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Limit of detection, limit of quantification and limit of blank

Elvar Theodorsson

LoB, LoD, LoQ

- Limit of blank (LoB), limit of decision, limit of detection (LoD) and limit of quantitation (LoQ) and are concepts and terms used to describe the lowest concentration of a measurand that can be reliably measured by a particular measurement procedure .
- The literature in this area has previously been and is unfortunately still confusing regarding concepts, nomenclature and methods.
- The approach recommended here is primarily based on recent recommendations by Eurachem
  https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf

CLSI - IFCC

EP17-A
Vol. 24 No. 34
Replaces EP17-P
Vol. 24 No. 10

Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

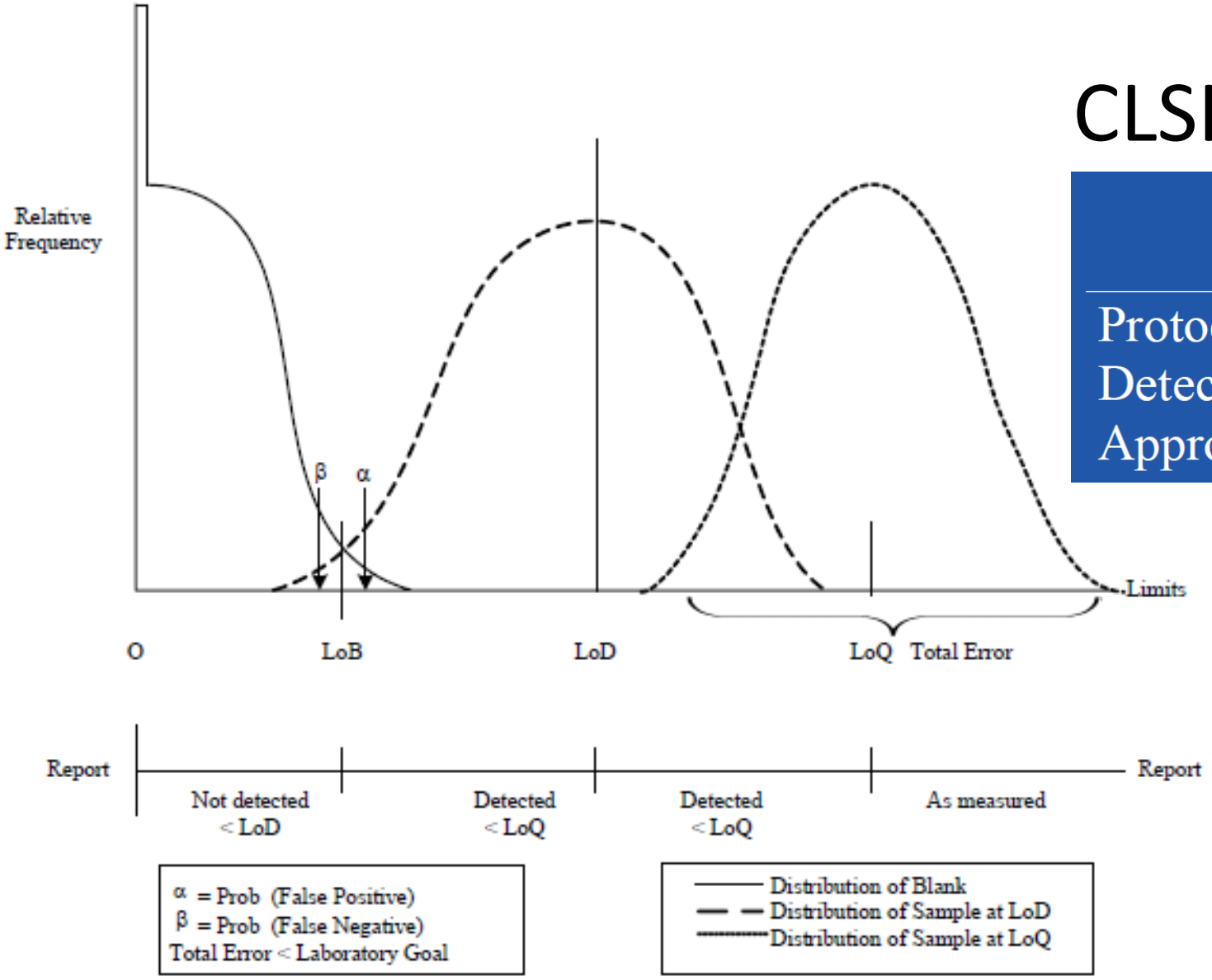

Figure 5. Distribution of Results for Blank, Low Positive at LoD, and Low Positive at LoQ (Report recommendations are shown for results at various points relative to limits.)

Imprecision profile

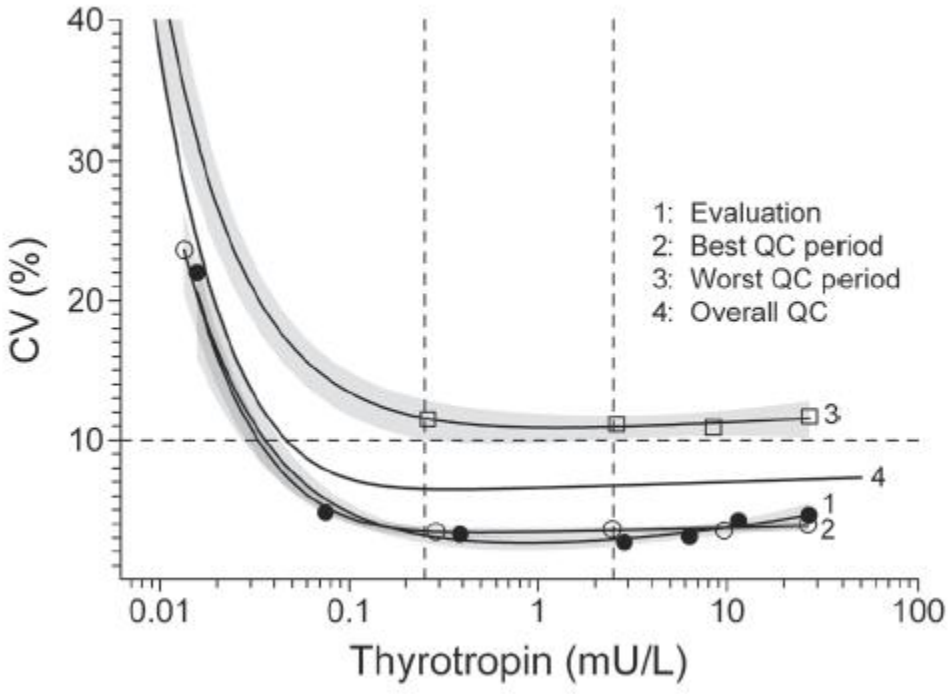

Figure 2. Total error imprecision profiles (eq. 5) for a TSH immunoluminometric assay on the Sanofi/Beckman/Coulter "Access" instrument (Beckman Coulter, Fullerton CA, USA). The profiles represent; 1: initial instrument evaluation, March through May 1997, seven specimens (solid circles), 245 degrees-of-freedom (df); 2: best QC period, February through August 2002 (QC #19), five specimens (open circles), 680 df; 3: worst QC period (QC #11), April through October 1998, five specimens (open squares – one data point off scale), 554 df; 4: overall QC profile (QC #9 through #29), September 1997 through July 2007, 105 specimens (data points omitted for clarity), 12884 df. All profiles reflect singleton measurement. Shaded areas are approximate 95% confidence intervals (note that confidence intervals for the overall profile are barely visible because they have virtually the same width as the plotted profile). In this case the assay reference range (0.25–2.5 mU/L) is defined by dashed vertical lines.

