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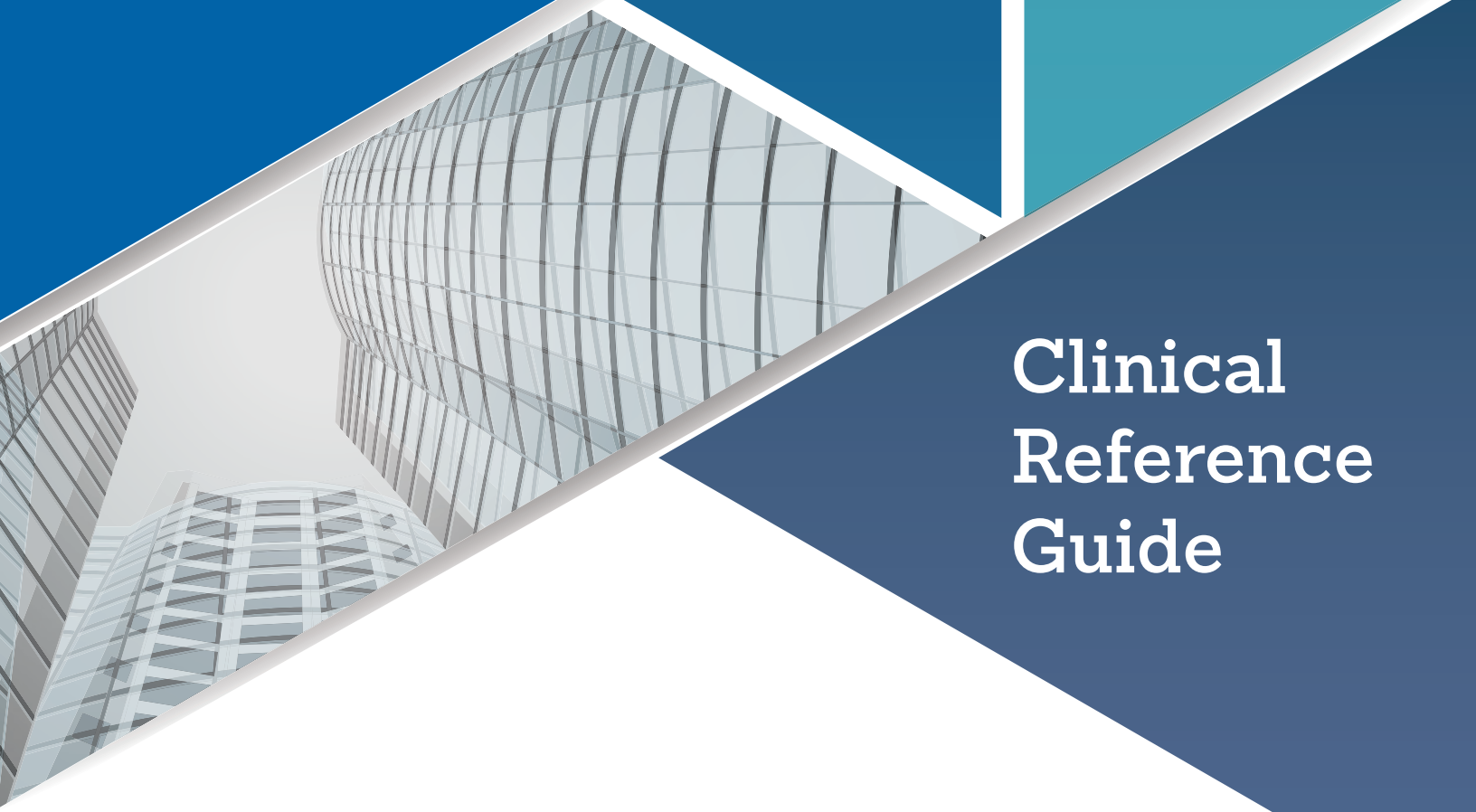

Clinical Reference Guide

Drug Stability and Toxicology Testing

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Drug Stability and Toxicology Testing

Drug stability is an important consideration when interpreting toxicology results, as conditions leading to poor stability/drug degradation may contribute to false negative results.

