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A Sanitation Assessment of Food Contact Surfaces in Child Care Centers using Microbiological Analysis and Rapid Sanitation Assays

Catherine Moore Cosby
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Catherine Moore Cosby entitled "A Sanitation Assessment of Food Contact Surfaces in Child Care Centers using Microbiological Analysis and Rapid Sanitation Assays." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

P. Michael Davidson, Major Professor

We have read this thesis and recommend its acceptance:

W.C. Morris, Carol A. Costello

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Sanitation Assessment of Food Contact Surfaces in Child Care Centers using Microbiological Analysis and Rapid Sanitation Assays

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Catherine Moore Cosby

December 2005

Dedication

This thesis is dedicated to my parents, Dr. and Mrs. Lincoln M. Moore, and my husband, Mr. Terrence Cosby. To my parents, who from at birth have always encouraged me to persevere through life's challenges and instilled in me the determination to want more and the confidence to try. To my husband, who has been my steady rock and source of strength during the joys and pains of this academic endeavor.

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Abstract

Food contact surfaces are a major concern for food service facilities in controlling the spread of foodborne pathogens. Food service areas within these facilities are considered critical to health, and therefore the microbiological quality of these surfaces within child care center food service areas must be surveyed and assessed. The main objectives of this study were: (1) to gather information as to the environmental microbiological quality of food service surfaces and a non-food contact surface in child care centers, and (2) to evaluate several rapid sanitation assays for determining the sanitation quality of food contact surfaces and to determine if the methods have potential for utilization within child care centers.

A microbiological survey was conducted in six (6) Knoxville, TN area child care centers. The facilities represented three large (> 100 children) and three small (< 50 children) centers. Each child care center was tested twice monthly over the course of an eight (8) month period for a total of 16 sampling periods per center. Four areas, one food service area, one diaper changing area, and two food preparation areas (n=288), within each center was sampled. The food service area and the food preparation areas consisted of three food contact surfaces, two food preparation surfaces (i.e. kitchen counters) and one food service surface (i.e. children's eating table), while the diaper changing area consisted of one non-food contact surface (i.e. diaper changing surface). All surfaces were tested three times daily, pre-opening, lunch time, and following final clean-up of the day, to monitor the microbiological quality of each surface throughout the day.

For the microbiological analysis, results were expressed as the mean aerobic plate count (APC) and coliforms per 50 cm^2 area and by prevalence rates of the presence of coliforms and *E. coli*, i.e., the percentage of total samples in which coliforms or *E. coli* were detected, per 50 cm^2 area. Mean log APC counts over the survey period were 3.04, 3.93, 3.08, 4.50, 3.45 and 4.16 $\log_{10}$ CFU/50 cm^2 for centers 1 through 6, respectively. Mean coliform counts were 1.35, 17.96, 5.59, 87.55, 10.22, and 20.43 CFU/50 cm^2 for the same respective centers. There was a significant difference between counts for center

size based on mean log APC with large centers having lower mean log APC (3.55 $\log_{10}$ CFU/50 cm^2) than small centers (3.81 $\log_{10}$ CFU/50 cm^2). As for coliform counts, there was no significant difference between coliform counts for center size with large centers having a mean coliform count of 35.62 CFU/50 cm^2 and small centers 10.72 CFU/50 cm^2. Coliforms were detected on 283 of 1,149 (24.7%) samples with counts ranging from 1 to 2,000 CFU/50 cm^2 while *E. coli* was detected on 18 of 1,149 (1.6%) samples with counts ranging from 1 to 35 CFU/50 cm^2.

For the rapid sanitation assays, microbial data and ATP, protein and glucose results were available on 1,129 samples. Correlations between rapid sanitation assays and microbiological analysis (APC) were determined using Spearman's rho analysis. Correlations ($p < 0.01$) were found, using Spearman's rho analysis between the APC and the ATP ($r = 0.26$) and protein assays ($r = 0.16$). Rapid sanitation assays were also compared with APC based upon percent agreement, i.e., the number of times the rapid sanitation assays and APC were in agreement of a "clean" or "dirty" surface or area. Surfaces and areas with < 1,000 CFU/50 cm^2 and ATP value of < 300 relative light units (RLU) or a negative protein or glucose reaction were defined as "clean." Surfaces with > 1,000 CFU/50 cm^2 and > 300 RLU or a positive protein or glucose reaction were considered "dirty". The overall percent agreement between APC and ATP bioluminescence, protein assay and glucose assay was 24.3%, 68.7% and 81.9%, respectively. Therefore, the protein and glucose assays were in agreement more often with the APC analysis. However, the level of RLU to define a "clean" surface using the ATP assay was based upon manufacturer recommendations used for food processing operations and could be modified for child care facilities. Using the manufacturer recommendations for RLU limits, the ATP bioluminescence assay classified only 16.2% of surfaces tested as "clean," i.e., RLU < 300. The greatest percentage of samples had RLUs between 1,000 and 5,000 (34.7%).

These findings demonstrate that microbial contamination is present on food contact surfaces of child care centers. Due to the high risk of foodborne illness associated with children, the possibility of cross-contamination from food contact or non-food

contact surfaces to foods is an aspect of food safety that requires more attention. The findings of the study indicate that rapid sanitation assays may be useful for monitoring the sanitation of food contact surfaces in child care centers. These tests could assist in improving sanitation and preventing cross-contamination by detecting unsanitary surfaces with food residues remaining on the surfaces.

Table of Contents

List of Tables

TABLE **PAGE**

List of Figures

FIGURE **PAGE**

1 INTRODUCTION

The cleanliness and sanitation of food contact surfaces within child care centers pose health risks to children due to their potential contribution to foodborne illness. Although many cases of foodborne illness have been attributed to inadequate cooking, temperature abuse, and the use of contaminated raw ingredients, cross-contamination between raw and cooked foods via food contact surfaces has also been identified as a significant risk factor (DeCesare *et al.,* 2003).

Child care center food service operations prepare food for one of the most susceptible groups at risk for foodborne illness. Due to underdeveloped immune systems, children are considered one of the highest risk populations for foodborne illness (Gerba *et al.,* 1996). The Children's Defense Fund (2000) reported that 60% of all preschoolers, toddlers and infants attend child care centers daily. With the increased number of working mothers over the past years, it is safe to expect that percentage will increase. Due to the increase, there is a need to survey and assess the sanitation of food contact surfaces within child care centers to ensure food safety for children.