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Common notion of limit of quantitation in clinical chemistry

- The concentration at which imprecision (coefficient of variation) of the method is 5%

However

- Medical laboratories are already and increasingly faced with the question of the possible presence of a measurand for medical (tumor markers, infectious agents, carcinogens, pollutants) or legal reasons (drugs of abuse). More formal understanding of the detection limit and limit of quantitation is therefore needed

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Limits of quantitation — Yet another suggestion

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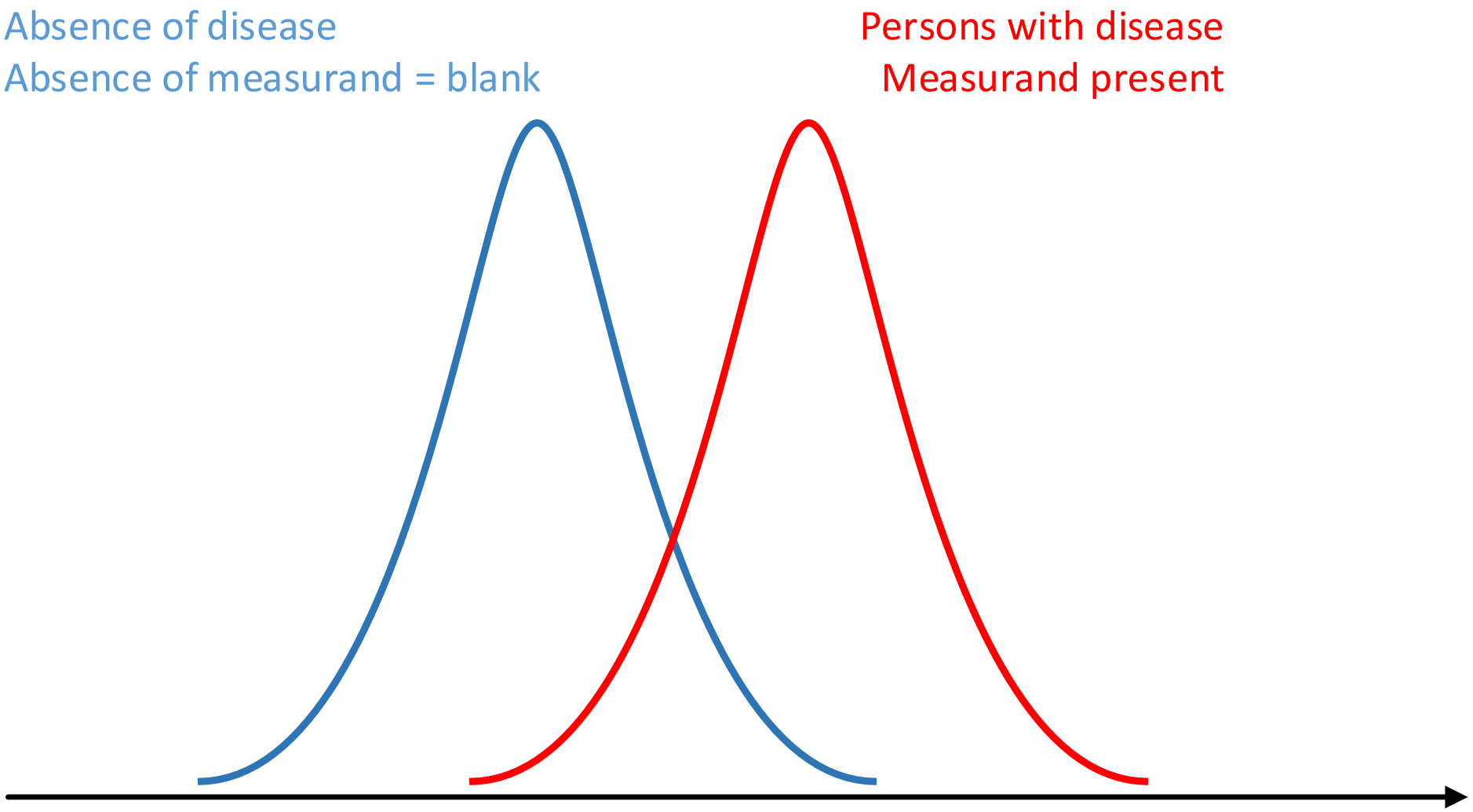
Limits of quantitation have always been overshadowed by decision levels and detection limits. The decision level is simply the level above which it is highly improbable to find a true net blank response, while, following Currie [1], the detection limit is such that analyte present at the detection limit is highly unlikely to go undetected. But the quantitation limit is far less well formed as a concept. Currie's "10σ" definition of it was simple, but has no fundamental justification: he simply referenced Adams et al. [2] as a reasonable source of the factor of 10. Likewise, the 1980 publication from the ACS Committee on Environmental Improvement simply defines the factor as 10 [3, p. 2247].

At the risk of adding yet another variant LOQ definition to the current collection, one possible route to standardization of the LOQ is to employ a prescient idea put forth by Coleman, Auses and Gram in 1997 [8, p. 78]: the LOQ "is the lowest concentration at or above which … measurements have at least 1.0 significant digit (at high confidence), and, equivalently, have limited relative measurement error, RME $\leq$ 5%." This, then, may constitute the fundamental reason for the formulation and usage of the LOQ concept, regardless of the specifics of any particular LOQ methodology. Clearly, if existing LOQ formalisms were brought into compliance with this requirement, it would facilitate meaningful comparisons of LOQs and promote their use as figures of merit.

Detection limit is basically a familiar concept



Detection limit is basically a familiar concept

	Participants			
	With disease	**Without disease**		
Positive test	True positives	False positives (type I error)	Total positive	**[PPV]**
Negative test	False negatives (type II error)	True negatives	Total negative	**[NPV]**
	Total with disease	Total without disease		
	[Sensitivity]	**[Specificity]**		

Three levels

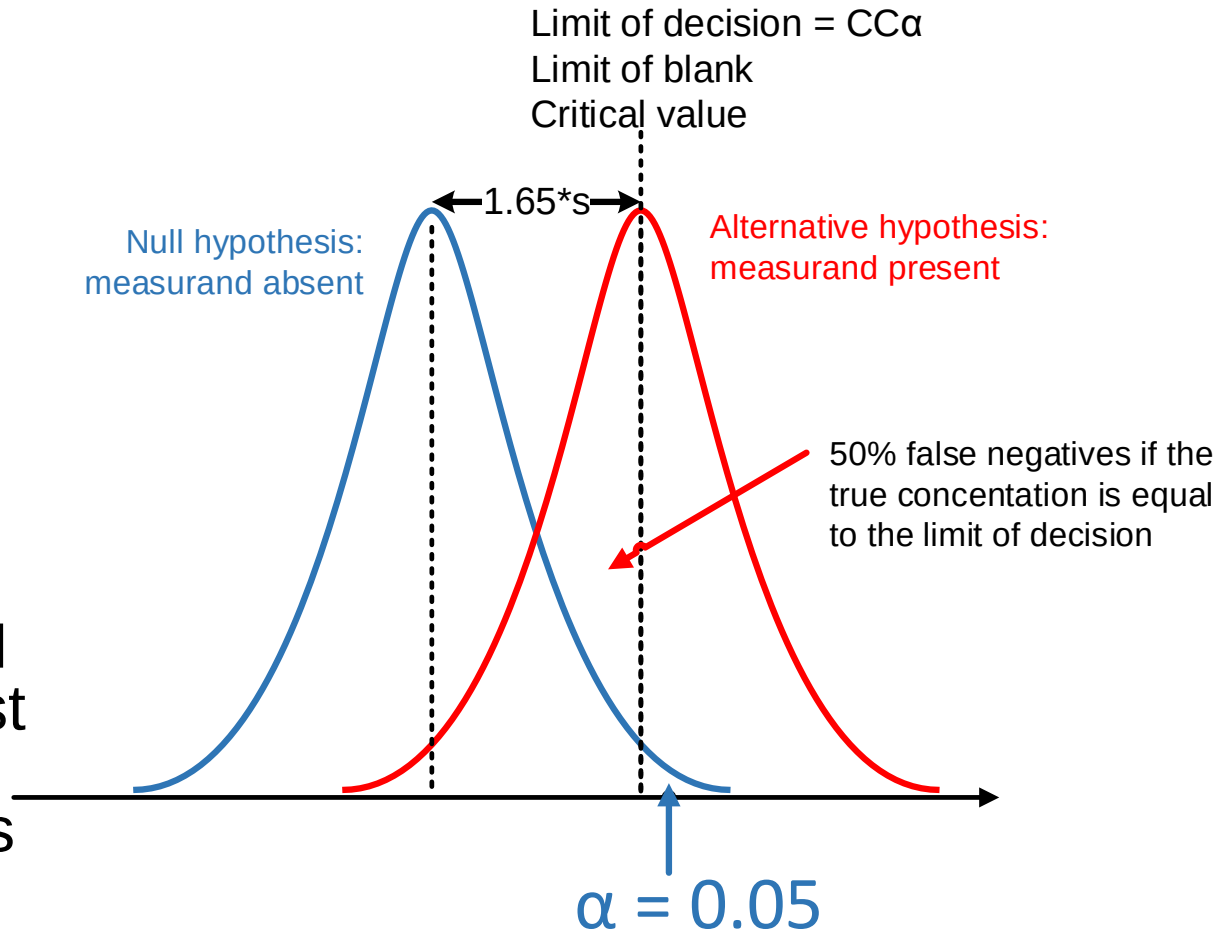
- Decision limit, limit of blank, critical value
  - **CCα** (term used in the EU directives)
- LOD
  - Limit of Detection, minimum detectable value, detection limit, **CCβ** (term used in the EU directives)
- LOQ
  - quantification limit, quantitation limit, limit of quantitation, limit of determination, reporting limit, limit of reporting and application limit.

