CAPTURE FILTRATION FOR
CONCENTRATION AND DETECTION OF
SELECTED MICROORGANISMS IN MILK

by

Robert Duane Byrne, Jr.

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APPROVED:

J. Russell Bishop, Chairman

S.E. Duncan

W.N. Eigel

C.R. Hackney

G.M. Jones

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(ABSTRACT)

The effectiveness of an adsorption filter in retaining bacteria present in milk was examined. Skim milk and whole milk (100ml) were separately filtered through a 47mm adsorption filter. No significant change in total solids, total fat, and solids-not-fat percentages of skim and whole milk permeates was observed after filtration. Adsorption of Pseudomonas fluorescens at target concentrations of $10^3$, $10^2$, and $10^1$ cells/ml was determined in 100ml of dairy standard methods buffer, nutrient broth, whole milk, and skim milk. The average percentage bacterial retentions were $95 \pm 5.5\%$, $95 \pm 2.6\%$, $28 \pm 22.1\%$, and $62 \pm 15.5\%$, respectively.

A treatment was developed for milk to increase the bacterial retention of P. fluorescens after filtration. The preferred treatment for 100ml of skim milk involved the following final concentrations (v/v): 0.80% disodium ethylenediamine-tetraacetic acid, 0.02% sodium dodecyl sulfate, pH to 7.5 with 1N sodium hydroxide. The average bacterial retention of P. fluorescens using the treatment
was 91 ± 7.1%.

Enumeration of bacteria adsorbed to the filter was then conducted using impedance microbiology. When milk was inoculated with *P. fluorescens* at target concentrations of $10^3$, $10^2$, and $10^1$ cells/ml, an average log bacterial increase of $1.4 ± 0.1$ (25x) was obtained. This method will allow for rapid detection of microorganisms in milk by increasing microbial load in the tested sample and eliminating the need for pre-enrichment.
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INTRODUCTION

Both spoilage and pathogenic microorganisms may be present in pasteurized milk as a result of post-pasteurization contamination. If present, the ability to detect these microorganisms within a short time is necessary to allow for effective corrective measures to be taken to ensure that a safe, high quality product reaches the consumer. Two problems exist when detection of microorganisms in milk is attempted. First, the level of microorganisms in a freshly pasteurized milk sample is usually very low. Many of the currently available rapid bacterial detection techniques are not sufficiently sensitive for detection at these low levels. Second, the current techniques can require a few days or weeks to detect the spoilage or pathogenic microorganisms. This time-frame does not allow for effective corrective measures to be taken if a quality or safety problem should occur. Therefore, the dairy industry needs a method for detecting spoilage and pathogenic microorganisms in a more timely manner.

The number of microorganisms in the tested sample must be increased in order to detect microorganisms in a shorter time-frame with adequate accuracy. An enrichment or preliminary incubation step may be used to increase the level of microorganisms in the test sample. These steps,
however, require additional time to increase the number of microorganisms to a detectable level.

The objective of this research was to develop a method for concentrating bacteria present in freshly pasteurized milk to a detectable level, making the time-consuming pre-enrichment step unnecessary. Such a concentration involves capture of bacterial cells in an adsorption filter (capture filtration) and subsequent detection of these bacteria using a rapid technique. If ≥90% of the microorganisms are concentrated onto the filter, the preliminary incubation and enrichment steps currently used to increase the level of microorganisms prior to detection may be eliminated. Subsequent use of rapid detection techniques to detect specific spoilage or pathogenic microorganisms can then be utilized.
LITERATURE REVIEW

Microorganisms In Milk

Milk is an ideal growth medium for bacteria. The approximate composition of whole bovine milk is 87-88% water, 3.1-4.0% fat, 4.5-4.8% lactose, 3.1-3.6% proteins, 0.1-0.8% ash, and a trace amount of vitamins and minerals (22,43,58,86,88,105,116,121). This composition readily supports microbial growth by supplying all the required nutrients. Microorganisms contaminate the milk either in the udder or after lactation. A cow with an udder infection (mastitis) may impart microorganisms directly into the milk prior to lactation. After lactation, microorganisms from milking equipment or personnel may contaminate milk.

Milk is pasteurized to ensure a safe milk supply that is free of pathogenic microorganisms. Pasteurization is a thermal process in which every particle of milk is heated to a specified temperature and held for a specified period of time. If pasteurization is properly performed, milk will be free of pathogens (114).

The bacteria that ultimately cause quality degradation of milk are those which are capable of growth at refrigeration temperatures. This group of bacteria was previously known as psychrophilic or cold-loving (13). This is a misleading term because these bacteria are not
cold-loving but cold-enduring (13, 59, 118). These cold-enduring bacteria are now classified as psychrotrophic microorganisms and are defined as bacteria that are capable of relatively rapid growth at refrigeration temperatures (27, 61, 115, 118). Several studies have shown that psychrotrophic microorganisms are the main cause of reduced shelf-life in milk (61, 91). Boyd (13) stated that flavor deterioration correlates with psychrotrophic bacterial growth and the primary bacteria isolated from milk with a poor keeping quality are gram-negative. Witter (118) reported that psychrotrophic bacteria are primarily responsible for limiting the keeping quality of milk and that most of the isolated microorganisms were gram-negative, non-sporeforming rods. Most of these isolates were \textit{Pseudomonas} species. \textit{Pseudomonas} are gram-negative, aerobic rods that do not ferment glucose. The genus has a strictly respiratory metabolism which makes \textit{Pseudomonas} inactive to many biochemical tests and difficult to characterize (33). Gyllenberg et al. (55) reported that pseudomonads may influence keeping quality. White et al. (117) reported \textit{Pseudomonas} species were present in 90% of 315 pasteurized milk samples that had reached the end of their shelf-life.

Psychrotrophs are present in water and soil and on dirty equipment (27, 118) but, for the most part, do not survive pasteurization (27). Psychrotrophic microorganisms
enter milk through post-pasteurization contamination (61, 67), from improperly cleaned and sanitized equipment, the air, or filling equipment (27, 56). Although reported data indicate that $5 \times 10^6$ (89), $>10^6$ (85), and $>10^7$ colony forming units per milliliter (cfu/ml) (80, 81, 95) are required before organoleptic changes are detected, low numbers of initial contamination will lead to a decreased keeping quality. Cousin (27) demonstrated that at 7.2°C, one psychrotrophic cfu/ml will multiply to $10^6$ psychrotrophic cfu/ml in 8 days or less. Carey (23) stated that psychrotrophs increased 5 logs from buy to pull date. Psychrotrophs will also influence keeping quality by secreting heat-stable enzymes such as proteinases and lipases (59, 65).

In addition to psychrotrophic microorganisms, some microorganisms that enter milk through post-pasteurization contamination may be pathogenic. Pathogenic microorganisms which have caused foodborne disease outbreaks involving dairy products include: Campylobacter jejuni, Salmonella, Clostridium botulinum, Brucella, Streptococcus, Staphylococcus aureus, Bacillus cereus, Versinia enterocolitica, Escherichia coli, and Listeria monocytogenes (5, 6, 28). This review will focus on Salmonella and Listeria due to the economic importance and product recalls associated with dairy products contaminated with either of
these two microorganisms. *Salmonella* caused the largest milkborne disease outbreak in recent history (66) and *Listeria* results in a large cost to a dairy plant when found in the plant or product (109). *Listeria* also has potentially lethal consequences for certain high-risk individuals (94).

*Salmonellae* are gram-negative, facultatively anaerobic, peritrichously flagellated rods that produce gas from glucose and utilize citrate as their sole carbon source. They produce hydrogen sulfide gas, decarboxylate lysine and ornithine, are urease-negative, and do not produce indole (33). Dairy products were implicated in approximately 6% of the *Salmonella* incidents in Canada between 1975-1984 and *Salmonella* was isolated from 3.9% of farm raw milk bulk tanks (110). Bryan (16) indicated that 41% of all milkborne disease outbreaks from 1970-1979 in the United States were due to *Salmonella* and that *Salmonella* was the cause of 50% of the milkborne disease outbreaks between 1980-1982. The United States Department of Agriculture lists milk as a major vehicle for *Salmonella* from 1977-1984 (112). In 1985 the largest outbreak of salmonellosis attributed to milk occurred in Illinois. More than 16,000 confirmed cases occurred as a result of the consumption of pasteurized milk contaminated with *Salmonella* (5,6,66). *Salmonella* continues to result in foodborne outbreaks.
Listeria emerged as a foodborne pathogen of importance in the 1980's (70,73,94). Listeria monocytogenes is a small, gram-positive, non-sporeforming, non-encapsulated, motile, multi-flagellated, rod. The microorganism is beta-hemolytic, catalase positive, and prefers microaerophilic conditions. The organism neither produces \( \text{H}_2\text{S} \) nor reduces nitrates and grows over a pH range of 5.6 to 9.8 and a temperature range of 1.0 to 42°C (94). L. monocytogenes utilizes rhamnose and gives a positive response to the Voges-Proskauer test (14,29). Individuals whose health is most at risk from L. monocytogenes are newborns, infants, the aged, pregnant women, and the immunocompromised (73,74).

Many researchers have reported the presence of Listeria in raw milk. Reported percentages of samples positive for Listeria vary from 3 to 12% (14,28,57,68,69,71,93). Raw milk may become contaminated with L. monocytogenes either from a cow carrying the microorganism or from the environment. Contaminated raw milk is one manner by which L. monocytogenes enters the dairy plant. Other sources of contamination include workers or contaminated materials and equipment (42). Listeria has been isolated from case washers, sanitizing floor mats, and foot baths within dairy plants at an overall 1.4% occurrence (63).

