



***CLSI Guideline EP17 and EP12:
Limits of Detection and
Performance Near Cutoff for
Qualitative Tests***

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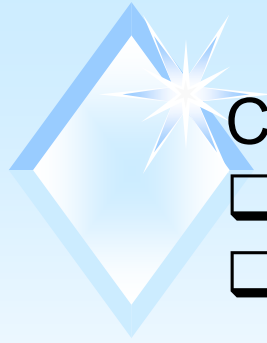


Discussion Topics

- ☐ EP12: Concepts C_5 and C_{95}
- ☐ EP17: Concepts of LoB (Limit of Blank) and LoD (Limit of Detection)
- ☐ How concepts of LoB and LoD are related to C_5 and C_{95}
- ☐ EP12: Laboratory Experiment for an Imprecision Curve (section 8.3, page 12-14).

1. *CLSI document EP12-A2 (2008): User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition*
2. *CLSI document EP17-A2 (2012): Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*

Qualitative test



Consider

- ☐ Test with two outcomes (pos, neg)
- ☐ Test has a numeric result

Cutoff

Example: numeric result is S/Co and Cutoff=1.0
sample from a patient

if $S/Co \geq 1.0$ then Test Outcome = Pos;

if $S/Co < 1.0$ then Test Outcome = Neg

Qualitative test discriminates between two classes:
“Non-diseased” and “Diseased”

←

A) Non-diseased subject
samples have detectable
analyte concentrations

→

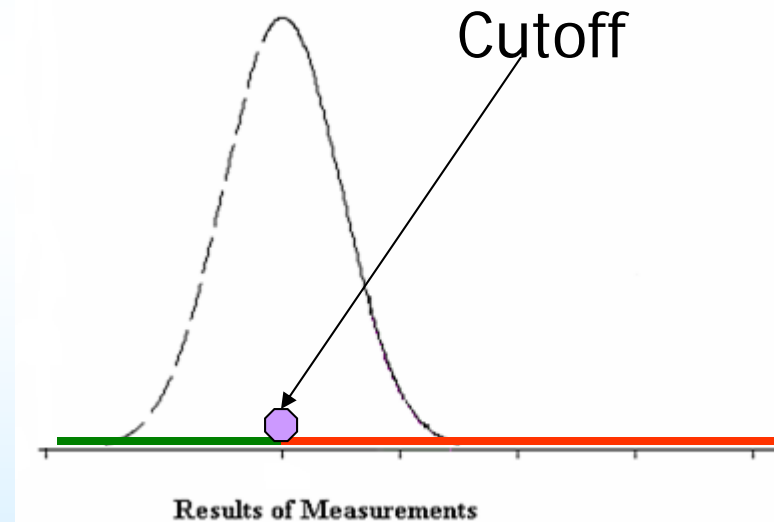
B) Non-diseased subject
samples have true ZERO
analyte concentrations

Scenario A (Non-diseased subject samples have detectable analyte concentrations)

Cutoff is chosen to optimize clinical sensitivity and clinical specificity based on a clinical data set.

Example: HPV test (Signal, cutoff=1.5)

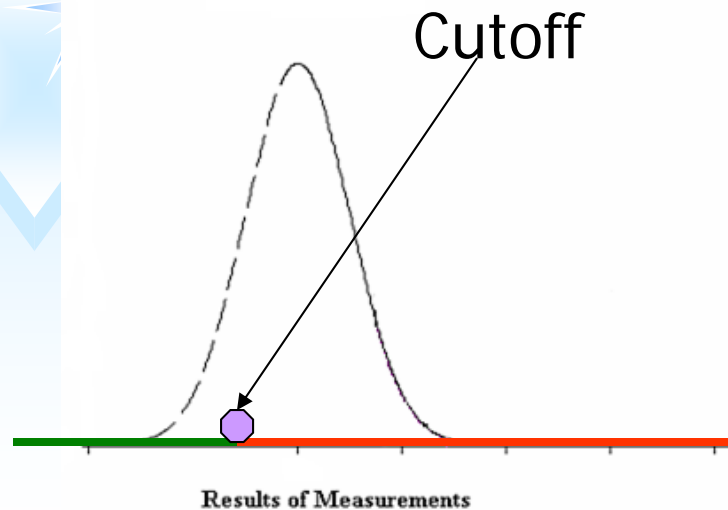
- ❖ Consider different testing conditions
 - a large series of repeated tests
- ❖ Concentration=average of numeric results
- ❖ A sample with concentration =Cutoff is 50% positive and 50% negative (C_{50})
- ❖ Distinguish between “result of measurement” vs “concentration”



See CLSI EP12-A2

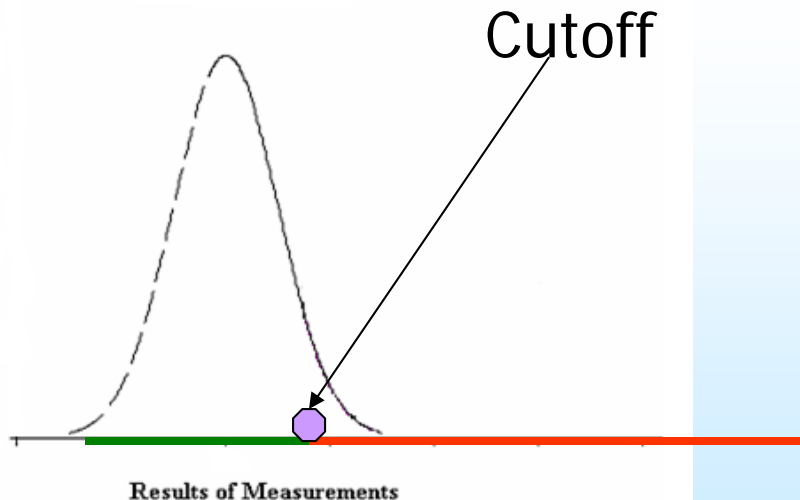
For sake of simplicity, consider that results of measurements have normal distribution