Critical value in clinical chemistry

- The concept **critical value** in the context of detection should be used with care in clinical chemistry since it is most commonly used to denote the concentration at which the laboratory needs to notify the clinic in an extraordinary manner

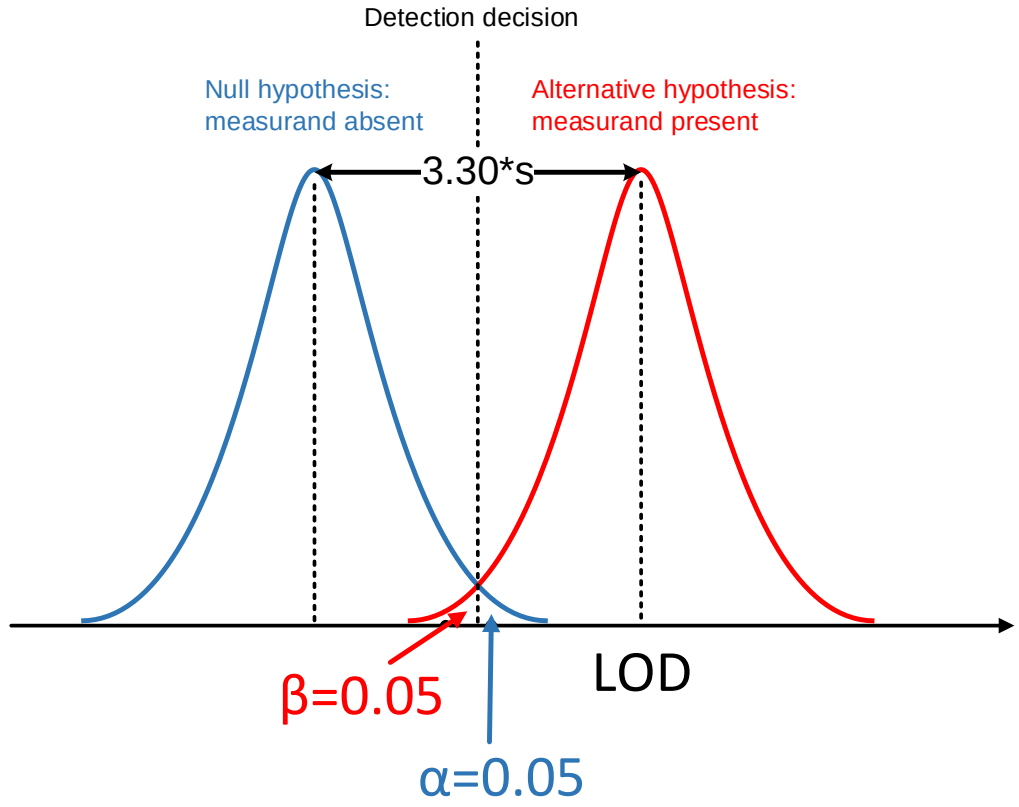
The limit of decision (CCα)

- The **limit of decision** (**CCα**) is the concentration of the measurand that is significantly different from zero.
- Limit of decision = CCα = s*1.65
- The concept is e.g. used when determining whether a material is contaminated or not.
- The **limit of blank** (**LoB**) or **critical value** is not a concept defined in VIM 3 but has been defined as the highest apparent concentration of a measurand expected when replicates of a blank sample containing no measurand are measured.



The limit of detection (LOD or CCβ)

- The limit of detection (LOD or CCβ) is the lowest concentration of the measurand that can be detected at a specified level of confidence.
- Limit of detection = LOD = s*3.3



Limit of detection (LOD or CCβ) – VIM 3

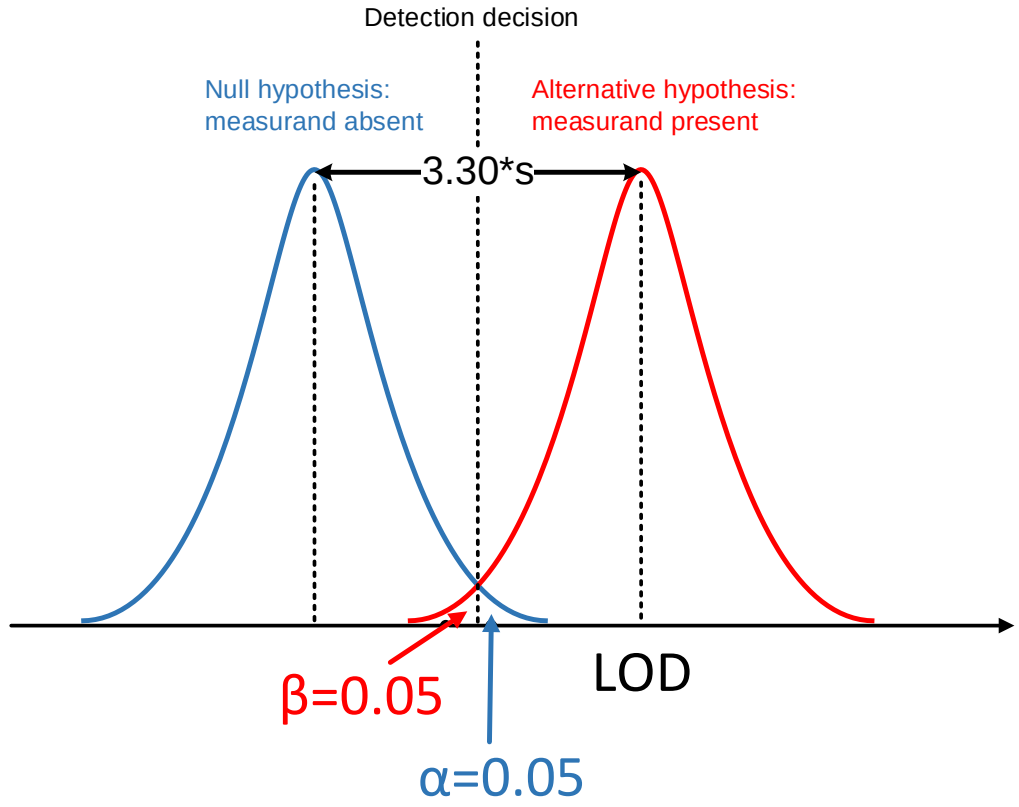
4.18 (4.15 Note 1)
detection limit
limit of detection

measured quantity value, obtained by a given **measurement procedure**, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence

NOTE 1 IUPAC recommends default values for α and β equal to 0.05.