Refrigeration will not stop growth of L. monocytogenes because the microorganism is psychrotrophic. Stanfield
(106) reported that Listeria survives for up to 14 days on plastic and waxed containers at refrigeration temperatures. Marth (74) reported that Listeria grows at 4°C. Meyer and Donnelly (79) reported that injured cells repair in 8-10 days at 4°C while generation times at 4°C have been reported as 1.2 to 1.7 days (32).

Early reports indicated that L. monocytogenes could survive pasteurization. Bearns and Girard (7) reported that the organism would survive pasteurization if greater than 50,000 cells/ml were present in raw milk. In one case, milk implicated in an outbreak of listeriosis showed no signs of improper pasteurization. Based on this outbreak, the possibility that the organism survived pasteurization was suspected (48). Doyle et al. (31,32) reported that L. monocytogenes could survive high-temperature, short-time pasteurization. However, Donnelly et al. (30), Bradshaw, et al. (15), Bunning et al. (18), and Lovett et al. (71) have contradicted this conclusion by reporting that L. monocytogenes does not survive pasteurization, even when present intracellularly.

Detection of Listeria is important to the dairy industry due to the microorganism’s lethality and economic implications. Recalls of milk and ice cream contaminated with Listeria cost $70 million in 1985 (111). In California, more than 150 individuals developed listeriosis
after consuming soft-style cheese made from raw milk. The overall death rate was 34% (6).

*Salmonella* and *Listeria* produced the highest number of foodborne disease-associated deaths reported from 1973-1987 (6,109). In 1983 and 1985, 25 of 35 and 72 of 76 deaths due to foodborne disease, respectively, were the result of illness from *Salmonella* and *Listeria* (5). From an industry and health-care cost perspective, these two microorganisms are also of much concern (92). Of the estimated 12.6 million cases of foodborne illness reported from 1973-1987, estimated to cost $8.4 billion per year, salmonellosis and listeriosis accounted for $4.0 billion and $313 million, respectively. *Salmonella* costs an estimated $700 per case, whereas *Listeria* costs approximately $135,000 per case (109). A zero tolerance level exists for both of these microorganisms in dairy products (120).

**Detection of Spoilage Microorganisms/Impedance Microbiology**

Impedance is the resistance to flow of an electrical current through a conducting material and is composed of conductance and capacitance. Conductance is the reciprocal of resistance (35). Capacitance occurs at the interface between the electrode and electrolyte and is affected by the adsorbed ions at the electrode as well as the solvated ions at the interface. Parsons and Sturges (84) stated that
conductivity could be used to detect metabolic changes in a medium. Allison et al. (1) stated that the electrical conductivity of solutions depends upon the concentration and mobilities of charged particles of varying sizes. As bacteria metabolize, they break down large molecules in the medium into smaller, charged molecules, which reduces the resistance to flow of the current, thereby increasing the conductance. At a concentration of approximately $10^7$ cfu/ml, a sharp inflection in the flow results. The point of inflection is the detection time.

Measurements of impedance have been used to detect post-pasteurization contamination of milk (8), contamination of cream (54), coliform contamination of milk (46), and for raw milk screening (21,83). Gnan and Luedcke (51) reported a correlation for Impedance Detection Time (IDT) to standard plate counts of -0.77 to -0.88. Firstenberg-Eden and Tricarico (47) obtained correlations for IDT to total, mesophilic, and psychrotrophic plate counts of -0.96, -0.95, and -0.96, respectively.

Martins et al. (75) found that impedance detection was superior to standard plate counts and psychrotrophic counts for predicting shelf-life of milk on the day of pasteurization since results could be obtained in 14 hours compared to 2 to 10 days. Kahn and Firstenberg-Eden (62) observed that IDT could be used as a substitution for
sensory evaluation for shelf-life determination. Bishop et al. (9), Bishop and White (10), and Byrne et al. (19) obtained correlations for IDT to shelf-life of milk of 0.88, 0.93, and 0.91, respectively.

Impedance has been used to detect both Salmonella and Listeria. Salmonella has been detected by impedimetric measurements in confectionery products (4), milk chocolate (17), and animal feeds (99). A pre-incubation for 18 hours at 37°C in buffered peptone water with trimethylamine oxide, followed by inoculation of test cells containing Easter and Gibson medium and incubation for 30 hours may be used in order to detect Salmonella in dairy products within 48 hours (26). Listeria has also been detected in cheese and ice cream after a preliminary incubation in enrichment broth for 24 hours at 37°C followed by incubation in conductance medium (12). Other selective agents for detecting Listeria that have been developed using impedance include: Rodriguez Listeria Medium and AC medium (87), and Listeria Enrichment Broth with lithium chloride and oxford antimicrobial supplement (82).

Detection of Salmonella

Culture methods have traditionally been used to detect Salmonella in foods. The main disadvantage of these methods is the time required to obtain a positive or negative
result. These methods usually require five steps for positive confirmation of *Salmonella*: pre-enrichment, enrichment, selective plating, biochemical characterization, and serological confirmation (2,3).

The pre-enrichment step is usually in a non-selective medium and functions to allow for repair of damaged cells. Typical media for *Salmonella* pre-enrichment include lactose broth, trypticase soy broth, nonfat dry milk, nutrient broth, and peptone water (2). The time required for pre-enrichment is usually 18-24 hours at 35°C.

The enrichment step is selective for the particular microorganism of interest. This step encourages growth of the pathogenic microorganism to a detectable level. Typical enrichment media for *Salmonella* include selenite cysteine broth and tetrathionate broth (2,3). A typical enrichment for *Salmonella* requires 24 hours at 35°C.

Enrichment cultures are streaked onto selective plating media to detect the presence of *Salmonella*. Media typically used for detection of *Salmonella* include xylose lysine desoxycholate agar, hektoen enteric agar, brilliant green agar, and bismuth sulfite agar (104). The plates are incubated for 24-48 hours at 35°C.

Typical colonies from selective medium plates are then characterized based on biochemical responses to obtain a presumptive confirmation for the presence of *Salmonella*.
Triple Sugar Iron slants are typically used for this purpose and require an additional 24-hour incubation at 35°C (104). Other biochemical tests used for characterization of Salmonella include various rapid biochemical characterization diagnostic kits such as Micro ID, API, and VIDAS. Colonies presumptively positive are then tested by serological methods to confirm the presence of Salmonella. Antisera specific for the K, pili, somatic, and flagellar antigens of the Salmonella serotypes of interest may be used (104) for confirmation.

Conventional culture methods for detecting Salmonella in foods are time-consuming and expensive. At least 4 days are required to complete adequate testing to obtain a negative result and several more days are needed to complete confirmatory testing for the presence of Salmonella (2). The overall cost in 1985 was approximately $10 per test. Additional costs associated with conventional culture methods include the expense companies incur as a result of holding and storing the product while waiting the 4–6 days for results. Mattingly (76) states that a simple, accurate, rapid method to detect Salmonella has been an elusive dream of the food industry for some time.

Many types of rapid tests have been developed to detect Salmonella in foods (44). Many of these methods continue to use the pre-enrichment or selective enrichment steps to
increase numbers of *Salmonella* to a detectable level. Swaminathan (107) indicated that rapid tests are advantageous because they reduce warehousing costs, give the ability to respond quickly to contamination problems, and allow for increased testing for *Salmonella*. The consumer will have more confidence in product safety and the opportunity to purchase pre-tested products. Feng (44) reported the sensitivity of the commercial rapid tests for detection of *Salmonella* is similar to that of conventional methods. Traditional pre-enrichment is necessary to resuscitate injured cells; enrichment is required to amplify cell numbers; and positive results must be confirmed through traditional culture methods with most of the rapid tests (44).

**Detection of *Listeria monocytogenes***

Isolation procedures for *L. monocytogenes* require enrichment, isolation, and identification steps. The level of *Listeria* in food is low and direct detection has proven unsuccessful (24,70). Enrichment procedures successfully used to detect *L. monocytogenes* include cold enrichment at 4°C for 1 week and 1 month (70), selective enrichments for 7 days at 30°C in University of Vermont Broth (70), and cold enrichment in tryptose broth (96). Isolation of *L. monocytogenes* has been accomplished by plating onto
selective media. McBride’s Listeria agar (77), Modified McBride’s Listeria agar (70), Martin’s Listeria agar (70), and Oxford agar (49) have been successfully used for isolation of Listeria. The official method employed by the United States Food and Drug Administration involves placing 25ml of sample into enrichment broth and incubating for 24-48 hours at 30°C. After incubation the sample is streaked onto Oxford and LPM agar and incubated at 35°C for 48 hours. Isolated colonies are streaked onto T-Scott Agar with Yeast Extract and incubated for 24 hours at 30°C. Typical colonies are then selected and confirmed. The total time required before confirmation is 4-10 days. Confirmation of the microorganism requires at least 2 weeks. Biochemical tests, a test for motility, and the CAMP test are then conducted to confirm Listeria (104). The main drawback of this method is the requirement for a minimum of 2 weeks for isolation and identification of the organism.

Filtration

Membrane filtration came into use in the United States after World War II to provide early detection of microorganisms in bacteriological warfare. This introduction initiated the development of filtration technology for use in bacteriological analysis. The small pores in membrane filters function to allow all liquid to
pass through the filter while microorganisms are retained on the membrane. A typical membrane filter is a screen or surface on which filtered particles are retained on the surface or within a 10-15μm depth. An average membrane filter has 400-500 million pores/cm² of filter surface area (34). Nutrients can soak through the thin, porous filter after the filter is placed on an agar surface. Microorganisms on the filter can then grow and produce visible colonies.

