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Partly Nonparametric Approach for Determining the Limit of Detection

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Background: **According to recent International Organization for Standardization (ISO) standards, the limit of detection (LoD) of an assay should be estimated taking both type I (α) and II (β) errors into account. The suggested procedure, however, supposes gaussian distributions of both blank and sample measurements and a linear calibration curve. In clinical chemistry, asymmetric, nongaussian blank distributions are common, and the calibration curve may be nonlinear. We present a partly nonparametric procedure that takes these aspects into account.**

Methods: **Using theoretical distribution models and simulation studies, we developed a LoD estimation procedure suitable for the field of clinical chemistry that is partly based on nonparametric statistics.**

Results: **For sample size n, the nonparametrically determined 95th percentile of the blank measurements {obtained as the value of the [n(95/100) + 0.5]th ordered observation} defines the limit for results significantly exceeding zero [limit of blank (LoB)]. The LoD is the lowest value that is likely to yield a result exceeding the LoB. LoD is estimated as: LoB + $c_\beta \times SD_S$, where SD_S is the analytical SD of a sample with a low concentration; $c_\beta = z_{1-\beta}/[1 - 1/(4 \times f)]$; $z_{1-\beta}$ is the standard normal deviate; and f is the number of degrees of freedom for estimation of SD_S. c_β is approximately equal to 1.65 for a type II error of 5%. Approaches and needed tabular values for calculation of confidence limits are presented as well as sample size. Worked examples are given to illustrate estimation and verification of the limit of detection. Simulation results are used to document performance.**

Conclusion: **The proposed procedure appears useful for application in the field of clinical chemistry and promotes a standardized approach for estimating LoDs of clinical chemistry assays.**

The limit of detection (LoD)[3] of an assay is a performance characteristic that is usually reported together with precision and bias. For some analytes, e.g., serum sodium, the range of clinical interest does not include the area close to zero, and the LoD is not a subject of clinical relevance. In other contexts, e.g., drugs-of-abuse testing, the LoD is an important characteristic. For quantitative assays, both a LoD and a lower limit of quantification (LoQ) may be considered. The latter specifies the lower limit at which the assay is able to provide quantitative results of a stated analytical quality. In this report, we focus on the LoD. Frequently, this limit is given as the lowest value that significantly exceeds the measurements of a blank sample. Thus, conventionally the limit may be estimated on the basis of repeated measurements of a blank sample and reported as the mean plus 2 or 3 SD of the blank measurements. When this approach is used, the question of whether a given measurement exceeds the blank value is addressed statistically. However, consideration of whether a given measurement exceeds a statistically derived limit for the blank measurements is only one part of the LoD question. An additional aspect concerns the lowest amount of analyte that is likely to yield a result that exceeds the limit of blank (LoB) measurements and therefore is declared larger than zero. Actually, repeated measurements of a sample with a true value exactly equal to the limit of statistical significance yield a distribution with 50% of values below and 50% above the limit because of random measurement error. Thus, the mean

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[3] Nonstandard abbreviations: LoD, limit of detection; LoQ, limit of quantification; LoB, limit of blank; ISO, International Organization for Standardization; and CI, confidence interval.

plus 2 or 3 SD only specifies a limit that should be exceeded for a result to be declared significantly higher than a blank measurement; it does not, however, address the lowest value that can be distinguished from a blank value with reasonable assurance. Only if the true value of the sample is higher than the significance limit can one be sure that a measured value will exceed the limit with a probability higher than 50%.

In a statistical sense, not only should the type I error (the significance test) be taken into account, but also the so-called type II error, i.e., the error of not detecting the presence of analyte. Recently, the International Organization for Standardization (ISO) published a set of guidelines concerning determination of the LoD that encompasses both type I and II errors related to detection *(1–4)*. This set of guidelines assumes (in line with most publications on determining the LoD) that distributions of values are gaussian, and accordingly, parametric statistical approaches are applied *(5, 6)*. Actually, in the field of clinical chemistry, the distribution of blank measurements is often truncated at zero and thus is asymmetric and nongaussian. This is so because the raw analytical signal is seldom provided. Instead, the user is presented with direct read-out or black-box systems providing concentration results that are nonnegative. Furthermore, the calibration function is often hidden in the apparatus; therefore, approaches based on specific forms of calibration functions (linear, four-parameter logistic, or other types of curves) often can not be applied *(2, 5, 7)*. With this in mind, we present here a nonparametric approach for estimation of the LoD that is suitable for the field of clinical chemistry. The procedure has the advantage of being generally applicable without relying on special distributional assumptions. The form of the calibration function is not relevant for the suggested procedure. The procedure is outlined, and we present tables with critical values, worked examples for estimation and verification of the LoD, and a performance study based on simulations.