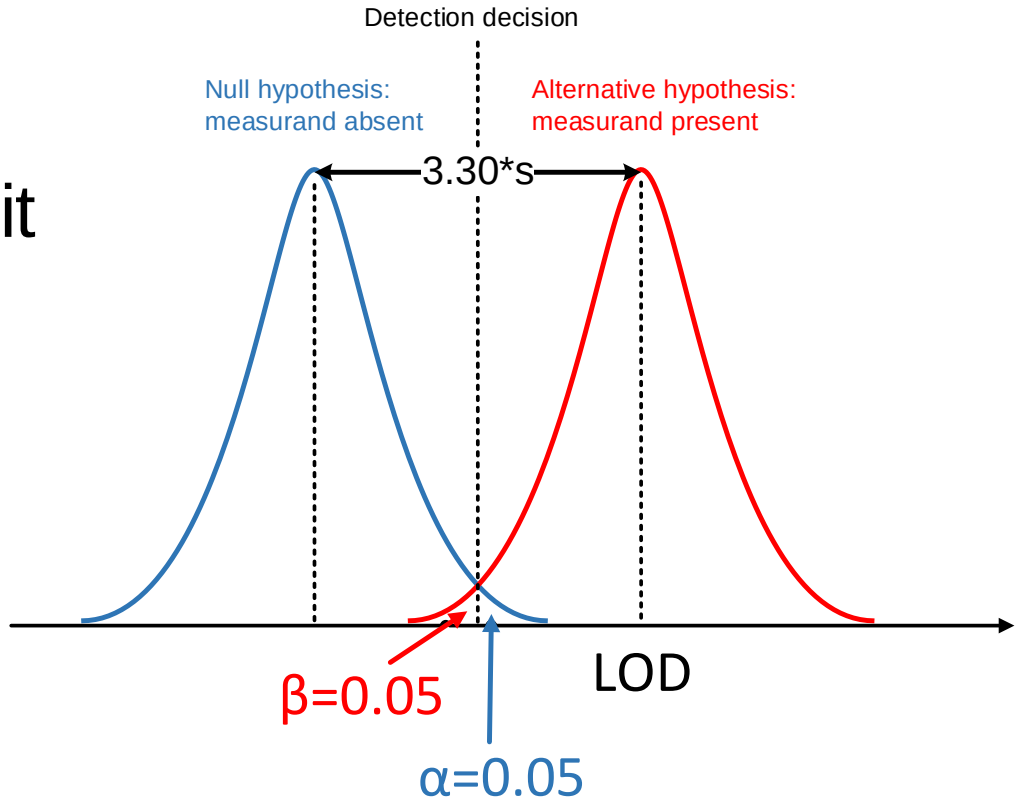
NOTE 2 The abbreviation LOD is sometimes used.

NOTE 3 The term "sensitivity" is discouraged for 'detection limit'.



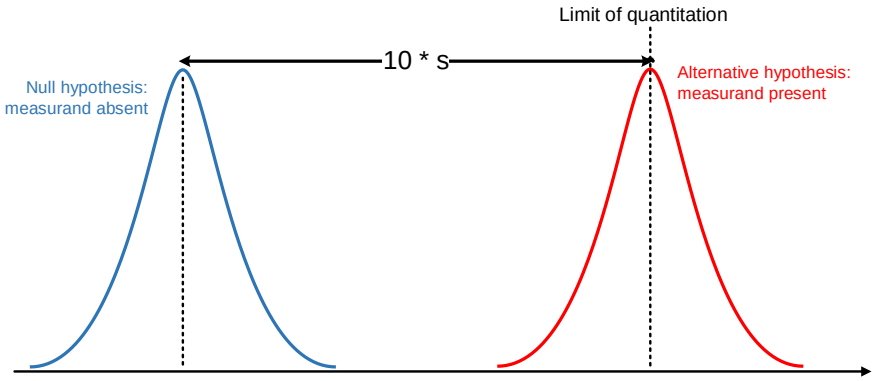
The limit of detection (LOD or CCβ)

- The detection limit of the measurement system/instrument and of the method should be kept apart. The detection limit of the *measurement system* is determined by presenting the system directly with the reagent blank or with other types of samples. When the detection limit of the *measurement method* is determined the sample is processed through the all steps of the measurement procedure.



Limit of quantitation (LoQ)

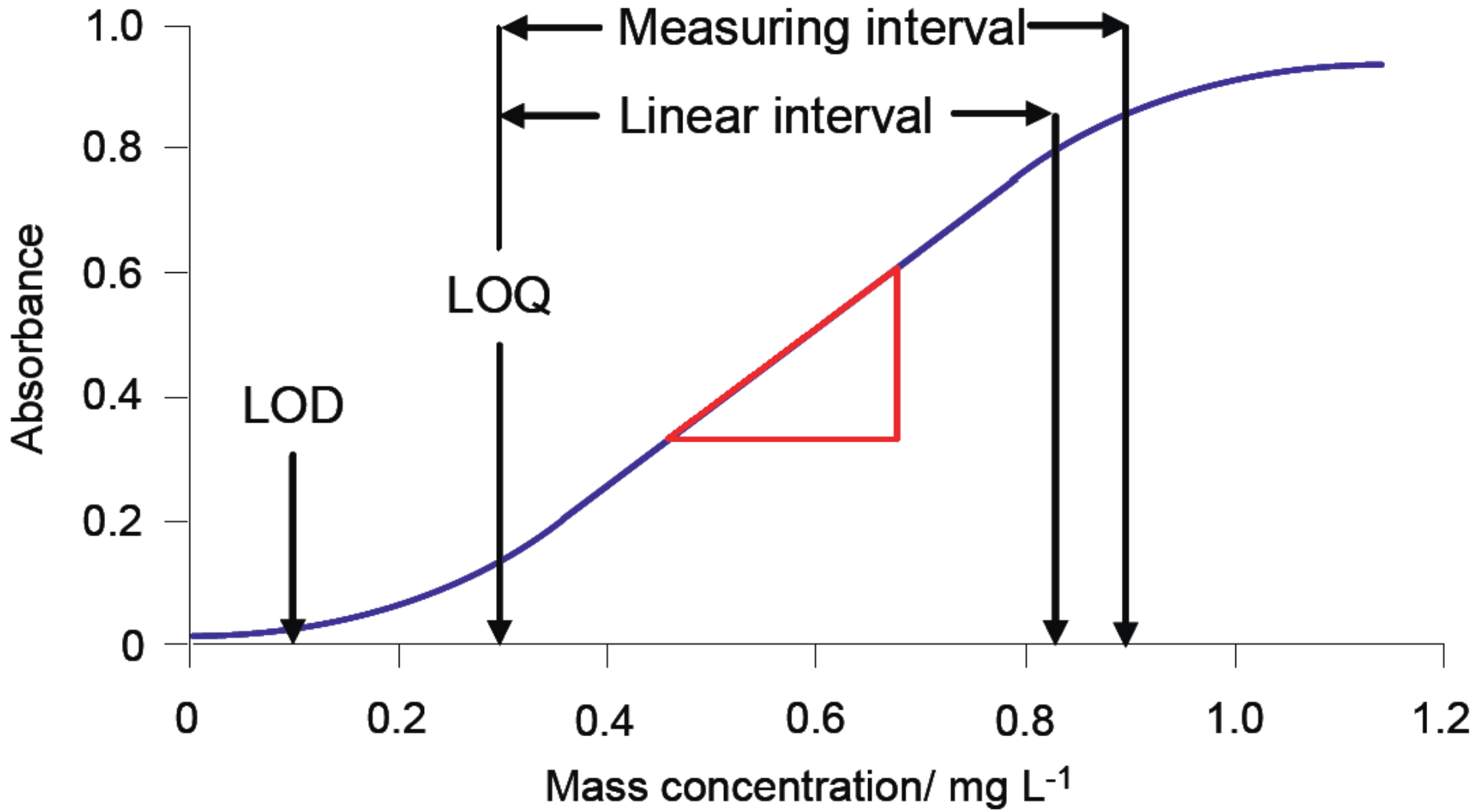
- The “limit of quantitation” (LoQ) is not a concept defined in VIM 3 but has been defined as “*the lowest concentration of measurand that can be determined with an acceptable level of repeatability precision and trueness*”.
- The limit of quantification (LOQ) is “*the lowest concentration at which the performance of a method or measurement system is acceptable for a specified use”*.
- Limit of quantitation = LOQ = s*10



