

**MEMO: How to calculate the limit of blank (LOB)?**

- a) **Definition:** the limit of blank  $LOB$  with a confidence level  $(1 - \alpha)$  is defined (in number of partitions in a well) as the maximum number of positive partitions expected in a well with a probability of  $1 - \alpha$  in a sample containing no target sequence.

In other words, this is the maximum number of false positives that are plausible with a  $1 - \alpha$  probability (typically 95% for  $\alpha = 5\%$ ).

b) **A calculation method:**

- 1) Make  $R$  sample replicates without any target sequence (take  $R \geq 30$  wells).

It is recommended to perform these replicates under the same experimental conditions as the test experiments, in particular with a quantity of nucleic acids which is representative of that of the tests (if this is not constant, then take the most unfavorable case with a large quantity of nucleic acids, for example between 1000 and 10000 copies /  $\mu\text{l}$ ).

- 2) Count the number  $x(i)$  of "false positive" partitions observed in the well of each replicate  $i$
- 3) Calculate the mean  $\mu$  and the standard deviation  $\sigma$  of the  $x(i)$
- 4) Calculate the corrected mean:  $\mu_{corr} = \mu + 1.645 \sigma / \sqrt{R}$
- 5) The LOB with 95% confidence level is determined as follows (calculation based on Normal Law approximation and Chernoff's inequality):

$\mu_{corr}$	$LOB(95\%)$ in number of positive partitions in well
$0 < \mu_{corr} \leq 0.180$	2
$0.180 < \mu_{corr} \leq 0.477$	3
$0.477 < \mu_{corr} \leq 0.863$	4
$0.863 < \mu_{corr} \leq 1.314$	5
$1.314 < \mu_{corr} \leq 1.813$	6
$1.813 < \mu_{corr} \leq 2.348$	7
$2.348 < \mu_{corr} \leq 2.913$	8
$2.913 < \mu_{corr} \leq 3.503$	9
$3.503 < \mu_{corr} \leq 4.115$	10

Special case: if  $\mu = 0$  (i.e. false positives are never present) then  $LOB(95\%) = 0$