



Detecting harmful bacteria from dairy products using the BAX[®] System

Whey is a natural byproduct of milk that is produced during the manufacturing of cheese. Despite being primarily composed of water, whey is highly nutritious and is used as an ingredient or supplement in several different foods, including sports nutrition, baked goods, seasonings and flavors. Due to the broad applications for whey protein it is tested quite often for bacterial contamination.

Recently, a large cheese manufacturer approached Hygiena with a challenge the company was facing. They were searching for an alternative rapid method to accurately detect and discriminate potentially pathogenic bacteria in the whey protein concentrate (WPC) the company produced. In response, the Applications scientists at Hygiena designed a validation study to screen WPC for the presence of *Salmonella* and *Listeria* species using the BAX[®] System Real-Time PCR Assays and the US Food and Drug Administration reference methods.

To accomplish this, the scientists independently inoculated samples of WPC 80 (% protein) with a low level of *Salmonella* Typhimurium or *Listeria ivanovii*. After enrichment, samples were simultaneously processed and analyzed using the BAX[®] System method and the reference method. The performance of each method was then compared to determine whether the results were equivalent.

The BAX[®] System PCR assay for *Salmonella* showed results identical to culture after incorporating a three-hour brain heart infusion (BHI) regrowth. When the BAX[®] system and reference method were compared, the BAX[®] System showed a higher proportion of positive results indicating a significant difference.

For *Listeria*, there was no significant statistical difference between the BAX® System presumptive and confirmed results or between BAX® System and the reference method.

Results showing low-spike comparisons are seen in Table 1, below.

Test methods compared	Organism	Most Probable Number/25g	BAX positive results	Reference positive results
BAX® System 24 h/reference	<i>Salmonella</i> Typhimurium	0.10	2	2
BAX® System 24 h + BHI regrowth/reference	<i>S. Typhimurium</i>	0.10	11	2
BAX® System 26 h/reference	<i>Listeria ivanovii</i>	0.25	6	4
BAX® System 48 h/reference	<i>L. ivanovii</i>	0.25	8	4

Table 1. Low-spike results for BAX® System and reference methods

The results of this study demonstrate that the BAX® System Real-Time PCR Assay for *Salmonella* can accurately detect *Salmonella* species in 375 g samples of WPC 80 after a three-hour BHI regrowth with superior performance to the reference method. The study also demonstrated that the Real-Time PCR Assay for Genus *Listeria* accurately detected *Listeria ivanovii* in 50 g samples of WPC 80, equivalent to the reference method.



Earlier studies by Hygiene scientists tested WPC 34(% protein) for *E. coli* O157:H7 and *L. monocytogenes*. The results for the BAX® System Real-Time PCR Assay for *E. coli* O157:H7 showed equivalent performance to the reference method in 375 g samples of WPC 34 after 22 hours of enrichment in modified tryptone soy broth (mTSB) with a regrowth. The results for the BAX® System Real-Time PCR assay for Genus *Listeria* and *L. monocytogenes* also showed equivalent performance to the reference method in 125 g samples of WPC 34 after a secondary enrichment in buffered *Listeria* enrichment broth (MOPS-BLEB).

Another dairy study by Hygiena compared the BAX® System Real-Time Assays for *Salmonella* and Genus *Listeria* with FDA reference methods on lactose powder, which is extracted from whey to be incorporated in breads, confections and as an important energy and calcium source in infant formula. This study found that the BAX® assays were statistically equivalent to the FDA reference method. Low-spiked samples with an MPN of 0.74/25 g found 10 positive *Salmonella* results using the BAX® System versus 13 positive results with the FDA method. Similarly, low-spiked samples with a most probable number (MPN) of 1.3/25 g found 12 positive *Listeria* species results versus 14 positive results using the FDA method.

PCR can accurately determine the presence or absence of certain pathogens in food enrichments, helping to maintain the safety of the food supply. However, like any pathogen test method, PCR can be hampered by factors in difficult matrices. Food matrices that may appear simple at first, can become difficult upon homogenization. Problematic food types can include fatty or greasy foods, powders and dried foods, and thick liquids. It is therefore important that the methods used to test products are validated fit for purpose.

To ensure that high quality dairy products such as whey protein are safe, reliable rapid methods are needed to screen these ingredients and products for foodborne pathogens. The BAX® System Real-Time PCR assays can deliver accurate, and sometimes superior results, in far less time than reference culture methods. As they were able to in this case study, Hygiena application scientists can design a validation study, investigate challenging matrices, and evaluate enrichment options to provide the level of confidence you need.