Measuring interval

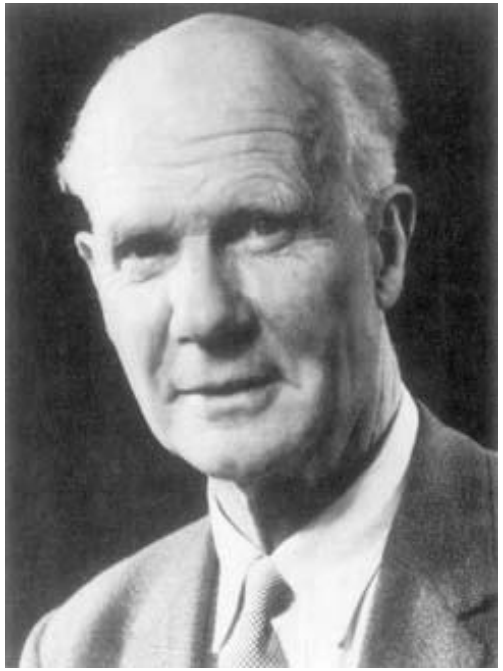
- Set of quantities of the same kind that can be measured by a given measuring instrument or measuring system with specified instrumental **uncertainty**, under defined **conditions**
- The measuring interval is dependent on the extent to which the measuring system can produce results which are **fit for the intended purpose**
- The lower limit of the measuring interval coincides with the limit of quantitation (LOQ) (the lowest concentration of the measurand that can be measured with an acceptable uncertainty) which is higher than the detection limit (LOD)

Measuring interval

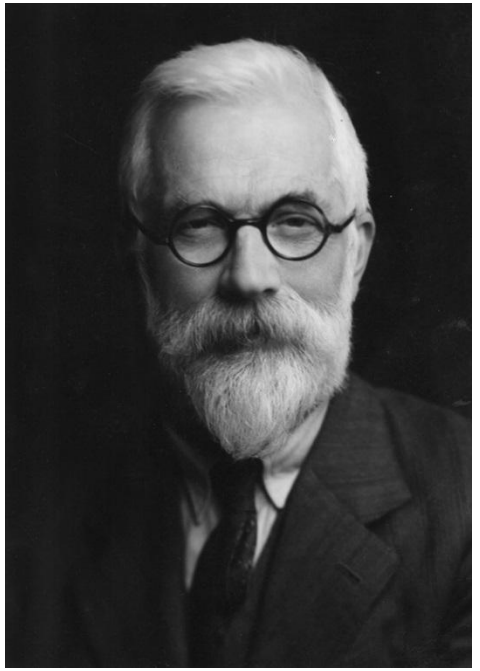


Theory of hypothesis testing

Jertzy Neyman



Egon Pearson

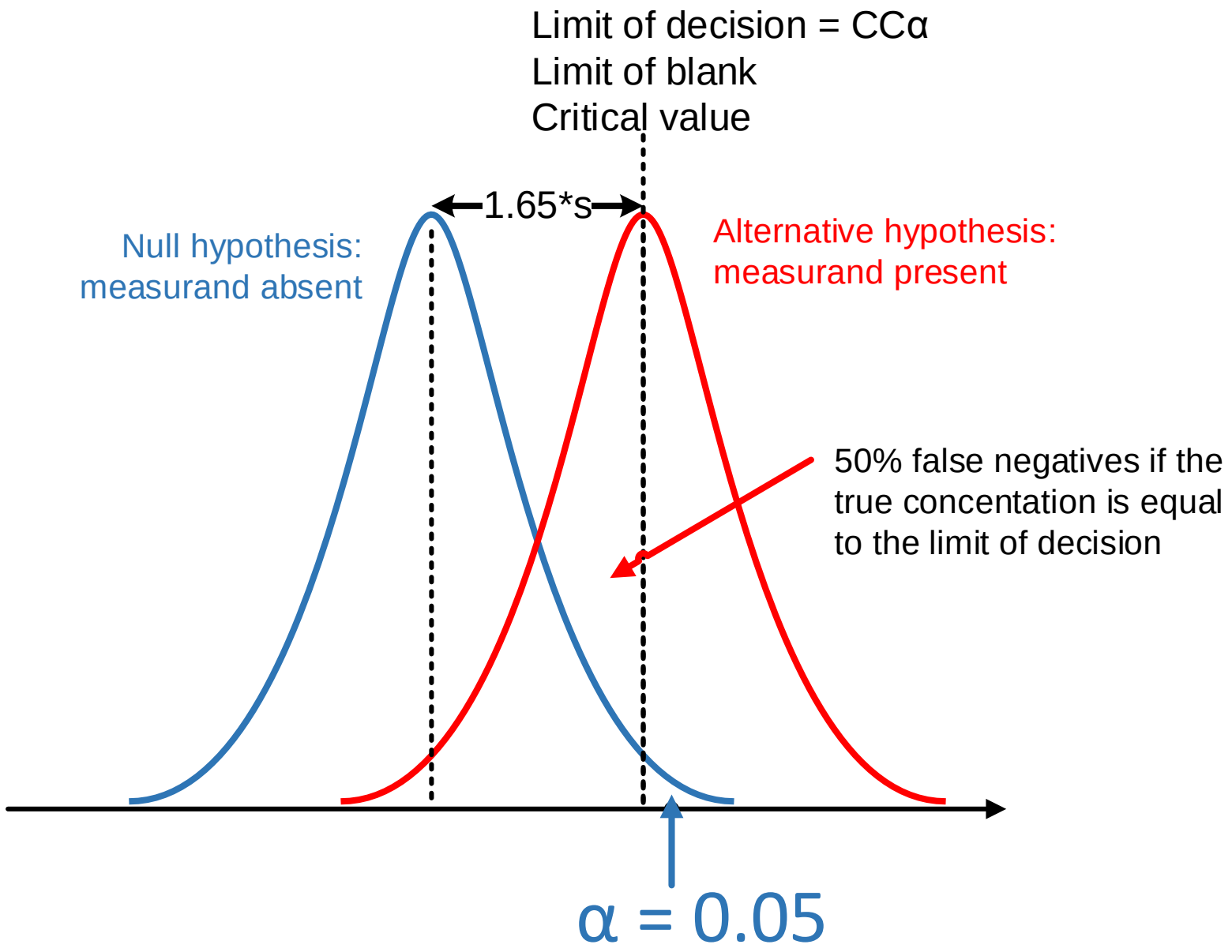


Roland Fisher

Erich L. Lehmann

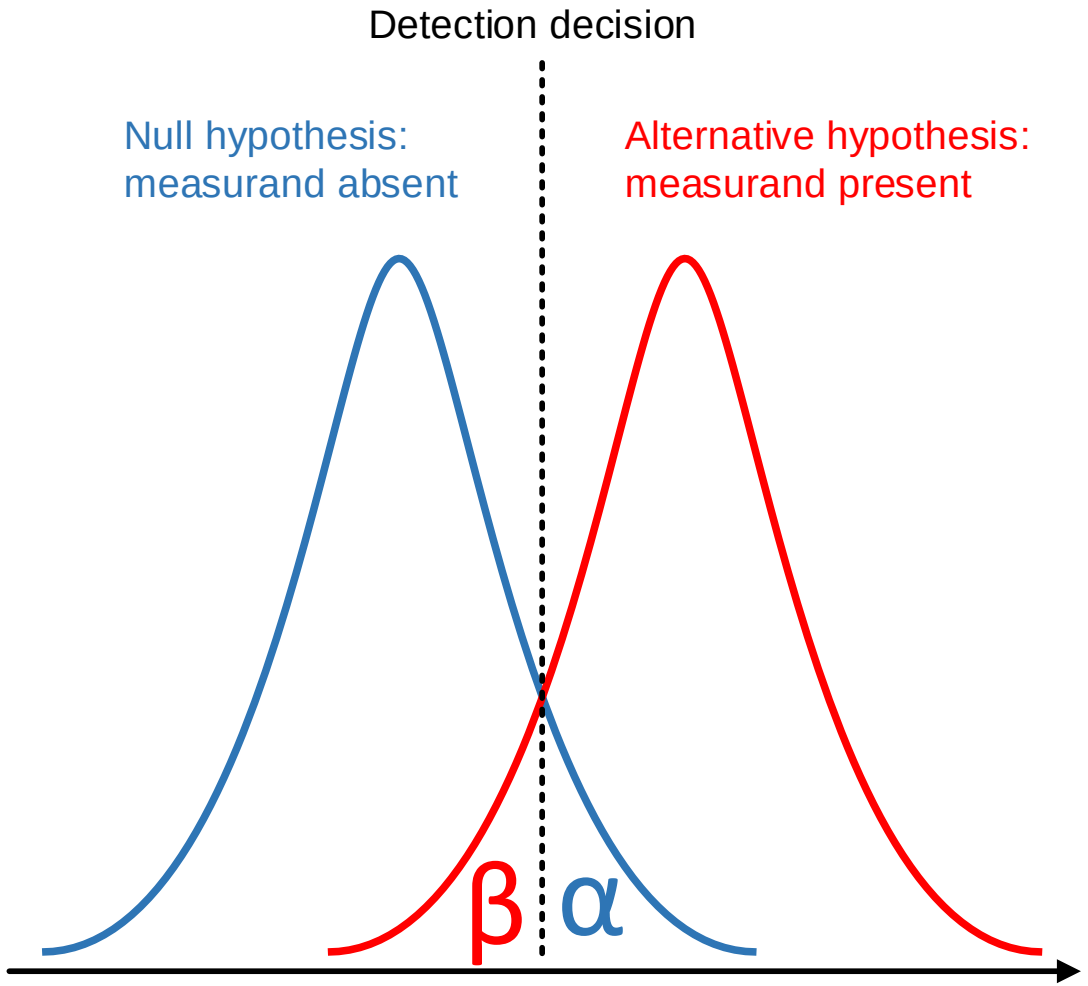
Fisher, Neyman, and the Creation of Classical Statistics