As opposed to the food processing and food service industries, sanitation in child care food service is generally much less defined. Several studies have examined food processing and foodservice establishments using microbiological testing. However, although there is an established amount of information available about the presence of bacterial contamination on food contact surfaces within food processing facilities and other commercial settings, similar information concerning the bacterial contamination of these surfaces within child care centers is lacking. Accordingly, the main objectives of this study were: (1) to gather information as to the environmental microbiological quality of food service surfaces and a non-food contact surface in child care centers, and (2) to evaluate several rapid sanitation assays for determining the sanitation quality of food contact surfaces and to determine if the methods have potential for utilization within child care centers.

A microbiological survey was conducted in six (6) Knoxville, TN area child care centers. The facilities represented three large (> 100 children) and three small (< 50 children) centers. Each child care center was tested twice monthly over the course of an eight (8) month period for a total of 16 sampling periods per center. Four areas, one food service area, one diaper changing area, and two food preparation areas, within the child care centers were sampled. The food service area and the food preparation areas consisted of three food contact surfaces, one food service surface and two food preparation surfaces, i.e., children's eating table and kitchen counters. The diaper changing area was the one non-food contact surface. All surfaces were tested three times daily, pre-opening, lunch time, and following final clean-up of the day, to monitor the microbiological quality of each surface throughout the day. Surfaces of each area varied with surface types including: plastic laminate for food service surfaces, stainless steel, grouted tile, plastic laminate, and wooden laminate for food preparation surfaces, and acrylic solid surface and plastic padding for diaper changing surfaces.

In order to complete each objective and reach the overall goals, it was necessary to use microbiological analysis and rapid sanitation assays to evaluate all surfaces surveyed in this study. Microbiological analysis included aerobic plate counts (APC), coliform counts, and *E. coli* counts of a 50 cm^2 area on all surfaces using standard microbiological swabbing methods. Microbiological analysis of surfaces was done by swabbing a 50cm^2 area using a sterile stainless steel template. Sampling was performed by swabbing the area horizontally, from one side of the template to the other, and repeating vertically, and then horizontally. Samples were transported to the laboratory and then plated onto Aerobic Count (AC) Plates and *E. coli*/Coliform Count Plates. Results were reported as mean log APC counts and mean coliform counts by center, center size, area, surface type, and time of day. The frequency of *E. coli* and coliform positive samples by surface type and area sampled were also reported.

Rapid sanitation assays included a commercial ATP bioluminescence assay, a protein detection assay, and a glucose detection assay. All rapid sanitation assays were done using a sterile, stainless steel template with a 50 cm^2 area exposed. Sampling was

performed by swabbing an adjacent 50 cm^2 area horizontally, from one side of the template to the other, and repeating vertically, and then horizontally again. Rapid assay results were read within 5 seconds to 10 minutes after sampling depending on manufacturer's instructions. The efficiency of the rapid assays was evaluated by comparing the results of each assay with APC counts for surface type and area sampled based on percent agreement, i.e., the number of times the rapid sanitation assays and APC were in agreement of a "clean" or "dirty" surface. Surfaces and areas with < 1,000 CFU/50 cm^2 and < 300 RLU as detected by the ATP assay or a negative protein or glucose reaction were considered "clean." Surfaces with > 1,000 CFU/50 cm^2 and > 300 RLU or a positive protein or glucose reaction were considered "dirty". Correlations between the rapid assays and APC using Spearman's rho analysis were also established to determine the efficiency of the rapid assays.

2 LITERATURE REVIEW

2.1 Introduction to Foodborne Illness (FBI)

The Centers for Disease Control and Prevention (CDC), estimates that approximately 76 million people within the United States contract a foodborne illness each year (CDC 2004). Of those 76 million cases, 325,000 hospitalizations, and 5,000 deaths result. The CDC's Preliminary FoodNet Data reports that in 2003, there were 15,600 diagnosed cases of foodborne illness caused by bacterial pathogens and of that total, 6,017 cases were attributed to *Salmonella*, 5,215 to *Campylobacter*, 3,021 to *Shigella*, 443 to *Escherichia coli*, and 138 to *Listeria* (CDC 2004).

Segments of the population, including the young, elderly, immunocompromised, and pregnant women, are among the highest risk groups for foodborne illness (Gerba *et al.,* 1996). The CDC estimated in 2000, that there were 3,513,694 cases of foodborne illness among children, with 33,711 children hospitalized and 1,604 deaths. Comparing the latter figure to the total deaths attributed to foodborne illness in 2003, approximately one-third of all deaths caused by foodborne illness are children. Incidence of foodborne illness is relatively higher for children than healthy adults because of their underdeveloped immune systems and lower body weight. Lower body weight means fewer pathogens are necessary to cause illness (Buzby 2001).

Most foodborne illnesses are classified as "acute". They are usually self-limiting and of short duration with symptoms including mild gastroenteritis. However, some illnesses progress to life-threatening neurological or renal syndromes called sequelae. The U.S. Food and Drug Administration (FDA) estimates that 2-3 percent of all acute cases result in sequelae and children are more prone to these symptoms (Buzby 2001). Examples of chronic sequelae of foodborne illnesses include hemolytic uremic syndrome (HUS) and Guillain-Barré Syndrome (GBS). These illnesses will affect children for the remainder of their lives and may result in premature death.

2.2 Contributing Factors to Foodborne Illness Associated with Food Handling

The CDC has identified improper handwashing, cross-contamination, and ineffective cleaning and sanitizing as some of the major contributing factors to the spread of foodborne illness (NRAEF 2005). Therefore, it is necessary to take the proper steps to ensure that these improper practices are avoided at all times.

2.2.1 Improper handwashing

Handwashing has long been known to be a beneficial public health practice for preventing the spread of infectious disease. According to the CDC, "handwashing is the single most important procedure for preventing the spread of infection" (Anonymous 2005). Bacteria, such as the foodborne pathogen *Staphylococcus aureus,* are found naturally on the human body and apparently healthy people may host foodborne pathogens, such as *Salmonella*, or viruses such as Hepatitis A. These people may be "carriers" and are capable of infecting others, yet they may not be aware that they are carriers because they may not show symptoms or become ill themselves. Therefore, it is necessary to utilize proper handwashing techniques after coughing, sneezing, blowing the nose, or touching any part of the body. Failure to use proper handwashing techniques increases the risk of transmission of foodborne illness.

