Question:

Guidance Documents for several PCR/RT-PCR based IVD devices discuss inclusion of a High Negative sample type in Precision studies. Please explain the rationale for extensive testing of high negative samples. Please explain the clinical relevance/value in obtaining results from high negative samples that can yield highly variable results (from 0-95% rates of detection), particularly in light of the fact that these samples are, by definition, below the Limit of Detection and therefore, by definition, known to not be reproducible.

Answer by Marina V. Kondratovich, Ph.D., OIVD,FDA

Qualitative Test with Two Outcomes

Cutoff for qualitative test:

- THRESHOLD for the OBSERVED result for a sample above which the result for a sample is reported as positive and below which the result is reported as negative.

There are three slightly different scenarios.

I) Cutoff is higher than LoB

(Non-disease subjects have some amount of analyte)

Cutoff =C

II) Cutoff is an analytical cutoff

(no analyte vs analyte present)

Cutoff =LoB

II.1) LoB >0

Samples with zero concentration have noisy results

II.2) LoB=0 Ultrasensitive assay

Samples with zero concentration have zero results

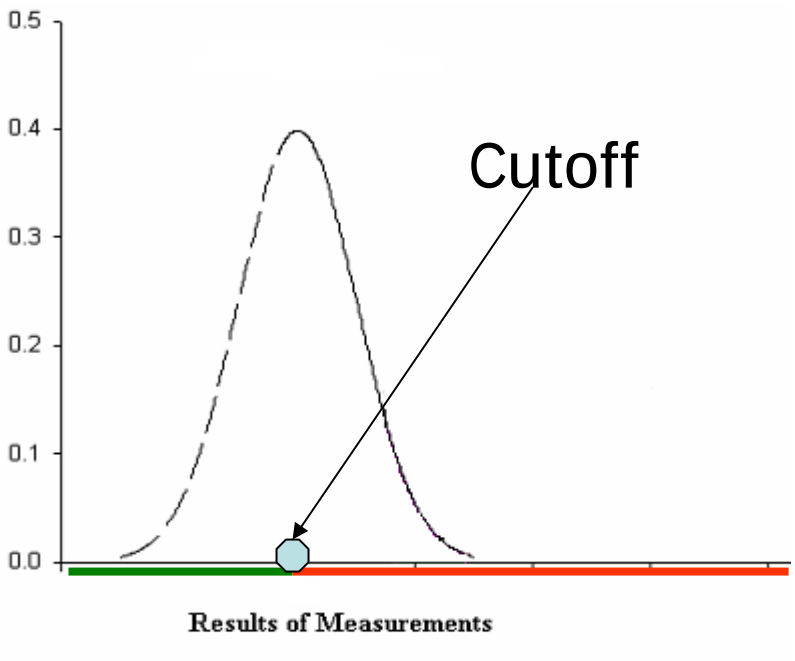
Answer is provided

- Scenario I
  (cutoff is higher than LoB)
- Scenario II.1 (Cutoff=LoB, LoB>0)
- Scenario II.2 (Cutoff=LoB, LoB=0)

Scenario I

Cutoff is based on clinical performance (Non-diseased and Diseased subjects have some amounts of analyte)

