# EP17 CLSI Guideline on Determination of Limits of Detection and Quantitation

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# Discussion Topics

- EP17: Analytical performance of a medical test at low levels of measurand:
  **Limit of Blank (LoB),**
  **Limit of Detection (LoD),**
  **Limit of Quantitation (LoQ),**

- Reporting and Interpreting Results

LoB and LoD are important metrological characteristics used to discriminate between the presence or absence of an analyte;
LoQ is used to reliably measure low levels of analyte.

# Problem Solving

- There are two true states of a system
  For every sample there are two possible states:
  "Analyte is absent (zero concentration)"
  "Analyte is present (any positive concentration)".

- Question: What results would mean that a sample has analyte?

- Binary decisions about true states of the measurand in the sample based on measurements:
  "Detected", "Not detected"

**•Distinguish "Result of measurement (device output)" vs "Concentration (true)"**

# Problem Solving

| | True State | |
|---|---|---|
| | Analyte is absent | Analyte is present |
| Not Detected (negative) | **Correct** | **Type II error** **(FN)** |
| Detected (positive) | **Type I error** **(FP)** | **Correct** |

**Type I error** is only possible if we obtained a positive result, and **Type II error** is only possible if we obtained a negative result.

# Analytical Limits at Low Levels: Limit of Blank

Blank samples – samples of pure matrix with no analyte present

Measurements of Blank samples

Normal distribution

- Consider different testing conditions

# Limit of Blank

In the clinical practice, the tests report usually only non-negative values (truncated at zero and thus non-normal).

$\alpha = 5\%$

LoB = 95$^{th}$ percentile of all measurements of blank samples

# Limit of Blank

Limit of Blank –a limit that is only exceeded with a probability of α for a Blank sample measurement.

**Measurement result ≤ LoB → "Not Detected"**
**Measurement result > LoB → "Detected"**

# Limit of Blank

- LoB is the lowest Result (measurment) that is unlikely to have been obtained from a blank sample.
- We consider a confidence level (95%) to define what is considered unlikely and what is not considered unlikely.
- So, any Result above LoB is considered as strong evidence that analyte is present (at least one molecule).
- Because of this implicit decision (that analyte is present), LoB sometimes called “decision threshold” (another term “Critical value”).

Unfortunately, LoB has sometimes confused with a limit of detection (LoD)

# Limit of Detection (LoD):

**The lowest amount of analyte in a sample that can be <u>detected</u> with stated probability (usually, 95%).**

The amount of analyte of LoB <u>is not</u> the LoD (**LoB < LoD**).

A sample with a concentration exactly at LoB will have **only a 50%** chance of returning a value that is interpreted as showing the presence of the analyte.

**With a sample having the concentration of LoD, only 5% of the measurements are erroneously declared "Not Detected" (not different from the Blank).**

# Limit of Detection (LoD):

- For sample with concentration at LoD, a lab test provides the most elementary form of information (a binary value) indicating whether the analyte is detected or not detected (at level of 95% confidence).

- At this concentration the lab test behaves as a qualitative test.

# Limit of Blank and Limit of Detection

**Limit of Blank (LoB):**

a limit that is only exceeded with a probability of 5% ($\alpha$ = 5%) for a blank sample measurement.

**Limit of Detection (LoD):**

the lowest concentration in a sample which is “detected” with 95% probability given 5% probability of falsely claiming “detected” for blank samples.

# Limit of Quantitation (LoQ)

Concept:
a Limit of Quantitation is the lowest concentration at which there is some confidence that the reported measurement is relatively close to the true value.

# Limit of Quantitation (LoQ)

**The lowest amount of an analyte in a sample that can be quantitatively determined with stated accuracy (as total error or as independent requirement for bias and/or precision).**

In reality, the LoQ is the lowest amount that can be reliably detected AND at which the total error meets requirements for accuracy.