Membrane filters rely on sieving action, adsorption, and coagulation to retain microorganisms. A membrane filter allows filtration of large volumes of liquid that contain a low concentration of microorganisms. The first extensive use of membrane filtration was in the analysis of water for the presence of coliform bacteria (34).

Membrane filtration has some limits, particularly for food systems. Sample characteristics may adversely affect the use of a membrane filter procedure (34) and particulate matter in a sample may limit the amount of sample filtered. Particulates greater in size than the 0.22-0.45μm pores in the membrane filter affect filtration by clogging the pores. A larger pore size filter could be used for food analysis but microorganisms would not be retained on the filter.

With respect to filtration of milk, the water will
readily pass through the small pores of the membrane filter but some constituents will not. Fat globules in raw milk range in size from 0.1 to 15μm in diameter and, therefore, may clog the pores of the filter. The casein micelles in milk range in size from 10-300nm. Consequently, they will also clog the pores of the filter. The globular proteins and lipoprotein particles range from 3-10nm and should pass through the filter's pores. Chloride ions, calcium ions, and lactose are all less than 1nm in diameter and should pass through the membrane filter (116). The larger constituents present in milk make large volumes of milk impossible to filter through the small pores of a membrane filter.

Another filtration procedure, the hydrophobic grid membrane filter, has been used in the food industry to concentrate microorganisms. This method uses a membrane filter that has a hydrophobic material imprinted in a grid pattern. The hydrophobic grid prevents spreading of colonies after incubation (38,39,40). This method is capable of detecting *Salmonella* in food in 2-3 days (37). Foods tested include semisweet chocolate, raw ground poultry meat, black ground pepper, cheese powder, egg powder, and nonfat dry milk (38). In this method, 25 grams of sample are pre-enriched and enriched before filtration of 1-10ml.
A limitation of this procedure is that very little of the original sample is filtered, thus decreasing the sensitivity of subsequent detection methods. The time required to detect microorganisms is also increased because of the required pre-enrichment and enrichment steps.

The hydrophobic grid membrane filter method has been used in conjunction with immunoassays to concentrate and detect specific microorganisms (41,98,107,119). Smith and Jones (98) used membrane filtration followed by an ELISA to detect Salmonella in milk, but found that the method was not effective. *Standard Methods for the Examination of Dairy Products* (90) includes a hydrophobic grid membrane filtration technique to concentrate coliforms in cheddar cheese. The method uses the sieving action of the small pores in the membrane filter to retain microorganisms. The type of food that may be filtered and sample size is limited. A sample dilution and pre-enrichment and/or enrichment procedure is also required.

**Adsorption**

The sieving action of membrane filters is not effective for retaining microorganisms in particulate solutions. The adsorption of cells to a filter material may be used to allow a sufficient volume of liquid to be filtered. Microorganisms in a liquid are considered living colloids.
and therefore follow physiochemical principles (11,36). The adsorption of colloids is influenced by the adsorption material, the solution, and the material being adsorbed (78). The adsorption material’s composition, temperature, surface area, availability, surface charge, and radius of curvature all influence the ability to adsorb particles (11,50,78,97). The solution’s temperature, pH, ionic strength, solutes, inorganic salt concentration, and contact time also affect adsorption. Cell concentration, polarity of cell surface, isoelectric point of proteins, net charge, size, and structure affect the ability of bacteria to adsorb (11,72,78,97).

The initial reaction between a bacterial cell and a surface is governed by long-range electrostatic and electrodynamic forces such as Van der Waals forces (50,60,64). As the cells move closer to the adsorption surface, short-range hydrophobic, cation bridging, and dipole-dipole forces may occur to promote adsorption of the bacterial cell to surfaces (50). Some researchers claim that the only requirements for successful adsorption are mutually attractive forces and a liquid medium (11). Adsorption may also occur between surfaces of similar charge if the repulsion is mediated by other factors (e.g. pili). Adsorption may occur as a result of anion exchange, a physical attraction, a chemical attraction, or a specific
attachment of functional groups (97).

The primary use of adsorption filtration to date has been for the detection of viruses. Initially, enteroviruses were recovered from water using adsorption. Sobsey et al. (100) filtered large volumes of water through epoxy-fiberglass and nitrocellulose filters and achieved a 77% recovery of enterovirus. Hepatitis A virus is retained from drinking water at a rate of 80% using electronegative filters and 97% using electropositive filters when the pH of the medium is adjusted to either 3.5, 4.5, or 5.5 and magnesium chloride and aluminum chloride are added to the solution before filtration (103). The overall recovery, after elution of the Hepatitis A Virus from the adsorbent, was 50%. Finance et al. (45) examined ground beef, oysters, and mussels and determined that both pH and conductivity affected the adsorption rates.

Sobsey (101) evaluated the ability of viruses to be recovered from oysters using adsorption. A pH of 5.5, aluminum chloride, and a low salt concentration was necessary to achieve a 63% recovery of the enterovirus. An overall recovery of 46% was obtained for Reovirus, Poliovirus, and Adenovirus using a pH of 5.0 and ≤2,000 mg of sodium chloride per liter (102). High conductivity resulted in lower adsorption rates. Sullivan et al. (108) recovered 55.4% of poliovirus from oyster samples, but found
that lowering the pH to 4.8 was necessary. Cole et al. (25) evaluated six methods to detect enteric viruses from oysters. They determined the best method required a pH of 5.0 for adsorption, followed by elution of the virus from the filter material. The overall recovery was 60.3%.

The above studies indicate that adsorption of viruses or bacterial cells onto a filter material is possible. The conditions of the solution containing the viruses or bacterial cells must be optimized to allow for successful adsorption. When high percentage retentions were obtained, an overall low recovery of viruses resulted after elution. Therefore, more efficient removal of the virus or bacterial cell from the filter material is necessary. Alternatively, the viruses or bacterial cells may be detected while adsorbed to the filter material.

The objective of this research was to develop a method for concentrating bacteria present in freshly pasteurized milk to a detectable level, making the time-consuming pre-enrichment step unnecessary. Such a concentration involved capture of bacterial cells in an adsorption filter (capture filtration) and subsequent detection of these bacteria using a rapid technique. If ≥90% of the microorganisms are concentrated onto the filter, the preliminary incubation and enrichment steps currently used to increase the level of microorganisms before detection may be eliminated.
Subsequent use of rapid detection techniques to detect specific spoilage or pathogenic microorganisms can then be utilized.
MATERIALS AND METHODS

Phase I - Filtration Effects on Composition

Skim milk and whole milk were obtained from local retail stores, transported directly to the laboratory, and held at 3°C until tested. A volume (100ml) of each was filtered separately through a 47mm adsorption filter (Biomérieux Vitek Systems, Inc., Hazelwood, MO) under 5 inches of mercury vacuum. The adsorption filter used was a fiberglass depth filter without a true pore size. In this filter system, efficient filtration was accomplished by the layers of fiberglass fibers and filler particles within the filter matrix. A tortuous path was formed which served to entrap particulate matter during filtration (52). Percent fat and total solids for whole and skim milk were determined using the Babcock test and the oven drying method, respectively (90). Fat and total solids were determined prior to filtration and on the filtrate. The study was replicated three times.

Phase II - Determination of Bacterial Loss after Filtration

The loss of Pseudomonas fluorescens ATCC 13525 from a liquid medium was examined by separately filtering 100ml of inoculated dairy standard methods buffer (90), nutrient broth (Difco Laboratories, Detroit, MI), whole milk, and
skim milk. Dairy standard methods buffer and nutrient broth (100ml) were sterilized in an autoclave at 121°C for 15 minutes. Milk was rendered free of bacteria by transferring 100ml into milk dilution bottles and steaming in an autoclave for 5 minutes at 100°C. The steamed milk was stored at 3°C until use. *P. fluorescens* culture was maintained on a nutrient agar slant at 3°C. A loop of culture was transferred to 9ml of sterile nutrient broth and incubated for 24 hours at 21°C. An inoculum from this culture (1ml) was transferred to 9ml of sterile nutrient broth and incubated at 21°C for 48 hours to obtain an approximate concentration of $5 \times 10^8$ cells/ml (20). The stationary phase culture was diluted in the appropriate liquid (sterile dairy standard methods buffer, sterile nutrient broth, steamed whole milk, or steamed skim milk) to obtain target concentrations of $10^3$, $10^2$, and $10^1$ cells/ml. The inoculated liquid was filtered through a 47mm adsorption filter (Biomérieux Vitek Systems, Inc., Hazelwood, MO) under 5 inches of mercury vacuum. A modified Psychrotrophic Bacteria Count (mPBC) (90) was conducted on the inoculated liquid prior to filtering and on the filtrate using Petrifilm AC (3M Corporation, St. Paul, MN). Bacterial loss from the liquid was calculated as the difference between the two bacterial plate counts. The study was replicated three times.
Phase III - Development of Method for Maximizing Bacterial Loss in Filtrate

Skim milk was obtained from local retail stores, steamed as previously described, and stored as described above. *P. fluorescens* ATCC 13525 was inoculated into 100ml of milk as described above. Treatments for altering milk component concentration; physiochemical, chemical, and biochemical characteristics of milk; or the interaction of microorganisms and filter material are given in Appendix A. The treatments were used to enhance the apparent bacterial retention on the filter as determined by bacterial loss in the filtrate. The inoculated, treated milk was filtered through a 47mm adsorption filter (Biomérieux Vitek Systems, Inc., Hazelwood, MO) under 5 inches of mercury vacuum. An MPBC was conducted on the inoculated, treated milk prior to filtering and on the filtrate using Petrifilm AC. The calculated difference between the bacterial plate counts indicated the promotion of apparent bacterial retention in the filter matrix. The preferred treatment was chosen based upon simplicity, repeatability, and a desired 90% average retention of *P. fluorescens*.