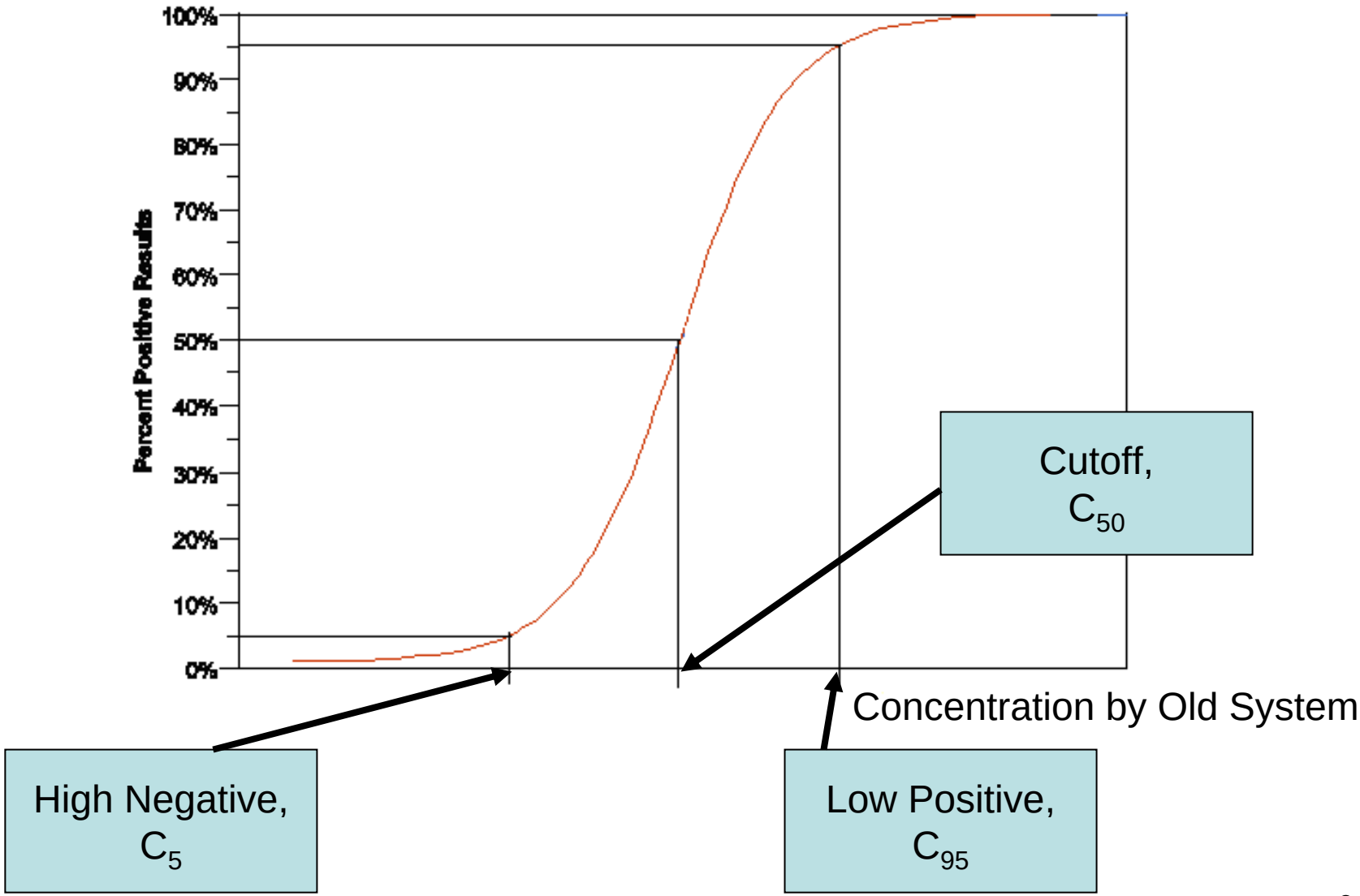
- Actual CONCENTRATION in a sample with this concentration is 50% positive and 50% negative (C_{50}) if a large series of repeated tests were performed



See CLSI EP12-A2

Assume that a distribution of measurement error is symmetrical.

Scenario I (cont.)



