RESEARCH REPORT
PORK SAFETY

Title: A Diagnostic for Rapid Detection of Pathogenic *Listeria monocytogenes* as an Indicator of Hygiene on Food Contact and Non-Food Contact Surfaces in the Pork Processing Environment - **NPB #06-178**

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Date Submitted: May 14, 2008

II. Industry Summary

During this project, a self contained test was developed, which was capable of rapid detection of *Listeria monocytogenes* on food contact and non-contact surfaces, as well as on samples of ready-to-eat (RTE) deli meat. The test was designed so that it could be completed directly in the food (pork) processing environment, with minimal need for equipment. When the test was evaluated using artificially contaminated stainless steel, acrylic and ceramic coupons, the test, termed the Phast Swab, was capable of detecting as few as 10^1 CFU/cm^2 of *Listeria monocytogenes*. When the Phast Swab was tested on RTE meat, as few as 10^2 CFU/100 cm diameter of deli meat was detectable. Test results were usually available within 15 to 18 hours, and the actual hands on time to complete the test (i.e the amount of time a person would need to spend doing the test) was 5-10 minutes. The Phast Swab is an easy to use visually interpreted test, that detects viable *L. monocytogenes* cells on a variety of surfaces, in a rapid and sensitive manner.

III. Scientific Abstract

Listeria monocytogenes is a ubiquitous pathogen and a major cause of food related illness. Current methods of detection are slow, laborious, and require expensive equipment, or technical expertise. A rapid, easy to use test to detect *L. monocytogenes* was developed, that requires little to no equipment or technical training. The Phast Swab is a self-contained test device, containing a sampling tool (swab), growth media, immunomagnetic separation (IMS) beads, and a colorimetric substrate, which when cleaved by the enzyme phosphatidylinositol-specific phospholipase C (PI-PLC), forms a visible indigo reaction. To conduct the test, a surface (food, contact and non-contact surfaces) to be tested is swabbed, and the swab is returned to the test device. Follwing a 10-13 hour enrichment, any *L. monocytogenes* cells present in the growth media are isolated and concentrated by IMS and the growth media is removed. Bacterial lysis buffer is added, followed by the enzyme substrate, which reacts with the PI-PLC to form the indicative indigo color for positive identification. The indigo reaction had to be visually detected within 5 hours to be considered positive.

The Phast Swab was evaluated using artificially contaminated RTE meat and food contact and non-contact surfaces.

A five-strain cocktail of *L. monocytogenes* was prepared so that the final concentration of the cocktail was 10^9 CFU/ml. The cocktail was serially diluted (10-fold) in a dilution range from 10^0-10^{-9}, and individual slices of deli turkey meat (100mm diameter) were inoculated with 1ml of each dilution. The meat was allowed to dry, and then each piece was swabbed with an individual Phast Swab, and the test was conducted as described.

The Phast Swab was capable of detecting 10^2 cells on the deli meat within 15 hours. The test detected 55 of 57 *L. monocytogenes* tested and none of the 15 non- *L. monocytogenes* bacterial strains tested. In pure culture, as few as 10^7 CFU/ml were directly detected within 3 hours. The addition of 428 mM KCl to the growth media significantly increased the sensitivity of the assay.

For detection of *L. monocytogenes* on food contact and non-contact surfaces, a five-strain cocktail of *L. monocytogenes* was prepared so that the final concentration of the cocktail was 10^9 CFU/ml with 10X serial dilutions to 10^0 CFU/ml. Individual stainless steel, acrylic and ceramic tile coupons (4"×4") (100 cm^2) chosen to simulate food contact and non-contact surfaces, were inoculated with 250µl of the 10^7, 10^6, 10^5, CFU/ml dilutions. Plate counts showed that after each surface dried for five hours, the actual *L. monocytogenes* counts on the surfaces were 2 logs lower than the initial inoculums. The coupons were allowed to dry and were swabbed with an individual Phast Swab device. The devices were incubated for 13 h, and the test was completed as described.

The Phast Swab was capable of detecting as few as 10^1 CFU/cm^2 that was present on the coupons.

Collectively, these data show that the Phast Swab can detect *L. monocytogenes* on food samples (RTE meat) and food contact and non-contact surfaces in the food processing environment in a rapid and specific manner without the need for major instrumentation to read the test result.

IV. Introduction

Listeria monocytogenes is a ubiquitous pathogen that continues to emerge as a major cause of food related illness. The immunocompromised, the elderly, and pregnant women in particular are at high risk for contracting listeriosis. Symptoms associated with listeriosis include fever, chills, headache, meningitis and septicemia, which may progress to abortion in pregnant women (Grahan and Collins 1991). While the infectious dose is unknown, the mortality rate is about 30% in the high risk groups and has been reported to be as high as 16% in non-risk groups (Goulet *et al*. 1993). Some strains of *L. monocytogenes* are avirulent, and expression of virulence factors is reported to be controlled by environmental factors including temperature and glucose (Datta 1994).

L. monocytogenes is frequently isolated from swine. The U.S. Department of Agriculture Food Safety and Inspection Service's (USDA-FSIS) nationwide microbial baseline survey of hogs reported the presence of *L. monocytogenes* on 7.4% of hog carcasses (FSIS 1996). The primary source of *L. monocytogenes* contamination of carcasses is assumed to be the contents of the large intestine, which may rupture at slaughter (Skovgaard and Norrung 1989). Several studies have reported the prevalence of *L. monocytogenes* in swine fecal samples to range from 0 to 47% (Fenlon *et al*. 1996, Wesley 1999). Various husbandry practices including rearing pigs in closed houses, maintaining specific pathogen free herds (a common practice in Europe), as well as differences in sampling sites (tonsils versus feces) geographical location, and seasonal variability may explain the differences in the reported variation in the incidence of *L. monocytogenes* in healthy pigs (Wesley 1999).

In research conducted at Colorado State University, Duffy *et al*. (2001) investigated the extent of microbial contamination in U.S. pork retail products. Three hundred and eighty four samples of retail pork were collected from 24 stores in various geographical locales. An additional 120 samples of freshly ground pork and pork sausage were collected from 6 different processing plants. The samples were analyzed for aerobic plate counts, total coliform counts, *Escherichia coli* counts, and the presence of bacterial pathogens including *Salmonella*

spp., *L. monocytogenes*, *Campylobacter jejuni*, *Campylobacter coli*, and *Yersinia enterocolitica*. Of importance to this proposal, the results indicated that the most common pathogen detected in ground pork samples collected from the processing plants was *L. monocytogenes*, which was found in 26.7% of the samples. The authors concluded that the use of external surface trim from carcasses contaminated with *L. monocytogenes* may have led to the high *L. monocytogenes* prevalence in the ground pork (Duffy *et al*. 2001). There was also a high incidence of *Listeria* spp. in retail pork samples, with an overall incidence of 41.9% across all retail products sampled. *L. monocytogenes* was the most prevalent pathogen detected in retail pork, with 19.8% of the samples testing positive. As with the studies described above, the authors reported that *L. monocytogenes* was present more frequently in ground products that in whole muscle products. In addition, pre-packaged ground pork and pork sausage products had the highest incidence of *L. monocytogenes* (Duffy *et al*. 2001). The authors concluded that the ground pork samples may have become contaminated from improperly cleaned grinding and processing equipment (Duffy *et al.* 2001). The contamination of processing equipment is a serious concern, because *L. monocytogenes* is a foodborne pathogen that possesses the ability to grow at refrigerated temperatures. This means that even when *L. monocytogenes* is present at low levels in biofilms in the processing plant, the pathogen can survive in pork products and become enriched during subsequent storage at refrigerated temperatures.