The Association for Professionals in Infection Control and Epidemiology (APIC) states that "handwashing causes a significant reduction in the carriage of potential pathogens on the hands" and recommends several steps for proper handwashing to prevent the spread of pathogens. During the handwashing procedure, failure to cover all surfaces on the hands because of poor techniques or use of insufficient cleansing agents may lead to subsequent contamination of surfaces. APIC indicates that handwashing associated with general patient care occurs in approximately half of the instances in which it is indicated and usually is of shorter duration than recommended. Pittet and others (1999) studied the compliance with handwashing guidelines in a teaching hospital. The authors concluded that compliance with handwashing guidelines was only 48

percent. The authors also concluded that the primary problem with handwashing was laxity of practice and that high workload among workers was associated with low compliance. Allwood and others (2004) conducted a survey of retail food establishments to investigate handwashing compliance among retail food establishment workers in Minnesota. According to the survey, only 52% of persons-in-charge could describe proper Minnesota Food Code handwashing techniques and only 48% of employees could demonstrate proper techniques. Results from the study indicated that the most common problems associated with handwashing practices were the lack of training and proper technique usage. As previously stated, failure to sufficiently cleanse hands can leave foods and food contact surfaces contaminated and this can lead to another contributing factor to foodborne illness, cross-contamination.

2.2.2 Cross-contamination

Cross-contamination between raw and cooked foods via food contact surfaces has also been identified as a significant risk factor for foodborne illness (DeCesare *et al.,* 2003). Cross-contamination is defined as the spread of bacteria between foods, surfaces or equipment. It is most likely to occur when raw food touches (or drips onto) other food, raw food touches (or drips onto) equipment or surfaces, or people touch raw food with their hands and then touch other surfaces or foods. Several studies indicate that various bacteria, including *Escherichia coli, Staphylococcus aureus* and *Salmonella* survive on hands for hours and days after initial contact with microorganisms (Scott and Bloomfield 1990). Zhao and others (1998) conducted a study to develop a model for evaluation of microbial cross-contamination in the kitchen. The authors concluded that the surfaces of cutting boards can be heavily contaminated with *Enterobacter aerogenes* B199A, an indicator bacterium with attachment characteristics similar to that of *Salmonella,* after cutting and handling contaminated poultry. The bacterial counts transferred to these surfaces can approximate those on contaminated meat (Zhao *et al.,* 1998). The U.S. Food and Drug Administration conducted a survey on the occurrence of selected food consumption and preparation behaviors which are associated with increased risk of foodborne diseases. The survey determined that 26% of American consumers do not

clean cutting boards after cutting raw meat or chicken (Klontz *et al.,* 1995; Zhao *et al.,* 1998). Failure to clean cutting boards can lead to an increased risk of cross-contamination which in turn increases the risk of foodborne illness.

2.2.3 Ineffective cleaning and sanitizing

Ineffective cleaning and sanitizing of food contact surfaces is another contributing factor to foodborne illness. Cross-contamination and therefore transmission of foodborne illness may be prevented through effective cleaning and sanitizing of food contact surfaces before, during, and after food preparation. Cleaning is defined as the complete removal of food soil using appropriate detergent chemicals under recommended conditions. Sanitizing is defined as the reduction of microorganisms to levels considered safe from a public health viewpoint (NRAEF 2005).

2.3 Prevention of Cross-Contamination via Food Contact Surfaces

In order to reduce or possibly eliminate the risk of cross-contamination it is necessary to properly clean and sanitize food contact surfaces before any type of food preparation takes place. Residues of raw meat, poultry exudates , and other food may remain on kitchen surfaces, serving as sources of microbial contamination for raw vegetables and other ready-to-eat (RTE) foods (DeCesare *et al.,* 2003). Therefore, all food contact surfaces must be cleaned and thoroughly rinsed to remove any residues before the sanitizing process can begin.

There are a number of sanitizers, such as quaternary ammonium compounds, iodophors, and chlorine, available for use in food processing and retail environments. However, the selection of a sanitizer depends on a number of factors, such as the type of equipment to be sanitized, the effectiveness of the sanitizer and cost. Quaternary ammonium compounds (QAC), in diluted form, are odorless, colorless, and nontoxic. They are stable at high temperatures, over a wide pH range, and in the presence of organic materials. QAC's are effective against some bacteria, but are slow-acting against some common spoilage bacteria. QAC solutions may leave films on equipment and are

not compatible with other common detergent compounds or chlorine sanitizers (Forwalter 1980).

Iodophors are a combination of iodine and a solubilizing agent that releases free iodine when diluted with water. Iodophors are fast-acting and effective against all bacteria and in diluted form, they are non-staining, relatively nontoxic, nonirritating to skin, and stable. Iodophors are widely used in hand sanitizing solutions (Forwalter 1980).

Chlorine-based sanitizers are the most commonly used sanitizers in food plants and are effective against all types of bacteria. In diluted form, chlorine-based sanitizers are colorless, relatively nontoxic, and non-staining. They are the easiest sanitizers to prepare and apply, are generally the most economical and they are best suited for food contact surfaces, water disinfection and smooth produce surfaces (Forwalter 1980).

2.3.1 Sanitization of food contact surfaces using chlorine

Sodium hypochlorite is the oldest and most widely used of the chlorine compounds employed in chemical sanitization (Lomander *et al.,* 2004). Chlorine is the most commonly used sanitizer because of its efficacy at low concentrations and low cost. However, the sanitizing activity of chlorine is dependent upon concentration, temperature, amount of organic material present, pH and contact time (Kusumaningrum *et al.,* 2003).

Parnes (1997) investigated the efficacy of manufacturer's recommended dilutions of sodium hypochlorite for the disinfection of inanimate surfaces contaminated with *Staphylococcus aureus* and *Escherichia coli* O157:H7. For this study, approximately 10^6 CFU of each test organism were dried onto 2x2 inch glazed ceramic tiles and formica squares and then wiped with a sponge dipped into a 5,250 ppm sodium hypochlorite (5.25%) solution. After wiping, surfaces were exposed to the sodium hypochlorite solution for two minutes and then wiped with a sterile gauze pad dampened with sterile water to retrieve bacteria remaining on the surface. Plate counts were made from the gauze to determine the number of bacteria that remained on the test surfaces. Results of