Springer


Limit of decision = CCα
Limit of blank
Critical value
1.65*s
Null hypothesis:
measurand absent
Alternative hypothesis:
measurand present
50% false negatives if the
true concentation is equal
to the limit of decision
α = 0.05

Errors in detection

- Alpha (**α**) error = **Type I** error = False positive – The probability of falsely rejecting the null hypothesis that a substance is not present when it is actually present
- Beta (**β**) error = **Type II** error = False negative – The probability of falsely accepting the null hypothesis that a substance is absent, when in fact the substance is present at the designated concentration



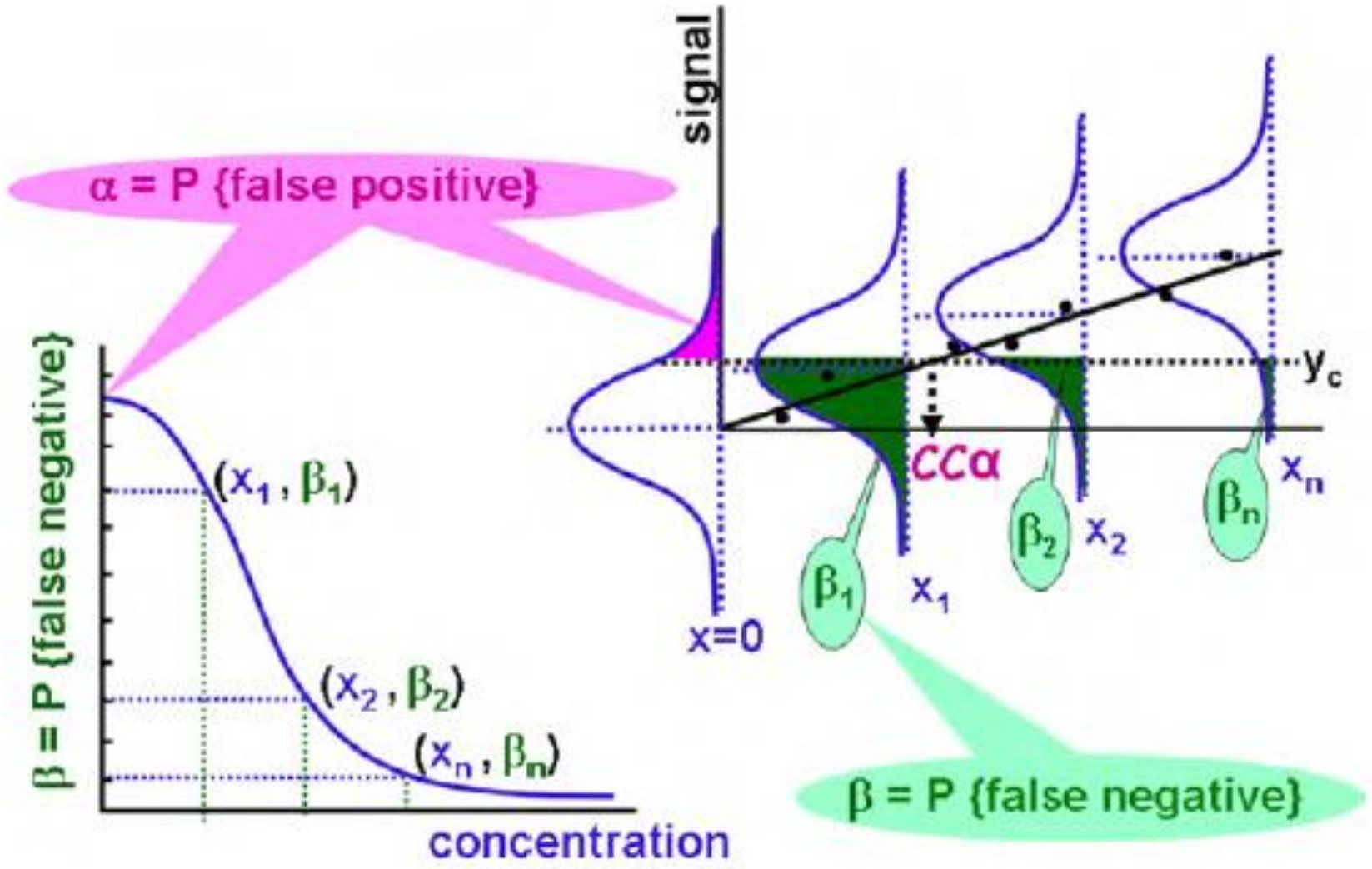


Fig. 3. Operating-characteristic curve of the hypothesis test to decide the presence of an analyte, Eq. (23). y_c is the decision signal. When the concentration is equal to CCα the probability of false negative, β, is 50% or even larger for a concentration, x_1, less than CCα.

M.C. Ortiz et al. / Analytica Chimica Acta *674 (2010) 123–142*

ISO-11843 standards (1997)

- The most common protocols for estimation of the LoD and LoQ including the ISO-standards assume that the instrument output at low concentrations can result in negative readings/concentrations


INTERNATIONAL STANDARD
NORME INTERNATIONALE

ISO 11843-1

Capability of detection —

Part 1:
Terms and definitions

Capacité de détection —

Partie 1:
Termes et définitions

ISO

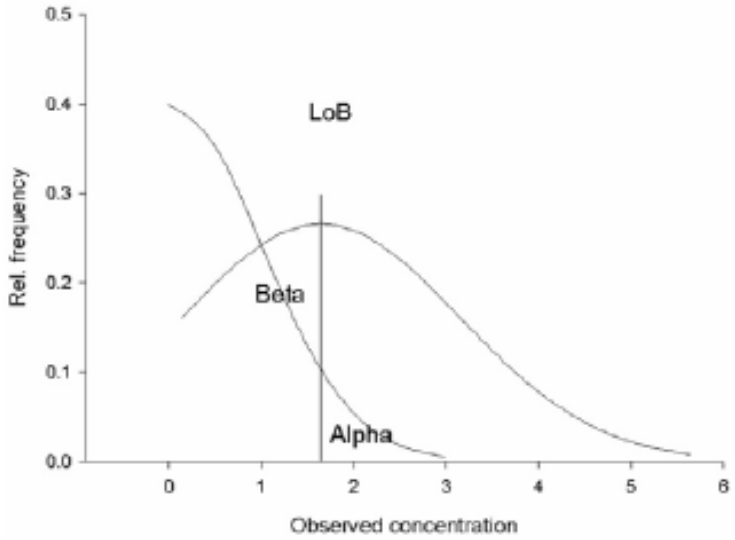


Figure 2a.

