changes in urine concentration as a contributing factor in the evaluation of urine toxicology results. In patients with unexpected positive buprenorphine results, providers may consider a history of Sublocade® injections in the evaluation of the results. Conversely, it is possible for patients being treated with Sublocade® to have negative toxicology results for buprenorphine. There is no specific study that investigates the use of Sublocade® and the length of detection in toxicology testing.

For individuals who are prescribed sublingual or buccal formulations and will submit oral fluid specimens, proper adherence to collection instructions and consideration of timing of last dose are both important factors. An oral depot effect is possible from recent drug administration, and high buprenorphine concentrations are possible for approximately one hour after a dose is administered. If an individual has taken a dose of buprenorphine within the last hour before sample collection, a urine sample may provide a better clinical picture of compliance, since oral fluid concentrations may be markedly high during this time frame.

Using Buprenorphine Concentrations and Buprenorphine/Norbuprenorphine Ratios

Utilizing the quantitative concentrations of buprenorphine and norbuprenorphine to assess patient behavior has both benefits and limitations. Buprenorphine/norbuprenorphine ratios are unrelated to dose.[6-7] Excretion patterns of parent drug and metabolite ratios may vary significantly depending on a patient's metabolism pattern or urine collection time relative to drug ingestion. Low concentrations of norbuprenorphine may be suggestive of drug abstinence or acute ingestion. In general, the buprenorphine/norbuprenorphine ratio will decrease over time following ingestion as buprenorphine is metabolized and norbuprenorphine concentrations increase.

Some investigators have reported that norbuprenorphine/buprenorphine ratios may be <1 for the first 7 hours post-dose.[8] However, norbuprenorphine-only results may also occur and can be consistent with chronic dosing.[9-10] CYP3A4 may be induced or inhibited by different drug-drug interactions, which may also impact parent/metabolite ratios.[11] Studies have indicated a range of buprenorphine to norbuprenorphine ratios between 0.04-15,with mean values between 0.25-0.5.[7,12-16] In such cases, norbuprenorphine concentrations exceeding buprenorphine would be typical. Of note, before mass spectrometry analysis at Aegis, samples are hydrolyzed to remove the glucuronide moieties, thereby adding to the detectable amounts of buprenorphine and norbuprenorphine.[7,17] As hydrolysis can vary slightly, this can affect the buprenorphine and norbuprenorphine concentrations that are reported.

The buprenorphine/norbuprenorphine ratio is extremely helpful in identifying situations of possible urine adulteration. High concentrations of buprenorphine with low concentrations of norbuprenorphine may indicate post-collection addition of the drug directly to the specimen. The reason for norbuprenorphine detection in cases of probable adulteration is unclear, but degradation of buprenorphine or its presence as a pharmaceutical impurity in buprenorphine have been proposed.

An analysis of Aegis data for approximately 2,500 buprenorphine-positive specimens indicated a range of buprenorphine/norbuprenorphine ratios from 0.015 to >5000. Most specimens exhibited low ratios, with 94.7% of all specimens falling below 5. A ratio of 35 was determined to be a statistical outlier, affecting approximately 5% of specimens. Urine specimens submitted to Aegis for analysis are evaluated for the relative ratio of buprenorphine to norbuprenorphine. If the ratio equals or exceeds 35, a comment will be placed on the report as a flag for potential adulteration to the practitioner. In these cases, further assessment of the patient is warranted and an observed specimen collection should be considered for future tests.

It is important to note that absence of norbuprenorphine in toxicology results is not always indicative of aberrant behavior. In situations of recent drug administration, metabolite may not be present at a detectable level. The same is possible with terminal elimination of the drug. Concentration of the urine may also impact metabolite detection, and dilute urine specimens may not have detectable amounts of norbuprenorphine. Drug concentrations following

transdermal administration may be extremely low as well, which can contribute to negative metabolite results. In oral fluid, parent drug concentrations typically exceed metabolite concentrations, so absence of metabolite in oral fluid is not necessarily unexpected. In general, a low parent buprenorphine concentration near the reporting threshold in any matrix with no metabolite detected is not indicative of aberrant behavior. Providers are welcome to contact the Aegis clinical team to discuss individual cases as drug interactions, time since last dose, dosage form, and specimen type are all important factors in evaluating this type of result.

Naloxone Testing and Interpretation

Naloxone testing is available in both urine and oral fluid to assess for compliance with a prescription. Naloxone detection is different from buprenorphine detection based on the unique pharmacokinetics of naloxone. The period of detection of naloxone in urine is up to 3 days though may not be detectable depending on time of last dose or formulation used.[1] In oral fluid, naloxone levels resulting from compliant use of a prescribed buprenorphine/naloxone product may not reach detectable levels, so a negative result does not necessarily indicate non-compliance. Before making clinical decisions regarding presence or absence of naloxone in a sample, it is recommended to ensure that naloxone testing was included. This testing is an add-on option and is not part of a testing profile. If it is not marked on the requisition, the testing will not be performed. If testing has been completed and results are unexpected, the Aegis Clinical Team is available to discuss the results. Naloxone may also be present in urine or oral fluid following administration of Narcan® or other emergency naloxone formulation. It is also thought to be an end metabolite of naloxegol (Movantik®) which is an oral medication indicated for opioid-induced constipation.[18]

Various buprenorphine/norbuprenorphine and buprenorphine/naloxone ratios have been postulated to characterize misuse of a buprenorphine combination product, such as altering sublingual films to create an injectable formulation. Drug concentrations in urine and oral fluid cannot be use to definitively state the route of administration of a drug. Factors such as amount of drug administered, time since last use, CYP3A4 enzyme activity, drug interactions, and other patient-specific factors can affect the amount of drug present in the specimen and alter the ratios. Even with compliant use of buprenorphine/naloxone combination products, the oral bioavailability of each ingredient is different as well as the renal excretion patterns. These differences also contribute to a wide possibility of buprenorphine/naloxone ratios among individuals. In an analysis of over 9000 urine specimens tested by Aegis, the median buprenorphine/naloxone ratio was 0.87, with an interquartile range of 0.56 to 1.47. This data was from samples from individuals who were prescribed buprenorphine, and samples with results suggesting adulteration were excluded. Factors such as renal function and total daily dose are unknown, which could also affect this analysis.

Unexpected Result Interpretation

In summary, providers have many considerations when evaluating buprenorphine and naloxone results. This is a simplified checklist, and additional considerations for individualized care may be necessary. Generally, a result evaluation should consider the following steps:

Unexpected Positive Results	Unexpected Negative Results

• Does the individual have a relevant prescription/medical history?
  ○ For unexpected positive buprenorphine results:
  ▪ Current or previous buprenorphine prescription (consider all dosage forms)
  ○ For unexpected positive naloxone results:
  ▪ Narcan® available for rescue treatment from a family member, law enforcement, or emergency personnel
  ▪ Naloxegol for opioid induced constipation
• Is the individual at risk for purchasing buprenorphine on the street?
	• When was the individual's last reported dose?
	• What specimen type was used for testing, and how does that impact the period of detection?
	• What dosage form is prescribed, and how does that impact the period of detection?
	• For unexpected negative naloxone results, is the individual at risk for using a non-prescribed buprenorphine monoproduct, or is time since last dose a more likely factor?

Discussion of unexpected toxicology results with the Aegis Clinical Team is recommended.	

NOTICE: The information above is intended as a resource for health care providers. Providers should use their independent medical judgment based on the clinical needs of the patient when making determinations of who to test, what medications to test, testing frequency, and the type of testing to conduct.

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