Overview of the LoD Concept

Type I and II errors in relation to the LoD concept

Schematic distributions of blank measurements and measurements of a sample with a low concentration of analyte for a given assay are presented in Fig. 1. In Fig. 1A, the mean of the blank measurements is zero with a distribution of negative and positive values to each side. Many instruments automatically convert negative values to zero or a small positive value so that only nonnegative concentration values are provided as output, which corresponds to the situation in Fig. 1B. In both cases, the 95th percentile of the distribution of blank values indicates a limit that is exceeded only with a probability of 5% for a blank sample. If we define the null hypothesis as the situation without analyte present, the 95th percentile of the distribution of blank values corresponds to the limit

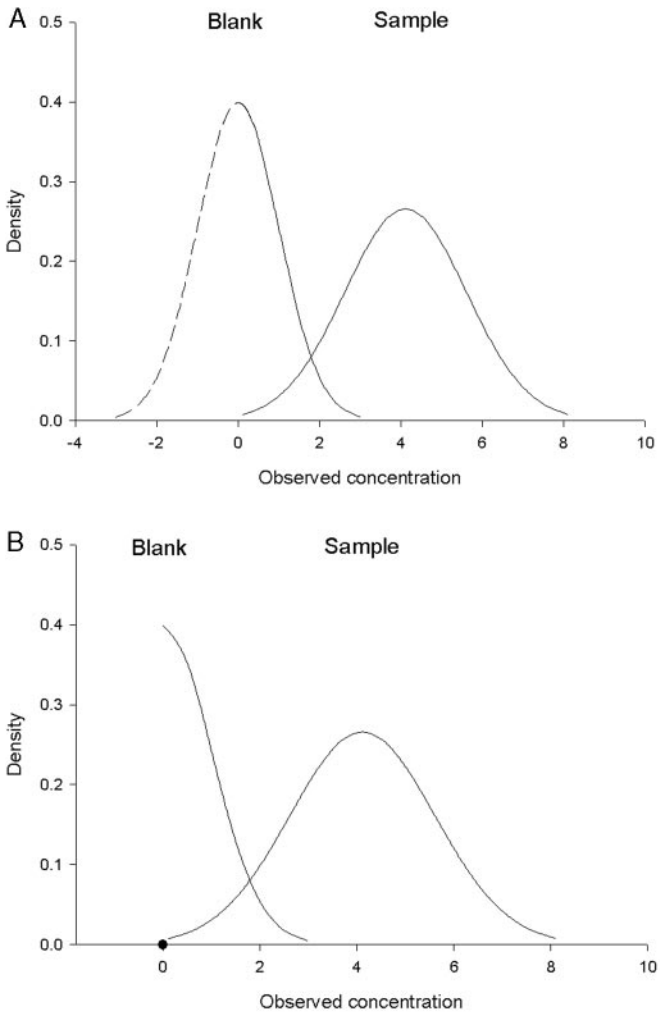

Fig. 1. Distributions of blank and sample values.

In *A*, the blank distribution is symmetric around zero, whereas in *B*, the blank values are truncated at zero. The ● in *B* represents the presence of zero values originating from negative values.

for rejection of the null hypothesis given a 5% significance level. Using this limit, we will falsely assume in 5% of all measurements of blank samples that the analyte concentration exceeds zero. This is the so-called type I error (α). On the other hand, we observe that some of the measurements for a sample with a low amount of analyte fall below this limit. Defining the alternative hypothesis as the case with analyte present implies that the alternative hypothesis is erroneously rejected in case of measurements of the low sample falling below the 95th percentile of blank measurements. Thus, we commit an error, the so-called type II error (β). We observe here that hypothesis testing is one-sided.

Recently, the ISO recommended a definition of the minimum LoD in relation to stated levels of type I and II errors *(1–4)*. The default level for these errors was set to 5%, i.e., $\alpha = \beta = 5\%$. An α value of 5% corresponds to using the 95th percentile of the distribution of blank values as the limit for declaring a measured value signif-

icantly higher than the blank. Given a gaussian distribution of blank values (Fig. 1A), this limit corresponds to:

$$\mu_B + 1.65\sigma_B$$

where μ_B and σ_B are the mean and SD of the blank measurements, respectively.

For the situation in Fig. 1B with an asymmetric distribution of blank values, the 95th percentile is not estimated correctly by a parametric approach. Using simulations, we estimated the error for a sample size of 25. If the true mean is 0 so that one-half of the blank values are negative and are given the value 0, the average estimated SD corresponds to 57% of the SD of the unmodified gaussian distribution. The average estimated mean is 0.40, and the average estimated 95th percentile corresponds to 1.37, i.e., 83% of the anticipated value of 1.65. For a true mean equal to 0.5 SD, the average estimated parametric 95th percentile amounts to 91% of the true value of 2.15.