1) Consider a sample with concentrations above the Cutoff



Percent pos results
 $>50\%$

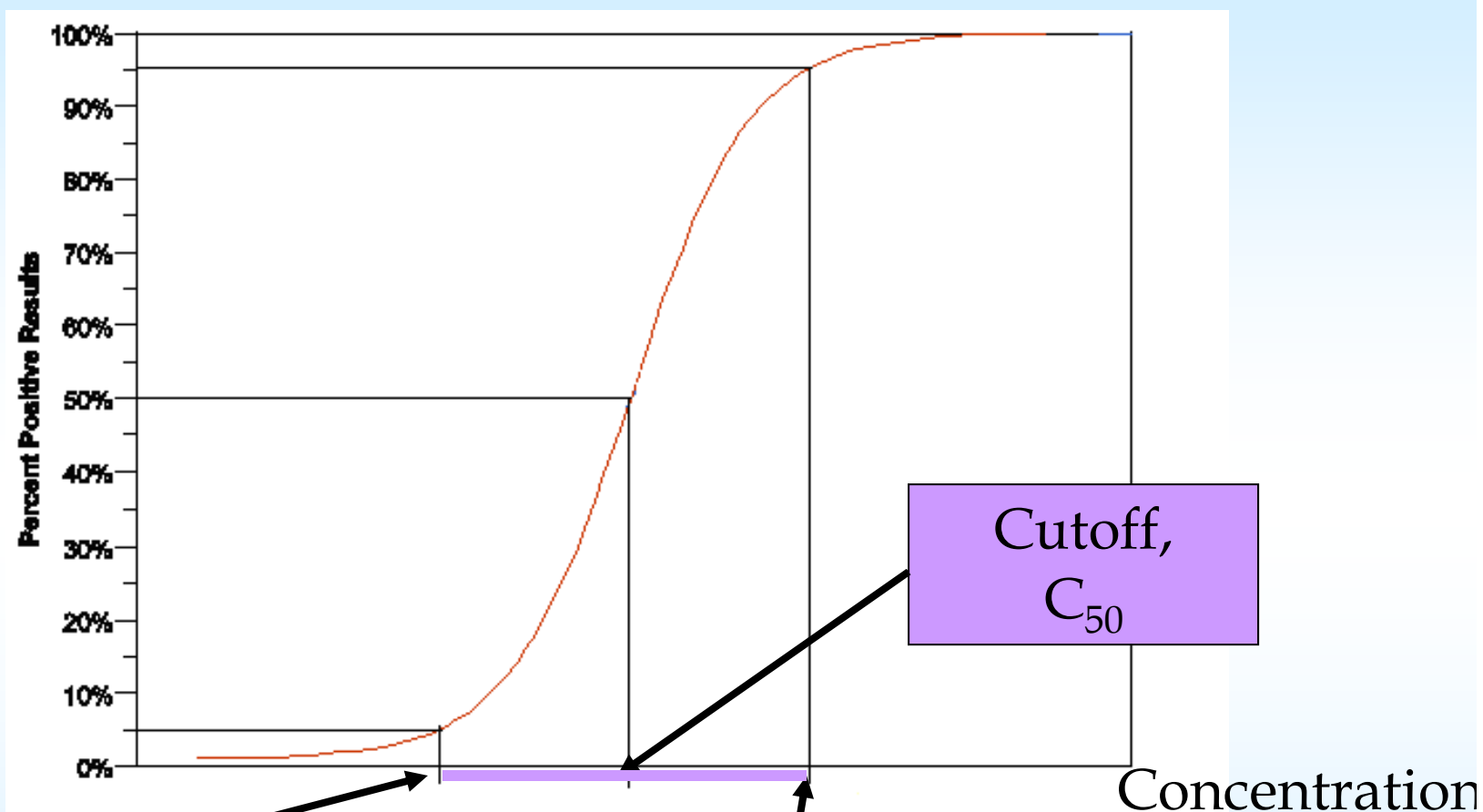
2) Consider a sample with concentrations below the Cutoff



Percent pos results
 $<50\%$



Imprecision Curve



C_5 and C_{95} are important performance characteristics for a qualitative test; length of the interval is $C_{95}-C_5$

EP17: Concepts of LoB (Limit of Blank) and LoD (Limit of Detection)

- ❑ For every sample there are two possible states:

“Analyte is absent (zero concentration)”

“Analyte is present (any positive concentration)”.

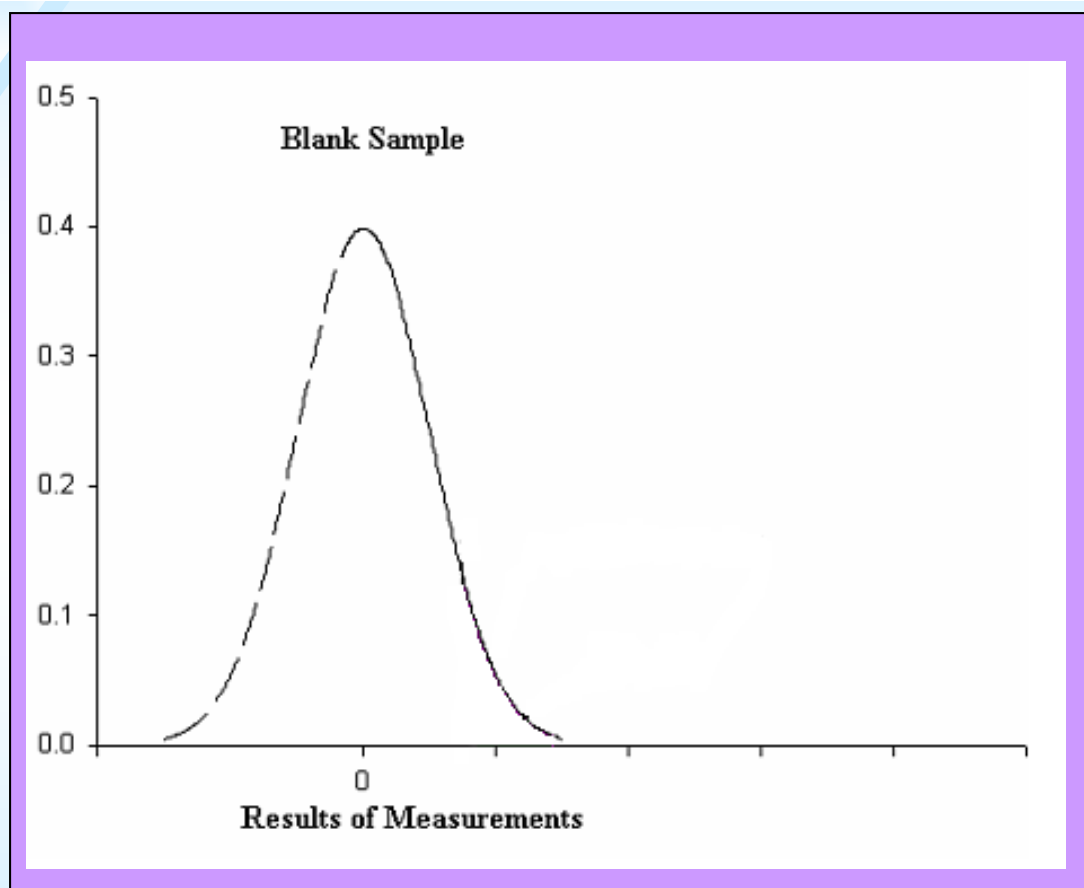
- ❑ Question:
What results constitute that a sample has analyte?