Phase IV - Enumeration Of Adsorbed Microorganisms

Steamed (as previously described) skim milk (15ml) was inoculated with either *P. fluorescens* ATCC 13525, *Salmonella*
typhimurium ATCC 14028, or Listeria monocytogenes Scott A 3b (obtained from Drs. Brackett and Beuchat, University of Georgia). P. fluorescens was maintained and inoculated as described previously. S. typhimurium was maintained and inoculated in the same manner as described for P. fluorescens except the incubation temperature was 32°C. L. monocytogenes was maintained on a trypticase-soy with 0.5% yeast extract agar slant at 3°C. A loop was transferred to 9ml of sterile trypticase-soy with 0.5% yeast extract broth and incubated at 32°C for 24 hours. An inoculum (1ml) from this culture was transferred to 9ml of sterile trypticase-soy with 0.5% yeast extract broth and incubated at 32°C for 48 hours to obtain an approximate concentration of $5 \times 10^8$ cells/ml (20). This stationary phase culture was diluted in steamed milk to the appropriate concentrations.

Inoculated milk and treated milk were enumerated by impedance microbiology by transferring 0.1ml to 1.5ml of Modified Plate Count Broth (Yeast Extract 20.0 g/l, Tryptone 20.0 g/l, and Dextrose 4.0 g/l) in a Bactometer module (Biomérieux Vitrek Systems, Hazelwood, MO). Inoculated, treated milk samples were filtered under 5 inches of mercury vacuum and viable cells on the adsorption filter were detected by transferring the filter to 1.5 ml of Modified Plate Count Broth the module. Each target concentration of inoculated milk was filtered through separate 13mm
adsorption filters (Biomérieux Vitek Systems, Inc., Hazelwood, MO). The filter size was reduced to 13 mm to accommodate the well size in the module. The volume of milk was decreased to 15 ml. A Bactometer M128 (Biomérieux Vitek Systems, Hazelwood, MO) was used to enumerate the microorganisms in the inoculated milk and on the filter. The instrument was operated at 21°C for P. fluorescens and 32°C for S. typhimurium and L. monocytogenes. Detection times generated by the instrument were converted to log cfu/ml using linear, quadratic, and hyperbolic standard curves. Standard curves for each microorganism were generated by adding 0.1 ml of serial dilutions to 1.5 ml of Modified Plate Count Broth in wells of the module. Detection times were compared to the appropriate plate count for each microorganism. The study was replicated three times.

**Statistical Analysis**

Comparison of means for possible compositional variation was conducted using Duncan's test of the Statistical Analysis System (SAS Institute, Cary, NC).
RESULTS AND DISCUSSION

Phase I - Filtration Effects on Composition

Effective use of the adsorption filter requires pores to remain free for trapping bacteria. Pores clogged with milk constituents could potentially reduce the number of bacterial cells captured. A comparison of milk composition before and after filtering was used to determine the extent of potential clogging of the pores. Whole or skim milk (100ml) was filtered through a 47mm adsorption filter. Fat, total solids, and solids-not-fat percentages were not significantly (p<0.05) changed in the filtrate relative to the composition of the unfiltered milk (Table 1). These results indicate that milk passed through the filter and that the pores of the filter were not fouled by the milk components. Fat globules in raw milk range in size from 0.1-15μm in diameter; casein micelles in milk range in size from 10-300nm; globular proteins and lipoprotein particles range from 1-10nm; chloride ions, calcium ions, and lactose are all less than 1nm in diameter. In homogenized milk the fat globules are decreased by a factor of approximately 10. Most milk constituents, therefore, should pass through the filter because they are smaller than 0.5-1.0μm, the normal porosity of the adsorption filter (52).
Table 1. Average composition of unfiltered and filtered whole and skim milk.

<table>
<thead>
<tr>
<th></th>
<th>UNFILTERED</th>
<th>FILTERED</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Fat</td>
<td>3.28±0.00</td>
<td>3.24±0.03</td>
</tr>
<tr>
<td>% Total Solids</td>
<td>12.05±0.04</td>
<td>11.99±0.10</td>
</tr>
<tr>
<td>% Solids-Not-Fat</td>
<td>8.77±0.04</td>
<td>8.75±0.10</td>
</tr>
</tbody>
</table>

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SKIM MILK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Fat</td>
<td>0.05±0.00</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>% Total Solids</td>
<td>9.12±0.05</td>
<td>9.12±0.05</td>
</tr>
<tr>
<td>% Solids-Not-Fat</td>
<td>9.07±0.05</td>
<td>9.07±0.05</td>
</tr>
</tbody>
</table>

n=3
Phase II - Determination of Bacterial Loss after Filtration

Bacterial loss after filtration was used to determine retention of *P. fluorescens* on the 47mm adsorption filter. The average percent bacterial retentions were 95 ± 5.5%, 95 ± 2.6%, 28 ± 22.1%, and 62 ± 15.5% for dairy standard methods buffer, nutrient broth, whole milk, and skim milk, respectively (Table 2). The high average percentage of retention (95%) after filtering dairy standard methods buffer indicated that *P. fluorescens* was adsorbed onto the filter material even though the normal porosity of the filter (0.5-1.0μm) was larger than the pore size (0.22-0.45 micron) of membrane filters typically used to retain microorganisms. An interaction such as adsorption between the bacterial cell and the filter material would explain the retention of bacterial cells on the filter. The high average percent retention for nutrient broth (95%) indicated that a more complex liquid solution would still allow adsorption of *P. fluorescens* onto the filter material.

Retention of *P. fluorescens* was low (28%) when whole milk was filtered indicating that some component of milk adsorbed to the bacterial cell or influenced the filter to create conditions unfavorable for bacterial adsorption. The higher retention for skim milk (62%) indicated that fat affected bacterial adsorption onto the filter material. However, fat was not the only factor involved since
retention for skim milk was still not as high as dairy standard methods buffer or nutrient broth (Table 2). These results indicate that one or more components of milk adversely affect the adsorption of microorganisms onto the filter material.

**Phase III - Development of Method for Maximizing Bacterial Loss in Filtrate**

Treatments developed to promote adsorption of microorganisms onto the filter material were designed to change the conditions of the solution, the bacterial cell, the filter material, or a combination of the above factors. Tables 3-23 illustrate the log count of bacterial cells before and after filtration and the subsequent percent not recovered in the filtrate (i.e., the percent retention plus any possible cell death). Initial treatments involved diluting the milk with dairy standard methods buffer to decrease the concentration of milk components in the solution, make the milk easier to filter, and allow greater contact of microorganisms with filter material. Data from these treatments indicate that dilution of the milk was not effective in promoting adsorption of microorganisms onto the filter material (Tables 3-4). A possible explanation is the ratio of milk components to bacterial cells remains the same after dilution, thus enabling milk components to continue
Table 2. Average percent retention* of *Pseudomonas* 
fluorescens on adsorption filter.

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Dairy Std. Methods Buffer (%)</th>
<th>Nutrient Broth (%)</th>
<th>Whole Milk (%)</th>
<th>Skim Milk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^3$</td>
<td>97±3.3</td>
<td>93±0.9</td>
<td>28±20.5</td>
<td>76±5.9</td>
</tr>
<tr>
<td>$10^2$</td>
<td>97±3.3</td>
<td>97±1.7</td>
<td>38±16.5</td>
<td>49±16.8</td>
</tr>
<tr>
<td>$10^1$</td>
<td>92±7.4</td>
<td>94±2.9</td>
<td>17±23.6</td>
<td>61±5.7</td>
</tr>
<tr>
<td>Average</td>
<td>95±5.5</td>
<td>95±2.6</td>
<td>28±22.1</td>
<td>62±15.5</td>
</tr>
</tbody>
</table>

n=3

* Calculated based on difference between bacterial counts of filtrate compared to unfiltered inoculated sample.
Table 3. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml whole milk, 400ml dairy standard methods buffer).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>7.0E3</td>
<td>3.1E3</td>
<td>56</td>
</tr>
<tr>
<td>1.0E2</td>
<td>7.9E2</td>
<td>4.3E2</td>
<td>46</td>
</tr>
<tr>
<td>1.0E1</td>
<td>7.1E1</td>
<td>3.7E1</td>
<td>48</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 50%.

Table 4. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml skim milk, 400ml dairy standard methods buffer).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>3.1E3</td>
<td>1.6E3</td>
<td>48</td>
</tr>
<tr>
<td>1.0E2</td>
<td>4.1E2</td>
<td>2.2E2</td>
<td>46</td>
</tr>
<tr>
<td>1.0E1</td>
<td>2.7E1</td>
<td>1.7E1</td>
<td>37</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 44%.
interaction with either the bacterial cell or filter material. Even though the milk was more easily filtered, dilution alone was not effective in promoting bacterial adsorption onto the filter material.