The most straightforward procedure to estimate the 95th percentile of an asymmetric distribution is to apply a nonparametric principle based on the ordered values *(8)*. Let n_B be the number of measurements of the blank sample. When we rank n_B values according to size, the 95th percentile may be estimated as the value of the $[n_B(95/100) + 0.5]$ ordered observation (see the *Appendix* that accompanies the online version of this article at http://www.clinchem.org/content/vol50/issue4/). In case of a non-integer value, interpolation is carried out between neighboring values (see the example later in the text). The limiting percentile (Perc) of the blank distribution, which cuts off the percentage α in the upper tail of the distribution, will in what follows be called the LoB, i.e.:

$$\text{LoB} = \text{Perc}_{1-\alpha}$$

To address the type II error level, one has to consider the minimum sample concentration that provides measured concentration values exceeding the LoB with a specified probability. If the type II error level, β, is set to 5%, 95% of the measurements should exceed the LoB. Fig. 2 illustrate two cases: one with a true sample concentration equal to the LoB, and another with a true sample concentration at a value so that the 5th percentile of the distribution of sample measurements equals the LoB. In the first case, 50% of the sample measurements are below the LoB with the other 50% of values exceeding the LoB. Only the latter 50% will be declared as significantly exceeding the blank value. In Fig. 2B, on the other hand, 95% of the measurements exceed the LoB and are declared significantly higher than the blank value. Thus, only 5% of the measurements are erroneously declared not significantly different from the blank, which is the type II error. According to the ISO definition, the true analyte concentration of this sample is the minimum detection limit, or the LoD. Usually, the sample distribution is gaussian, and in this case the 5th percentile of the distribution can be derived from the mean and SD as:

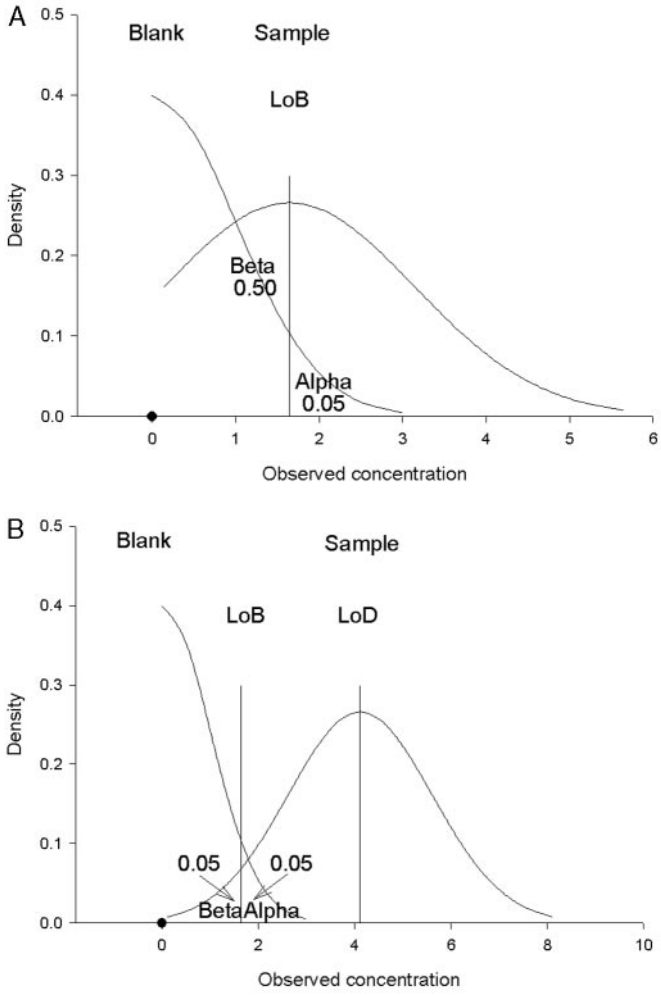

Fig. 2. Effect of location of the sample distribution.

When the mean of the sample distribution equals the LoB, 50% of the measurements exceed LoB (*A*). At a sample concentration equal to the LoD, (100% − β) of the sample measurements (here 95%) exceed the LoB (*B*). The ● represents the presence of zero values originating from negative values.

$$\mu_S - 1.65\sigma_S$$

where μ_S and σ_S are the mean and SD of the sample measurements, respectively. Overall, we have:

$$\text{LoD} = \text{LoB} + 1.65\sigma_S$$

If the sample distribution is not gaussian, the 5th percentile of the sample distribution can be estimated nonparametrically in the same way as the LoB. However, parametric estimation is more efficient and should be used when possible (see the online *Appendix*).

ESTIMATION OF LoB AND LoD

The outline given above was based on theoretical (population) distributions. In practice, one has to deal with real sample distributions. Thus, LoB must be estimated from repeated (n_B) measurements of one or several blank samples, and the SD of sample measurements from repeated measurements (n_S) of sample(s) with a relevant

concentration. Thus, an estimate (indicated with the subscript EST) of the LoD is then obtained as:

$$LoD_{EST} = LoB_{EST} + c_\beta \times SD_S$$

where SD_S is the estimated SD of the sample distribution with f degrees of freedom, and c_β is $z_{1-\beta} \times \sigma/E(SD_S)$. $z_{1-\beta}$ is the $(1 - \beta)$ percentile of the standard gaussian distribution, and $E(SD_S)$ is the mean value of SD_S. If the number of repeated measurements (n_S) is not too small, the mean value of SD_S can be approximated by $\sigma \times [1 - 1/(4 \times f)]$ (for details, see the section on "Estimation of SD_S" in the online *Appendix*), and $LoD_{EST} = LoB_{EST} + z_{1-\beta}/[1 - 1/(4 \times f)] \times SD_S$ is an unbiased estimate of $LoD = LoB + z_{1-\beta} \times \sigma_S$.