- ❑ Binary decisions (“Not Detected”, “Detected”) about true states of the measurand in the sample based on one measurement

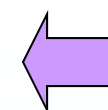
	True State	
	Analyte is absent	Analyte is present
Not Detected (negative)	Correct	Type II error (FN)
Detected (positive)	Type I error (FP)	Correct

Analytical Limits at Low Levels: Limit of Blank

Blank samples – patient samples with zero concentration of analyte



Measurement
of Blank
samples

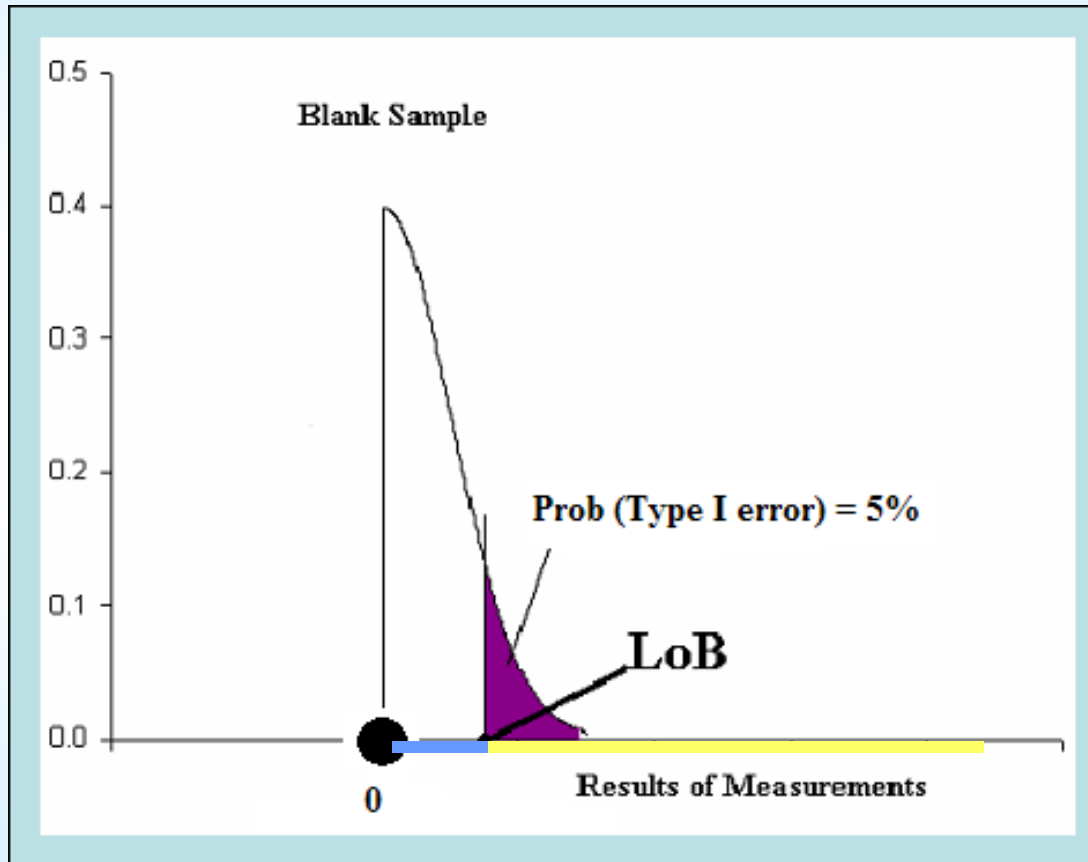


Normal
distribution

- Consider different testing conditions
- Distinguish “Result of measurement (device output)” vs (true) concentration

Limit of Blank

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



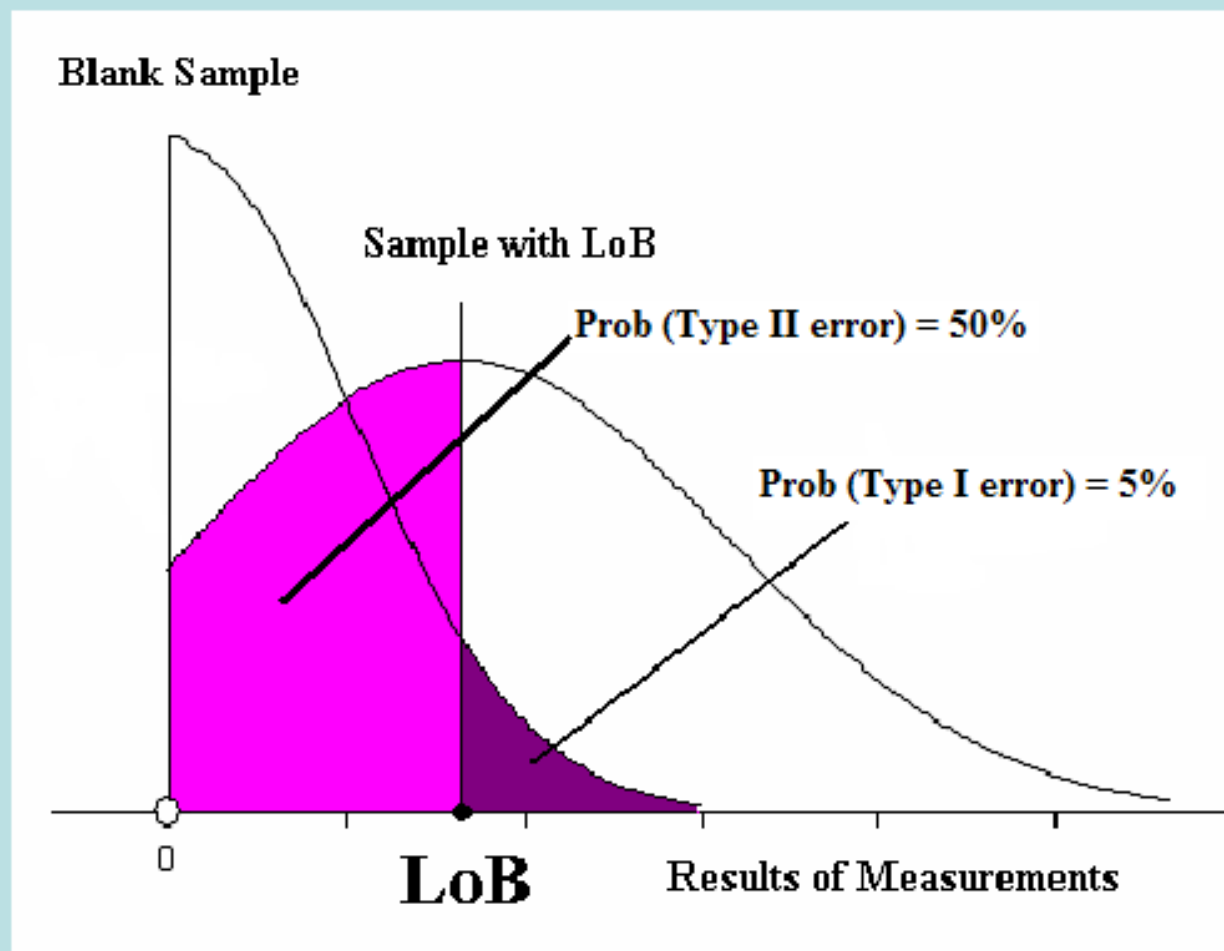
$\alpha = 5\%$
LoB = 95th percentile of
all measurements of
blank samples