The collective literature has demonstrated that processed pork products may be heavily contaminated with *L. monocytogenes*, and that the contamination source is likely improperly sanitized processing equipment. Therefore it is clear that measures aimed at reducing the incidence of *L. monocytogenes* in pork should be directed at reducing the presence of this pathogen on processing equipment. Borch *et al*. (1996) has suggested that because bacteria such as *L. monocytogenes* can be endemic in the swine processing environment, and since these bacteria are effectively controlled with proper sanitation, *L. monocytogenes* would be useful as an indicator of the success of pork processing equipment cleaning and disinfection protocols (Borch *et al*. 1996). This last statement provides the rationale for this project, in which we proposed to develop a integrated sampling and testing device that permitted the rapid detection of pathogenic strains of *L. monocytogenes* on food contact (processing equipment) and non-food contact (drains) surfaces as an indicator of acceptable hygiene.

V. Objectives

The goal of this proposal was the development of a rapid assay, that was capable of detecting *L. monocytogenes* on food contact and non-food contact surfaces a rapid time period. The specific objectives of this research were to: 1) Develop a detection method that integrates a sampling method (swabbing), immunomagnetic separation, bacterial enrichment, and detection (either visual detection, or luminescent detection) in one easy to use device; 2) To use the newly created assay to effect rapid detection of *L. monocytogenes* on food contact and non-food contact surfaces within the pork processing environment; and 3) To evaluate and validate the detection assay using artificially contaminated food contact and non-food contact surfaces.

VI. Materials and Methods

Bacterial Strains

Five strains of *L. monocytogenes* were used in this study. The strains were C1-056 (serotype 1/2a, human isolate), J1-177 (serotype 1/2b, human isolate), N1-227 (serotype 4b, food isolate), N3-013 (serotype 4b, food isolate) and R2-499 (serotype 1/2a, human isolate). These strains were combined in a cocktail for all experiments. The strains were chosen because they represent the serotypes of the most common human pathogens. The strains were collected from various human epidemic outbreaks from contaminated human blood, and contaminated food samples. Stock bacterial cultures were maintained in 30% glycerol and were frozen at -70^{o}C.

Isolation and preparation of the *L. monocytogenes* strains

For all experiments, the inocula consisted of stationary phase cells that were obtained by inoculating Tryptic Soy Broth (TSB) with a single colony from an overnight TSA plate and incubating the preparations overnight with shaking at 37°C. The overnight (18 h) cultures were combined into a 5 strain cocktail by standardizing the OD_{600nm} of each culture, and then combining an equal volume of each culture into a sterile test tube. The concentration of the cocktail was then determined by plate count on TSA. Inoculum levels used in all experiments were determined by serial dilution in lambda diluent, followed by plating onto TSA.

Growth Curves

Evaluation of Different Growth media

Several different media were evaluated to determine the best growth medium to use during enrichment of *L. monocytogenes* in the Phast Swab. The media evaluated were Buffered Peptone Water (BPW), Universal Preenrichment Broth (UPB), Brain Heart Infusion (BHI) broth, BHI supplemented with 428 mM potassium chloride (KCl), Tryptic Soy Broth (TSB) and TSB supplemented with 428 mM KCl. To evaluate each growth medium, the *L. monocytogenes* isolates that comprised the cocktail were individually subjected to growth curve experiments in each broth. To conduct the growth curves, each isolate was streaked from frozen culture onto a Tryptic Soy Agar (TSA) plate, followed by overnight incubation at 37°C. A well isolated colony from the overnight TSA plate was inoculated into a test tube containing 10 ml of the growth media to be evaluated, followed by overnight incubation at 37°C with shaking at 250 rpm. The following day, 100 µl of the broth culture was subcultured into a test tube containing 10 ml of the growth media to be evaluated. One milliliter of the bacterial suspension was added to a well of a 24 well microtiter plate, and in addition, 1 ml of the growth media to be evaluated was added to the well. The microtiter plate was placed into a BioTek Synergy II microplate reader, and the reader was programmed to take OD_{600nm} readings at 30 second intervals for a total time period of 12 hours. The microplate was also shaken continuously at 37°C. Each growth curve was repeated in triplicate.

Enrichment of Stressed Cells

Preliminary results from the growth curve assay indicated that TSB supplemented with 428 mM KCl resulted in the fastest enrichment of the *L. monocytogenes* cells. Since it is likely that any *L. monocytogenes* present in the food production environment may be sublethally stressed, an experiment was conducted to determine if *L. monocytogenes* cells could be enriched in TSB (KCL) after being subjected to several types of stresses, including high pH (pH 9.0), low pH (pH 3.0), heat, and high osmolarity.

Each strain of *L. monocytogenes* was grown separately on TSA plates overnight. A well isolated colony was transferred from the TSA into 50ml of TSB (KCl), followed by overnight incubation for 18 hours at 37°C and 250 RPM. After the incubation, 5 ml of the broth culture was subcultured into 45 ml of TSB (KCl), which was previously adjusted to reflect the type of stress to be evaluated. For the pH studies, the TSB (KCL) was adjusted to the required pH (3 or 9) with the use of hydrochloric acid (HCl) or sodium hydroxide (NaOH). For the osmolarity studies, the TSB (KCL) broth was supplemented with sodium chloride (NaCl) to a final concentration of 15% (w/v). Each flask was incubated (at room temperature) for 2 hours. The heat stress challenge was performed by transferring 5 ml of the overnight culture into 45 ml of TSB (KCl), followed by incubation for approximately 1.75 hours at 37°C with shaking (250 rpm). Next, the flask was placed into a 48°C water bath for 15 minutes (once the final temperature of 48°C was reached). After the various incubations described above, 500 µl of each broth culture was transferred into a individual flask containing 49.5 ml of TSB (KCl), mixed, and then 250 µl of these suspensions were transferred into separate wells of a BioScreen honeycomb plate. The plate was placed into the BioScreen machine, which was set to take readings at 30 minute intervals for 1020 minutes. The honeycomb plate was also shaken continuously at 37°C for the duration of the experiment. Each growth curve was repeated in triplicate.