the study indicated that the bleach solution was effective against both test organisms, with approximately 10^1 CFU remaining on both surfaces after only two minute exposure times to sodium hypochlorite solution. Results from this study indicate that commercial disinfectant products such as sodium hypochlorite can eliminate microorganisms such as *S. aureus* and *E. coli.* Mafu and others (1990) studied the efficiency of sodium hypochlorite against *Listeria monocytogenes* on stainless steel, glass, polypropylene and rubber surfaces. Ten millimeter cylinders of each surface type were dipped for 15 minutes in 10 ml of bacterial suspension according to the AOAC use-dilution method and dried for 30 minutes. After drying each contaminated surface was exposed to sodium hypochlorite at concentrations between 5 and 500 ppm at 50 ppm intervals and greater than 10,000 and maintained for 10 minutes at 4° C or 20° C. Cylinders were transferred to culture medium and results were reported as positive or negative for growth after incubation. The critical sanitization point, or minimum concentration required to sanitize each surface, was obtained by testing the range of concentrations of sodium hypochlorite. Higher and lower concentrations were tested until the lowest limit value of five negative tubes was obtained indicating no bacterial growth. Results of the study indicated that lower concentrations of sodium hypochlorite were needed for the sanitization of surfaces to achieve this five tube limit. For stainless steel and glass a concentration of 200 ppm, the manufacturer's recommended concentration, was more effective for inactivation of *L. monocytogenes* and for polypropylene and rubber a concentration of 800 ppm was more effective. The temperature of the sodium hypochlorite solution had an effect on the sanitizing performance, with 20°C requiring 130 ppm and 115 ppm for sanitization on stainless steel and glass, respectively, versus 450 ppm and 300 ppm at 4°C. Frank and Chmielewski (1997) conducted a study to evaluate the effectiveness of chlorine on the sanitization of stainless steel and other domestic food preparation surfaces. Squares (7.5 x 11 cm) of stainless steel, polycarbonate, and mineral resin surfaces, either smooth and unused or abraded, were submerged into 1.0 liter of *Staphylococcus aureus* culture, to obtain an initial population of 10^4 to 10^5 CFU/cm^2 attached to each surface. Upon completion of attachment, test surfaces were immersed into a 200 ppm sodium hypochlorite solution for 20 seconds. Surfaces were exposed to the sodium hypochlorite

solution for a total of 45 seconds. Direct surface agar plating was used to enumerate the amount of bacteria remaining on the surfaces. Results from the study indicated that the sodium hypochlorite solution was most effective on smooth stainless steel and polycarbonate surfaces by reducing cell populations to less than 1.0 log CFU/cm^2. Cell populations remained greater than 1.0 log CFU/cm^2 on abraded stainless steel and mineral resin surfaces.

In general, the longer time a sanitizer is in contact with the food contact surface, the more effective the sanitizing effect. Chlorine sanitizers should remain on the surface for at least two minutes before rinsing or wiping. Assanta and others (1996) conducted a study that evaluated the efficiency of the sanitizing agents, Dettol, a sanitizer with the active agents of chloroxylenol, Sterol, and Tor, at three time periods, 2, 5, and 10 minutes, to destroy *Listeria monocytogenes, Yersinia enterocolitica* and *Staphylococcus aureus* on10 mm stainless steel surfaces. The authors found that contact time was important, with all three sanitizing agents being more effective in inactivating the three pathogens on stainless steel after 10 minutes of exposure time. These findings indicate that adequate contact time is necessary to inactivate pathogens that may be present on food contact surfaces. Researchers in this study also concluded that various species of foodborne pathogens may show varying resistance to sanitizers. For instance, the sanitizer Dettol, in order to inactivate *L. monocytogenes*, a concentration of 0.9% was required with a 10 minute contact time; however, for *Y. enterocolitica* and *S. aureus*, concentrations of 0.5% and greater than 25% were required for inactivation of these microorganisms, respectively. These findings are similar to those found in previous studies (Best *et al.,* 1990).

2.4 Food Contact Surface Microbiological Sampling Methods

2.4.1 Comparison of sampling methods for pathogen detection

Analyzing foods and food contact surfaces for the presence of both pathogenic and spoilage bacteria is a standard practice for ensuring food safety and quality (DeBoer

and Beumer 1999). If microorganisms are able to survive and grow on food contact surfaces, the risk for cross-contamination and foodborne illness is increased. In order to ensure that proper cleaning and sanitizing of food contact surfaces has occurred, there are a number of microbiological sampling methods available to monitor the presence of microorganisms or soil. Microbiological monitoring of food processing environments may be conducted to meet one or more of the following objectives: "(1) verification of the effectiveness of cleaning and disinfection practices; (2) determination of the frequency required for cleaning and disinfection; (3) determination of the presence of foodborne pathogens in the environment; (4) discovery of environmental sources of spoilage organisms; (5) determination of the frequency required for special maintenance procedures; (6) evaluation of hygienic design and fabrication of food processing equipment and facilities" (Evancho *et al.,* 2001).

Traditionally, methods such as swabbing and plating on microbiological media or agar contact plates have been used to detect bacteria on food contact surfaces. The most commonly used methods for food contact surface assessment in food operations are the Swab/Swab-Rinse Method and the Contact Plate Method (Jay *et al.,* 2005). The swab-rinse method was developed by W.A. Manheimer and T. Yabanez and is the oldest and most widely used method for the microbiological examination of surfaces in the food and dairy industry and in hospitals and restaurants (Jay *et al.,* 2005). The swab-rinse method utilizes either cotton or calcium-alginate swabs to examine a defined area of a surface. This method should be used for surfaces with cracks, corners, or crevices, areas where the swab will be more effective in recovering organisms. With this method, a sterile template is placed over the surface to be sampled and the area is swabbed thoroughly with the moistened swab in horizontal and vertical directions, reversing direction between strokes. The exposed swab is then returned to its holder containing the appropriate buffer solution and the buffer solution is surface or pour plated to enumerate the microorganisms.

An alternative to agar pour plates is the rehydratable dry film method. This method can be used for nonselective as well as, selective methods to detect for specific

groups such *E. coli* and coliforms. In comparison to the pour plate and spread plate methods, the Petrifilm™ method has been found to be not significantly different in the detection and enumeration of coliforms and aerobic bacteria in studies with dairy products, such as milk, and other foods (Ginn *et al.,* 1986; Morton 2001). High correlation coefficients, for APC, 0.99, 0.95, and 0.94, and coliforms, 0.83, 0.96, and 0.81 for pork, beef, and chicken samples respectively, were found between the Petrifilm plate method and standard aerobic count method and violet red bile agar method for meat products (Park *et al.,* 2001). A comparison between the Petrifilm rapid coliform count (RCC) plate method and the conventional method of violet red bile agar (VRBA) for enumerating coliforms concluded that the Petrifilm plate counts were not significantly different from VRBA counts, ($p > 0.05$) in surimi-based imitation crab slurry (Chung *et al.,* 2000). For food contact surfaces associated with ground beef processing, no significant differences ($p > 0.05$) between swab-rinse methods coupled with pour plating techniques and Petrifilm™ methods was found in the recovery of aerobic bacteria, coliforms, or *E. coli* (Linton *et al.,* 1997).