The LoQ may be equivalent to the LoD or it could be at a much higher concentration.

$$LoB < LoD \leq LoQ$$

# Total Error

Error = Result of Lab test -True

# Total Error

Lab test

**Bias=0**

Pr (True ≤ Result -1.96*SD or
True ≥ Result +1.96*SD) =95%

**Total Error with 95% confidence =$TE_{95}$ = 1.96 *SD**

Meaning of $TE_{95}$:
95% of the time, the True value is not further than $TE_{95}$ from the Result of the Lab test.

# Total Error

## Bias is larger than SD

Pr (True ≤ Result –Bias -1.645*SD) =95%

**Total Error with 95% confidence =$TE_{95}$ = Bias+1.65 *SD**

Meaning of $TE_{95}$:
95% of the time, the True value is not further than $TE_{95}$ from the Result of the Lab test.

# Total Error

$TE_{95}$: 95% of the time, the True value is not further than $TE_{95}$ from the Result of the Lab test.

If Bias is small,
then Total Error $(TE)_{95} = |Bias| + 1.96 * SD$

If Bias is large,
then Total Error $(TE)_{95} = |Bias| + 1.65 * SD$

Bias is small (large): compare to SD

# %Total Error

**$\%TE_{95} = TE_{95}$ /True.**

$TE_{95}$ = |Bias| +1.96* SD
%Bias = Bias/True
SD/True=(SD/Mean) * (Mean/True) = %CV*(1+%Bias)

If %Bias is small,
then $\%TE_{95}$ = |%Bias| +1.96 *%CV* (1+%Bias)

If %Bias is large,
then $\%TE_{95}$ = |%Bias| +1.65*%CV* (1+%Bias)

# Allowable Errors

**Bias affects:**

- Location of reference interval;
- More than 5% will be outside of the reference limits;
- Comparison of the result to a decision threshold.

Both imprecision and bias affect clinical sensitivity and clinical specificity.

# Imprecision affects:

- The dispersion of a single test result;
- The dispersion of the reference interval;
- The change value that should be used to assess the significance of a change in serial results

Two measurements $X_1$ and $X_2$

Question: what changes in measurements would mean a true change in the measurand?

Two measurements $X_1$ and $X_2$

True change = 0 vs

True change > 0 (it can be true change ≠ 0)

*Example 1*

Consider that measurement X is Gaussian, SD is constant;

Difference $=X_2-X_1$

Two measurements $X_2$ and $X_1$ from the SAME sample:

Difference is $N(0, 2*SD^2)$

Pr (-1.96*sqrt(2)*SD≤Difference≤1.96*sqrt(2)*SD) =95%

**Threshold for Significant change=**1.96*sqrt(2)*SD= **2.77*SD**

$X_2-X_1$

For example, if significant change=0.7 then SD<0.25 (=0.7/2.77)

*Example 2*

Consider that measurement X is Gaussian, %CV is constant;

A) Relative Difference $=(X_2-X_1)/X_1$

B) Relative Difference $= (X_2-X_1)/((X_2+X_1)/2)$

Two measurements $X_2$ and $X_1$ from the SAME sample:

**%CV=10%**

$\Pr(-24.6\% \le (X_2-X_1)/X_1 \le 32.4\%) = 95\%$

**Threshold for Significant increase = 32.4%**

$\Pr(-28.0\% \le (X_2-X_1)/((X_2+X_1)/2) \le 28.0\%) = 95\%$

**Threshold for Significant increase = 28.0%**

If I observed an increase more than 28% then I am confident (95%) that there is a true increase.

Zhang L., Mathew T., Yang H., K. Krishnamoorthy, Iksung Cho I. (2009): Tolerance Limits for a Ratio of Normal Random Variables, Journal of Biopharmaceutical Statistics, 20:1, 172-184

# Allowable Errors

Consensus hierarchy of approaches to establish criteria for analytical Performance:
ISO Technical Committee 212 Working Group on Analytical Performance Goals Based on Medical Needs (Stockholm conference in 1999)

***1) Assessment of the effect of analytical performance on specific clinical decision-making.***

Well-designed clinical outcomes studies are the highest standard.
Clinical outcome studies are difficult to perform.