Development of the Colorimetric Phast Swab

The Phast Swab (Figure 1) is an integrated device that contains all diagnostic components in a single device. To develop the Phast Swab, 1 ml of TSB (KCl) was placed in the bottom of the device, along with 40 µl of IMS beads. The top of the device contained bacterial lysis buffer and the colorimetric PI-PLC substrate (X-inp). The completed Phast Swab was evaluated for specificity and sensitivity.

Development of the Luminescent Phast Swab

In an attempt to increase the sensitivity of the assay, we also developed a version of the Phast Swab test that utilizes a luminescent substrate for detection. The substrate was obtained from Paradigm Diagnostics (St. Paul, MN), and detects a *Listeria* specific enzyme in the cell wall (as opposed to PI-PLC). The luminescent test was identical to the colorimetric one, except for the fact that a luminescent substrate and a hand held luminometer were used to complete the test.

Specificity Tests

Fifty-seven strains of *L. monocytogenes* and 15 non-*L. monocytogenes* isolates were tested to determine the specificity of the Phast Swab assay. The non-*L. monocytogenes* strains that were tested included: 3 strains of *E. coli* O157:H7; 4 strains of non pathogenic *E. coli*; 1 strain each of *Salmonella* Typhimurium, *S.* Anatum, *S.* Enteriditis and *S.* Newport; 1 strain of *Listeria ivanovii* and 2 strains of *Listeria innocua.* The 57 strains of *L. monocytogenes* that were tested were obtained from the International Life Sciences Institute (ILSI) collection at Cornell University. The specificity of the Phast Swab was tested by adding 1 ml of an overnight culture of each bacterial isolate to an individual test device, followed by addition of 50 µl of X-inp. After incubation for 5 hours at 37^{o}C (with shaking), the Phast Swabs were evaluated for a color change (to indigo) indicating a positive result.

Sensitivity Tests

The five strain cocktail of *L. monocytogenes* was produced as described, and adjusted to a final concentration of 10^9 CFU/ml. The cocktail was serially diluted (10-fold) in a range from 10^0-10^{-9}. One milliliter of each dilution was placed into an individual Phast Swab, and incubated for 10 minutes to allow any *L. monyctogenes* cells to attach to the IMS beads. The Phast Swab device was placed on a magnet to separate the beads from the broth, which was removed with a disposable transfer pipette. The IMS beads were resuspended in 100 µl of lysis buffer, and 50µl of X-Inp was added. The Phast Swabs were allowed to incubate for 5 hours with shaking (250 rpm) at 37°C and then 100µl from each Phast Swab was pipetted into a separate well of a 96 well microtiter plate and evaluated at OD_{450nm} in the BioTEK Synergy II plate reader to determine the detection limit of the assay.

Inoculation procedures.

Inoculation of RTE meats

The five strain *L. monocytogenes* cocktail was prepared as described above. The cocktail was serially diluted (10-fold) in a dilution range from 10^0-10^{-9}, and individual slices of deli turkey meat (100 mm diameter) were inoculated with 1ml of each dilution. The meat was allowed to dry for 20 min, and then each piece was swabbed with an individual Phast Swab, followed by a10 hour enrichment.

Inoculation of Coupons

The five strain *L. monocytogenes* cocktail was prepared as described above. Individual stainless steel, acrylic and ceramic tile coupons (4"×4") were chosen to simulate food contact and non-contact surfaces, and were inoculated with 250µl of the 10^7, 10^6, 10^5, and the CFU/ml dilutions. The coupons were allowed to dry for 5 hours and were swabbed with an individual Phast Swab device. The devices were incubated for 13 h. Following completion of the test, each sample was transferred from the respective Phast Swab and placed into an

individual well of a 96 well microtiter plate. The plate was placed into a BioTEK II Synergy Plate reader and the absorbance was read at $OD_{450nm.}$

VII. Results

Evaluation of Different Enrichment Broths

Several broths were evaluated for their ability to rapidly increase the numbers of *L. monocytogenes* during enrichment. These broths included Buffered Peptone Water (BPW), Universal Preenrichment Broth (UPB), Brain Heart Infusion (BHI) broth, BHI supplemented with 428 mM potassium chloride (KCl), Tryptic Soy Broth (TSB) and TSB supplemented with 428 mM KCl. The results are shown in Figures 2-7 and indicated that TSB (KCl) allowed for the best enrichment of *L. monocytogenes* (Figure 7). While the lag phages of *L. monocytogenes* cells grown in TSB (KCL) was slightly extended compared to growth in TSB, the TSB (KCl) resulted in a slightly higher concentration of cells after the enrichment (as compared to TSB), and several studies have shown that production of PI-PLC is enhanced when the cells are grown in media supplemented with KCl (Myers *et al*. 1993). Both BPW and UPB were shown to result in very slow and low enrichment of *L. monocytogenes* (Figures 2 and 3).

Enrichment of Stressed Cells

We were interested in assessing the ability of the TSB (KCl) media to recover stressed *L. monocytogenes* cells. The results are shown in Figures 8-11. While the lag phases for each isolate was markedly increased, the media led to the recovery of *L. monocytogenes* regardless of stress, and maximum OD_{600nm} readings were achieved for all strains within 10 hours.

Production of the *Listeria monocytogenes* Phast Swab (colorimetric and luminescent)

We have developed a rapid assay for *L. monocytogenes* that addresses the limitations observed with other testing methods. The assay is called the Phast Swab, and integrates sampling and testing in a simple self contained device, which contains a swab for sampling, enrichment broth, *L. monocytogenes* immunomagnetic particles, a bacterial lysis agent, and a enzyme substrate (Figure 12).

To test a sample for the presence of *L. monocytogenes*, the swab is removed from the device, the surface is swabbed, and the swab is returned to the device, followed by enrichment for 10 hours (in the colorimetric version of the test)(Figure 12, #1). Following enrichment, the entire device is placed in a magnet, and any *L. monocytogenes* cells are specifically captured and concentrated by immunomagnetic separation for 10 minutes, and any background flora in the enrichment broth is removed (Figure 12, #2). Next, the cap of the swab device is broken, releasing a bacterial lysis agent which permeabilizes the cells, releasing their contents, and a colorimetric or luminescent substrate which will be specifically cleaved by the enzyme phosphatidylinositol-specific phospholipase C (PI-PLC) (Figure 12, #3). If the sample contains *L. monocytogenes*, a blue precipitate forms, while in a negative test, the color remains brown (Figure 12, #4).

