# The Statistics of Retesting a BAX® System for screening Real-Time *Listeria monocytogenes*

## Introduction

With a sensitivity/specificity rate of 98%, the BAX System for screening Real-Time *Listeria monocytogenes* assay offers the best science-based solution available for food safety/food quality testing. The remaining 2% can often be attributed to random events, such as those caused by system noise, operator error, nonspecific PCR product or low-level laboratory contamination, which are occasionally interpreted as positive results by the BAX System Q7. Some of these events could be reduced, but only at the cost of reducing overall assay sensitivity. Hygiena™ is committed to maintaining the integrity of BAX System assays to assure optimum sensitivity. Customers who encounter positive results with the BAX System must determine the appropriate action to take according to their own Standard Operating Procedures (SOPs). In our experience, companies most often make these decisions based on several factors, including historical rate of positive results, shelf-life of the tested product, or amount of tested product affected. For example, companies testing product where more than 1% of samples might typically be found positive will usually either take direct action or culturally confirm the result, depending on the amount of product at risk. Other companies testing product that typically yields less than 1 per 1000 positive results may choose to retest the sample before taking direct action.

## Retest

Some businesses include retesting the sample before taking action as part of their SOPs because random events will not usually produce the same results. A good lab practice is to repeat the BAX System *Listeria monocytogenes* Real-Time assay in quadruplicate from the sample's final enrichment. If all retest results are negative, the sample can be considered negative. If one or more of the retest results are positive, the sample should be treated as positive and handled in accordance with SOPs. Retests are started as soon as possible, and care must be taken to assure the integrity of the enrichment. Retesting is performed on the same enrichment that generated the original BAX System *Listeria monocytogenes* Real-Time suspect. It is important that a new lysate is prepared for each replicate. Repeating the lysis step helps to ensure any errors from the initial result are not propagated in the retesting process.

## The Math

Hygiena claims a sensitivity/ specificity of 98% for the BAX System for screening Real Time *Listeria monocytogenes* assay. The remaining 2% are attributed to random events such as system noise, operator error, non-specific PCR product or low-level contamination. The detection limit is 10,000 cfu/ml after enrichment. At this level there would be 7.5 calculated cells in a PCR reaction. According to the Poisson Sampling Statistics (refer to table 1), the chance that this test would actually test negative is 0.06%. If the cell level in the enrichment is 1,000 cfu/ml, there would be 0.75 calculated cells in a PCR (table 1) reaction. Again, the statistics indicate that the chance that this test would be negative is approximately 47.24%.

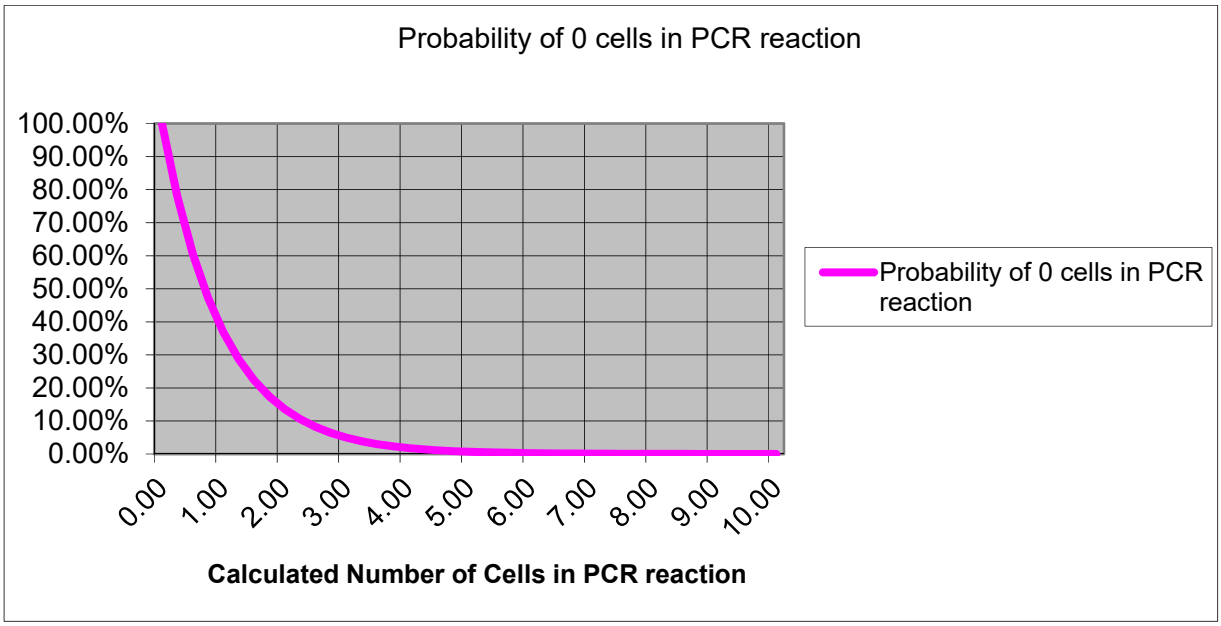
5ul from an enrichment (with 10,000 cfu/ml) = 50 Cells

50 Cells/ml is divided by 6.66 (because we only 30ul of the 200ul lysate to hydrate the PCR tablet) = 7.5 Cells/PCR tablet

Thus, in a sample at the threshold of detection with 1,000 cfu/ml or 0.75 calculated cells in a PCR reaction, the probability of a negative test is the product of the probability of each individual test (0.4724x0. 4724x0. 4724 = 0.1050 or 10.5%). Therefore, with the additional three retests results there is a 10.5 % chance that all three will be negative if there are 1,000 cfu/ml in the enrichment. Conversely, there is a 89.5% chance that we would find the true positive by re-testing in triplicate. Using this logic if one were to run additional replicates the chance that all testing replicates would report a negative continue to decrease by the product of the same 0.4724 probability. For example (0. 4724*0. 4724*0. 4724*0. 4724 = 0.0498 or 4.98%) a fifth replicate results in 2.4% probability that all replicates are negative and the “true” result was positive.

It is our recommendation that if a customer wishes to retest an existing result they should retest the last enrichment with 4 new lysates. If any of the results are positive the lab should follow their SOP for positive results. The probability of a positive result at the assays’ threshold would be 95%.

Table 1




10e4 CFU/mL enrichment would have 7.5 "calculated number of cells in PCR reaction". The probability of actually getting 0 cells in that PCR reaction is 0.06%. Conversely a 10e3 CFU/mL enrichment would have 0.75 "calculated number of cells in PCR reaction". The Probability of having 0 cells in that PCR reaction is 47.24%.