In contrast to the swab-rinse method, the Contact Plate Method utilizes the replicate organism direct agar contact (RODAC) Petri plate. This method is used only on flat, impervious surfaces that are relatively easy to clean and disinfect and when quantitative data are desired. Disposable plastic RODAC plates may be purchased or they can be filled at the laboratory. With this method, the plastic cover of the RODAC plate is removed and the surface of the agar is gently pressed onto the surface being sampled using a rolling, uniform pressure to ensure the entire agar surface contacts the surface. After contact, the cover is replaced and the plate is incubated in an inverted position under the required time and temperature guidelines for the microorganism in question and the colonies are enumerated (Evancho *et al.,* 2001).

As with any method, there are advantages and disadvantages of using one method over another. The swab-rinse method has been shown to be a timely, simple, and inexpensive way to assess the microbiological quality of food contact surfaces allowing for results to be obtained within 24 to 48 hours. This method has been found to be most

suitable for flexible, uneven, and heavily contaminated surfaces (Jay *et al.,* 2005; Niskanen and Pohja 1977). The ease of removal of organisms depends on the texture of the surface and the nature and types of microorganisms present on a surface. The contact plate method has been shown to be most effective on surfaces that are smooth, firm, nonporous, and have low numbers of microorganisms present. The most serious drawbacks to this method are the covering of the agar surface by spreading colonies, and its ineffectiveness for heavily contaminated surfaces (Jay *et al.,* 2005).

2.4.2 Rapid methods importance and usage

Traditional agar-based testing methods rely solely on microbiological media for isolation, detection, and enumeration of viable bacterial cells and require incubation periods of 24 to 48 hours. These methods are sensitive and provide both qualitative and quantitative information on the number and types of microorganisms present in a sample. However, preventative risk-based food safety management systems, such as Hazard Analysis Critical Control Points (HACCP), require that hygiene monitoring provide results rapidly and in time for remedial action so as to be able to regain control of a process and/or product (Griffiths 1997; Moore and Griffith 2001). Therefore, it may be necessary to implement rapid hygiene monitoring systems that allow for results to be obtained in a shorter time than conventional microbiological methods such as swabbing/plating or agar contact methods which require incubation for 24-48 hours (Davidson *et al.,* 1999).

The presence of microorganisms on food contact surfaces is important, but the hygienic status of the surface also depends on the presence or absence of product residues (Mackintosh 1990; Moore and Griffith 2001). If a surface is unclean because of residual food debris, this residue can become a good source of nutrients for microorganisms and therefore encourage and facilitate their growth and increase the risk for cross contamination. If sanitation results regarding total organic load can be obtained more rapidly than with traditional testing methods, this would be beneficial for initial hygiene monitoring in the case of receiving results faster.

There are several requirements which must be considered before adapting a new rapid method (DeBoer and Beumer 1999):

1. Accuracy- false-positive and false-negative results must minimal, preferably zero.
2. Validation- rapid methods should be validated against standard tests and evaluated by collaborative studies.
3. Speed- should provide accurate results within hours and at the most within 8 hours. Must provide information in time for corrective actions to be taken.
4. Automation and computerization- ability to test many samples at one time.
5. Sample matrix- new systems should give a good performance of environment to be tested.
6. Cost- purchasing, reagents, supply, operational costs, upkeep. The initial financial investment may be high because many systems require expensive instruments.
7. Simplicity- methods should be user friendly and easy to operate.
8. Training and support is essential.

The ATP bioluminescence method has been used as an estimation of microbial load as well as an index of general cleanliness of food production environments (Entis *et al.,* 2001). The bioluminescence reaction, used to measure ATP, is based on the biological reaction that occurs naturally in fireflies to produce light (Figure 2.1). The bioluminescence reaction is catalyzed by the enzyme luciferase and occurs when this enzyme utilizes the chemical energy contained in the ATP molecule to drive the oxidative decarboxylation of luciferin (Hawronskyj and Holah 1997).

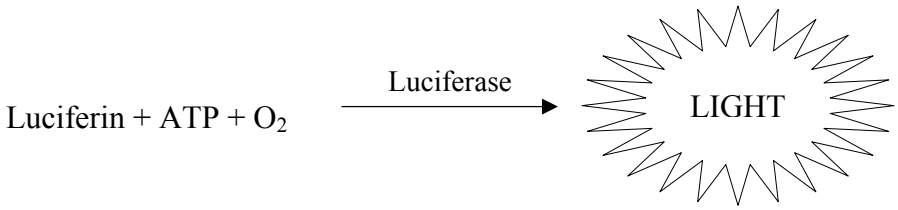

Figure 2.1: ATP Bioluminescence Reaction

The quantity of light is expressed in relative light units (RLUs) and directly correlates with the amount of ATP present and thus with the level of biological load on the sampled area (Deshpande 2001). The major drawback to the ATP bioluminescence method for determination of microorganisms is that it does not differentiate between sources of ATP. Therefore the ATP bioluminescence method may be better utilized for sanitation monitoring in the food production environment as an indicator of total biomass present. In sanitation monitoring both microbial ATP and ATP present from food debris are of relevance thus a total ATP estimation is desirable to evaluate hygiene levels and cleanliness. If an unsatisfactory surface is found it can immediately be re-cleaned and the ATP measurement repeated.

Since the amount of light produced can indicate the amount of soil present on the surface, ATP bioluminescence does not necessarily indicate the amount of bacterial contamination present. The limit of detection of some ATP monitoring systems may be higher than the amount of microbial ATP present on the surface; therefore, there is a risk for false negative results. The general amount of ATP per cell is generally constant at 3.6 x 10^{-18} mole per bacterial cell. This number corresponds to approximately 3.6 x 10^{-13} M ATP/10^5 CFU bacteria (Thore *et al.,* 1975). A number of studies have reported that the limit of detection for ATP bioluminescence assays is approximately 10^4 to 10^5 CFU (Davidson *et al.,* 1999).

