editorial

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Defining Limit of Detection and Limit of Quantitation as Applied to Drug of Abuse Testing: Striving for a Consensus

The minimum concentrations of an analyte that can be reliably detected or measured in an analytical procedure are important performance characteristics of the assay. Over the years, several methods have been described for assessing assay detection limits or quantification limits (*1–4*). These concentration limits have been described by various names. The term sensitivity, though frequently used, is unfortunate because it was earlier defined by IUPAC as the slope of the calibration curve. The terms limit of detection (LOD) and limit of quantitation (LOQ), used by many analytical chemists, have become fairly commonplace in laboratories that test for abused drugs. The LOD is the minimum concentration of an analyte that can be distinguished from the assay background (i.e., the "response" given by a blank sample known to be free of the analyte) at a specified level of confidence. The LOQ is the minimum concentration that can be quantified at a specified level of precision or accuracy (or both). Thus, LOD sets the lower concentration limit for assessing, qualitatively, the presence or absence of analyte; LOQ sets the lower limit for quantifying the analyte as well. Under normal circumstances, the LOQ equals or exceeds the LOD. The importance of accurately defining the LOD for a given assay depends on how the results will be used. In forensic analyses, which include testing for abused drugs, it is important that the laboratory define the LOD for each analyte in question. Because the test results may be reported as either positive or negative, depending on whether the assayed value exceeds or falls below the LOD, the ultimate assessment of guilt or innocence may depend on the assigned value of the LOD, which, in turn, depends on the method used to establish the LOD.

The LOD and LOQ should not be confused with the cutoff concentrations (e.g., those recommended by the Department of Defense) used by many laboratories to delineate positive from negative results in actual practice. These so-called administrative cutoff concentrations are generally well above the analytical detection limits that can be achieved by contemporary gas chromatography–mass spectrometry (GC-MS) confirmation methods. These cutoffs have been established to maximize specificity at the expense of some loss of sensitivity, to reduce the possibility of false-positive results to an absolute minimum. The LOD can become relevant when a specimen that tested positive for a drug in one laboratory is submitted to a second laboratory for reconfirmation. For laboratories enrolled in the National Laboratory Certification Program (NLCP) under the direction of the US Department of Health and Human Services, a recognized entity in setting standards for drug of abuse testing, a specimen submitted for a retest is considered reconfirmed as positive if the second laboratory detects the drug at a concentration that equals or exceeds the LOD as documented by the laboratory. Lowering the critical decision level to the LOD allows for any possible drug degradation that may occur over time between the first and second confirmation tests, thereby protecting the first laboratory from reporting a positive result that could not be confirmed by the second laboratory. Although this requirement strictly applies only to those laboratories regulated by the NLCP, it is important that any laboratory performing forensic drug testing in urine establish detection limits for all of its procedures, regardless of the cutoff concentrations used during routine testing. The laboratory should determine—and document—LODs in the event that unusual situations arise in which the laboratory is required to test at concentrations at its absolute detection limit. The importance of developing some guidelines for establishing LODs is evident when we realize that significant differences may be encountered, depending on which method is applied.

Despite what in principle seems relatively straightforward, considerable confusion surrounds the concepts of LOD and LOQ as they relate to confirmation assays for abused drugs. In part, this confusion is related to the methodology specific to GC-MS analysis. A consensus as to how the LOD and LOQ should be operationally defined and established would contribute significantly to standardization in the field of drug testing. In this issue of *Clinical Chemistry,* Armbruster et al. (*5*) describes two methods for establishing LOD and LOQ as applied to the GC-MS confirmation assays for abused drugs performed in their laboratory, a statistical method and an empirical method. The statistical method requires repetitive analysis of a certified negative urine (blank) and subsequent calculation of the mean and standard deviation of the blank "response" (*1–3*). For GC-MS assays in which data are acquired in the selected-ion monitoring mode, the "response" represents the ion current, which registers in the specific ion channel of interest. The LOD is then defined as the mean response obtained for the blank plus two or three standard deviations, depending on whether the desired confidence level for distinguishing a positive sample from the blank is 95% or 99%, respectively.