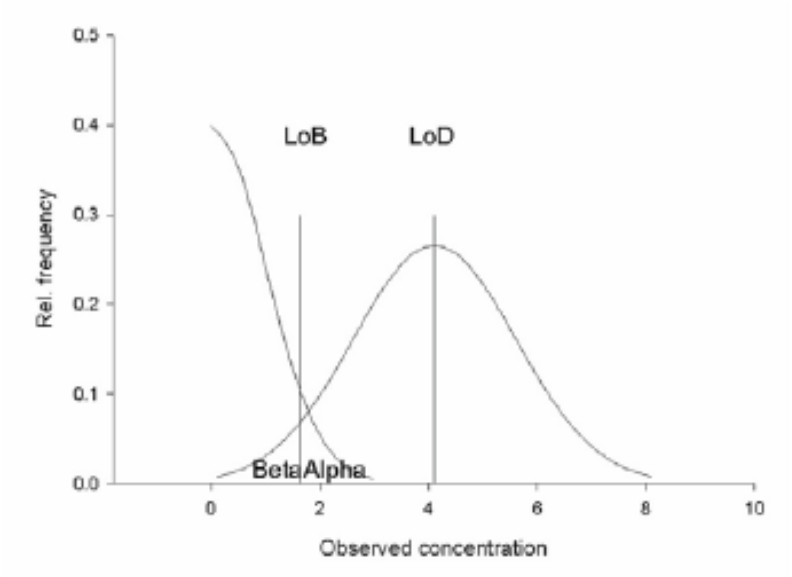


Figure 2b.

Figure 2. Distributions of Replicates for Blank Samples (left curve in both figures) and Two Hypothetical Low-Level Positive Samples (2a and 2b). When the actual concentration of analyte in the sample equals LoB, 50% of the measurements exceed LoB (a). With an actual sample concentration equal to LoD, (100% - β) (95%) of the sample measurements exceed LoB (b).

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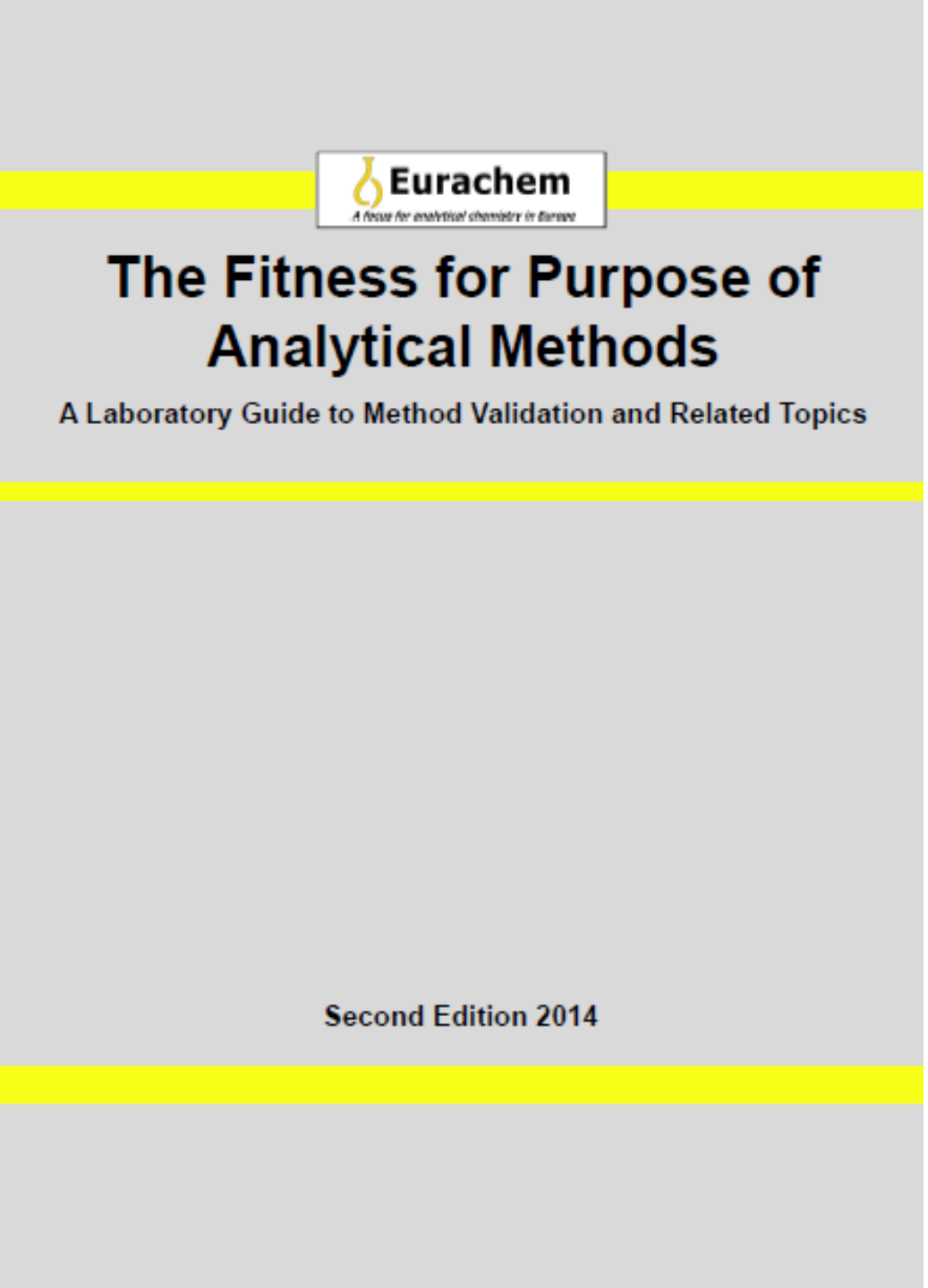
Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline



https://eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf

Limit of decision (CCα) – straightforward procedure applicable in clinical chemistry

- Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measurand
- Limit of decision = CCα = $s * 1.65$

Limit of detection (LOD= CCβ) – straightforward procedure applicable in clinical chemistry

- Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measurand
- Limit of detection = LOD = s*3.3

Limit of quantitation (LOQ) – straightforward procedure applicable in clinical chemistry

- Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measurand
- Limit of quantitation = LOQ = $s*10$
- The multiplier is usually 10, but that number is historical and arbitrary. Other values such as 5 or 6 are commonly used (based on ‘fitness for purpose’ criteria).
- Consider the fitness of purpose of using the concentration at which imprecision (coefficient of variation) of the method is 5%

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Review

Over a century of detection and quantification capabilities in analytical chemistry – Historical overview and trends

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ABSTRACT

The detection limit (L_D) and the quantification limit (L_Q) are important parameters in the validation process. Estimation of these parameters is especially important when trace and ultra-trace quantities of analyte are to be detected. When the apparatus response from the analyte is below the detection limit, it does not necessarily mean that the analyte is not present in the sample. It may be a message that the analyte concentration could be below the detection capabilities of the instrument or analytical method. By using a more sensitive detector or a different analytical method it is possible to quantitatively determine the analyte in a given sample. The terms associated with detection capabilities have been

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General Clinical Chemistry

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 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

Linnet & Kondratovich 2004

- In clinical chemistry, asymmetric, nongaussian blank distributions are common, and the calibration curve may be nonlinear.
- 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 + $c_\beta x SD_S$, where SD_S is the analytical SD of a sample with a low concentration c_β is approximately equal to 1.65 for a type II error of 5%.

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Receiver operating characteristic-curve limits of detection ☆

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ABSTRACT

Using a simple UV LED-excited ruby fluorescence measurement system, we demonstrate that it is easily possible to obtain unbiased detection limits, despite the system deliberately having non-linear response function and non-Gaussian noise. Even when the noise precision model is heteroscedastic, but otherwise only roughly linear, the receiver operating characteristic (ROC) method readily yields results that are in accordance with *a priori* canonical specifications of false positives and false negatives at the detection limit. The present work demonstrates that obtaining unbiased detection limits is not abstruse and need not be mathematically complicated. Rather, detection limits continue to serve a useful purpose as part of the characterization of chemical measurement systems.

Limit of Blank, Limit of Detection and Limit of Quantitation

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Clin Biochem Rev Vol 29 Suppl (i) August 2008 I S49

Summary

- Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) are terms used to describe the smallest concentration of a measurand that can be reliably measured by an analytical procedure.
- LoB is the highest *apparent* analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested.