Surfactants were used to enhance adsorption of bacteria to the filter material by intensifying and changing charges on the filter or in the solution (53). A change in charge is necessary because adsorption may be prevented when the solute and solid surface hold the same charge. Treatments using Triton X-100 (nonionic), Tween 80 (nonionic), and sodium dodecyl sulfate (anionic) were unsuccessful in promoting adsorption when used alone (Tables 5-7) and in conjunction with diluting milk (Tables 8-10). Data indicate that the charge is not the only factor involved in the low adsorption rate of bacteria onto the filter material and that other factors in milk or the solution are inhibiting adsorption.

The pH of the solution was altered to change the ionic conditions in the solution and around the bacterial cell. A change in these conditions may influence the adsorption of bacterial cells onto the filter material by eliminating any competing components, thus allowing enhanced bacterial adsorption. The pH was lowered to 6.0 using both an inorganic acid (hydrochloric acid) and an organic acid (lactic acid). At lower pH values, more hydrogen ions are
Table 5. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk with 0.1% sodium dodecyl sulfate).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>5.3E3</td>
<td>2.0E3</td>
<td>62</td>
</tr>
<tr>
<td>1.0E2</td>
<td>6.4E2</td>
<td>4.1E2</td>
<td>36</td>
</tr>
<tr>
<td>1.0E1</td>
<td>2.8E2</td>
<td>2.2E2</td>
<td>21</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 40%.

Table 6. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk with 0.1% Tween 80).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>4.2E3</td>
<td>2.0E3</td>
<td>52</td>
</tr>
<tr>
<td>1.0E2</td>
<td>5.3E2</td>
<td>3.8E2</td>
<td>28</td>
</tr>
<tr>
<td>1.0E1</td>
<td>3.9E2</td>
<td>3.1E2</td>
<td>21</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 34%.

Table 7. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk with 0.1% Triton X-100).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>2.9E3</td>
<td>1.6E3</td>
<td>45</td>
</tr>
<tr>
<td>1.0E2</td>
<td>7.6E2</td>
<td>3.8E2</td>
<td>50</td>
</tr>
<tr>
<td>1.0E1</td>
<td>3.6E2</td>
<td>3.7E2</td>
<td>0</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 32%.
Table 8. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 400ml dairy standard methods buffer, 0.1% sodium dodecyl sulfate).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>5.0E4</td>
<td>4.8E4</td>
<td>4</td>
</tr>
<tr>
<td>1.0E2</td>
<td>5.3E3</td>
<td>7.7E3</td>
<td>0</td>
</tr>
<tr>
<td>1.0E1</td>
<td>8.2E2</td>
<td>1.1E3</td>
<td>0</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 1%.

Table 9. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 100ml dairy standard methods buffer, 0.1% Tween 80).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>5.3E3</td>
<td>5.6E3</td>
<td>0</td>
</tr>
<tr>
<td>1.0E2</td>
<td>5.9E2</td>
<td>2.4E3</td>
<td>0</td>
</tr>
<tr>
<td>1.0E1</td>
<td>1.3E2</td>
<td>1.1E3</td>
<td>0</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 0%.

Table 10. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 100ml dairy standard methods buffer, 0.1% Triton X-100).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>2.0E3</td>
<td>1.5E3</td>
<td>25</td>
</tr>
<tr>
<td>1.0E2</td>
<td>2.4E2</td>
<td>2.2E3</td>
<td>0</td>
</tr>
<tr>
<td>1.0E1</td>
<td>9.7E1</td>
<td>9.2E2</td>
<td>0</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 8%.
present in solution to neutralize negatively charged components that may be interfering with adsorption. Bacterial adsorption may be increased by removing the competing negative charges. However, data indicate that adsorption was not promoted by adjusting the pH of milk to 6.0 using either hydrochloric or lactic acid (Tables 11-12). The addition of hydrogen ions to neutralize competing negative charges was not effective in increasing bacterial adsorption so other factors in the solution such as components of milk must also influence the rate of bacterial adsorption.

A warm solution can promote adsorption by allowing greater contact and mobility between the adsorbent and adsorbate. Too much warming may adversely affect adsorption because the environment of the solution may change through increased solubility or binding of extraneous material to the adsorption surface. Sub-lethal time and temperature combinations for *P. fluorescens* were selected for their effect on adsorption. Data indicate that incubation of inoculated milk at 47°C for 5 minutes (Table 13) or at 40°C for 15 minutes (Table 14) were not successful in promoting adsorption.

Because of the lack of success at promoting adsorption by changing solution characteristics, the constituents of milk were examined for their role in preventing adsorption
Table 11. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, pH to 6.0 with 1N hydrochloric acid).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>5.3E3</td>
<td>4.2E3</td>
<td>21</td>
</tr>
<tr>
<td>1.0E2</td>
<td>2.9E2</td>
<td>5.4E2</td>
<td>0</td>
</tr>
<tr>
<td>1.0E1</td>
<td>1.7E1</td>
<td>1.0E0</td>
<td>94</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 38%.

Table 12. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, pH to 6.0 with 1N lactic acid).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>5.5E3</td>
<td>3.0E3</td>
<td>45</td>
</tr>
<tr>
<td>1.0E2</td>
<td>3.7E2</td>
<td>2.5E2</td>
<td>32</td>
</tr>
<tr>
<td>1.0E1</td>
<td>4.1E1</td>
<td>3.0E1</td>
<td>27</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 35%.
Table 13. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 5 minutes at 47°C).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>9.0E2</td>
<td>3.4E2</td>
<td>62</td>
</tr>
<tr>
<td>1.0E2</td>
<td>1.0E2</td>
<td>4.5E1</td>
<td>55</td>
</tr>
<tr>
<td>1.0E1</td>
<td>1.4E1</td>
<td>4.0E0</td>
<td>71</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 63%.

Table 14. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 15 minutes at 40°C).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>1.2E4</td>
<td>7.9E3</td>
<td>34</td>
</tr>
<tr>
<td>1.0E2</td>
<td>1.7E3</td>
<td>8.5E2</td>
<td>50</td>
</tr>
<tr>
<td>1.0E1</td>
<td>9.2E1</td>
<td>5.7E1</td>
<td>38</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 41%.
of bacterial cells to the filter material. Organic and inorganic compounds present in milk may alter surface charges. Minerals in milk, such as calcium and magnesium and proteins, such as casein, also carry charges and may adversely affect adsorption.

Ethylene diamine tetraacetic acid (EDTA), a chelating agent with a binding affinity for metal ions, was added to reduce the influence of magnesium and calcium on adsorption of bacterial cells to the filter material. In addition to chelating minerals, EDTA will also affect casein by removing calcium. Destabilized casein micelles may influence adsorption by altering the charge or position of amino acids on the casein molecule as secondary and tertiary structures are altered. The addition of EDTA alters the solubility of casein by causing precipitation; as a result the milk would not pass through the adsorption filter. To increase filterability, the pH was increased with sodium hydroxide. Addition of sodium hydroxide added negatively-charged hydroxide and positively-charged sodium ions to the solution, thus affecting the hydrogen and hydrophobic bonding that occurs within casein. This treatment allowed filtration of the desired volume of milk (100ml).

None of the above factors (dilution, surfactants, pH change, and warming) were able to enhance adsorption to the desired level when used individually. Subsequent treatments
focused on combinations of individual factors to enhance adsorption. After testing many combinations of the above factors (Appendix A), the most successful treatments for promoting bacterial adsorption onto the filter material involved milk with the following final concentrations (v/v): 0.8% EDTA, 0.02% SDS, pH values of 5.5, 6.5, and 7.5, and warming the solution at 21°C or 37°C for 5 minutes (Tables 15-23). Similar adsorption percentages resulted from each treatment. The treatment used for the next phase of this research (Table 17) - 100ml milk, 0.8% EDTA, 0.02% SDS, and adjusting the pH to 7.5 with sodium hydroxide - was chosen over other similar treatments because of the percent not recovered in filtrate and the simplicity since no warming step is required. Upon subsequent replication with this treatment (n=3), the average percent not recovered in filtrate was lower but maintained the set criteria of ≥90% (Table 24). The percent not recovered for this treatment was 91 ± 7.1%. The other similar treatments were all within this range, therefore, the preferred treatment was selected due to the ease of use.

Phase IV - Enumeration of Adsorbed Microorganisms

The selected treatment was used to capture *P. fluorescens* onto the filter material. Subsequent detection of the microorganism was accomplished by impedance
Table 15. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 0.80% EDTA, 0.02% SDS, pH to 5.5 with 1N HCl).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>6.6E3</td>
<td>2.5E2</td>
<td>96</td>
</tr>
<tr>
<td>1.0E2</td>
<td>5.1E2</td>
<td>3.0E1</td>
<td>94</td>
</tr>
<tr>
<td>1.0E1</td>
<td>5.1E1</td>
<td>1.0E0</td>
<td>98</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 96%.

Table 16. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 0.80% EDTA, 0.02% SDS, pH to 6.5 with 1N HCl).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>3.6E3</td>
<td>1.6E2</td>
<td>96</td>
</tr>
<tr>
<td>1.0E2</td>
<td>4.5E2</td>
<td>1.6E1</td>
<td>96</td>
</tr>
<tr>
<td>1.0E1</td>
<td>3.6E1</td>
<td>2.0E0</td>
<td>94</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 95%.

Table 17. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 0.80% EDTA, 0.02% SDS, pH to 7.5 with 1N NaOH).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>2.6E3</td>
<td>3.2E1</td>
<td>99</td>
</tr>
<tr>
<td>1.0E2</td>
<td>2.8E2</td>
<td>3.0E0</td>
<td>99</td>
</tr>
<tr>
<td>1.0E1</td>
<td>3.9E1</td>
<td>4.0E0</td>
<td>90</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 96%.