Specificity

The Phast Swab was tested for specificity. The test detected 55 of the 57 *L. monocytogenes* strains tested. The two strains that the test did not detect have severe genetic mutations that affect virulence, (Nightingale 2008). The putative mutations are believed to be in *plc*A, which affects production of PI-PLC. None of the non-*L .monocytogenes* gave a false positive result, with the exception of one strain of *L. ivanovii*, which did produce a positive result due to the fact that this species produces PI-PLC.

Sensitivity

In pure culture, as few as 10^7 CFU/ml were directly detected within 3 hours, with the colorimetric version of the assay. When a luminescent substrate was used, as few as 10^5 CFU/ml were detected within 1 hour (Figure 13). However, the luminescent substrate proved to be unstable and the development of the luminescent assay was

subsequently discontinued. The addition of 428 mM KCl to the growth media significantly increased the sensitivity of the assay.

Detection of *L. monocytogenes* on Ready to Eat Deli Meat

The results indicated that the Phast Swab was capable of detecting 10^2 cells on the deli meat within 15 hours (Figure 14).

Detection of *L. monocytogenes* on Food Contact and Non Contact Surfaces

The Phast Swab was capable of detecting as few as 10^1 CFU/cm^2 on the ceramic, stainless steel, and acrylic coupons (Figures 15-17).

VIII. Discussion

Rapid detection of *L. monocytogenes* can be effected with the use of the Phast Swab Assay. The assay is capable of detecting *L. monocytogenes* on food contact and non-contact surfaces, as well as on RTE meats. The Phast Swab is completely integrated and takes between 15 and 18 hours to complete, depending on the type of test being conducted. For example, if RTE meat samples are being tested, results will be reported within 15 hours. If food contact and non-contact surfaces are being tested, then the test results will take 18 hours. The difference in total test time is due to the fact that *L. monocytogenes* isolated from food production surfaces is enriched for 3 more hours (13 hours) than cells from RTE meat samples (10 hours). The increased enrichment time allows for the cells (which may be stressed) to more fully recover.

Collectively, the results of this project indicate that that the Phast Swab can detect *L. monocytogenes* on food contact and non-contact surfaces in the food processing environment, and on RTE meats in a rapid and specific manner without the need for instrumentation to read the test result.

IX. References

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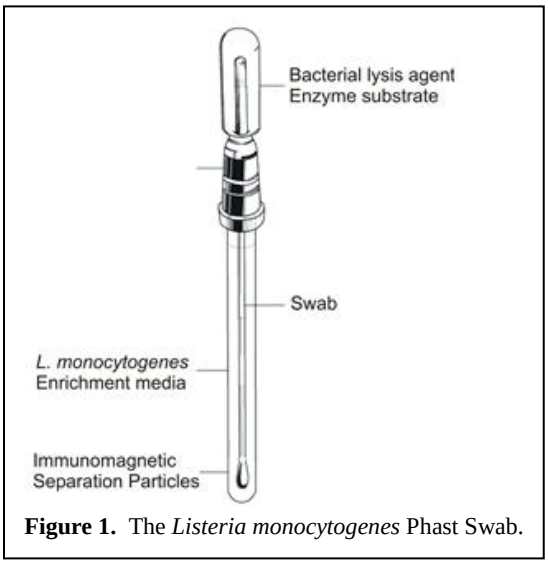


Figure 1. The *Listeria monocytogenes* Phast Swab.

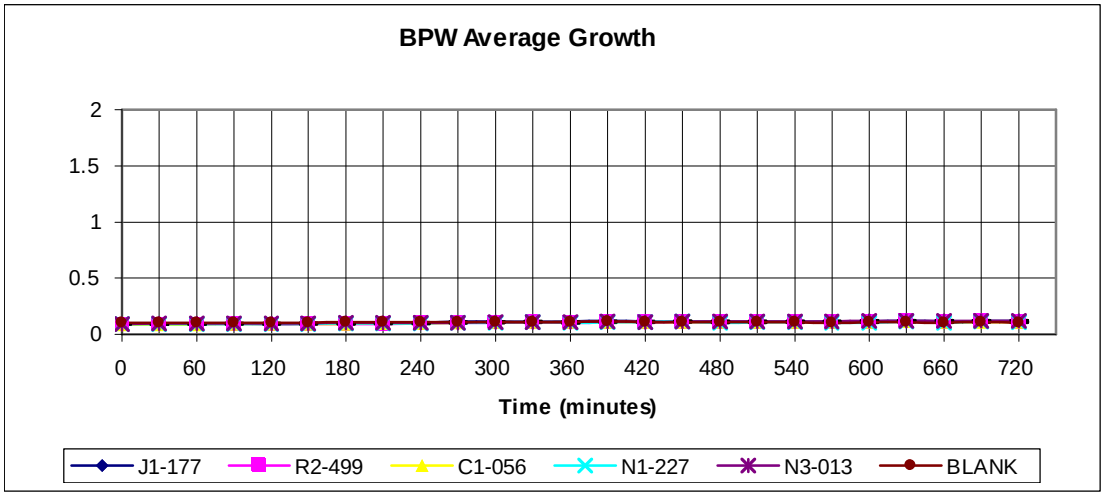


Figure 2. Growth of *Listeria monocytogenes* in Buffered Peptone Water. Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.

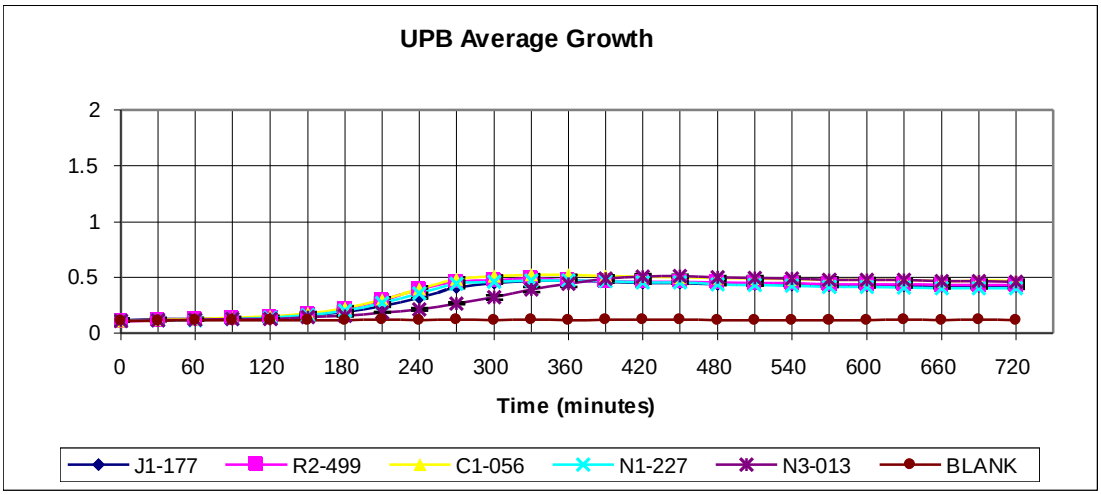


Figure 3. Growth of *Listeria monocytogenes* in Universal Preenrichment Broth. Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.

