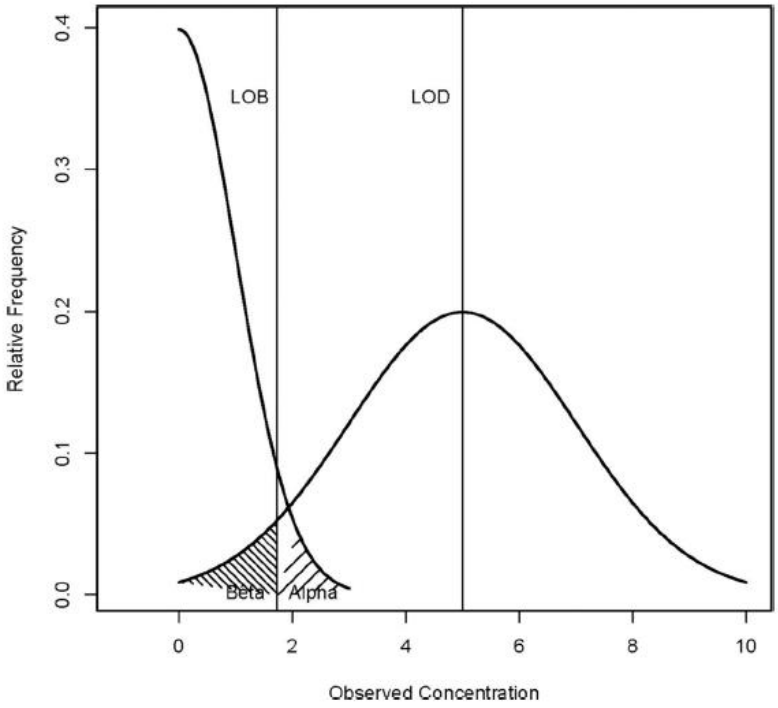
MEMO - How to calculate the limit of detection (LOD)

For automated computation, see the statistical tools available on www.gene-pi.com

a) Definition: the limit of Detection LOD with a confidence level $(1-\beta)$ is defined (in concentration unit) as the minimum concentration for which detecting the target sequence in a well is possible with a probability of $1-\beta$.

In other words, this is the minimum concentration that can be said to be non-zero and statistically higher than the limit of blank LOB with a 1- β probability (typically 95% for $\beta = 5\%$).



b) Calculation method:

1) Determine the limit of blank $LOB(95\%)$ of your experiment by following the method "How to calculate the limit of blank".

2) Calculate $$p_0 = \frac{2b+z^2+z\sqrt{z^2+4b(1-b/N)}}{2N(1+z^2/N)}$$

where:

- $b = LOB(95\%)$ is the 95% limit of blank
- $z = 1.645$ is the "one-tail" quantile at 95%
- N is the total number of partitions that are generated on average in a well (typically $N = 28000$)

- p_0 is the higher-value solution of the following equation (which can be simplified as a second degree equation in p):

$$p = b/N + z\sqrt{p(1-p)/N}$$

3) The LOD with a 95% confidence level is determined as follows (calculation based on the Normal Law approximation and the Poisson Law):

- In concentration in the well (cp / uL):

$$LOD(95\%) = -N\ \ln(1-p_0)/V$$

$$LOD(95\%) = -\ln(1-p_0)/v$$

where v is the average partition volume in μL (for example $v =$ 0,00058592 μL in a Sapphire chip), so that $V = N\,v$ is the analyzed volume (i.e. the cumulative total volume of all the partitions generated in a well).

- In number of copies included in the volume analyzed in the well:

$$LOD_{cp}(95\%) = \lceil -N\ \ln(1-p_0) \rceil$$

knowing that this unit in number of copies can be approximated as "number of positive partitions" in the well.

- For example: if $LOB(95\%) = 2$ and $N = 28000$, then $LOD(95\%) = 0.37$ cp / uL and $LOD_{cp}(95\%) = 7$ copies (or "positive partitions")

- Special case: if $LOB(95\%) = 0$ (i.e. false positives are never present) then $LOD(95\%) = 3/V = 0.18$ cp / uL and $LOD_{cp}(95\%) = 3$ copies.
  In fact, if there are never false positives, then the 95% detection limit is equal to the 95% sampling limit which is: $LOS(95\%) = 3/V$ cp / uL.

  - **Definition of the sampling limit** LOS at a confidence level $(1-\alpha) = 95\%$: the lowest concentration in the well for which the probability of having no target sequence in the analyzed sub-sample is less than $\alpha = 5\%$.