42
Table 18. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 0.80% EDTA, 0.02% SDS, 5 minutes at 21°C, pH to 5.5 with 1N HCl).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>5.9E3</td>
<td>4.7E2</td>
<td>92</td>
</tr>
<tr>
<td>1.0E2</td>
<td>7.5E2</td>
<td>4.0E1</td>
<td>95</td>
</tr>
<tr>
<td>1.0E1</td>
<td>5.6E1</td>
<td>1.0E0</td>
<td>98</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 95%.

Table 19. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 0.80% EDTA, 0.02% SDS, 5 minutes at 21°C, pH to 6.5 with 1N HCl).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>4.4E3</td>
<td>1.7E2</td>
<td>96</td>
</tr>
<tr>
<td>1.0E2</td>
<td>3.3E2</td>
<td>1.4E1</td>
<td>96</td>
</tr>
<tr>
<td>1.0E1</td>
<td>4.3E1</td>
<td>1.0E1</td>
<td>77</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 90%.

Table 20. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 0.80% EDTA, 0.02% SDS, 5 minutes at 21°C, pH to 7.5 with 1N NaOH).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>3.9E3</td>
<td>6.2E1</td>
<td>98</td>
</tr>
<tr>
<td>1.0E2</td>
<td>4.7E2</td>
<td>8.0E0</td>
<td>98</td>
</tr>
<tr>
<td>1.0E1</td>
<td>4.8E1</td>
<td>1.0E0</td>
<td>98</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 98%.

43
Table 21. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 0.80% EDTA, 0.02% SDS, 5 minutes at 37°C, pH to 5.5 with 1N HCl).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>1.4E4</td>
<td>5.8E3</td>
<td>59</td>
</tr>
<tr>
<td>1.0E2</td>
<td>1.1E3</td>
<td>1.0E2</td>
<td>91</td>
</tr>
<tr>
<td>1.0E1</td>
<td>8.8E1</td>
<td>1.0E0</td>
<td>99</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 83%.

Table 22. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 0.80% EDTA, 0.02% SDS, 5 minutes at 37°C, pH to 6.5 with 1N HCl).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>6.4E3</td>
<td>7.6E2</td>
<td>88</td>
</tr>
<tr>
<td>1.0E2</td>
<td>6.1E2</td>
<td>5.7E1</td>
<td>91</td>
</tr>
<tr>
<td>1.0E1</td>
<td>6.9E1</td>
<td>6.0E0</td>
<td>91</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 90%.

Table 23. Apparent retention of *Pseudomonas fluorescens* in treated milk on adsorption filter (100ml milk, 0.80% EDTA, 0.02% SDS, 5 minutes at 37°C, pH to 7.5 with 1N NaOH).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>Before filtration (cells/ml)</th>
<th>After filtration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0E3</td>
<td>3.3E3</td>
<td>8.3E1</td>
<td>97</td>
</tr>
<tr>
<td>1.0E2</td>
<td>4.0E2</td>
<td>1.8E1</td>
<td>96</td>
</tr>
<tr>
<td>1.0E1</td>
<td>4.9E1</td>
<td>2.0E0</td>
<td>96</td>
</tr>
</tbody>
</table>

Average Not Recovered in Filtrate = 96%.
Table 24. Average apparent retention of *Pseudomonas fluorescens* using the preferred treatment (100ml milk, 0.80% EDTA, 0.02% SDS, pH to 7.5 with 1N NaOH).

<table>
<thead>
<tr>
<th>Target Concentration (cells/ml)</th>
<th>% Not Recovered in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^3$</td>
<td>93±4.5</td>
</tr>
<tr>
<td>$10^2$</td>
<td>96±2.2</td>
</tr>
<tr>
<td>$10^1$</td>
<td>84±6.3</td>
</tr>
<tr>
<td>Average</td>
<td>91±7.1</td>
</tr>
</tbody>
</table>

n=3
microbiology which allowed detection of microorganisms adsorbed to the filter material by placing the filter directly into a module, thus eliminating an elution step required to remove adsorbed microorganisms from the filter material prior to detection by traditional techniques. A 13mm filter was used for filtration and the volume of milk was adjusted to 15ml (the largest volume that would pass through the 13mm filter).

Detection times generated by the impedance instrument were converted to log cfu/ml using linear, quadratic, and hyperbolic standard curves. Log values were used because the bacterial numbers were determined by bacterial metabolism in the module, not by counting colony forming units on a Petri plate.

Standard curves for *P. fluorescens* demonstrate a high correlation between detection time and modified psychrotrophic bacteria count (r= -0.99, -0.99, and -0.98 for linear, quadratic, and hyperbolic, respectively) (Figures 1-3). Therefore, each of the standard curves may be used to give an accurate estimate of the number of *P. fluorescens* present. The average log bacterial numbers determined before filtration (tz), after treatment (tz1), and on the filter after filtering treated milk (filter) indicate the level of *P. fluorescens* present at each stage (Table 25). The comparison of pre-treated inoculated milk
Figure 1. Scattergram of the linear relationship of log modified Psychrotrophic Bacteria Count to Impedance Detection Time for *Pseudomonas fluorescens*.
Figure 2. Scattergram of the quadratic relationship of log modified Psychrotrophic Bacteria Count to Impedance Detection Time for *Pseudomonas fluorescens*.
Figure 3. Scattergram of the hyperbolic relationship of log modified Psychrotrophic Bacteria Count to Impedance Detection Time for *Pseudomonas fluorescens*.
Table 25. Average log cfu/ml for the linear, quadratic and hyperbolic standard curves for Pseudomonas fluorescens.

<table>
<thead>
<tr>
<th>Log Target Concentration</th>
<th>Linear</th>
<th>Quadratic</th>
<th>Hyperbolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>tz 3.0</td>
<td>3.4±0.2</td>
<td>3.3±0.1</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>tz1 filter</td>
<td>2.9±0.05</td>
<td>2.9±0.05</td>
<td>2.8±0.05</td>
</tr>
<tr>
<td>filter</td>
<td>4.9±0.05</td>
<td>4.8±0.05</td>
<td>4.5±0.05</td>
</tr>
<tr>
<td>tz 2.0</td>
<td>2.0±0.1</td>
<td>2.1±0.1</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>tz1 filter</td>
<td>1.8±0.2</td>
<td>1.9±0.2</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>filter</td>
<td>3.8±0.2</td>
<td>3.7±0.2</td>
<td>3.6±0.2</td>
</tr>
<tr>
<td>tz 1.0</td>
<td>0.6±0.1</td>
<td>1.0±0.1</td>
<td>1.4±0.05</td>
</tr>
<tr>
<td>tz1 filter</td>
<td>0.4±0.3</td>
<td>0.7±0.3</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>filter</td>
<td>3.0±0.2</td>
<td>3.0±0.2</td>
<td>2.9±0.1</td>
</tr>
</tbody>
</table>

tz = inoculated milk
tz1 = inoculated, treated milk
filter = 13mm adsorption filter after filtration of inoculated, treated milk.

Table 26. Average log increase in Pseudomonas fluorescens retained on the filter for the linear, quadratic, and hyperbolic standard curves.

<table>
<thead>
<tr>
<th>Log Target Concentration</th>
<th>Linear</th>
<th>Quadratic</th>
<th>Hyperbolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>1.5±0.2</td>
<td>1.4±0.2</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>2.0</td>
<td>1.8±0.08</td>
<td>1.7±0.1</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>1.0</td>
<td>2.4±0.2</td>
<td>2.0±0.2</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>Average</td>
<td>1.9±0.4</td>
<td>1.7±0.2</td>
<td>1.4±0.1</td>
</tr>
</tbody>
</table>
(tz) and post-treatment (tz1) log bacterial numbers indicates that the treatment caused little bacterial cell death. While the log values may be slightly different between untreated and treated milk, they are within a practical significance for microbiological data of 0.5 log values.

The comparison of pre-treated inoculated milk (tz) and the filter (filter) log bacterial numbers indicates average log increases of 1.9, 1.7, and 1.4 in number of *P. fluorescens* retained on the filter after filtration using the linear, quadratic, and hyperbolic standard curves, respectively (Table 26). The results also demonstrate that the hyperbolic curve was preferred because log values obtained from the hyperbolic curve were close to the highest possible bacterial increase that could have occurred when 15ml of milk was filtered. Using the hyperbolic curve, at log target concentrations of 3.0, 2.0, and 1.0 cells/ml, increases of 20x, 25x, and 32x, respectively, occurred. Mathematically, the largest increase that should have occurred, based on 15ml filtered and 91% average retention, was 13.6x (1.1 log values). The resulting increases observed using the hyperbolic standard curve, however, are within 0.5 log values of the ideal 13.6x increase.

Data in Appendix B suggests the possible use of this system for detecting *S. typhimurium*. The average increase
using the hyperbolic standard curve was 25x (1.4 log values). However, adsorption studies were not conducted with this microorganism. Therefore, the apparent retentions and detection of \textit{S. typhimurium} can only be hypothesized to be as result of adsorption of the microorganism onto the filter material.