Rapid hygiene monitoring kits which utilize ATP bioluminescence are available to the food industry and can provide a real-time estimate of total surface contamination and/or overall cleaning efficacy (Griffiths 1997; Moore and Griffith 2002). One of the first applications of ATP bioluminescence to detect microorganisms was in aerospace water systems. In the dairy industry ATP bioluminescence has been used to assess dairy processing cleaning and sanitizing programs as well as estimate and predict the shelf-life of milk and dairy products (Bautista *et al.,* 1992; Hawronskyj and Holah 1997). Many attempts have been made to correlate ATP levels with total bacterial count methods, with mixed success. A study conducted in 2003 compared conventional swabbing methods for the detection of microorganisms to ATP bioluminescence on 225 kitchen table surfaces in

homes (Larson *et al.,* 2003). No significant correlation between ATP and colony forming units (CFUs) from total plate counts ($r = 0.004$, $P = 0.58$) were found. Researchers in the study concluded that ATP bioluminescence is not a reliable substitute for conventional swabbing methods when the amount of microbial contamination on a surface is desired. Tebbutt (1999) compared conventional swabbing methods for the detection of microorganisms to ATP bioluminescence on 139 cutting boards used in hotel kitchens. A positive correlation was found between the bacterial count and the amount of ATP detected ($r = 0.58$). Due to the low correlation coefficient, researchers concluded that ATP bioluminescence is not fully reliable for highlighting the bacterial risk associated with food contact surfaces; however, this method does allow for on the spot-remedial action to be taken in the case of an unsanitary surface. Illsley and others (2000) compared standard surface swabbing techniques to ATP bioluminescence to determine the adequacy of the ATP methods for evaluating sanitation in a baking facility. The standard swabbing techniques and the ATP bioluminescence method were compared as to the percentage of times both methods agreed to pass or fail a surface, and based on percent agreement (81.6% - 83.3%), the study concluded that there was a good correlation between the results obtained using the plate count method and ATP bioluminescence and that the latter were reliable alternatives to traditional surface swabbing and plate count methods (Illsley *et al.,* 2000). Seeger and Griffiths (1994) assessed the practical use of the ATP bioluminescence method to evaluate the effectiveness of cleaning and sanitizing food contact surfaces in eight health care institutions. In comparison to conventional swabbing techniques, results from the study indicated that there was an overall agreement of 74% for all surfaces tested between results obtained using ATP bioluminescence and standard plate count methods. The study concluded that ATP bioluminescence is a reliable alternative to standard plate count methods for evaluating the cleanliness and sanitation of food contact surfaces. In contrast to earlier studies, Moore and Griffith (2002) concluded that there was a significant difference ($p < 0.05$) between the results obtained using ATP bioluminescence method and a traditional agar-based microbiological method, dip slides, to evaluate the cleanliness of food contact surfaces within four different food processing environments. While there was a 55.6% agreement

between the ATP bioluminescence method and the traditional microbiological method, 28.8% (13 of 45) of samples were considered unacceptable for food production using the ATP method despite being 'passing' by the dip slide method.

In addition to ATP bioluminescence assays, there are also assays that detect either protein or glucose. These rapid detection methods are designed to be presence/absence tests that detect food residues containing proteins or sugars that may remain on a surface following cleaning. These methods utilize color indicators to indicate residues. Tebbutt (1999) compared conventional swabbing methods for the detection of microorganisms to protein detection methods on 139 cutting boards used in hotel kitchens. A positive correlation was found between the bacterial count and the amount of protein detected ($r = 0.67$) and researchers concluded that the presence or absence of protein could not always be relied upon as an indicator of microbial contamination on surfaces. Moore and Griffith (2001) conducted a study to compare a rapid protein detection method to traditional agar-based methods for standard plate counts. There was a 68.9% agreement between the results of the protein detection method and those obtained using the standard plate counts.

These studies have demonstrated the value of rapid methods which allow for on the spot-remedial action to be taken in the case of an unsanitary food contact surface when food residues are found. As seen from the varying results from these studies, it is reasonable to conclude that rapid methods can be used as an indicator of cleanliness and sanitation on food contact surfaces.

2.5 Food Safety in Child Care Centers (CCC)

Child care facilities have become an integral part of today's society. In 2000, the Children's Defense Fund (2000) reported that 60 percent of all preschoolers, toddlers and infants attended child care facilities daily. Sixty five percent of mothers in the labor force have children under age six, and 78 percent have children age six to 13. Additionally, 51 percent of mothers with infants (children under age one) are in the labor force (U.S. Department of Labor 2004).

While child care facilities provide a necessary and important service, they may serve as a focal point for certain types of infectious diseases. The increasing number of mothers of young children in the work force has resulted in an increase in use of child care facilities and has affected the epidemiology of infectious diseases in young children. Children attending child care are generally at higher risk for gastrointestinal tract illnesses than other youth (Young 1989). The Centers for Disease Control and Prevention have estimated that there are 3,713,000 cases of infectious diseases associated with day care facilities each year (Bennett *et al.,* 1987; Gibson *et al.,* 2002). Infants and children under the age of 10, have the highest reported incidence rates of *E. coli* O157:H7, shigellosis, campylobacteriosis, salmonellosis, and listeriosis. Children can be exposed to these pathogens through contaminated foods as well as secondary sources of exposure such as other ill children in child care facilities

2.5.1 Characteristics of foodborne pathogens most associated with child care centers

E. coli O157:H7 and its link to food became well known to the public as a result of the 1993 *E. coli* O157:H7 outbreak caused by contaminated hamburgers. Over 700 people became ill from this outbreak and 4 children died (Buzby 2001). *E. coli* O157:H7 can be found on cattle farms and the pathogen can live in the intestines of healthy cattle. During slaughter, the pathogen can be passed to the beef thus contaminating the meat. *E. coli* O157:H7 may be acquired through the consumption of meat that has not been sufficiently cooked, unpasteurized milk, and person-to-person transmission can occur via the fecal-oral route (Belongia *et al.,* 1993). Once either of these factors occur, like other foodborne pathogens, *E. coli* O157:H7 can be found in the diarrheal stool of infected persons. The pathogen can then be spread if personal hygiene and handwashing procedures are inadequate. Young children typically shed this organism in their feces between one to two weeks after their illness; therefore, precaution and appropriate personal hygiene measures must be taken in order to ensure the prevention of this pathogen even if symptoms have resided.