Drug characteristics, environmental conditions, storage time, the collection device, and specimen factors may all affect stability (refer to Table 7.1).[1-7] Although urine is considered sterile, bacterial contamination of urine specimens easily occurs due to urinary tract infections, genital bacterial contamination, and/ or contamination during the collection process and handling of the specimen.[4] Oral fluid is naturally colonized with bacteria; over 700 bacterial species have been detected in the oral cavity.[8] Bacteria may contribute to the instability of a drug through breakdown by bacterial enzymes or by increasing the pH of the specimen over time, thus contributing to transformation of the drug.[4,9]

Light exposures and increases in temperature can also speed up the degradation process.[2,3] Oxidation of certain drugs may occur when specimens undergo prolonged exposure to air; therefore, specimen containers should be tightly sealed as quickly as possible following sample collection to minimize drug decomposition.[2,3] In addition, some lipophilic drugs (e.g. marijuana) may be prone to concentration loss due to adsorbing to the storage container;[1,2,6,7] this resulted in losses up to 15% in one study.[7] Some collection devices, especially oral fluid devices, may contain a preservative or buffer to minimize stability issues. Aegis currently uses the Quantisal™ device for oral fluid collection which comes with a buffer solution for storage during transit to the laboratory. Overall, the degradation of drugs due to stability concerns may contribute to lower levels of the parent drug or increases/decreases of metabolites in biological specimens. Depending on the amount of drug present and the degree of degradation, these changes may lead to drug concentrations below testing thresholds and false negative results.

Most drugs involved in toxicology testing are relatively stable under refrigerated or frozen conditions. However, the drugs discussed below are more prone to stability issues. These drugs and their metabolites are subject to a variable amount of degradation during storage.

Table 7.1: *Poor Drug Stability*

FACTORS CONTRIBUTING TO POOR DRUG STABILITY
Adsorption to collection or storage device[1,2,6,7]
Air amount/quality in container[2,3]
Bacterial/fungal contamination[4,5]
High pH[4,5]
Increased temperature[2,3]
Lack of preservatives/buffer[2-4]
Natural and UV light[2]
Prolonged storage[3]

A. Clonazepam

- Losses of 7-amino-clonazepam in urine, the main clonazepam metabolite, averaging 43% have been reported under refrigerated conditions at 2 months.[10]
- A 76% drop in clonazepam concentration has occurred in an oral fluid specimen stored at room temperature overnight.[11]
- Complete loss of clonazepam in oral fluid has occurred in 7 days at room temperature.[12]
- In oral fluid, losses of 7-aminoclonazepam up to 20% and 33% have been observed at room and refrigerated temperatures, respectively, over a week.[13]

B. Cocaine

- Cocaine can spontaneously hydrolyze in solution to benzoylecgonine (common metabolite tested in urine) and methylecgonine.[4]
- Urinary cocaine losses as much as 80% have been observed in bacteria-contaminated urine at 14 days with complete loss by 30 days at room temperature.[4]
- In oral fluid, losses of cocaine up to 66% and 18% at room and refrigerated temperatures, respectively, have been observed at one week; however, doubling of the cocaine metabolite (benzoylecgonine) concentration has been observed.[13,14]

C. Heroin

- Heroin spontaneously hydrolyzes in aqueous solutions.[15]
- A 50% loss of heroin was demonstrated within six hours in an alkaline aqueous solution (pH 9) when stored at room temperature.[15]
- Losses in urine of 6-acetylmorphine (6-AM, heroin metabolite) have been reported up to 65% at one day, 94% at seven days, and 100% at fourteen days under room temperature.[16]
- In oral fluid, losses of heroin as much as 97% at room temperature over one day and 85% at refrigerated temperatures over three days have been reported.[17]
- Losses at one week of 6-AM in oral fluid up to 53% and 46% at room temperature and refrigerated conditions, respectively have been reported.[13]
- Once 6-AM hydrolyzes to morphine, it can be difficult to distinguish if heroin, codeine, or morphine was ingested.

D. Marijuana

- THC, the active constituent of marijuana, is highly lipophilic and may adhere to storage containers, especially plastic.[7]
- In deteriorated urine specimens at room temperature, total losses of carboxy-THC metabolite averaged 40% at 10 days.[18]
- In urine, losses up to 34% of carboxy-THC have been reported even under frozen conditions at 45 days.[7]
- In oral fluid, losses of THC at one week up to 24% and 26% under room and refrigerated conditions, respectively have been reported.[13]
- Specimens for THC testing should be protected from light.[2]

In order to minimize the likelihood of false negative results due to instability, it is recommended to protect specimens from light, keep specimens refrigerated between 2 and 8 °C before shipping, and ship to the laboratory for testing within 24 hours of collection. Samples that cannot be shipped within 48 hours of collection should be immediately frozen at < -10 °C. Utilization of frost-free (automatic defrost) freezers with frequent freeze-thaw cycles may contribute to sample degradation. Frozen samples should be shipped to the laboratory with a cold pack if possible. If specimens are exposed to extreme conditions such as high temperatures, high bacterial contamination (e.g., urinary tract infection), and/or prolonged storage before testing, the potential for false negatives must be considered. Of note, the alcohol metabolite ethyl glucuronide (EtG) is subject to both degradation and formation post-collection; EtG may be formed post-collection in the presence of yeast and bacteria in diabetic patients due to the fermentation of glucose into ethanol and subsequent formation of EtG.[9]