Data in Appendix C indicates that detection of \textit{L. monocytogenes} cannot be successfully accomplished using this system. The large increases obtained are well above the 13.6x (1.1 log value) increase that is mathematically possible. Adsorption studies were not conducted using \textit{L. monocytogenes}. Therefore, the level of adsorption of the microorganism onto the filter material is unknown. The treatment used for the gram-negative microorganisms may not be successful for gram-positive microorganisms. One possible explanation is the presence of sodium dodecyl sulfate, which may bacteriocidal, particularly for gram-positive microorganisms.
CONCLUSIONS

This research was successful in developing a treatment that allowed for adsorption of Pseudomonas fluorescens in fluid milk and subsequent enumeration of pure cultures of this microorganism using impedance microbiology. If this adsorption and subsequent enumeration procedure could be further developed to allow for selective adsorption or selective detection of a particular microorganism, a more rapid and more sensitive method to detect these spoilage or pathogenic microorganisms in dairy products would result. Current standard testing procedures for detecting spoilage and pathogenic microorganisms in milk involve time-consuming preliminary incubation, pre-enrichment, and enrichment steps which require between 18 hours and 1 month to allow the microorganism to grow to a detectable level. Many of these procedures also use very little of the actual sample for testing after the incubation steps as a sub-sample of the pre-enriched or enriched sample is used for the final testing. The Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture recently released characteristics that are desirable for in-plant testing methods (113). While FSIS is not involved in the testing of fluid milk products, this clearly demonstrates the mind-set in regulatory agencies regarding the desire for
new, more sensitive testing procedures.

The method developed for adsorption of Pseudomonas fluorescens onto the capture filtration material offers many advantages over the standard procedures for detecting microorganisms in milk. The capture filtration procedure developed requires approximately 15 minutes, a considerable decrease from the 18 hours to 1 month required for standard procedures. Subsequent detection or enumeration utilizing impedance microbiology requires less than 24 hours. The total time for detection is 1 day. Another advantage of the developed method is the sample size subjected to detection. The entire sample is being filtered, resulting in a test sample of 15ml. This increases the sensitivity of the testing procedures and decreases the time required to detect the microorganisms.

The data for the log increase in number of microorganisms is representative when filtering 15ml of milk inoculated with pure cultures of P. fluorescens. A 15-fold increase in bacterial numbers would result in a 1.2 log increase on the filter. The average log increase for P. fluorescens was 1.4 for the hyperbolic standard curve. This log increase is well within a normal 0.5 log variation. The data indicated that a hyperbolic standard curve must be used to determine cell numbers because a hyperbolic curve is the most representative of possible cell numbers.
Impedance detection data obtained for *Salmonella typhimurium* suggests the possible use of adsorption for detecting this microorganism. However, impedance detection data obtained for *Listeria monocytogenes* indicates that the system does not function properly for this microorganism. The disparity in results indicates that adsorption studies must be conducted to ensure that the microorganism of concern will adsorb to the filter material using the prescribed method.
REFERENCES


APPENDIX A - Treatments used to increase adsorption

Decrease concentration of milk components through dilution
100ml milk, 400ml dairy standard methods buffer

Add surfactants
100ml milk, 0.1% SDS
100ml milk, 0.1% Tween 80
100ml milk, 0.1% Triton X-100
100ml milk, 0.1% SDS, 400ml dairy standard methods buffer
100ml milk, 0.1% Tween 80, 400ml dairy standard methods buffer
100ml milk, 0.1% Triton X-100, 400ml dairy standard methods buffer

Similar to DEFT
100ml milk, 0.04% Triton X-100, 10ml trypsin
100ml milk, 5ml trypsin, 50°C, 5min.
100ml milk, 0.02% Triton X-100, 5ml trypsin
100ml milk, 0.02% Triton X-100
100ml milk, 0.02% SDS

Change pH
100ml milk, pH to 6.0 with 1N HCl
100ml milk, pH to 6.0 with lactic acid

Combine pH change with DEFT
100ml milk, pH to 6.0 with lactic acid, 5ml trypsin, 50°C, 5min.

Add EDTA
100ml milk, 0.1M EDTA
100ml milk, 0.09% EDTA
100ml milk, 0.009% EDTA
100ml milk, 0.0009% EDTA

Combine EDTA with changing pH and adding SDS
100ml milk, 1.67% EDTA, pH to 8.5 with 1N NaOH
100ml milk, 0.83% EDTA, pH to 7.0 with 1N NaOH
100ml milk, 0.80% EDTA, 0.02% SDS, pH to 7.0 with 1N NaOH
100ml milk, 0.80% EDTA, 0.02% SDS, pH to 8.0 with 1N NaOH

Combine EDTA, changing pH, adding SDS, and diluting milk
100ml milk, 400ml dairy standard methods buffer, 0.19% EDTA, pH to 7.0 with 1N NaOH
100ml milk, 400ml dairy standard methods buffer, 0.19% EDTA, pH to 8.0 with 1N NaOH
100ml milk, 400ml dairy standard methods buffer, 0.19% EDTA, 0.005% SDS, pH to 7.0 with 1N NaOH
100ml milk, 400ml dairy standard methods buffer, 0.19% EDTA, 0.005% SDS, pH to 8.0 with 1N NaOH

Warm milk
100ml milk, 5min. at 47°C
100ml milk, warm to 47°C
100ml milk, 15min. at 40°C

Warm milk and change pH
100ml milk, 10min. at 47°C, 2ml 1N NaOH
100ml milk, 10min. at 47°C, pH to 8.0 with 1N NaOH

Warm milk and dilute
100ml milk, 400ml dairy standard methods buffer, 10min. at 47°C

Warm milk, add EDTA and SDS or Triton X-100, warm milk, change pH
100ml milk, 0.83% EDTA, 10min. at 47°C, pH to 8.0 with 1N NaOH
100ml milk, 0.80% EDTA, 0.02% SDS, 5min. at 21°C, pH to 8.0 with 1N NaOH
100ml milk, 0.80% EDTA, 0.02% SDS, 5min. at 47°C, pH to 8.0 with 1N NaOH
100ml milk, 0.80% EDTA, 0.02% SDS, 15min. at 40°C, pH to 8.0 with 1N NaOH
100ml milk, 0.80% EDTA, 0.02% SDS, 10min. at 21°C, pH to 8.0 with 1N NaOH
100ml milk, 0.83% EDTA, 0.004% SDS, 15min. at 40°C, pH to 9.0 with 1N NaOH
100ml milk, 0.83% EDTA, 15min. at 40°C, 3ml 1N NaOH
100ml milk, 1.25% EDTA, 0.02% SDS, 10min. at 21°C, pH to 8.0 with 1N NaOH
100ml milk, 1.25% EDTA, 0.004% SDS, 10min. at 37°C, pH to 8.0 with 1N NaOH
100ml milk, 0.96% EDTA, 0.02% Triton X-100, 7min. at 21°C, pH to 7.0 with 1N NaOH
100ml milk, 0.96% EDTA, 0.02% SDS, 7min. at 21°C, pH to 7.0 with 1N NaOH
100ml milk, 0.96% EDTA, 0.02% Triton X-100, 7min. at 21°C, pH to 8.0 with 1N NaOH
100ml milk, 0.96% EDTA, 0.02% Triton X-100, 10min. at 21°C, pH to 7.0 with 1N NaOH
100ml milk, 0.96% EDTA, 0.02% Triton X-100, 10min. at 47°C, pH to 7.0 with 1N NaOH
Warm sample, pre-treat filter, add EDTA and SDS

100ml milk, 5min. at 47°C, pre-treat filter with 20ml of 47°C water
100ml milk, 0.80% EDTA, 0.02% SDS, 5min. at 47°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.83% EDTA, 10min. at 47°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.83% EDTA, 10min. at 47°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 47°C SDS
100ml milk, 0.83% EDTA, 10min. at 47°C, pH to 9.0 with 1N NaOH, pre-treat filter with 5ml of 47°C SDS
100ml milk, 0.80% EDTA, 0.02% SDS, 10min. at 47°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.80% EDTA, 0.02% SDS, 10min. at 47°C, pH to 9.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.80% EDTA, 0.02% SDS, 10min. at 47°C, pH to 9.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.80% EDTA, 0.02% SDS, 10min. at 47°C, pH to 9.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.83% EDTA, 10min. at 47°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.83% EDTA, 10min. at 47°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.83% EDTA, 10min. at 47°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.83% EDTA, 10min. at 47°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.80% EDTA, 0.02% SDS, 10min. at 47°C, pH to 9.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.80% EDTA, 0.02% SDS, 10min. at 47°C, pH to 9.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.80% EDTA, 0.02% SDS, 10min. at 47°C, pH to 9.0 with 1N NaOH, pre-treat filter with 5ml of 47°C water
100ml milk, 0.83% EDTA, 10min. at 47°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 21°C water
100ml milk, 0.80% EDTA, 0.02% SDS, 10min. at 21°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 21°C water
100ml milk, 0.77% EDTA, 0.04% SDS, 10min. at 21°C, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 21°C water

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100ml milk, 0.77% EDTA, 0.04% SDS, 10min. at 21°C, pH to 9.0 with 1N NaOH, pre-treat filter with 5ml of 47°C Triton X-100