Measurement result \leq LoB \rightarrow "Not Detected"
Measurement result $>$ LoB \rightarrow "Detected"

Limit of Detection (LoD):

The lowest actual amount of analyte in a sample that can be detected with stated probability (usually, 95%)

The amount of analyte of LoB is not the LoD (**LoB < LoD**)

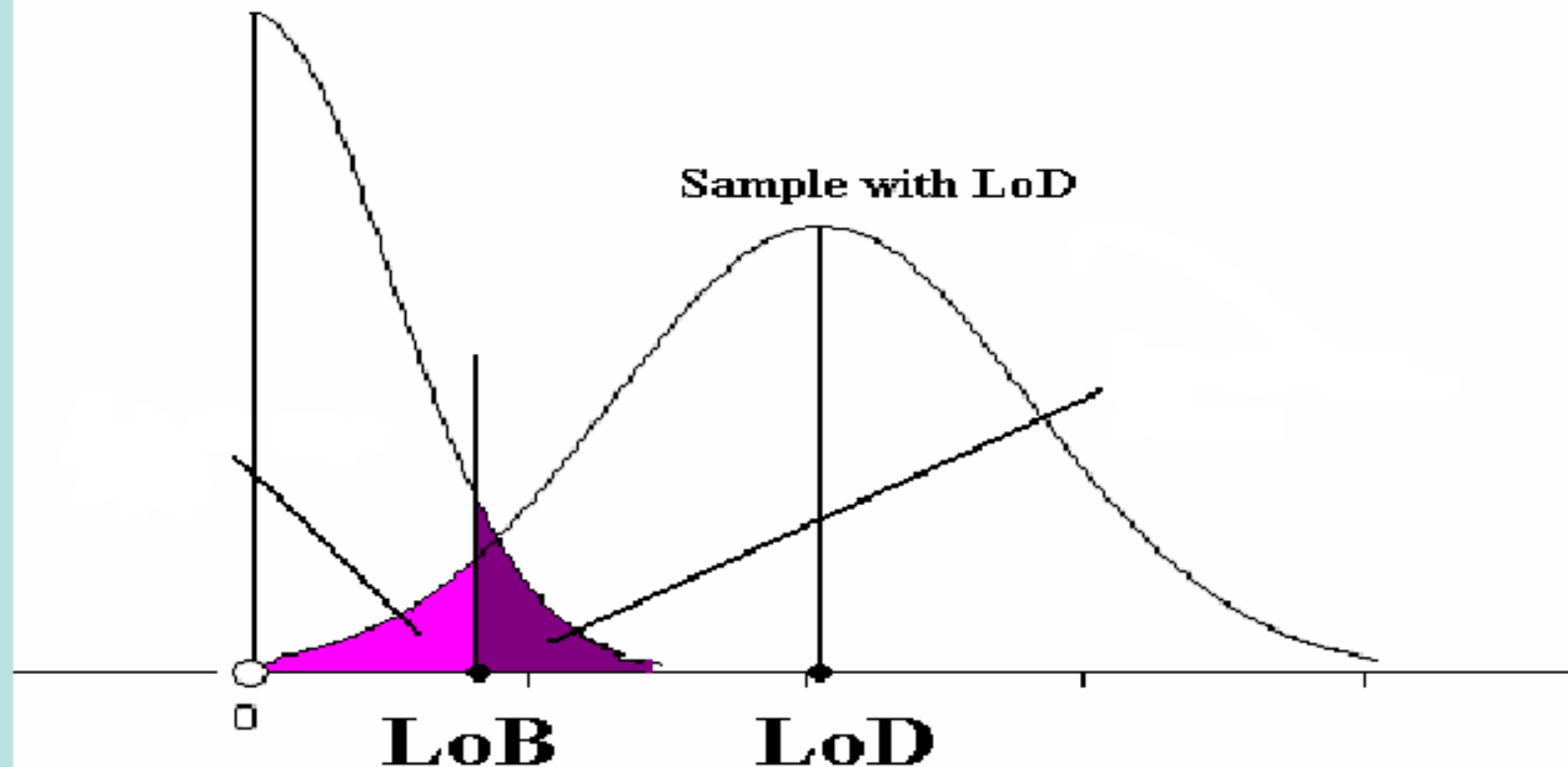


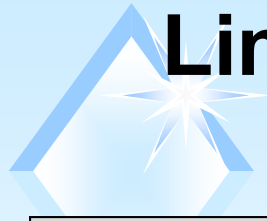
A sample with a true concentration = LoB will have **50% “Detected”** and 50% “Not Detected”

With a sample having the actual concentration of LoD, only β of the measurements are erroneously declared “Not Detected” (not different from the Blank)

Blank Sample

Sample with LoD





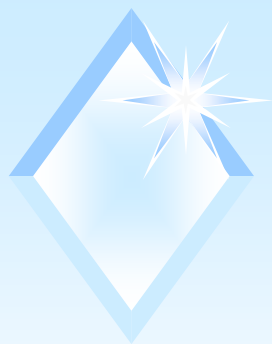
Limit of Blank and Limit of Detection

Limit of Blank (LoB):