Most people infected with *E. coli* O157:H7 develop bloody diarrhea and abdominal cramps and the illness normally resolves in 5 to 10 days. However, in some people, especially children under the age of 5, a complication called hemolytic uremic syndrome (HUS) can develop. HUS is a severe, life-threatening sequellae associated with *E. coli* O157:H7 and its toxins and is a predominantly pediatric condition that consists of the simultaneous triad of hemolytic anemia, thrombocytopenia and acute renal failure. HUS develops after ingestion of *E. coli* O157:H7 and its Shiga toxin enters the body's circulation by binding to special receptors. These Shiga-toxin receptors are distributed in major body organs, such as the kidneys, brain, and pancreas, allowing distinct thrombotic (blood clotting) effects. The greatest Shiga-toxin receptor concentration appears to be in the kidneys, especially in children. As the inflammatory reaction process accelerates, red blood cells are destroyed and the body's clot breaking mechanisms are disrupted. Damaged red blood cells (Fig. 2.2) clog the tiny blood vessels in the kidneys, making them work harder to remove wastes and extra fluid from the blood. The body's inability to rid itself of excess fluid and wastes may in turn cause high blood pressure or swelling of parts of or the entire body resulting in acute renal failure (NKUDIC 2003).

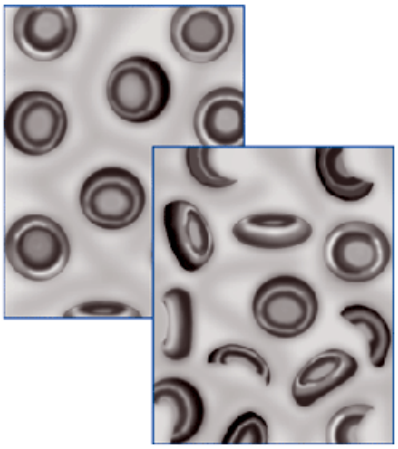

Figure 2.2: Destruction of red blood cells by Hemolytic Uremic Syndrome (HUS). Healthy red blood cells (left) are smooth and round. Red blood cells destroyed by HUS are misshapen and broken (right).

Approximately 5-10% of individuals infected with *E. coli* O157:H7 develop HUS. HUS is now recognized as the principal cause of kidney failure in previously healthy children in the United States. Some children infected with HUS have abnormal kidney function many years later and a few require long-term dialysis (CDC-DBMD 2004). HUS has a mortality rate of 5–15%; however, approximately 85% of children recover if given supportive care. Children who survive kidney failure from HUS, often go on to develop other lifelong complications, such as high blood pressure, seizures, blindness, paralysis, and the effects of having part of their bowel removed. The high incidence rate of HUS in children could reflect the smaller infective dose and immune system development of children (Buzby 2001).

Child care centers are an important channel for the transmission for *E. coli* O157:H7. Approximately 200,000 cases of foodborne illness associated with this pathogen occur each year in the U.S., of which 5% are estimated to be associated with child care centers (Van *et al.,* 1991). Rangel and others (2004) studied the epidemiology of *E. coli* O157:H7 outbreaks in the United Sates from 1982 to 2002. The results from their study discovered a total of 350 outbreaks were reported from 49 states, accounting for 8,598 cases of *E. coli* O157:H7. During these years, 50 outbreaks were spread by the fecal-oral route and 40 (80%) of the outbreak settings were child day care centers. These settings were the most frequent carriers of person-to-person outbreaks. Belongia and others (1993) researched the transmission of *E. coli* O157:H7 in nine Minnesota child day care centers from July 1988 through December 1999 to assess the occurrence of person-to-person transmission. According to the Minnesota Department of Health, between July 1988 and December 1989, there were 110 cases of *E. coli* O157:H7 infection reported. There were 44 HUS cases, including 19 (43%) which tested positive for *E. coli* O157:H7. The outbreak investigation occurred in nine child care facilities where an infected child attended after the onset of symptoms. The children who attended day care after the onset of symptoms were classified as “primary cases”. During the study, a case of *E. coli* O157:H7 was defined as an individual who had *E. coli* O157:H7 isolated from a stool specimen, or a child who developed either HUS or bloody diarrhea while attending a day

care facility with other culture-confirmed cases. Results of the outbreak study indicated that of the 254 preschool children that attended the nine facilities, 38 (15%) met the case definition. Five (13%) of the infected children had HUS, 22 (56%) had bloody diarrhea, five (13%) had non-bloody diarrhea, and six (16%) were asymptomatic. At two facilities, the primary case child continued to attend at least one week after the onset of diarrhea and transmission may have occurred at any point during this time. At two of the facilities, children continued to shed the organism anywhere from 2 to 26 days after symptoms resided. The study concluded that person-to-person transmission of *E. coli* O157:H7 is common when infected preschool children attend day care while symptomatic.

Shigella is a Gram-negative bacteria that is similar in behavior and habitat to *Escherichia coli*. This pathogen was discovered over 100 years ago by a Japanese scientist named Kiyoshi Shiga, for whom they are named. There are several different species of *Shigella* including: *Shigella sonnei,* also known as "Group D" *Shigella, Shigella flexneri,* or "Group B" *Shigella*. There are other types of *Shigella* that are rare in the U.S. but important in developing countries. For example, *Shigella dysenteriae* type 1 causes deadly epidemics in developing countries (CDC-DBMD 2004). *Shigella* are rarely found in animals and are principally a disease of humans and other primates such as monkeys and chimpanzees. The pathogen is frequently found in water that has been polluted with human feces. *Shigella* are mostly associated with salads such as potato, tuna, macaroni, or chicken, raw vegetables, dairy products and poultry. Contamination of these foods is usually via the fecal-oral route and is most commonly due to fecally contaminated water and unsanitary handling by food handlers (CDC-DBMD 2004). As few as 10 cells depending on the age and condition of the host are necessary to cause disease. As with *E. coli* O157:H7, *Shigella* are present in the diarrheal stool of infected persons and can be transmitted during infection as well as one to two weeks after symptoms subside. Most infections that occur are the result of the bacterium passing from stools or soiled fingers of one person to the mouth or fingers of another person. Children are susceptible to this type of transmission within child care facilities due to children constantly placing their fingers in their mouths.

Shigellosis, is an infectious disease caused by *Shigella sonnei* or "Group D" *Shigella*. Approximately 300,000 cases occur each year in the U.S. Approximately 25% of cases are associated with child care centers and *Shigella* is the bacterium most frequently associated with outbreaks of infectious intestinal disease in child care settings (Gibson *et al.,* 2002; Van *et al.,* 1991). Most people infected with *Shigella* develop mild diarrhea, fever, and stomach cramps and usually recover within 5 to 7 days; however, in young children these symptoms, especially the diarrhea, can be so severe that the patient needs to be hospitalized. A severe infection with high fever may also be associated with seizures in children less than 2 years old (CDC-MMWR 2004).