As Armbruster et al. noted, the statistical method has an inherent limitation when applied to GC-MS confirmation assays. Most current methods are designed to monitor simultaneously at least three ion fragments that are characteristic of the drug or metabolite in question. Typ-

ically, one ion is used for quantitative purposes (quantitating ion) and the other ions are monitored to assure specificity (confirming or qualifying ions). For a specimen to be reported as positive, all ion fragments must be observed, and their relative abundance ratios must be in accord (within specified limits) with the corresponding ratios given by a calibrator included in the same batch. The overall procedure essentially consists of a series of individual, independent assays for each specified ion. It is a simple matter to apply the statistical method by analyzing, repetitively, a blank sample; however, data analysis is problematic, given the extremely improbability that the low-level, random background signals acquired in each ion channel will yield ion *ratios* characteristic of the drug. An LOD *can* be assigned based on the mean plus two or three standard deviations obtained from the signals acquired in the quantitating ion channel, but the signals acquired in the confirming ion channels are meaningless. As Armbruster et al. emphasized, under such conditions the LOD will be assigned from analytical data that fail to meet a critical criterion (i.e., demonstration of characteristic ion ratios) for demonstrating the presence of the drug. Because the gold standard status attributed to GC-MS in confirming the presence of abused drugs depends on the ion ratioing procedures, it is specious to assign LODs by using data that fail the test. This point has been stressed previously by Needleman and Romberg (*4*). For analytical procedures with a one-dimensional detection system (e.g., a spectrophotometric reading, single chromatographic peak, radioactivity determination), however, the statistical method is completely valid.

The empirical method for determining LOD and LOQ is performed by analyzing a series of samples prepared to contain decreasing but known concentrations of drug or metabolite (*4*). The LOD is defined as the lowest concentration at which the ion ratios meet specified acceptance criteria. The LOQ is defined as the lowest concentration at which the ion ratios meet acceptance criteria *and* the assayed and target concentrations agree within a specified tolerance. The advantage of the empirical method, as contrasted with the statistical method, is that the criteria used in establishing LOD and LOQ are identical to the criteria that must be met to report a confirmed positive result on an actual specimen. Armbruster et al. (*5*) strongly advocate the empirical method over the statistical method. Consistent with tolerances required by the NLCP, they recommend that, for LOD determination, ion ratios agree within ±20% relative to those obtained for a calibrating standard assayed in the same batch; and that in addition, for LOQ determination, the assayed and target concentrations agree within ±20% as well. Although the GC-MS confirmatory assays the authors used are designed to monitor three ions and two ion ratios (a common practice in many drug-testing laboratories), the empirical method is not restricted to this format. It can also be applied in cases in which multiple ions are monitored and the data are utilized in a matching algorithm to calculate an identity index. The important point is that the same criteria used to establish positivity in an actual sample are also satisfied when the LOD is determined empirically.

Even if this or a similar procedure were to be accepted as a standard method for establishing the LOD and LOQ in GC-MS confirmation assays, a certain degree of uncertainty would remain. Unfortunately, LOD and LOQ are not static parameters. It is noteworthy that Armbruster et al. (*5*) extended their study over 5 weeks to account for potential variability over time. The detection sensitivity of a given GC-MS instrument depends on many variables, including lens and electron multiplier voltages, column condition, injection port and ion source cleanliness, small vacuum fluctuations, etc. For low-volume laboratories, the LOD and LOQ may vary during the week, depending on the maintenance schedule; for high-throughput reference laboratories, the LOD and LOQ may conceivably vary over the course of 24 h. Such variability, which is inherent in the methodology, means that the LOD and LOQ attainable at any given time may differ somewhat from values established during the preceding LOD/LOQ validation run. Moreover, method-dependent variables such as the volume of sample extracted or calibrator concentration can also affect LOD and LOQ (*4*).

Despite these caveats, establishment of some degree of standardization for determining LOD and LOQ in drug-testing laboratories is a worthy goal. Testing for abused drugs, as properly performed, has achieved a high level of credibility, due in large part to standardization of technology. It is a source of frustration when laboratory professionals who test for abused drugs cannot agree on an accepted method for determining LOD and LOQ. This is not a trivial concern because, as shown here (*5*), differences between the established and empirical methods can exceed an order of magnitude. It is unlikely that the approach recommended by Armbruster et al. will be the last word on LOD and LOQ as applied to confirming abused drugs by GC-MS. Nonetheless, they have proposed a reasonable, logical, and self-consistent scheme for establishing LODs and LOQs. Their study should contribute to and stimulate further dialog on this subject, and perhaps a consensus will emerge in the near future.

References

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