C_5 and C_{95} are important performance characteristics for qualitative test

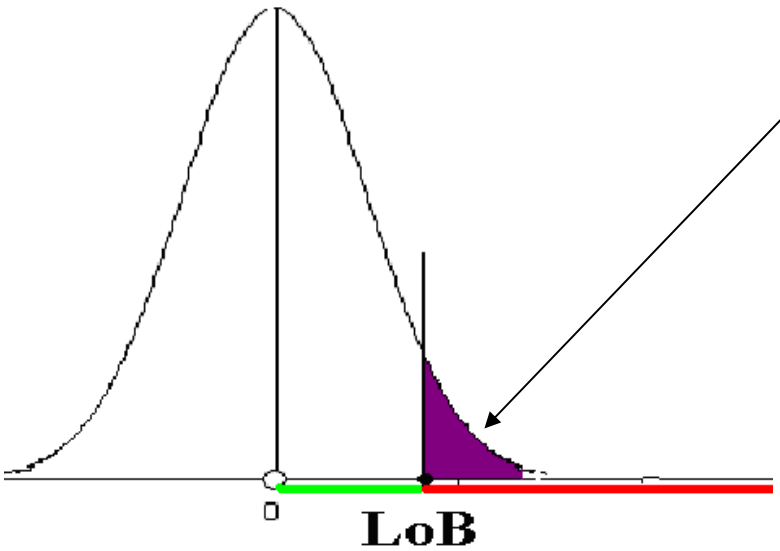
Scenario I (cont.)

Samples in precision study for qualitative test:

1) Sample below the cutoff (around C_5)
2) Sample above the cutoff (around C_{95})
3) Sample truly negative (zero analyte)
4) Sample moderate positive

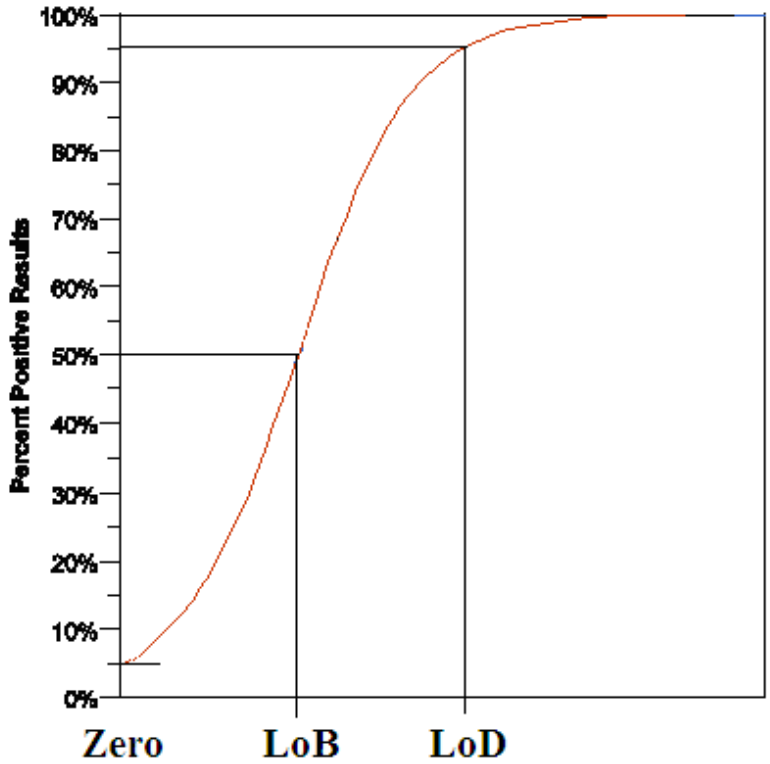
Scenario II.1: Cutoff =LoB

Samples with zero concentration have noisy results



- Cutoff is based on the performance of the samples with zero concentration, Cutoff=LoB

- Percent of positive results for the samples with zero concentration is 5%;
- Percent of positive results for the samples with LoB concentration is 50%;
- Percent of positive results for the samples with LoD is 95%



C_5=zero, C_{95}=LoD

Scenario II.1(cont.)