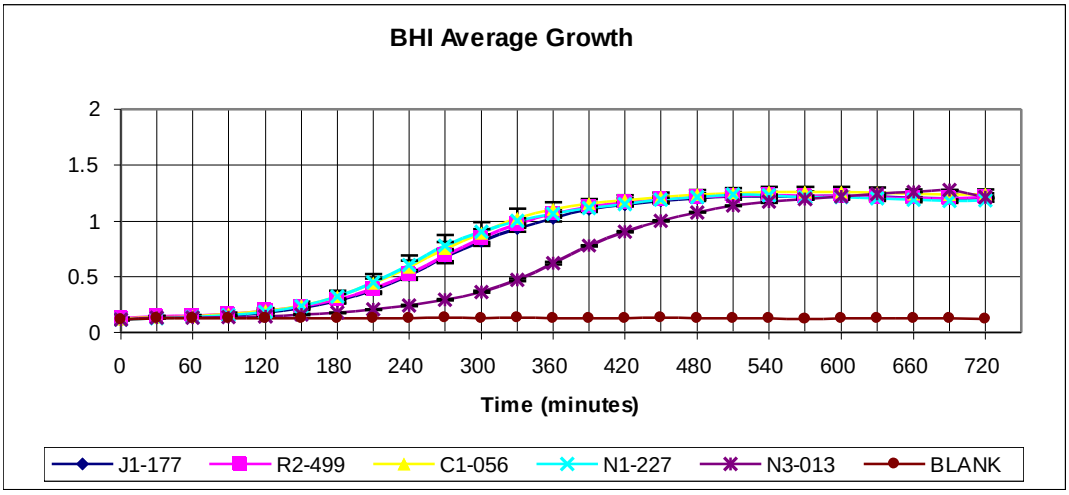


Figure 4. Growth of *Listeria monocytogenes* in Brain Heart Infusion Broth. Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.

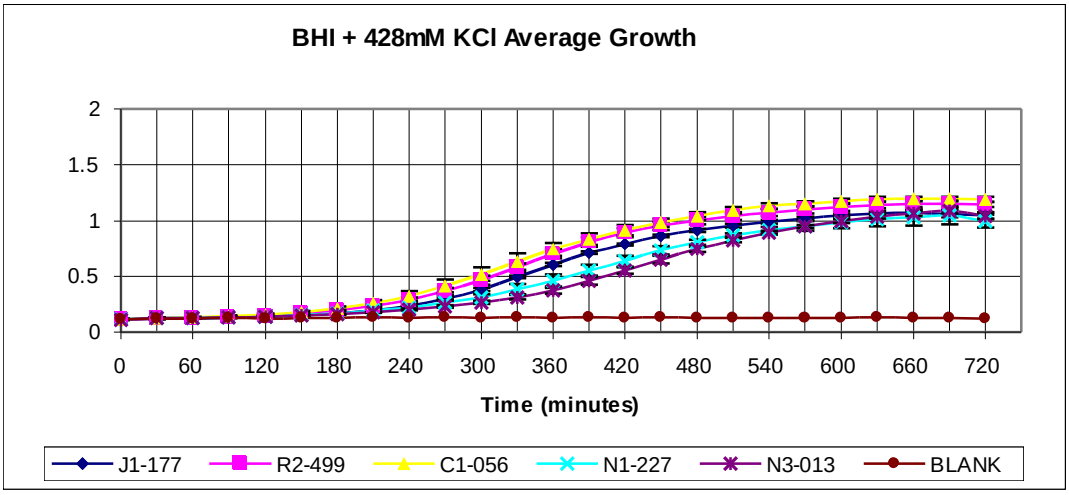


Figure 5. Growth of *Listeria monocytogenes* in Brain Heart Infusion Broth supplemented with 428 mM potassium chloride. Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.

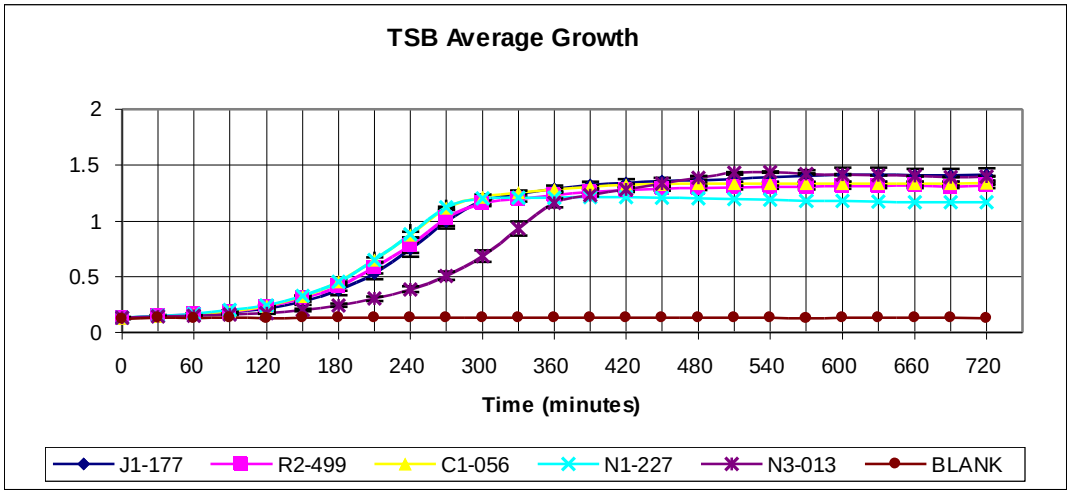
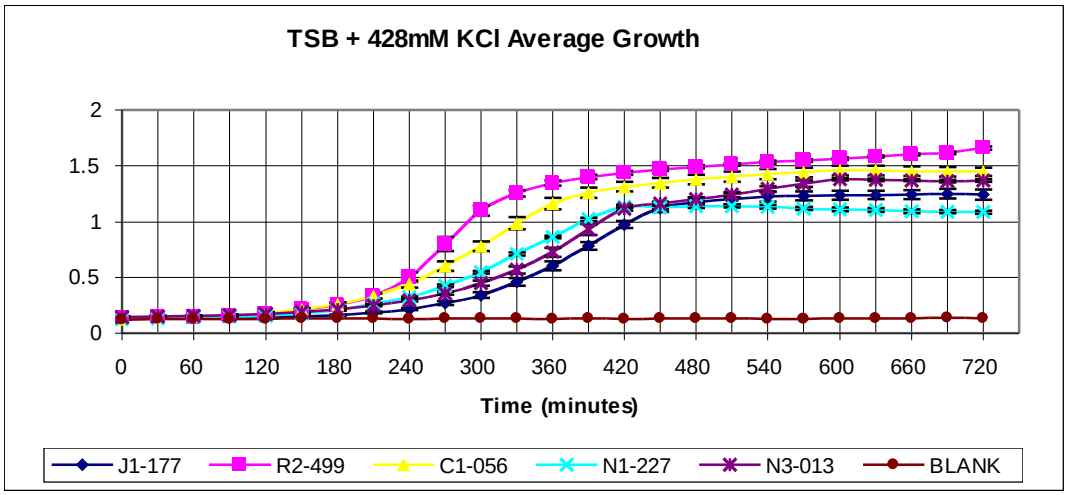


Figure 6. Growth of *Listeria monocytogenes* in Tryptic Soy Broth. Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.