# Allowable Errors

***2) General quality specifications based on medical opinions (questionnaire)***

***3) General quality specifications based on biological variation.***

Example of logic - *Imprecision*

If the subject is undergoing monitoring of analyte, the variation from measurement to measurement consists of BOTH Analytical and Biological components (within-subject)).

$(SD_{Measurement})^2 = (SD_{within\text{-}subject})^2 + (SD_{analytical})^2$

If $SD_{analytical} \leq SD_{within\text{-}subject} * 0.5$

$(SD_{Measurement})^2 \leq (SD_{within\text{-}subject})^2 + (SD_{within\text{-}subject})^2 * 0.25$

→ $SD_{Measurement} \leq SD_{within\text{-}subject} *sqrt(1.25)$

$SD_{Measurement}$ only exceeds $SD_{within\text{-}subject}$ by less than **12%.**

# Allowable Errors

***4) General quality specifications based on fractions of reference intervals (e.g.,Tonk's limits)***

***5) General Guidelines from national or international expert groups***

***6) Specifications from set by regulation or other accrediting bodies***

Proficiency testing requirement for acceptable performance

***7) Published data on the "state- of- the- art"***

# LoQ

Example : Assay for viral load

Requirements for LoQ

a) Total Error should be less than 0.7

b) Test should have a difference more than 0. 7 as an indicator that there is a true change (SD≤0.25).

LoQ is the lowest concentration with these requirements.
Bias=0.1, SD =0.24 (TE=0.57)

# Example

Assay for the quantitation of viral load in the serum

**Limit of Blank** ($\alpha$=5%, samples from the negative patients, blood donors)

**2,500 copies/mL**

**Limit of Detection** ($\beta$ = 5%)

**5,500 copies/mL**

**Limit of Quantitation** (requirement for accuracy were %CV$\leq$ 20%, %bias $\leq$2%)

**10,200 copies/mL**

# Example 3

If it is <u>impossible</u> to evaluate bias

LoQ: precision is acceptable

deviation from linearity is acceptable

LoD

LoQ
%CV≤20%

In labeling, ALWAYS provide requirements for LoQ.

# Reporting the Results

Reports of results depend on where the observed result lies relative to the analytical limits at low levels.

**LoB and LoQ** are important limits for reporting the results

# Interpretation of the Results

1) **Result ≤ LoB,**

- "Not Detected",
- concentration can be zero or any positive below **LoD**

2) **LoB<Result ≤LoD**,

- "Detected",
- concentration is positive (can be as low as <LoB, can be as high as above LoD),
- if sample is tested a second time, can be "Not detected"

3) **LoD<Result ≤LoQ,**

- "Detected",
- concentration is positive (higher than LoB),
- if sample is tested a second time, it will be "Detected"

4) **Result >LoQ**,

- numerical value
- the true value of the sample is not further than $TE_{95}$ from the Result for this sample with 95% confidence

# Second Edition of EP17

EP17-A2 “Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition”, June 2012

Study designs are the same as in EP17-A

Within-laboratory variability

(4 different samples, 3-5 days, 2 lots)

# Notes

- Problems with studies design
  - blank samples (zero calibrator??)
  - sources of variability (1 run???)
  - if PMA - different lots??

- Calculations are usually correct

- Labeling: information about requirements for LoQ??

  Advice: if there is space in labeling, provide
  - definition of LoB and value,
  - definition of LoD and value and
  - definition of LoQ and value.

# References


1. **CLSI document EP17-A2 (2012): *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideleine – Secnd Edition***

2 Currie A.L. (1968). Limits for qualitative detection and quantitative determination. *Analytical Chemistry*. Vol. 40 (3): 586-593.

3. Linnet K., Kondratovich M. (2004). Partly nonparametric approach for determining the limit of detection. *Clinical Chemistry.* 50: 732-740.

4. 24 papers about The Stockholm Consensus Conference on Quality Specifications in Laboratory Medicine published in Scandinavian Journal of Clinical and Laboratory Investigation, 1999

# Thank you for your attention !!!

Questions!

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