The uncertainty of the estimates is considered next. With regard to LoB, the standard error of estimates for theoretical distributions can be derived (see the online *Appendix*). With actual data, the user may obtain the 95% confidence interval (CI) limits from the ranked blank values according to Table 1 *(9)*. The uncertainty of the LoD estimate is composed of a component originating from the uncertainty of the LoB estimate and a part from the sample measurements. As detailed in the online *Appendix*, an approximate 95% CI of the LoD can be derived by combining the 80% CI for the LoB and SD_S, supposing $n_B = n_S$. The 80% CI limits for the LoB can be derived from the ranked values by use of Table 1, and multiplication factors that provide the 80% CI limits for SD_S are presented in Table 2. The suggested procedure can be applied down to a sample size of $n_B = n_S = 50$. An alternative to the described procedure that also may apply at lower sample sizes is the bootstrap principle *(10)*.

In relation to estimation of the LoD, a problem may be that σ_S often is nonconstant because it frequently increases with sample concentration. However, over the limited range of low concentration values that are of interest in the present context, σ_S may be approximately constant, and the outlined procedure is then straightforward (see the example later in the text). Otherwise, a more complicated approach can be undertaken in which it is assumed that the sample SD is a function of the concentration *(2)*.

Table 1. Rank number corresponding to upper and lower 80% and 95% CI limits of the LoB.[a]

	80% CI		95% CI	
n	Lower	Upper	Lower	Upper
50	n − 4	n	n − 5	n
60	n − 5	n	n − 6	n
70	n − 5	n − 1	n − 7	n
80	n − 6	n − 1	n − 8	n
90	n − 7	n − 1	n − 8	n
100	n − 7	n − 2	n − 9	n
150	n − 11	n − 3	n − 12	n − 1
200	n − 14	n − 5	n − 16	n − 3
250	n − 17	n − 7	n − 19	n − 5
300	n − 20	n − 10	n − 22	n − 7

[a] LoB = 95th percentile of the distribution of blank measurements.

Table 2. Factors corresponding to 80% and 95% CI limits of an estimated SD.

	80% CI		95% CI	
n	Lower	Upper	Lower	Upper
50	0.881	1.140	0.835	1.243
60	0.897	1.138	0.848	1.217
70	0.904	1.126	0.857	1.198
80	0.909	1.116	0.865	1.183
90	0.914	1.109	0.872	1.171
100	0.918	1.102	0.878	1.161
150	0.932	1.082	0.898	1.128
200	0.941	1.070	0.911	1.109
250	0.947	1.062	0.919	1.096
300	0.951	1.056	0.926	1.087

[a] The number of degrees of freedom is n − 1.

VERIFYING A CLAIMED LoD

In addition to estimation of the LoD, one may also be interested in primarily verifying a claimed LoD, e.g., a laboratory may want to assure that an assay fulfills the claim of a given LoD stated by a manufacturer. In that case, it is necessary to distinguish between a full and a partial verification procedure. A full procedure consists of estimating the LoB, performing repeated measurements of sample(s) with a concentration equal to the claimed LoD, and estimating the proportion of results exceeding the LoB. If the recorded proportion is in agreement with the expected 95%, then the data support the claim of the LoD. A partial procedure consists of applying the LoB stated by the manufacturer and recording the proportion of results exceeding the LoB for sample(s) with a concentration equal to the claimed LoD. Shown in Table 3, for sample sizes of 20–1000, are the lower bounds for the recorded proportion that are in accordance with $\alpha = \beta =$ 5% for the full and partial procedures. The interval is slightly wider for the full verification procedure because, in this case, the LoB is a random variable (see the online *Appendix*). Notice that the bounds for partial verification of the LoD can also be applied as bounds for verification of a claimed LoB. If the proportion of blank measurements below a claimed LoB is equal to or higher than the proportion listed in Table 3, the claimed LoB is supported. An example of verification is shown in the *Examples* section.

CHARACTERISTICS OF BLANK AND SAMPLE

The blank sample(s) should be as similar as possible to the natural patient samples, e.g., for a drug assay a suitable blank sample would be a serum or plasma sample free of drug, and not just a buffer solution. To assure that the measurements are representative, compilation of measurements on several blank samples is preferable. Thus,

Table 3. Lower bounds (one-sided 95% CI) of observed proportions of results exceeding the LoB in a verification procedure that are in accordance with the hypothesis of $1 - \beta = 95\%$.[a]

	Proportion of results exceeding the LoB, %	
n	Full verification	Partial verification
20	85	85
30	87	87
40	88	90
50	88	90
60	88	90
70	89	90
80	89	91
90	90	91
100	90	91
150	91	92
200	92	93
250	92	93
300	92	93
400	93	93
500	93	93
1000	94	94