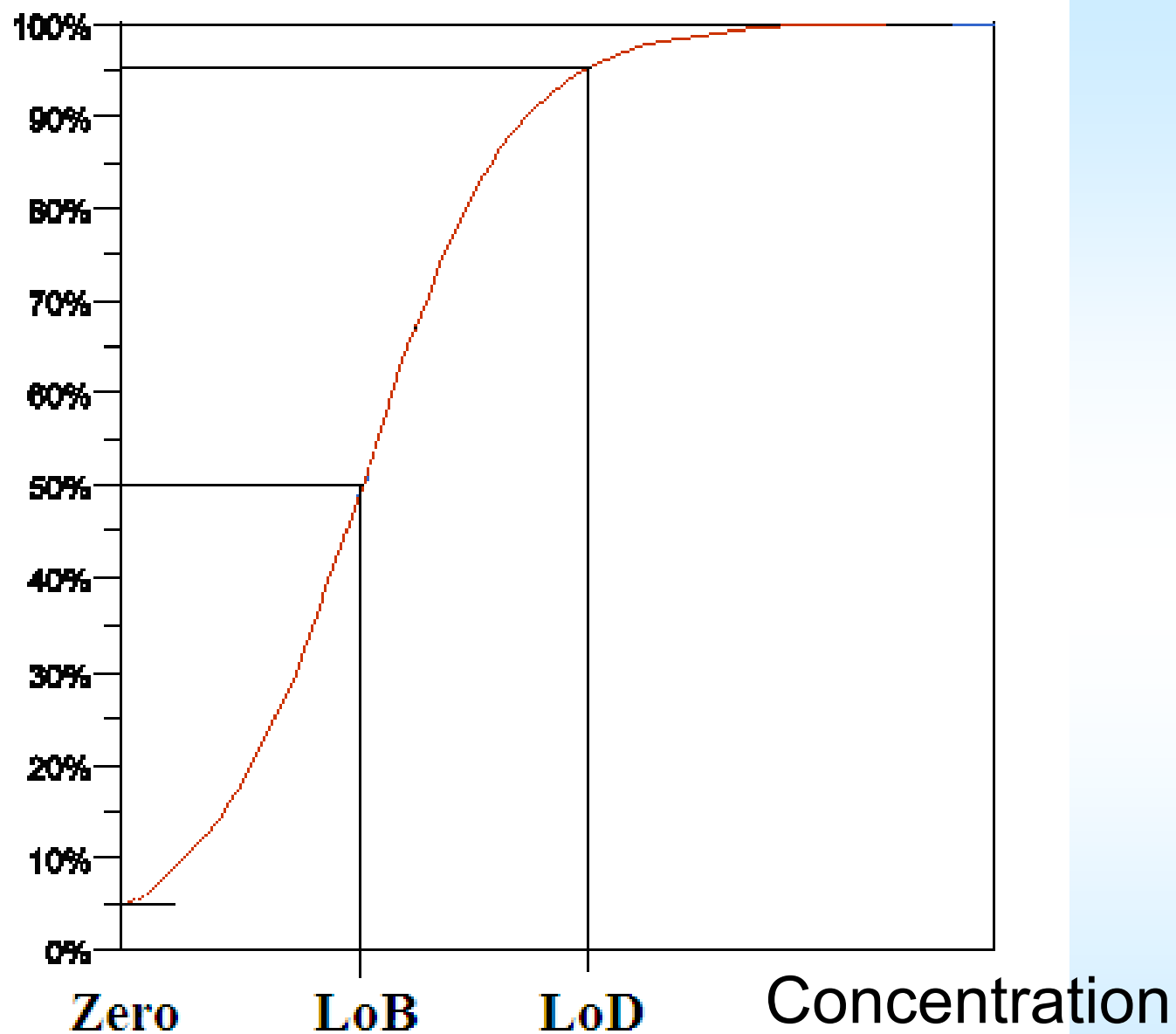
a limit that is only exceeded with a probability of 5% ($\alpha = 5\%$) for a blank sample measurement.

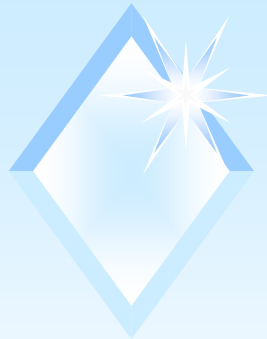
Limit of Detection (LoD):

the lowest actual (true) concentration in a sample which is “detected” with probability $1-\beta$ (usually, 95%) given a probability α (usually, 5%) of falsely claiming “detected” for blank samples.