Samples in precision study for qualitative test:

1) Sample above the cutoff (around LoD)
   (C_{95}=LoD)
2) Sample truly negative (zero analyte)
   (C_5=0)
3) Sample moderate positive

Scenario II.2

LoB=0, Ultrasensitive test

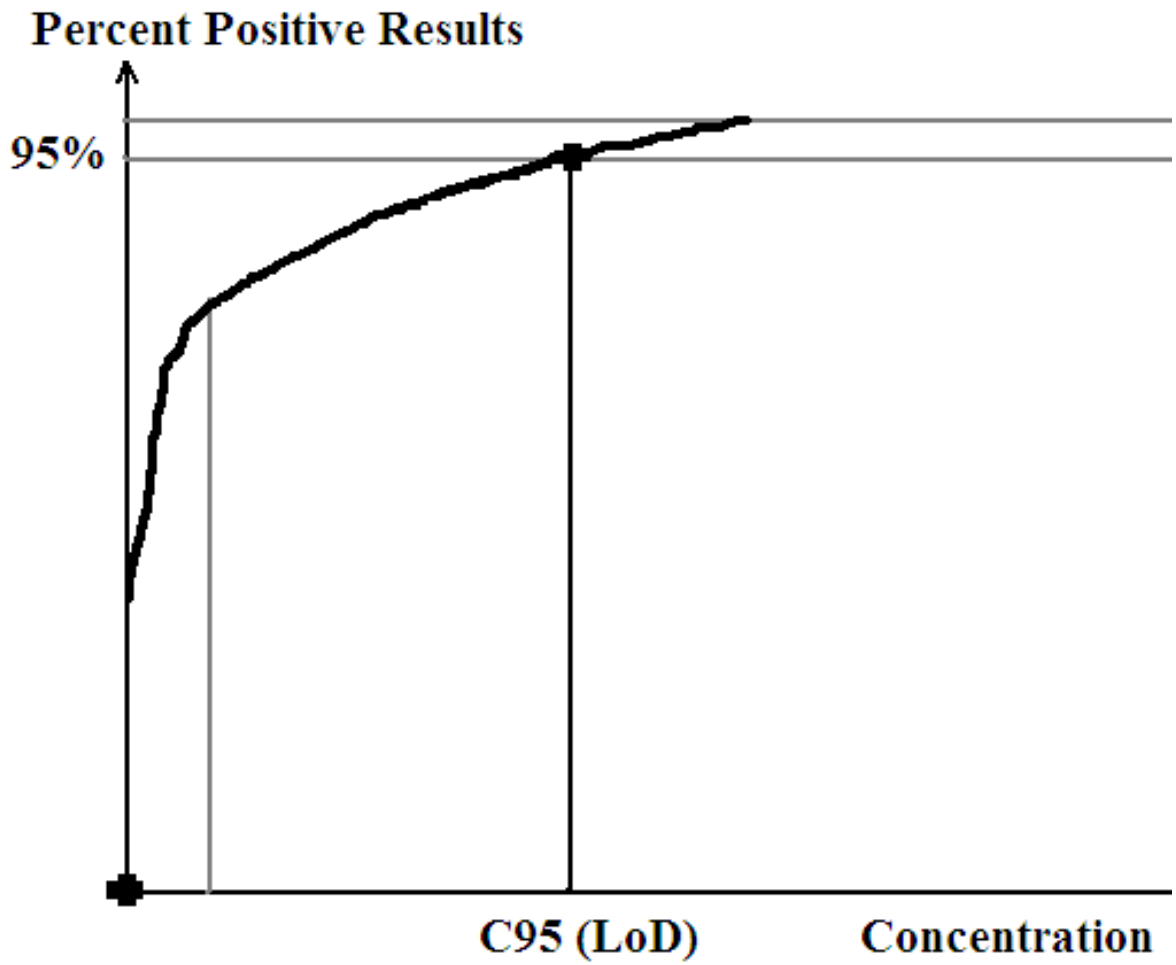
Example of Ultrasensitive Test**:** RT-PCR

Consider that Cutoff =45 cycles;

- If samples are truly negative, all results are "Negative" => Type I error is close to zero.
- Cutoff is not established based on the truly negative samples (samples with zero concentration);