[a] For the full verification procedure, it is assumed that $\sigma_S/\sigma_B = 1.5$ and $n = n_B = n_S$. Table values are based on 10 000 simulation runs for each parameter combination.

instead of repeated measurements of only one particular serum sample, a set of 5–10 or more blank serum samples would be preferable because matrix differences exist from sample to sample. With regard to endogenous compounds, it may be difficult to obtain blank samples. In some situations, e.g., for tumor markers, samples from nondiseased individuals may be appropriate. For hormones, blank samples may in some cases be provided from diseased individuals or individuals with suppressed hormone concentrations attributable to pharmacologic treatments, assuming that these samples have characteristics otherwise similar to the routine test samples. Otherwise, blank samples might be samples stripped of the component, e.g., by precipitation by an antibody, by enzymatic degradation, or by adsorption to charcoal. However, such treatments may also remove potential interfering compounds and thus give a too optimistic picture of assay performance.

With regard to the sample(s) with low analyte concentrations, it may be preferable to add analyte, e.g., a drug, to a set of serum samples from different patients rather than to just one serum sample or a serum pool. For endogenous compounds, ideally a set of patient samples with concentrations in the low range might be used. A pooled SD_S estimate can then be derived from repeated measurements of the set of samples, e.g., 5–10 measurements of each of 5–10 samples (see the example presented later). Measurements on different days should be carried out, so that SD_S reflects the total analytical variation.

For the estimates of LoB and LoD to be meaningful, the measurements by the method in question should be unbiased. When a reference method for the analyte exists, comparison of measurements in the low range with this method should be undertaken. Alternatively, measurements of samples to which analyte has been added might be used to demonstrate that realistic measurements are obtained. This prevents a spuriously low LoD from being reported because if the assay provides values that are lower than the true analyte concentration, other factors being equal, a too-low LoD is going to be estimated.

SAMPLE SIZE CONSIDERATIONS

The optimum ratio between the number of blank and sample measurements in the estimation procedure is related to the uncertainties of the estimated LoB and SD_S of the sample measurements. Nonparametric estimation of LoB is roughly half as effective as a parametric estimation procedure (see the online *Appendix*). The uncertainties of percentile or SD estimates will also tend to be proportional to the dispersion of the distributions. Thus, nonparametric estimation of the LoB would suggest that the number of blank measurements should exceed that of the sample measurements. However, the dispersion of sample measurements is likely to exceed that of the blank measurements. Overall, an approximately equal number of blank and sample measurements is likely to be near optimal in many cases (see the online *Appendix*). Concerning the precision of estimates of LoB and LoD in relation to the number of measurements, see the *Simulation* section.

REPORTING OF RESULTS

In a laboratory, the LoB may be used to determine how patients' results will be reported: i.e., as substance detected or not detected. It should be kept clear what the exact meanings of "detected" and "not detected" are. Not detected, i.e., a result below the LoB, means that the true concentration is likely to be less than the LoD. "Likely" refers to the type II error level (β), which often is set to 5%. Thus, a result less than the LoB should be reported as "<LoD" and not as "<LoB" or "zero". A result exceeding the LoB, i.e., detected, means that the true concentration is likely to exceed zero, and the reporting could be ">zero" or "detected". Likely refers here to the type I error (α), which often is set to 5%.

A modification of the above-mentioned principles for reporting results might be considered in cases in which results are to be used in scientific studies. Here, unbiased results for the groups of individuals being investigated are obtained by reporting the concentrations actually measured irrespective of whether the values are below or above the LoB. Otherwise, biased results for groups may be obtained.

The relative uncertainty of measurements at or just exceeding the LoD is often large, and usually a quantitative result is not reported. The lower limit for reporting quantitative results (LoQ) relates to the relative impreci-

sion (CV) considered acceptable by the laboratory. From a precision profile for the assay, the LoQ may be determined, e.g., corresponding to a CV of 10% or 20% *(5)*. The possible bias of the method at this level might also be taken into consideration, so that an upper limit of the total error determines the LoQ. The LoQ will constitute the lower limit of the reportable range for quantitative results for the assay. The LoQ is not considered further here.

Examples

EXAMPLE OF ESTIMATING THE LoD OF AN ASSAY

We consider an assay based on HPLC with ultraviolet detection for an antidepressant drug, mirtazapine, in serum that is used in the laboratory of one of the authors. The method is based on automated solid-phase extraction with online injection of the eluate into the HPLC apparatus. A serum pool with the drug added is used for calibration. Trimipramine serves as internal standard. The peaks for the analyte and internal standard are well separated, and no background peaks interfere. When deriving the LoD, the default values $\alpha = \beta = 5\%$ were used. The blank measurements consisted of four measurements on each of five sera from patients not taking the drug, and the low-sample measurements were based on four measurements on each of five other sera from patients not taking the drug, to which 10 nmol/L of the drug had been added (Fig. 3). The distribution of blank measurements deviated significantly from the normal form ($P = 0.04$, Anderson–Darling test), and the LoB was estimated nonparametrically as the 95th percentile of the measurements. The 95th percentile corresponds to the 19.5th ordered observation [$20 \times (95/100) + 0.5$]. Linear interpolation between the 19th and 20th observations yielded a LoB estimate of 6.85 nmol/L. A pooled estimate of the SD_S was 2.85 nmol/L (see the online *Appendix*). An estimate of the LoD was then obtained:

$$\mathrm{LoD_{EST}} = \mathrm{LoB_{EST}} + \frac{z_{1-\beta}}{1 - 1/(4 \times f)} \times \mathrm{SD_S}$$

$$= 6.85 + \frac{1.645}{1 - 1/60} \times 2.85 = 11.6$$

Notice that the degrees of freedom for SD_S are $5 \times (4 - 1) = 15$.

This is a point estimate, and the true LoD may be somewhat lower or higher. The 95% CI for the LoD can not be derived as described above because of the small sample sizes used here. The usual steady-state serum concentrations for patients in treatment with the drug extend from 50 to 350 nmol/L. Accordingly, a LoD of 11.6 nmol/L is acceptable for clinical use of the assay.

EXAMPLE OF VERIFYING A CLAIMED LoD OF AN ASSAY

A given analytical procedure is claimed by the manufacturer to have a LoD of 45 U/L with $\alpha = \beta = 5\%$. The user decides to carry out a full verification procedure on the basis of 25 blank measurements (5 measurements of 5 blank samples over 5 days) and 25 measurements of samples to which 45 U/L of the analyte had been added (5 measurements of 5 samples over 5 days; Fig. 4). Visual inspection reveals that the distribution of blank values is asymmetric (nine 0 values), and accordingly the LoB is estimated nonparametrically. The blank values are ranked according to size, and the 95th percentile corresponds to the 24.25th ordered observation ($25 \times 0.95 + 0.5$). Linear interpolation between the 24th and 25th observations yields 19.17 U/L [$18.01 + 0.25 \times (22.65 - 18.01)$].

The proportion of sample measurements that exceed the LoB is 96% (24 of 25). From Table 3 we see that 96% is above the lower bound for agreement (87%; full verification procedure). Thus, the observed proportion is in accordance with the expected one of 95% according to the

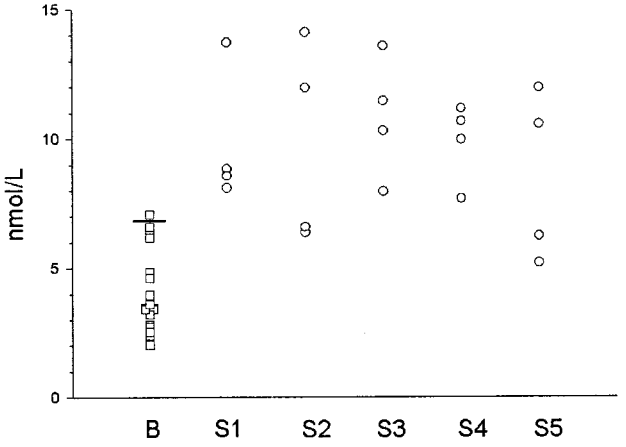

Fig. 3. Blank and low sample values for the LoD estimation example.

Four measurements of each of five blank sera are displayed together (*B*). The LoB limit (*horizontal bar*) was estimated to be 6.85 nmol/L (95th percentile of the blank distribution). The ○ for *S1–S5* indicate the four values obtained for sera 1–5, to which 10 nmol/L drug had been added.

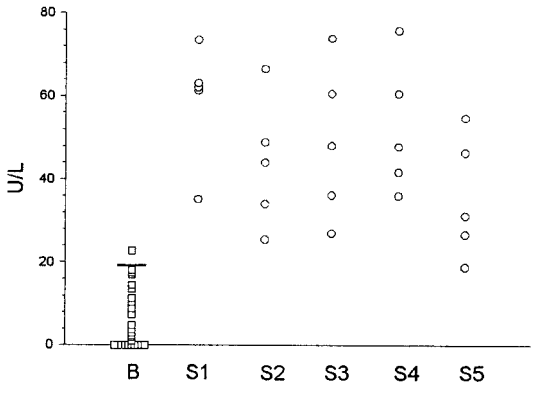

Fig. 4. Example illustrating the LoD verification procedure.

The 25 blank measurements (*B*) and 5×5 measurements of samples to which 45.0 U/L of the analyte had been added (*S1–S5*) are shown. The LoB limit (*horizontal bar*) was estimated to be 19.17 U/L (95th percentile of the blank distribution). Ninety-six percent (24 of 25) of the sample measurements exceeded the LoB.

claim, and the present evaluation does not contradict this claim.

Evaluation of the Performance of the LoD Estimation/Verification Procedures by Simulation

ESTIMATION OF LoB AND LoD

The parameters of the model outlined in Fig. 2B are considered as a basis.