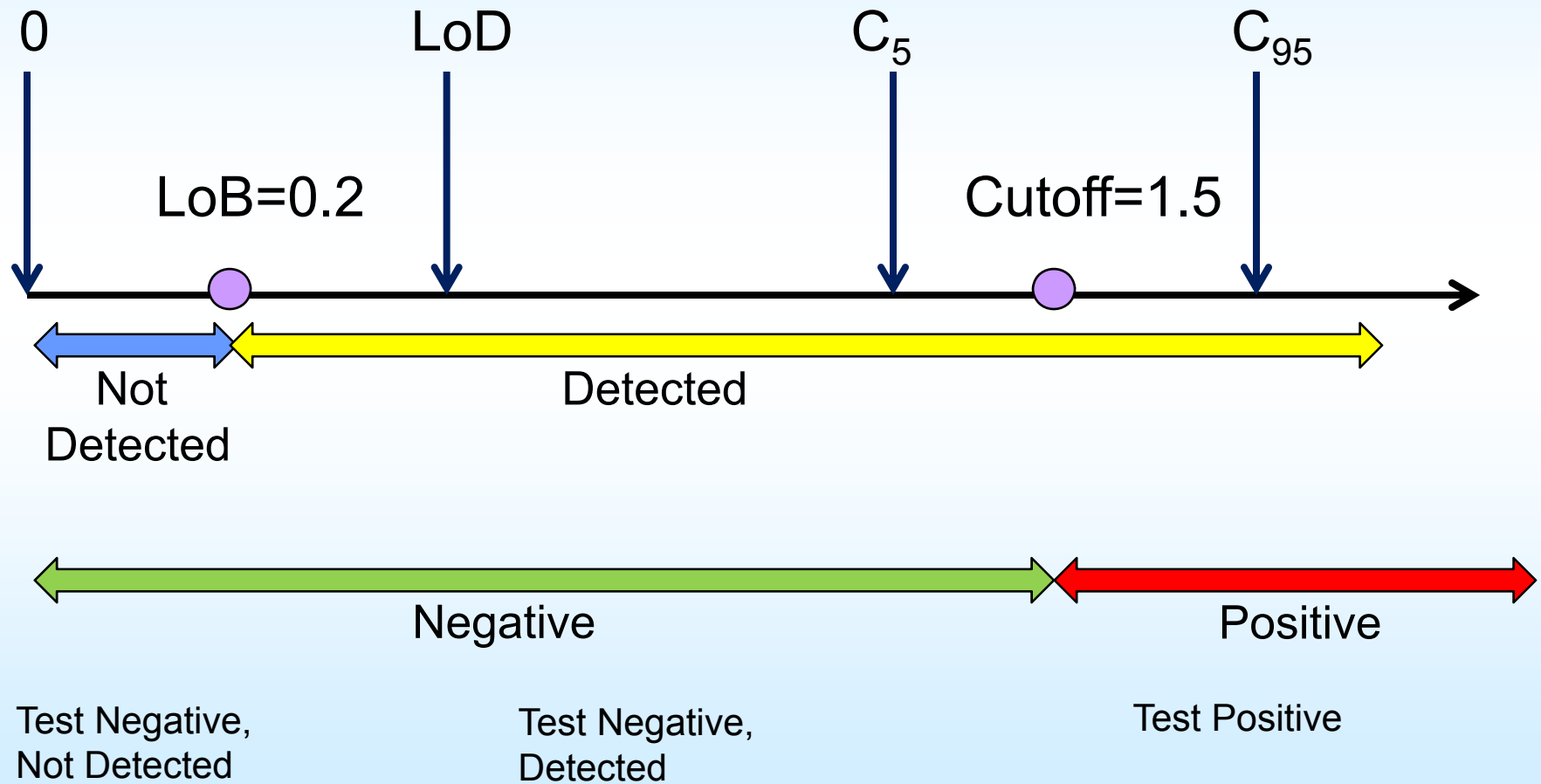


Percent
of “Detected”





Example: HPV test



If one has numerical signals

How concepts of LoB and LoD are related to C_5 and C_{95}

A) Clinical cutoff is higher than the analytical cutoff (LoB)

(Non-Diseased subjects have some amount of analyte)

Cutoff = C

B) Clinical cutoff is the analytical cutoff (LoB)
(no analyte vs analyte present)

Cutoff = LoB

B.1) LoB > 0

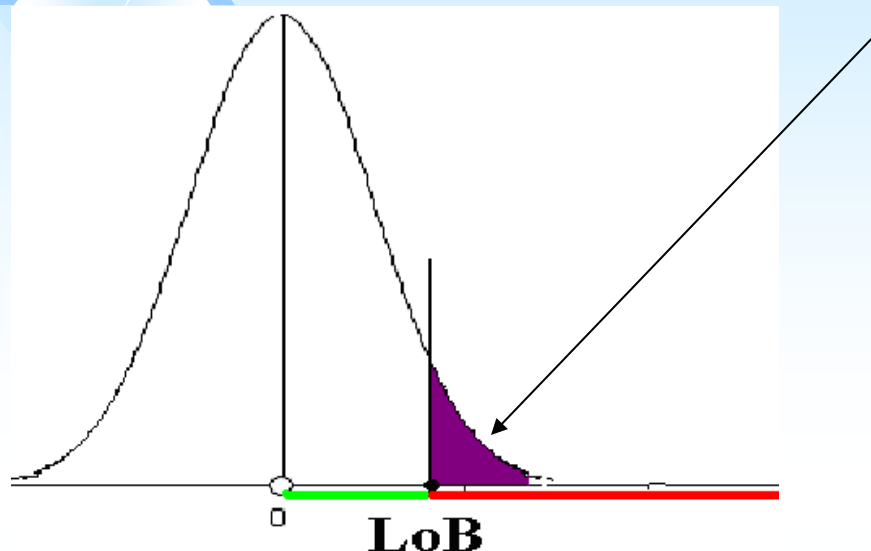
Samples with zero concentration have noisy results

B.2) LoB = 0

For example:
Ultrasensitive assay
Samples with zero concentration have negative results

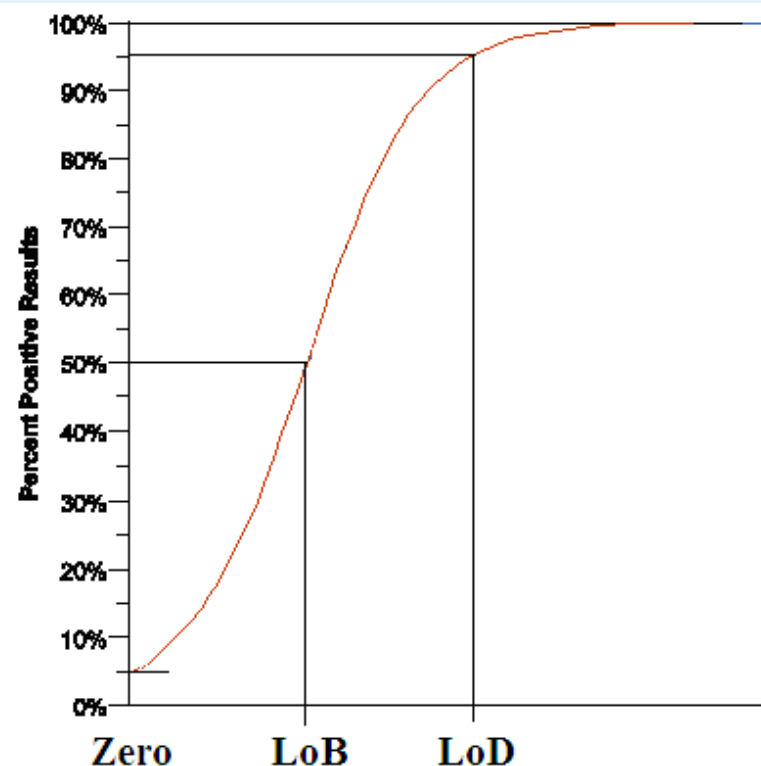
Scenario B.1: Cutoff = LoB > 0

Samples with zero concentration have noisy results



- Percent of positive results for the samples with zero concentration is 5%;
- Percent of positive results for the samples with LoD is 95%;

LoD is used for metrological interpretation of “Negative” result (below the cutoff = LoB).



$$C_5 = \text{zero}, C_{95} = \text{LoD}$$

Scenario B.2: Cutoff = LoB=0

Samples with zero concentration have negative results

Ultraseensitive test

Example of Ultraseensitive Test: PCR

Consider that Cutoff =45 cycles;

