PIERCING PUMPKIN PRODUCTS

USING THE BAX® SYSTEM TO DETECT PATHOGENS IN DIFFICULT MATRICES

Using the polymerase chain reaction (PCR) method to detect pathogens is a common and powerful tool, but detecting bacteria in food can be very challenging. Food matrices are often complex and contain substances that inhibit the ability of available methods to detect pathogens. In the case of pumpkin meat and pumpkin seed oil, the very antioxidant properties that make these products popular nutraceuticals can also inhibit or inactivate pathogens, making them more difficult to detect. Nevertheless, if contamination occurs, the risk must be assessed.

A large US fruit and vegetable processor approached Hygiena's Applications Group scientists to develop an appropriate enrichment and testing method to detect bacterial pathogens in pumpkin products. The company was interested in detecting *E. coli* O157:H7, *Salmonella* species and *Listeria monocytogenes*, all common and serious pathogens found in food.

HYGIENA'S SCIENTISTS CONDUCTED THREE SEPARATE STUDIES FOR EACH BACTERIAL TARGET, USING THE BAX® SYSTEM Q7 INSTRUMENT AND BAX® SYSTEM REAL-TIME PCR ASSAYS:

STUDY 1:

A matrix control that contained no target bacteria was performed to assess the performance of the BAX® System internal positive control to make sure that the matrix composition of the enriched samples would not inhibit with a PCR signal. The use of an internal positive control helps to discern the possibility of matrix interference resulting in a false negative.

STUDY 2:

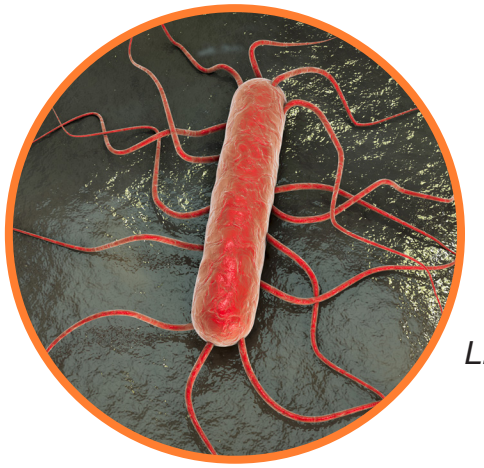
A post-enrichment spike study was performed to confirm that if the bacterial target is present at or above the limit of detection (LOD), the BAX® System will accurately detect it. This involved adding varying levels of target bacteria after matrix enrichment and then processing samples.

STUDY 3:

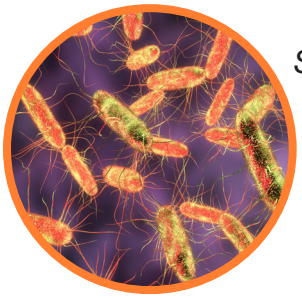
An artificial spike study was performed to determine if pumpkin and pumpkin oil could support bacterial growth and be subsequently detected by PCR methods. Samples of pumpkin were inoculated with three levels of bacteria, enriched and tested with the BAX® System.

From these studies above, enrichment and testing parameters including media, temperature, dilution ratio and regrowth were evaluated to optimize a protocol that work in both pumpkin matrices for all three target bacteria.

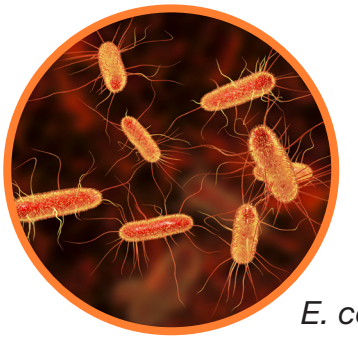
- For *E. coli*, no PCR interference was seen in pumpkin oil enriched in modified tryptone soya broth (mTSB, which is selective for *E. coli*), while a three-hour regrowth in Brain Heart infusion (BHI) broth was required to overcome inhibition in dry pumpkin. Post-enrichment spikes could be detected in pumpkin oil with or without a BHI regrowth, but again, BHI was needed to overcome matrix inhibition in dry pumpkin.
- For *L. mono*, no PCR interference was seen, and post- enrichment spikes were detected, in dry pumpkin or seed oil enriched in Demi-Fraser broth (often used for selective *Listeria* enrichment), then transferred to MOPS-BLEB media (also selective for Listeria growth).
- For *Salmonella*, no PCR interference was seen in oil enriched in buffered peptone water (BPW, selective for *Salmonella*), but a three-hour BHI regrowth was needed to overcome inhibition in dry pumpkin. Post enrichment spikes could be detected in oil enriched 1:10 in BPW but needed a 1:20 BPW enrichment and three-hour BHI regrowth to detect bacteria enriched in pumpkin oil.



L. mono



Salmonella



E. coli

BASED ON THESE RESULTS, THE FOLLOWING ENRICHMENT METHODS ARE RECOMMENDED:

TABLE 1. RECOMMENDED ENRICHMENT STEPS FOR DETECTING BACTERIA IN PUMPKIN PRODUCTS

Product	***E. coli O157:H7***	***Salmonella spp.***	***L. mono***
Pumpkin seed oil	mTSB enrichment, followed by a 3 hour BHI regrowth	1:10 BPW enrichment	Primary 1:10 enrichment in DemiFraser broth, secondary 1:100 transfer to MOPS-BLEB
Dry pumpkin		1:20 BPW enrichment, followed by a 3 hour BHI regrowth	

Like any pathogen test method, PCR can be hampered by factors in certain difficult matrices. Some of the problems encountered include indeterminate results, inhibition of the internal positive control, positive control bacteria sample yielding negative results, false negatives or false positives, PCR shutdown, and cross-contamination. It is therefore imperative that a thorough performance evaluation be conducted with each company's specific products and protocols to ensure the pathogen method is capable of detecting the target organisms.

Hygiena's Applications Group can investigate your issue, perform key experiments and recommend specific protocols to detect your target organisms in your specific food matrix. These solutions have included protocol development for new matrices and first-time customers, evaluation of enrichment methods, multi-level inoculation studies to determine the limit of detection, and validations of customer specific products.