  $LoB = mean_{blank} + 1.645(SD_{blank})$
- LoD is the lowest analyte concentration likely to be reliably distinguished from the LoB and at which detection is feasible. LoD is determined by utilising both the measured LoB and test replicates of a sample known to contain a low concentration of analyte.

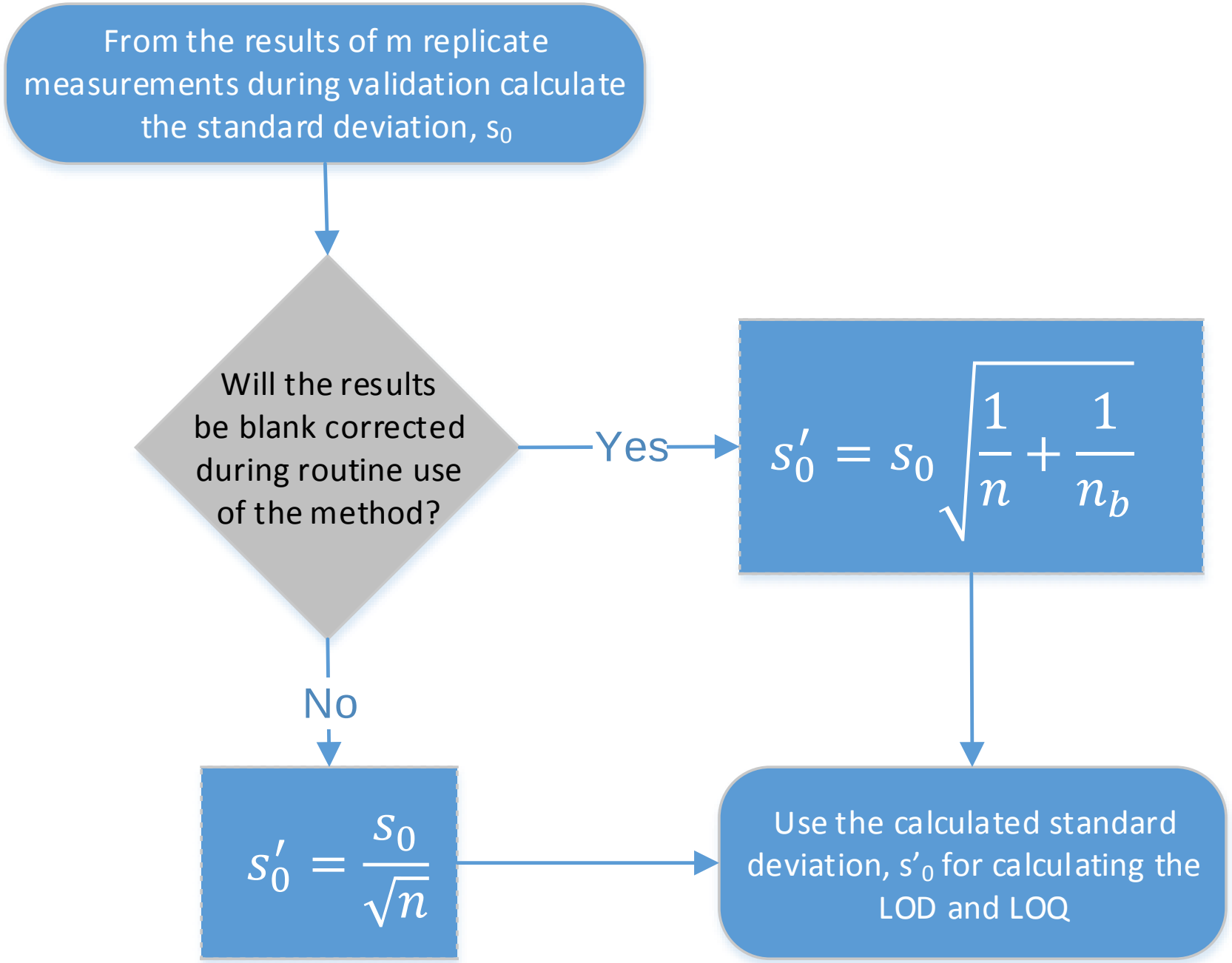
  $LoD = LoB + 1.645(SD_{low\ concentration\ sample})$
- LoQ is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met. The LoQ may be equivalent to the LoD or it could be at a much higher concentration.

s_0= The estimated standard deviation of m single results at or near zero concentration

s'_0= The standard deviation used for calculating LOD and LOQ

n = The number of replicate observations averaged when reporting results where each replicate is obtained following the ientire measurement procedure

n_b= The number of blank observations averaged when calculating the blank correction according to the measuremernt procedure



Calculation of LOD

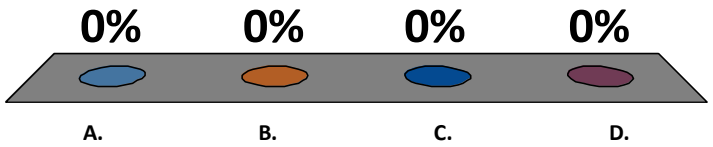
What to do	How many times	What to calculate from the data	Comments
a) Replicate measurements of blank samples, i.e. matrices containing no detectable measurand or replicate measurement of test samples with low concentrations of the measurand	10	Calculate the standard deviation, s0 of the results. Calculate s'_0 from s_0 as shown in Fig. 1. Calculate $LOD=3* s'_0$	
b) Replicate measurements of reagent blanks or replicate measurements reagent blanks spiked with low concentrations of measurand	10	Calculate the standard deviation, s_0 of the results. Calculate s'_0 from s_0 as shown in Fig. 1. Calculate $LOD=3* s'_0$	Approach b) is acceptable, when it is not possible to obtain blank samples or test samples at low concentrations. When these blanks do not go through the whole measurement procedure the calculation will give instrumental LOD

Calculation of LOQ

What to do	How many times	What to calculate from the data	Comments
a) Replicate measurements of blank samples, i.e. matrices containing no detectable measurand or replicate measurements of test samples with low concentrations of analyte.	10	Calculate s'_0 from s_0 as shown. Calculate LOQ as $LOQ = kQ \times s'_0$.	The value for the multiplier kQ is usually 10, but other values such as 5 or 6 are commonly used (based on ”fitness for purpose” criteria).
b) Replicate measurements of reagent blanks or replicate measurements of reagent blanks spiked with low concentrations of measurand.	10	Calculate the standard deviation, s_0 of the results. Calculate s'_0 from s_0 as shown in Fig. 1. Calculate LOQ as $LOQ = kQ \times s'_0$.	Approach b) is acceptable, when it is not possible to obtain blank samples or test samples at low concentrations. When these reagent blanks are not taken through the whole measurement procedure and are presented directly to the instrument the calculation will give the instrument LOQ.

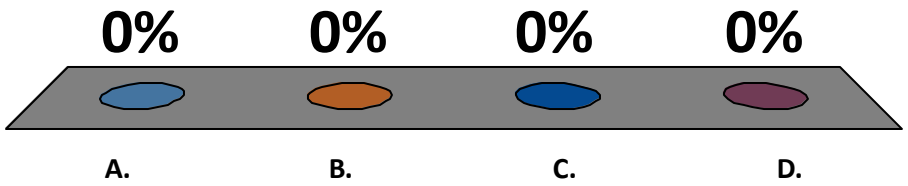
The limit of detection (LOD) is...

A. The lowest concentration that can be measured
B. The concentration at which we can decide whether an element is present or not
C. The point where we can just distinguish a signal from the background
D. The lowest concentration that should be repoted



The limit of quantification (LOQ)

A. Is a purely statistical concept
B. Is based on fitness of purpose criteria – only
C. Combines fitness of purpose criteria with statistical criteria
D. Can be lower than LOD



Take a home message

- Decision limit, limit of blank, critical value, CCα (term used in the EU directives)
  - Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measurand. Limit of decision = CCα = s*1.65
- LOD, Limit of Detection, minimum detectable value, detection limit, CCβ (term used in the EU directives)
  - Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measurand Limit of detection = LOD = s*3.3
- LOQ, quantification limit, quantitation limit, limit of quantitation, limit of determination, reporting limit, limit of reporting and application limit.
  - Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measurand Limit of quantitation = LOQ = s*10. Consider the fitness of purpose of using the concentration at which imprecision (coefficient of variation) of the method is 5%