It is important to consider stability concerns when requesting a retest for drugs that are notably unstable. Additionally, drugs at very low concentrations have the potential to not reconfirm, as just minor stability decreases may drive a low concentration below threshold. Retest requests should be communicated as quickly as possible following reporting to minimize stability concerns. Due to the instability of heroin and 6-AM, Aegis does not retest heroin past 7 days of the date it was originally analyzed.

REFERENCES:

1. Ventura M, Pichini S, Ventura R, et al. Stability of drugs of abuse in oral fluid collection devices with purpose of external quality assessment schemes. *Ther Drug Monit*. 2009;31(2):277-80.
2. Anizan S, Bergamaschi MM, Barnes AJ, et al. Impact of oral fluid collection device on cannabinoid stability following smoked cannabis. *Drug Test Anal*. 2014;7(2):114-20.
3. Mandić-Radić S, Džingalašević G, Luković N. Stability of ethanol in blood and urine samples. *J Med Biochem*. 2007;26(3):241-4.
4. Zaitsu K, Miki A, Katagi M, Tsuchihashi H. Long-term stability of various drugs and metabolites in urine, and preventative measures against their decomposition with special attention to filtration sterilization. *Forensic Sci Int*. 2008;174(2-3):189-96.
5. Skopp G, Pötsch L. An investigation of the stability of free and glucuronidated 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid in authentic urine samples. *J Anal Toxicol*. 2004;28:35-40.
6. Desrosiers NA, Lee D, Scheidweiler KB, Concheiro-Guisan M, Gorelick DA, Huestis MA. In vitro stability of free and glucuronidated cannabinoids in urine following controlled smoked cannabis. *Anal Bioanal Chem*. 2014;406(3):785-92.
7. Paul BD, McKinley RM, Walsh JK Jr, Jamir TS, Past MR. Effect of freezing on the concentration of drugs of abuse in urine. *J Anal Toxicol*. 1993;17:378-80.
8. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol*. 2005;43(11):5721-32.
9. Helander A, Olsson I, Dahl H. Postcollection synthesis of ethyl glucuronide by bacteria in urine may cause false identification of alcohol consumption. *Clin Chem*. 2007;53(10):1855-7.
10. Gonzales E, Ng G, Pesce A, et al. Stability of pain-related medications, metabolites, and illicit substances in urine. *Clin Chim Acta*. 2013;416:80-5.
11. Hart BJ, Wilting J, de Gier JJ. The stability of benzodiazepines in saliva. *Methods Find Exp Clin Pharmacol*. 1988;10(1):21-6.
12. Kempf J, Wuske T, Schubert R, Weinmann W. Pre-analytical stability of selected benzodiazepines on a polymeric oral fluid sampling device.

Forensic Sci Int. 2009;186:81-5.
13. Lund HM, Øiestad EL, Gjerde H, Christophersen AS. Drugs of abuse in oral fluid collected by two different sample kits--stability testing and validation using ultra performance tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2011;3367-77.
14. Ventura M, Pichini S, Ventura R, Zuccaro P, Pacifici R, de la Torre R. Stability studies of principal illicit drugs in oral fluid: preparation of reference materials for external quality assessment schemes. *Ther Drug Monit*. 2007;29(5):662-5.
15. Barrett DA, Dyssegaard ALP, Shaw PN. The effect of temperature and pH on the deacetylation of diamorphine in aqueous solution and in human plasma. *J Pharm Pharmacol*. 1992;44:606-8.
16. Rop PP, Grimaldi F, Burle J, De Saint Leger MN, Viala A. Determination of 6-monoacetylmorphine and morphine in plasma, whole blood and urine using high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Appl*. 1994;661(2):245-53.
17. Concheiro M, Gray TR, Shakleya DM, Huestis MA. High-throughout simultaneous analysis of buprenorphine, methadone, cocaine, opiates, nicotine, and metabolites in oral fluid by liquid chromatography tandem mass spectrometry. *Anal Bioanal Chem*. 2010;398(2):915-24.
18. Skopp G, Pötsch L, Mattern R, Aderjan R. Short-term stability of lysergic acid diethylamide (LSD), N-demethyl-LSD, and 2-oxo-3-hydroxy-LSD in urine, assessed by liquid chromatography-tandem mass spectrometry. *Clin Chem*. 2002;48(9):1615-8.