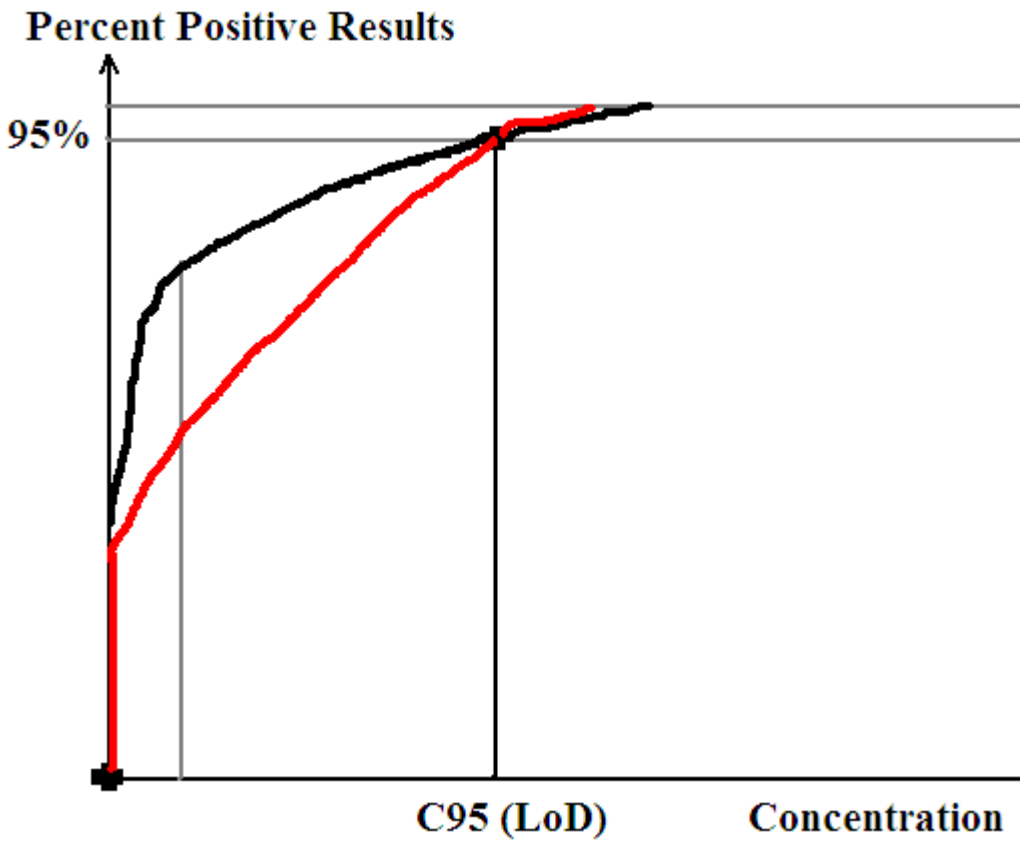
Scenario II.2 (cont.) Ultrasensitive test

- Zero concentration has zero percent positive results;
- Concentration corresponding to Ct=45 is close to zero;
- C_{95} (LoD)- concentration corresponding to Ct=38.



Scenario II.2 (cont.)

Problem : C_5 not easy to prepare.
C_5 is very close to 0
where large variability.
If only two points:
Zero concentration
percent of positive
results is 0
•**LoD concentration**
percent of positive
results is 95%
then curves of %
positive results of two tests
can be different



(much uncertainty between two curves if only two points on these curves are similar)

Scenario II.2 (cont.)

Modified Approach Case A

If the percent of Diseased subjects from the intended use population with the test results less than LoD is less than **10%** of all Diseased subjects of the intended use population **,** then no need for C_5.

Modified recommended concentrations for precision studies :

- truly negative sample
- C_{95} (LoD) sample
- moderate positive

Scenario II.2 (cont.)

Modified Approach Case B

If the percent of Diseased subjects of the intended use population with test results less than LoD is greater than or equal to **10%** of all Diseased subjects of the intended use population**,** then a sample from the range C_{20}-C_{80} is recommended for testing.

Modified recommended concentrations for precision studies :

- truly negative sample
- C_{95} (LoD) sample
- Sample from range C_{20}-C_{80}
- Moderate positive