For sample: Cycles <45, result = positive

Cycles = 45, result = negative

- ❑ If samples are truly negative, all results are “Negative” => Type I error is close to zero.
- ❑ Cutoff of 45 corresponds to zero concentration

Ultrasensitive test

❑ Zero

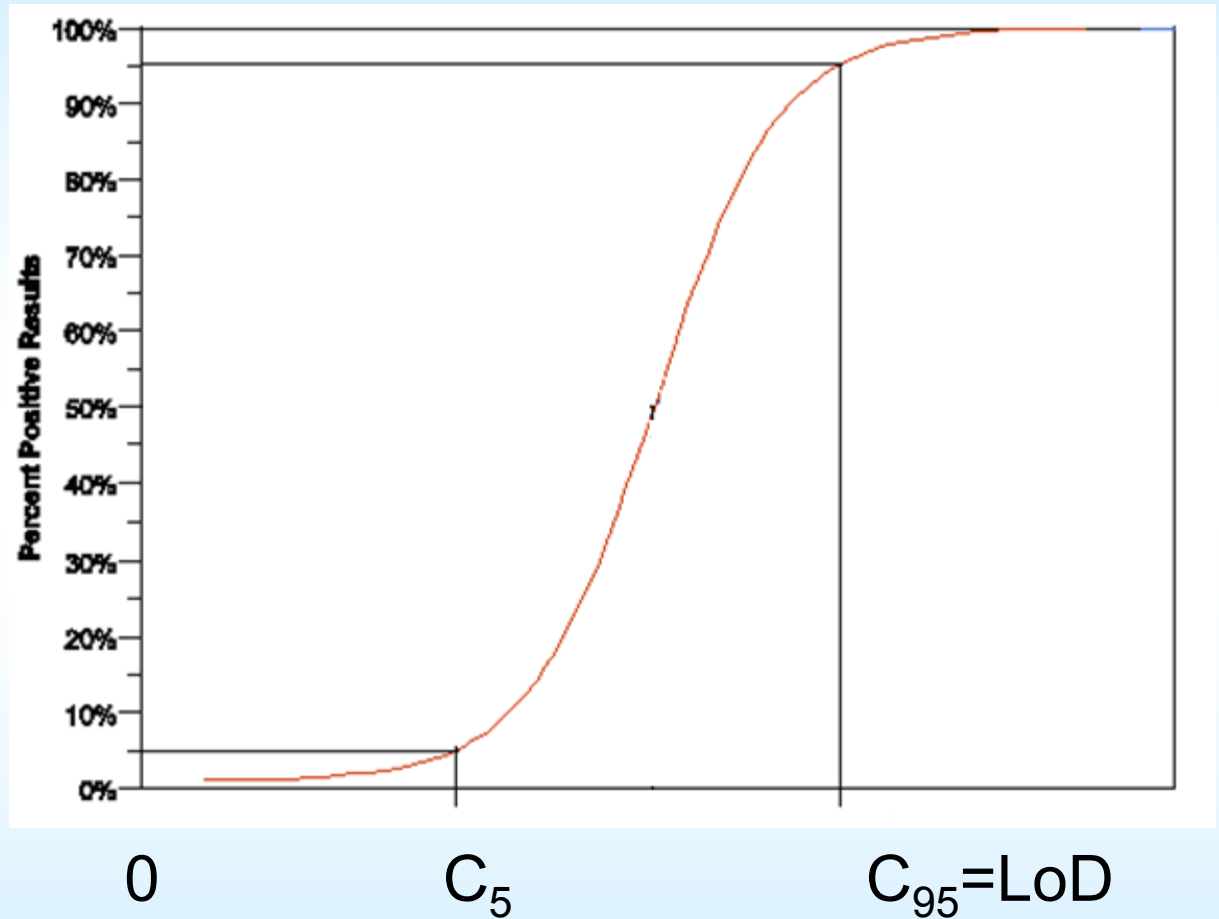
concentration
has zero percent
positive results;

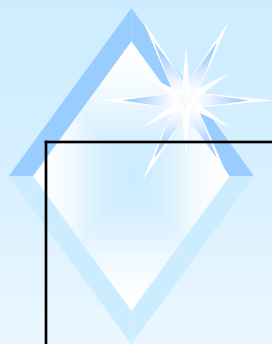
❑ C_{95} (LoD)-

concentration
corresponding to
 $C_t=38$.