Similar to treatments used for adsorption of viruses

100ml milk, 0.005M MgCl₂, 2ml 1N HCl
100ml milk, 2ml 1N HCl
100ml milk, 100ml 37°C dairy standard methods buffer, pH to 5.75 with 1N HCl
100ml milk, 20ml glycine-saline buffer, pH to 5.75 with 1N HCl, pre-treat filter with 5ml of 21°C 0.3% beef extract
100ml milk, 20ml glycine-saline buffer, pre-treat filter with 5ml of 0.3% beef extract
100ml milk, 0.77% EDTA, 10ml glycine-saline buffer, pH to 6.0 with 1N HCl
100ml milk, 0.77% EDTA, 10ml glycine-saline buffer, pH to 6.0 with 1N HCl, pre-treat filter with 5ml of 21°C 0.3% beef extract
100ml milk, 0.77% EDTA, 10ml glycine-saline buffer, pH to 6.0 with 1N NaOH
100ml milk, 0.77% EDTA, 10ml glycine-saline buffer, pH to 8.0 with 1N NaOH, pre-treat filter with 5ml of 21°C 0.3% beef extract
100ml milk, 0.0002M AlCl₃, pH to 6.5 with 1N HCl
100ml milk, 0.0002M AlCl₃, pH to 7.5 with 1N HCl
100ml milk, 0.0002M AlCl₃, 5min. at 21°C, pH to 5.5 with 1N HCl
100ml milk, 0.0002M AlCl₃, 5min. at 21°C, pH to 6.5 with 1N HCl
100ml milk, 0.0002M AlCl₃, 5min. at 21°C, pH to 7.5 with 1N NaOH

Combinations of EDTA, SDS, and pH changes

100ml milk, 0.80% EDTA, 0.02% SDS, pH to 5.5 with 1N HCl
100ml milk, 0.80% EDTA, 0.02% SDS, pH to 6.5 with 1N HCl
100ml milk, 0.80% EDTA, 0.02% SDS, pH to 7.5 with 1N NaOH
100ml milk, 0.80% EDTA, 0.02% SDS, 5min. at 21°C, pH to 5.5 with 1N HCl
100ml milk, 0.80% EDTA, 0.02% SDS, 5min. at 21°C, pH to 6.5 with 1N HCl
100ml milk, 0.80% EDTA, 0.02% SDS, 5min. at 21°C, pH to 7.5 with 1N NaOH
100ml milk, 0.80% EDTA, 0.02% SDS, 5min. at 37°C, pH to 5.5 with 1N HCl
100ml milk, 0.80% EDTA, 0.02% SDS, 5min. at 37°C, pH to 6.5 with 1N HCl
100ml milk, 0.80% EDTA, 0.02% SDS, 5min. at 37°C, pH to 7.5 with 1N NaOH
DEPT = Direct Epifluorescent Filter Technique
EDTA = ethylene diamine tetraacetic acid
SDS = sodium dodecyl sulfate
HCl = hydrochloric acid
NaOH = sodium hydroxide
MgCl₂ = magnesium chloride
AlCl₃ = aluminum chloride
APPENDIX B - Impedance detection of Salmonella typhimurium using the developed treatment

Figure B-1. Scattergram of the linear relationship of log Standard Plate Count to Impedance Detection Time for Salmonella typhimurium.
Figure B-2. Scattergram of the quadratic relationship of log Standard Plate Count to Impedance Detection Time for *Salmonella typhimurium*. 
Figure B-3. Scattergram of the hyperbolic relationship of log Standard Plate Count to Impedance Detection Time for *Salmonella typhimurium*. 

Calibration Code: saim  # Samples: 37  Spec CFU/ML: 1.00e03  
Cutoff Time: 7.0  Caution Time: 11.3  
\[ \text{LOG DT} = -0.12 \times \text{LOG CFU/ML} + 1.32 \]  
Correlation: -0.98
Table B-1. Average log cfu/ml for the linear, quadratic and hyperbolic standard curves for *Salmonella typhimurium*.

<table>
<thead>
<tr>
<th>Log Target Concentration</th>
<th>Linear</th>
<th>Quadratic</th>
<th>Hyperbolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 tz</td>
<td>3.5±0.05</td>
<td>3.4±0.05</td>
<td>3.5±0.0</td>
</tr>
<tr>
<td>tzl</td>
<td>3.5±0.0</td>
<td>3.4±0.0</td>
<td>3.5±0.0</td>
</tr>
<tr>
<td>filter</td>
<td>5.4±0.1</td>
<td>5.2±0.1</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>2.0 tz</td>
<td>2.5±0.2</td>
<td>2.5±0.2</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>tzl</td>
<td>2.4±0.0</td>
<td>2.5±0.0</td>
<td>2.8±0.0</td>
</tr>
<tr>
<td>filter</td>
<td>4.3±0.05</td>
<td>4.2±0.05</td>
<td>4.1±0.05</td>
</tr>
<tr>
<td>1.0 tz</td>
<td>0.9±0.2</td>
<td>1.5±0.1</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>tzl</td>
<td>1.3±0.2</td>
<td>1.8±0.1</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>filter</td>
<td>3.5±0.05</td>
<td>3.4±0.05</td>
<td>3.4±0.0</td>
</tr>
</tbody>
</table>

tz = inoculated milk
tzl = inoculated, treated milk
filter = 13mm adsorption filter after filtration of inoculated, treated milk.

Table B-2. Average log increase in *Salmonella typhimurium* retained on the filter for the linear, quadratic, and hyperbolic standard curves.

<table>
<thead>
<tr>
<th>Log Target Concentration</th>
<th>Linear</th>
<th>Quadratic</th>
<th>Hyperbolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>1.9±0.2</td>
<td>1.8±0.2</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>2.0</td>
<td>1.9±0.3</td>
<td>1.6±0.2</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>2.5±0.2</td>
<td>1.8±0.1</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Average</td>
<td>2.1±0.3</td>
<td>1.7±0.09</td>
<td>1.4±0.1</td>
</tr>
</tbody>
</table>
APPENDIX C - Impedance detection of \textit{Listeria monocytogenes} using the developed treatment

![Graph](image)

Calibration Code: list  
# Samples: 45  
Spec CFU/mL: 1.00e03  
Cutoff Time: 18.7  
Caution Time: 21.5  
LOG CFU/ML: \(-0.33 \times DT + 9.70\)  
Correlation: -0.98

Figure C-1. Scattergram of the linear relationship of log Standard Plate Count to Impedance Detection Time for \textit{Listeria monocytogenes}.
Figure C-2. Scattergram of the quadratic relationship of log Standard Plate Count to Impedance Detection Time for *Listeria monocytogenes*.
Figure C-3. Scattergram of the hyperbolic relationship of log Standard Plate Count to Impedance Detection Time for *Listeria monocytogenes.*
Table C-1. Average log cfu/ml for the linear, quadratic and hyperbolic standard curves for *Listeria monocytogenes*.

<table>
<thead>
<tr>
<th>Log Target Concentration</th>
<th>Linear</th>
<th>Quadratic</th>
<th>Hyperbolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 tz</td>
<td>4.2±0.1</td>
<td>3.9±0.05</td>
<td>4.0±0.1</td>
</tr>
<tr>
<td>tzl</td>
<td>4.4±0.1</td>
<td>4.0±0.1</td>
<td>4.1±0.05</td>
</tr>
<tr>
<td>filter</td>
<td>6.2±0.1</td>
<td>5.9±0.1</td>
<td>5.9±0.1</td>
</tr>
<tr>
<td>2.0 tz</td>
<td>3.0±0.4</td>
<td>3.0±0.2</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>tzl</td>
<td>3.6±0.3</td>
<td>3.3±0.2</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>filter</td>
<td>6.6±0.1</td>
<td>6.4±0.6</td>
<td>6.5±0.1</td>
</tr>
<tr>
<td>1.0 tz</td>
<td>2.7±0.2</td>
<td>3.3±0.6</td>
<td>3.4±0.6</td>
</tr>
<tr>
<td>tzl</td>
<td>2.5±0.2</td>
<td>2.7±0.1</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>filter</td>
<td>6.7±0.0</td>
<td>6.5±0.05</td>
<td>6.6±0.05</td>
</tr>
</tbody>
</table>

tz = inoculated milk

tzl = inoculated, treated milk

filter = 13mm adsorption filter after filtration of inoculated, treated milk.

Table C-2. Average log increase in *Listeria monocytogenes* retained on the filter for the linear, quadratic, and hyperbolic standard curves.

<table>
<thead>
<tr>
<th>Log Target Concentration</th>
<th>Linear</th>
<th>Quadratic</th>
<th>Hyperbolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>1.9±0.2</td>
<td>2.0±0.1</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>2.0</td>
<td>3.6±0.3</td>
<td>3.4±0.1</td>
<td>3.3±0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>3.4±0.8</td>
<td>3.3±0.6</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td>Average</td>
<td>3.0±0.8</td>
<td>2.9±0.6</td>
<td>2.8±0.7</td>
</tr>
</tbody>
</table>
VITA

The author, Robert Duane Byrne, Jr., was born July 15, 1964 in Silver Spring, Maryland. He grew up in Richmond, Virginia and graduated from Monacan High School in June of 1982. In September of 1982 he enrolled at Virginia Polytechnic Institute and State University. In June of 1986 he received a Bachelor of Science degree from Virginia Polytechnic Institute and State University with a major in Biology (Microbiology option) and a minor in Chemistry.

In September of 1986, Rob began work towards a Master of Science degree in Food Science and Technology under the direction of Dr. J. Russell Bishop. In December of 1989 he received his Master of Science degree in Food Science and Technology. In January of 1990, Rob began working towards a Doctor of Philosophy degree in Food Science and Technology at Virginia Polytechnic Institute and State University under the direction of Dr. J. Russell Bishop. His area of research is dairy microbiological quality control. Rob is a member of the Institute of Food Technologists, the American Dairy Science Association, and the International Association of Milk, Food, and Environmental Sanitarians.

Robert Duane Byrne, Jr.