F**igure 7.** Growth of *Listeria monocytogenes* in Tryptic Soy Broth supplemented with 428 mM potassium chloride. Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.

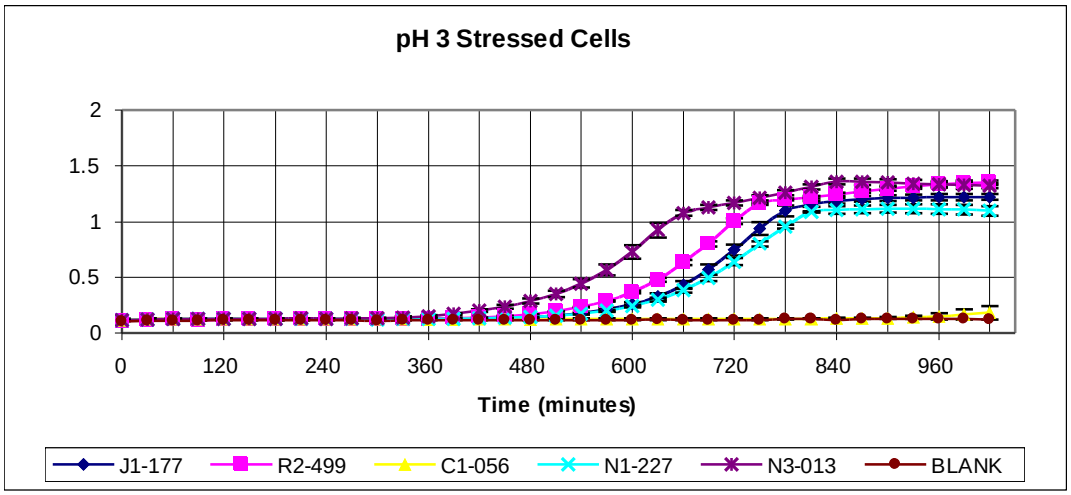


Figure 8. Growth of *L. monocytogenes* following incubation at pH 3. Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.

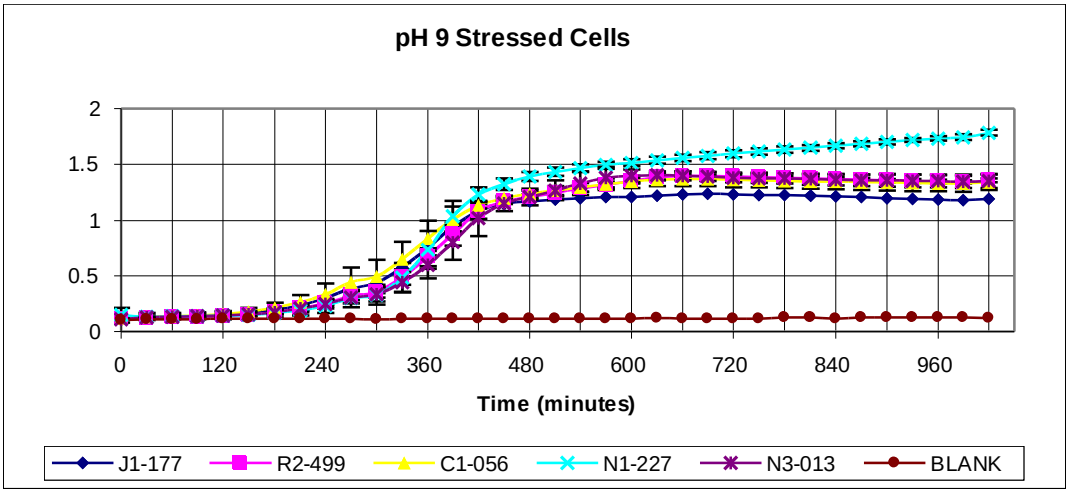


Figure 9. Growth of *L. monocytogenes* following incubation at pH 9. Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.

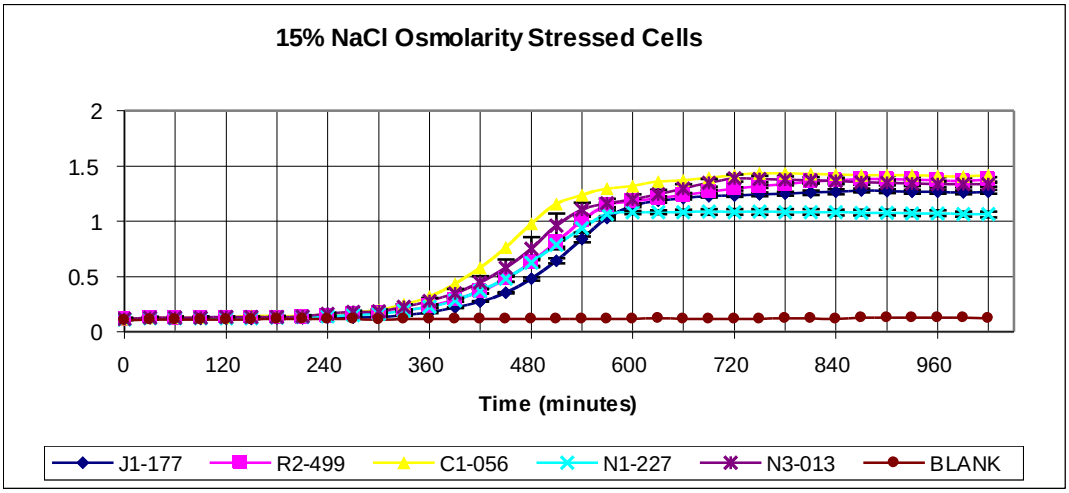


Figure 10. Growth of *L. monocytogenes* following incubation in high osmolarity conditions. Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.