❑ Concentration

corresponding to
 $C_t=45$ is close to
zero; $LoB=0$

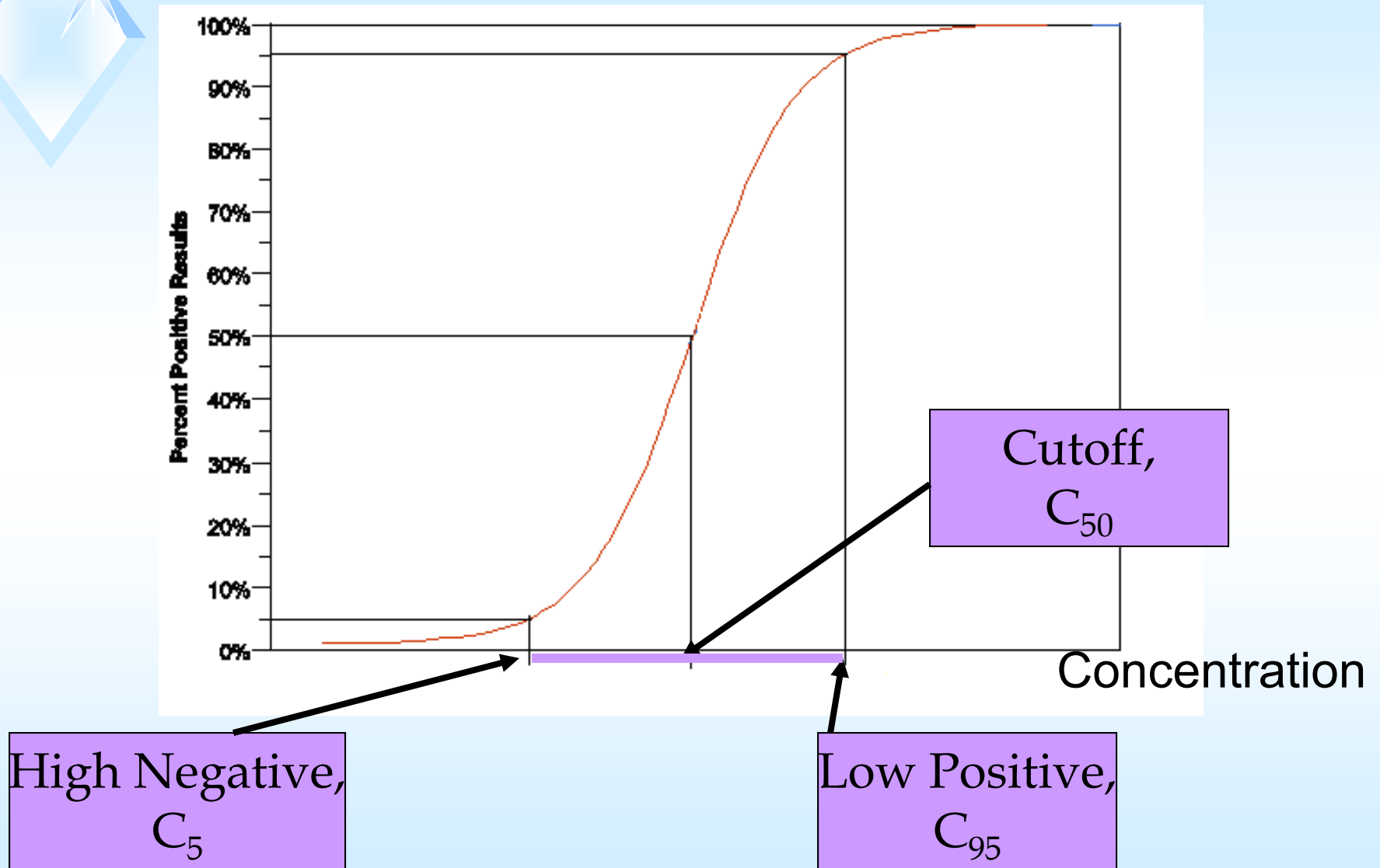




	High Negative	Low Positive	Comment
Scenario A (Clinical cutoff is higher than analytical cutoff)	C_5	C_{95}	$C_{50} = C$ (cutoff) $C_5 < C < C_{95}$
Scenario B.1 (Clinical cutoff = analytical cutoff, $LoB > 0$)	$C_5 = 0$	$C_{95} = LoD$	$C_{50} = C = LoB$ $C_5 < C < C_{95}$
Scenario B.2 (Clinical cutoff = analytical cutoff, $LoB = 0$)	C_5	$C_{95} = LoD$	$LoB = 0 = C_0$ $0 < C_5 < C_{95}$

Contact OIR for more discussions about samples near the cutoff.

❑ EP12: Laboratory Experiment for an Imprecision Curve
(section 8.3, page 12-14).



Simple experiment that provides information about C_5 and C_{95} .

Study Design:


Prepare 3 samples

- 1) Sample C_{50} =Cutoff
- 2) Sample S1 with concentrations 20% below C_{50}
- 3) Sample S2 with concentration 20% above C_{50}

Test every sample 40 times under different testing conditions

Note:

- One can choose not $\pm 20\%$ but $\pm 10\%$ or $\pm 30\%$
- One can have different number of replicates
- Different testing condition: sources of variability as different operators, different temperature or light conditions, different lots, different reading times and so on.



1) Is the cutoff as stated in the labelling,
was the sample C_{50} prepared correctly?

One anticipate percent of positive for this
sample around 50%.

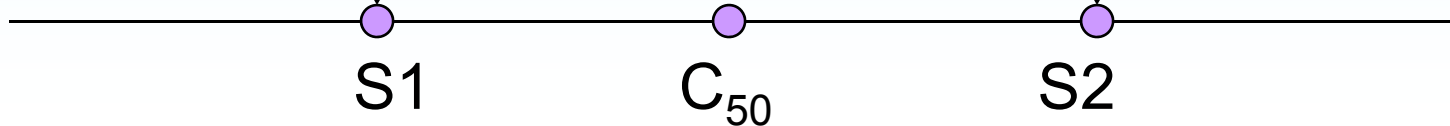
C_{50} : percent of positive
from 35% (14/40) to 65% (26/40)
then it is OK.

2) Does interval $[S1, S2]$ contain interval $[C_5-C_{95}]$?

a)

$\leq 87.5\%$ neg
(35/40)

$\leq 87.5\%$ pos
(35/40)

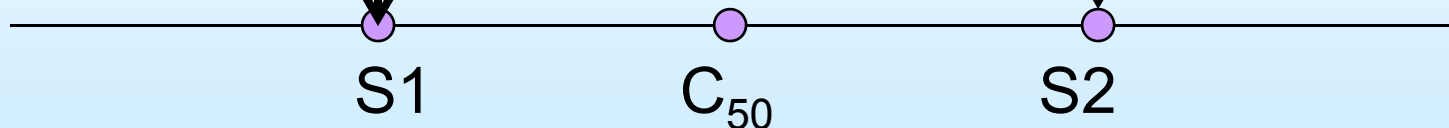


Conclusion: $[C_5-C_{95}]$ is wider than $[S1, S2]$

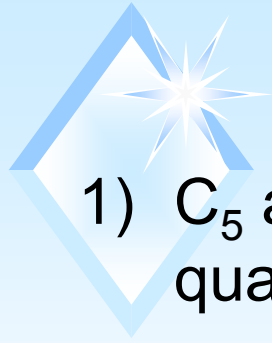
b)

$\geq 90\%$ neg
(36/40)

$\geq 90\%$ pos
(36/40)

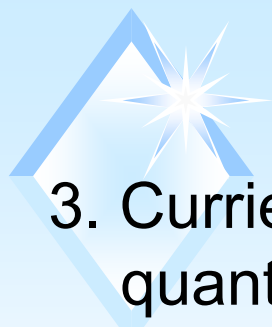


Conclusion: data do not contradict that $[C_5-C_{95}]$ is inside of $[S1, S2]$



Summary

- 1) C_5 and C_{95} are important metrological characteristics of a qualitative test describing imprecision around the cutoff.
- 2) LoB and LoD are important metrological characteristics used to discriminate between the presence or absence of an analyte.
- 3) For qualitative test with cutoff at LoB (analytical cutoff), C_{95} and LoD are the same concepts.
- 3) Laboratory experiment with three samples to obtain information about C_{50} , C_5 and C_{95} .



Additional References

3. Currie A.L. (1968). Limits for qualitative detection and quantitative determination. Analytical Chemistry. Vol. 40 (3): 586-593.
4. Linnet K., Kondratovich M. (2004). Partly nonparametric approach for determining the limit of detection. Clinical Chemistry. 50: 732-740.

Thank you for your attention !!!

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