The blank distribution follows a standard gaussian distribution ($\mu_B = 0$ and $\sigma_B = 1$) with negative values assigned the value zero. The sample distribution is gaussian with $\mu_S = 4.1125$ and $\sigma_S = 1.5$. Under this model, the population LoB is 1.645, and the LoD is 4.1125 [1.645 + 1.5 × 1.645 ($\alpha = \beta = 5\%$)]. It is assumed that σ_S is constant in a region around the LoD. Thus, the estimation procedure consists in estimating the LoB and adding c_β SD_S.

The performance of the suggested LoD estimation procedure can be evaluated as a function of the sample size by drawing pseudo-random numbers from these distributions. The bias and precision of LoB and LoD estimates are recorded (Table 4).

Observe that the nonparametric LoB estimate has considerable uncertainty. At a sample size of 25, the SE amounts to 38% of σ_B in the model, decreasing to ~20% of σ_B at a sample size of 100. The SE of the LoD exceeds that of the LoB for a given sample size, illustrating that both the uncertainty with regard to the LoB estimation and that associated with SD_S estimation contribute to the overall uncertainty. Thus, if a manufacturer wishes to assure a reasonably precise estimate of the LoD, a fairly large sample size is needed. At least 100 blank measurements and 100 measurements of samples with low amounts of analyte should be considered. Given an automated assay, this may not constitute a problem, but for manual assays, e.g., a hormone assay, a large number of repetitions may represent a considerable task. When estimating the LoD, one should ideally keep the conditions near routine operating conditions, e.g., the measurements should be dispersed over several days and not restricted to a single run.

Table 4. Performance of the LoD estimation procedure as a function of sample size evaluated by simulation for the given data model.[a]

	LoB			LoD	
Sample size[b]	Mean	SE	SE of SD_S	Mean	SE
10	1.53	0.58	0.35	4.02	0.84
25	1.63	0.38	0.21	4.10	0.52
50	1.63	0.29	0.15	4.10	0.39
100	1.64	0.21	0.11	4.10	0.27
200	1.64	0.15	0.075	4.11	0.19
500	1.65	0.094	0.047	4.11	0.12

[a] Table values are based on 10 000 simulation runs for each parameter combination.
[b] $n_B = n_S$.

Table 5. Power to detect that the claimed LoD is 25% less than the true LoD (claimed LoD = 0.75 × true LoD).

	σ_S/σ_B			
Sample size,[b] n	1.0	1.5	2.0	5.0
20	56%	45%	38%	24%
30	64%	54%	47%	32%
40	73%	64%	56%	38%
50	77%	69%	62%	43%
60	83%	74%	67%	48%
70	85%	78%	71%	51%

[a] Table values are based on 10 000 simulation runs for each parameter combination.
[b] $n = n_B = n_S$.

PERFORMANCE OF THE VERIFICATION PROCEDURE

The performance of the full and partial verification procedures can be studied by simulations. Two aspects are of interest here. One is the ability to verify a claimed LoD that is equal to or higher than the true LoD, and the other is to discover a claimed LoD that is too low. Concerning the first point, the use of CIs in the verification procedures defines the probability of verifying a true LoD. A one-sided 95% CI assures 95% probability of verifying a claimed LoD equal to or higher than the true LoD, according to the either partial or the full verification procedure. We studied the other problem, discovering a claimed LoD as being too low, by simulations.

The ability (power) of detecting a claimed LoD as being too low depends on the sample size and the ratio between the dispersions in the sample and the blank, σ_S/σ_B. We chose to study the detection of a claimed LoD that was 25% lower than the true LoD according to the full procedure (Table 5). For sample sizes ranging from 20 to 70, the power of detection ranged from 24% to 85%. The highest range of power (56–85%) was observed for $\sigma_S/\sigma_B = 1$. Given a slightly higher ratio, $\sigma_S/\sigma_B = 1.5$, the range of power decreased to 45–78%. For the highest ratio studied, $\sigma_S/\sigma_B = 5$, the range of power was lowest, 24–51%.

EVALUATION OF THE COVERAGE OF THE SUGGESTED PROCEDURE FOR ESTIMATION OF THE CI OF LoD

The suggested procedure for estimation of the 95% CI for a LoD relies on some approximations outlined in the online *Appendix*. To study the performance of the procedure, we evaluated the coverage by simulations (10 000 runs for each parameter combination). The same model situations as considered above were investigated. The coverage indicates the frequency with which the true value of the LoD for a given model is included in the estimated 95% CI. Ideally, the frequency should be 95%. As may be observed from Table 6, the actual coverage ranged from 87% to 96%. The best results were obtained for low σ_S/σ_B values. Thus, the estimated limits were approximately correct. An alternative to the suggested procedure is to apply the bootstrap approach, which, however, requires computerized calculations.

Table 6. Coverage of the approximate procedure for estimation of 95% CI for the LoD.[a]

	σ_S/σ_B			
Sample size,[b] n	**1.0**	**1.5**	**2.0**	**5.0**
50	93%	93%	93%	90%
100	96%	96%	95%	91%
200	95%	95%	95%	89%
300	92%	93%	92%	87%

[a] Table values are based on 10 000 simulation runs for each parameter combination.