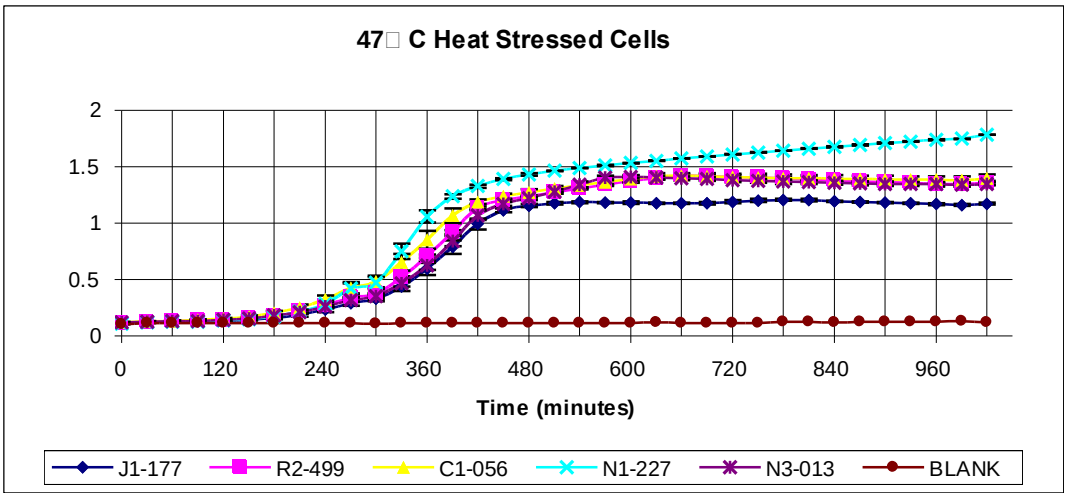


Figure 11. Growth of *L. monocytogenes* following incubation at an elevated temperature (47°C). Each *L. monocytogenes* isolate was individually evaluated. The individual isolates are denoted in the legend.

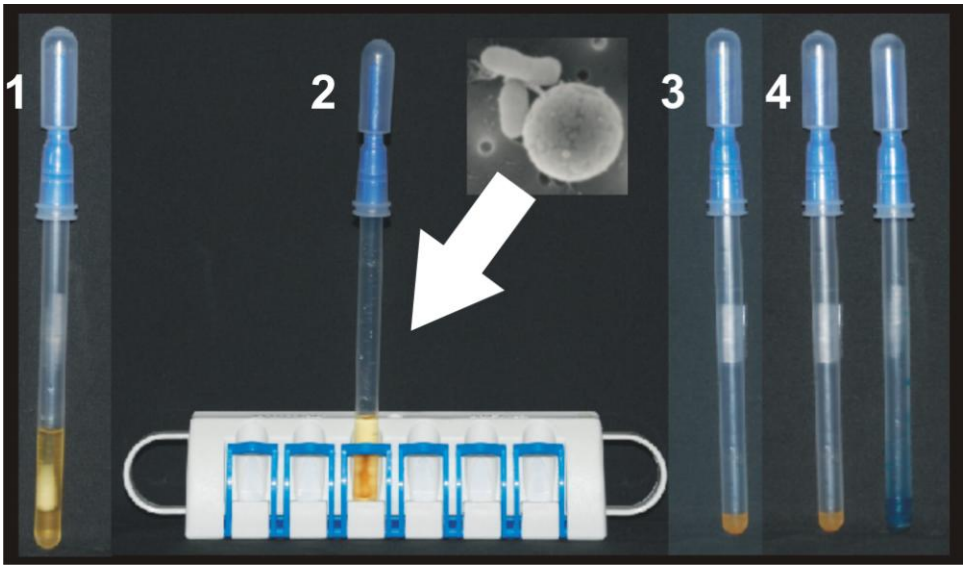


Figure 12. The Colorimetric Phast Swab.

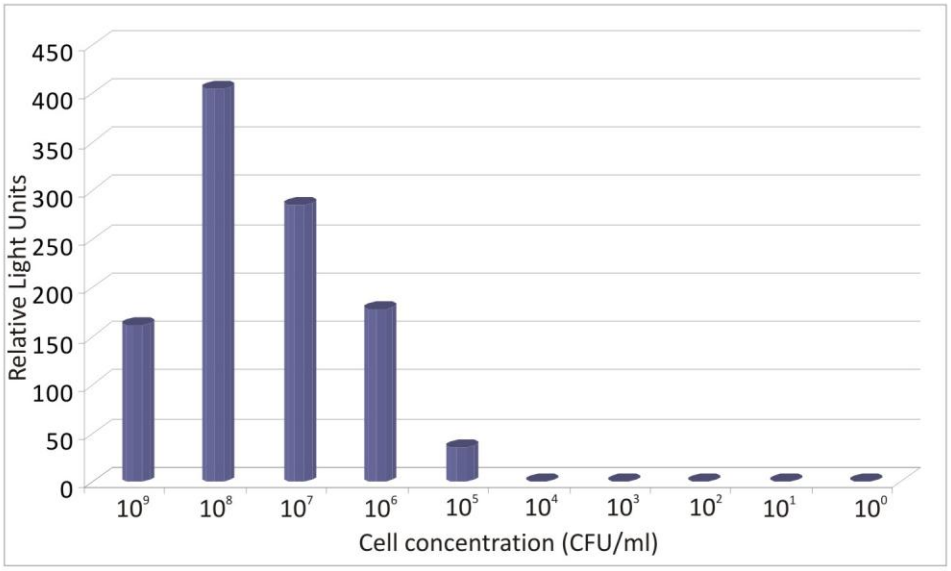


Figure 13. Sensitivity of the Luminescent Phast Swab

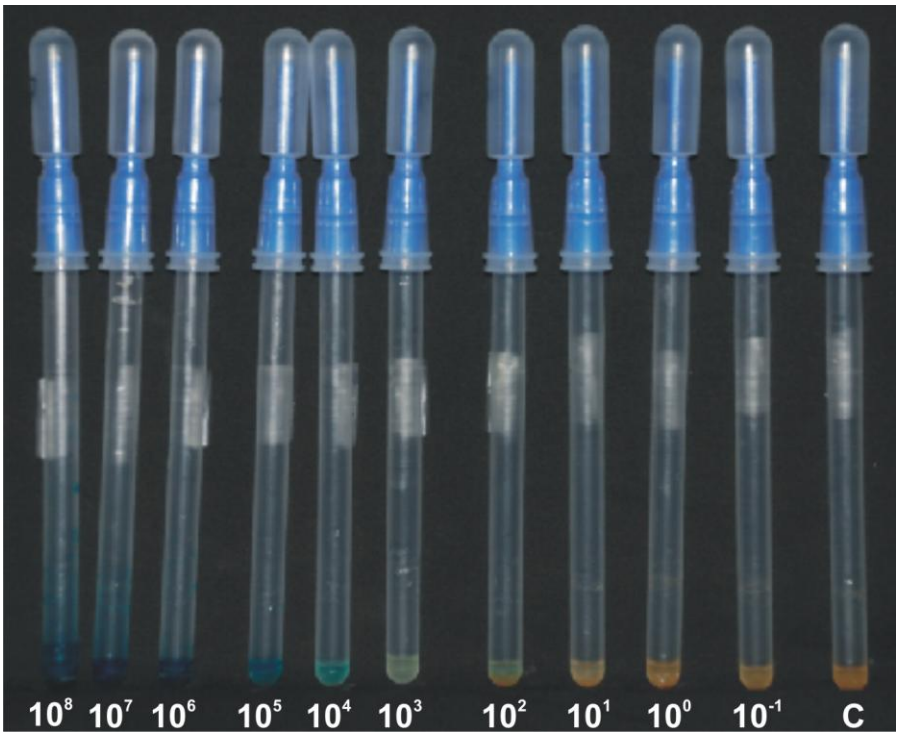


Figure 14. Results of the Phast Swab based detection *of Listeria monocytogenes* on RTE meat.. The detection limit was an initial inoculum of 10^2 CFU/slice of deli meat. Key: C, negative control. The initial inoculum (in CFU/100mm diameter of deli meat) is indicated beneath each Phast Swab.

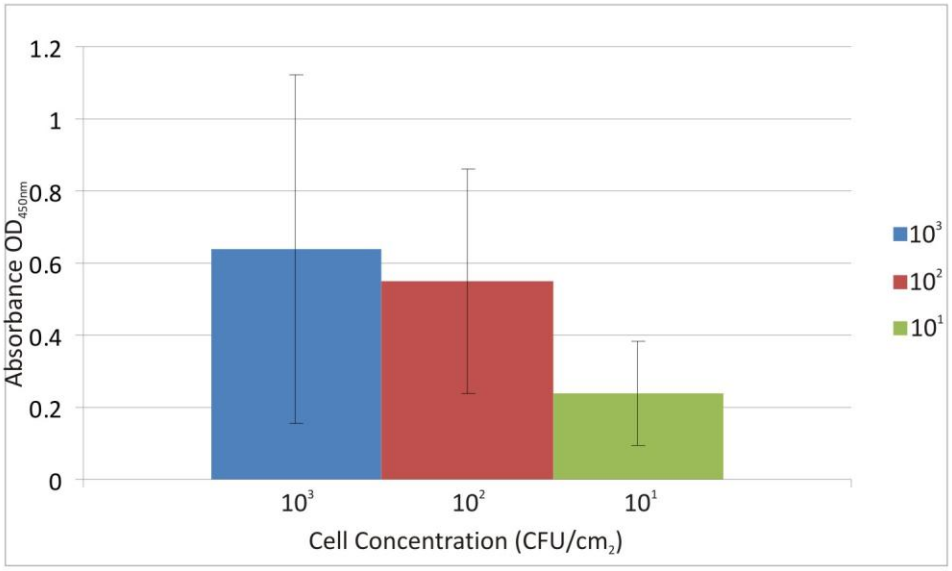


Figure 15. Detection of several concentrations of *Listeria monocytogenes* after inoculation onto 4 x4" steel coupons.

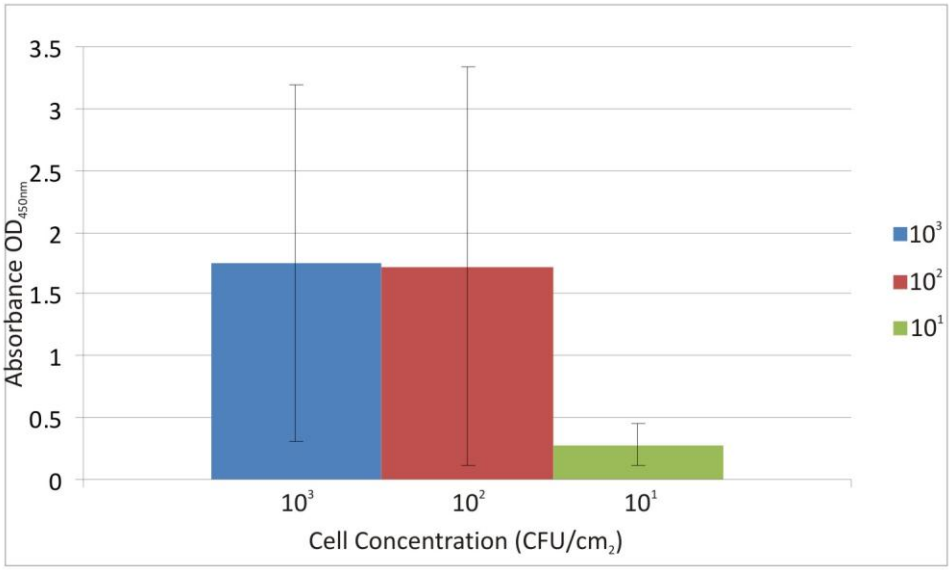


Figure 16. Detection of several concentrations of *Listeria monocytogenes* after inoculation onto 4 x4" ceramic coupons.

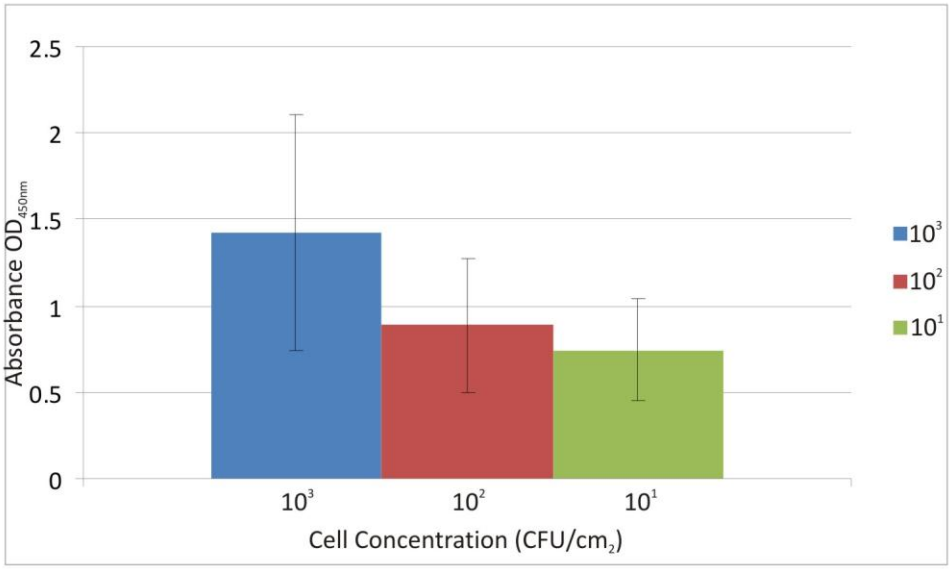


Figure 17. Detection of several concentrations of *Listeria monocytogenes* after inoculation onto 4 x4" acrylic coupons.