[b] $n = n_B = n_S$.

Discussion

The LoD concept has been the subject of considerable interest over the years. The focus has generally been on how to declare a measurement result significantly higher than a blank measurement. Kaiser *(11)* addressed this issue, suggesting that the LoD be given as the mean plus the SD of blank measurements multiplied by a factor, conventionally set to 2 or 3, initially without clear-cut probability considerations. Thus, in the early literature on the subject, the focus was on the question of significance in relation to blank measurements. This approach is probably still the most widely used procedure in practice, as supported in the "Instructions to Authors" sections of many laboratory journals, including *Clinical Chemistry*. Later it was recognized that in addition to the significance aspect, one should also take into account the lowest amount of analyte that was likely to be declared significantly higher than zero, i.e., the type II error *(12–15)*. Recently, the latter concept has been recommended in a set of ISO guidelines incorporating procedures reported in the more recent literature *(1–4)*.

Although one part of the problem is the general concept of the LoD, another part involves the statistical techniques applied. Somewhat surprisingly, all of the publications cited here, except Brown et al. *(7)*, suppose gaussian distributions of both blank and sample measurements. The background may be that most authors deal with the signal response (y) rather than the concentration scale (x). Having determined the statistical limits on the response scale, the authors may subsequently transform values to the concentration scale on the basis of the calibration function. Accordingly, procedures linked to linear or nonlinear calibration functions have been described, the latter mainly in connection with immunoassays *(2, 5, 7, 14, 15)*. When one is working on a response scale, symmetric distributions of blank responses may occur frequently, and a fully parametric approach is appropriate. In clinical chemistry, however, the instrument response is usually hidden, and one has access only to the final concentration output. Given a symmetric distribution of blank responses, transformation to concentration values may give both positive and apparently negative concentration values. The latter are usually converted to zero or a small positive number by the instrument, which leads to asymmetric distribution of blank concentration values. As mentioned earlier, this is the background for the procedure suggested here. To apply the proposed procedure, it is necessary to have concentration values down to zero available. If the instrument software truncates results at a higher value, it is not possible to carry out a meaningful assessment of the LoD.

The present procedure has the advantage of being able to deal with asymmetric blank distributions. In addition, the procedure conforms to currently accepted concepts for establishing the LoD, as outlined in the ISO guidelines, primarily in regard to both type I and II errors. Furthermore, by focusing on concentration or amount of analyte directly, the procedure becomes general and does not depend on a particular type of calibration function. Variations in terminology that exist in this field can create confusion. Several reports, including the ISO guidelines, use the term critical limit (x_c) for the significance limit of blank measurements. Because "critical" in laboratory medicine often has another meaning, i.e., an alarm value that should be reported immediately, we prefer another term, the LoB. With regard to LoD, many variations exist, e.g., minimum detectable level (MDL) and lower limit of detection (LLoD); these variations are of minor significance as long as the precise definition is clear and the probability levels are indicated. A drawback of the present procedure is that it gives a less precise estimation of the LoB values than may be obtained by fully parametric procedures. It is possible to increase the precision of the nonparametrically estimated percentile slightly by applying modern computer-based statistical methods, e.g., a resampling principle (bootstrap method) *(10, 16)*, weighted percentile estimation *(17)*, or smoothing techniques *(18)*. The bootstrap principle is the simplest and most generally applicable procedure of those mentioned and has the advantage of also providing confidence limits for the percentile estimate. The gain in precision corresponds to saving 10–15% of the observations compared with the simple nonparametric procedure *(16)*. The bootstrap method also represents an alternative procedure for estimation of the CI of the LoD.

The LoD of a method should not be confused with the so-called sensitivity. According to ISO, the analytical sensitivity relates to the slope of the calibration function *(19)*. The steeper the slope, the more sensitive the assay is to slight changes in the amount of analyte. This definition of sensitivity expresses an entirely different concept than the LoD. The distinction between the LoD and the LoQ should also be kept clear *(5)*. A laboratory may choose to report results at or only slightly above the LoD as being detected and restrict the reporting of quantitative results to values at or exceeding the LoQ *(20)*. The LoQ indicates the lowest value at which the analytical procedure fulfills claimed imprecision or total error specifications and will often be a more important characteristic for a quantitative analytical procedure than the LoD. The LoD may be of

particular interest in relation to detection of drugs of abuse and in relation to tumor markers.

It is important that measurements of blanks and samples be carried out in a comparable way. If only a buffer solution is used as a blank, a falsely low LoB may be obtained because the dispersion of measurements often is larger in the real matrix *(21)*. Concerning sample(s) with low concentrations, estimation of the dispersion (SD_S) from several samples is an advantage to assure that average performance is assessed. Finally, total assay variation should be reflected by measurements performed on different days.

Manufacturers may compete with regard to producing assays with the lowest possible LoD. Thus it is important to have a standardized procedure with expressed levels of type I and II errors for determining the capability of detection. Use of this procedure could allow a fair comparison of competing assays.

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