Shigella sonnei account for over two-thirds of the shigellosis in the United States (CDC-MMWR 2004). Between June 2001 and March 2003, outbreaks of *Shigella sonnei*, with approximately 3,081 laboratory-confirmed cases, were reported in six states, Delaware, Maryland, New Jersey, North Carolina, South Carolina, and Virginia, with median age ranges of patients being 4, 6, 5, 5, 5, and 7 years of age for their respective states. These outbreaks occurred in multiple child care facility settings that became prolonged and communitywide. Delaware reported a total of 506 culture-confirmed cases between June 2002 and March 2003, with a total of 200 (40%) of cases being day-care related. Between the months of November 2001 and March 2003, a total of 1,222 culture-confirmed cases were reported in Maryland, with a total of 250 (20%) known day care related cases. During an outbreak between October 2002 and March 2003, in Mecklenburg County, North Carolina, 729 cases of shigellosis were reported. Epidemiological investigations from this outbreak indicated that these outbreaks originated in day care facilities and then progressed to elementary schools. One hundred seventy-two culture-confirmed cases of shigellosis were reported in South Carolina between the months of June 2002 to March 2003. Approximately 55% of the cases were day care related. In Virginia, a day care related outbreak of shigellosis was attributed to *S. sonnei* being found in southeastern Virginia and subsequently becoming regional with a total of 876 laboratory-confirmed cases being reported. In all cases, health departments

excluded children with diarrhea from day care facilities and did not allow them to return until symptoms had subsided (CDC-MMWR 2004).

Campylobacter jejuni has been recognized as a cause of disease in animals since 1909. However, it is only in the last two decades of the twentieth century that the bacteria were identified as the cause of the human disease campylobacteriosis. *Campylobacter* bacteria naturally inhabit the intestinal tract of many animals, including swine, cattle, dogs, shellfish and poultry. These animals are often asymptomatic. The prevalence of *Campylobacter jejuni* in poultry carcasses results from the contamination of the meat by the intestinal contents of the bird (including the bacteria) when an infected bird is slaughtered (CDC-DBMD 2004). Studies monitoring poultry carcasses have demonstrated that over 30% of retail chickens are contaminated with *Campylobacter* (FDA 2004). A principle reason for the wide distribution of *Campylobacter* is the ability of the bacteria to survive anywhere there is moisture, food source, less than an atmospheric level of oxygen and room temperature conditions.

Campylobacter is the most common cause of bacterial diarrhea in the United States with more occurrences than S*almonella* (CDC 2004). According to active surveillance via FoodNet, approximately 15 cases of campylobacteriosis are diagnosed each year per 100,000 persons in the population; however, due to undiagnosed or unreported cases, it is estimated that over 1 million persons are infected every year (CDC-DBMD 2004). Unlike other pathogens, such as *E. coli* O157:H7 and *Salmonella*, *Campylobacter* is not usually spread from person-to-person; however, this can happen if the infected person is a small child or producing a large volume of diarrhea. Smaller outbreaks of campylobacteriosis, which are more common, are typically associated with handling raw poultry or eating raw or undercooked poultry; whereas, larger outbreaks typically occur from drinking unpasteurized milk or contaminated water.

Fewer than 500 *Campylobacter* organisms are needed to cause illness in humans, and even one drop of juice from raw poultry can infect one person. Most people who become ill with campylobacteriosis, develop diarrhea, cramping, abdominal pain, and

fever within 2 to 5 days after exposure to the organism. The diarrhea may be bloody and can be accompanied by nausea and vomiting and the illness normally lasts one week. Sequela from this illness are rare; however, they have been associated with Guillain-Barre syndrome (GBS). GBS is a neurological disorder that occurs when a person's own immune system begins to attack the body's own nerves. This disorder affects the peripheral nerves and causes weakness, paralysis, and occasionally death. It has been estimated that one in every 1,000 cases of campylobacteriosis leads to Guillan-Barre syndrome (CDC-DBMD 2004). Isolation of *Campylobacter* is much more common from children under five and young adults more frequently than other age groups. In children, diarrhea persisting for more than one week can lead to nutrient losses, which result in dehydration, and can also compromise their immune system (Young 1989). Outbreaks of campylobacteriosis in child care facilities are rare; however, in the United States, infants have the highest reported incidence of campylobacteriosis.

Olsen and others (2001) reported an outbreak of *Campylobacter jejuni* infections associated with food handler contamination during a school luncheon. In 1998, the Kansas Department of Health and Environment was notified of an outbreak of *C. jejuni* infections and in order to determine the cause of the outbreak a case study was performed. Trace back studies were completed by investigation of the source of food items eaten during the luncheon. For the study, a clinical case patient was defined as having diarrhea more than three times within a 24 hour period, while a confirmed case was defined as a clinical case whose stool sample tested positive for *C. jejuni*. During the study, 129 persons were identified as case patients, 33 had stool samples obtained where 27 samples were positive for *C. jejuni*. There were no particular foods linked to the outbreak; however, illness was traced back to a food handler who had severe abdominal cramps and profuse diarrhea during the time frame of the luncheon. Despite symptoms, the food handler continued to work during illness.

Salmonella is a genus of Gram-negative bacteria that was discovered over 100 years ago by an American scientist named Salmon, for whom they are named. There are a number of different strains of *Salmonella;* however, *Salmonella* serotype Typhimurium

and *Salmonella* serotype Enteritidis are the most common in the United States. Concerning food poisoning, *Salmonella* Enteriditis is of particular concern because this strain causes gastroenteritis and other problems because of several virulence factors the organism is armed with. One of the most important virulence factors displayed by *Salmonella* is the production of an enterotoxin. This is especially important due to the fact that the enterotoxin remains inside the bacteria, so the toxin concentration increases with the increase in bacterial numbers (CDC 2004).

Salmonella spp. are typically found in animals, especially in poultry and swine and have also been linked to hamsters and reptiles. There are various environmental sources that include water, soil, factory surfaces, kitchen surfaces, and animal feces only to name a few. Foods associated with *Salmonella* spp. include poultry, eggs, red meat, diary products, processed meats, cream-based desserts, and salad-type sandwich filling (such as tuna salad or chicken salad) as these are prime targets for colonization by species of *Salmonella*. Inadequate cooking of eggs are a major concern due to the fact that poultry may be asymptomatic carriers of the bacteria, and due to vertical transmission, i.e., deposition of the bacteria in the yolk by an infected layer hen prior to shell deposition, eggs become carriers of the bacteria (FDA 2004). Raw eggs may be unrecognized in some foods such as ice cream, homemade mayonnaise and salad dressings, cookie dough and frostings. Ice cream, cookie dough and frostings are of concern with children since these are foods that are generally consumed by this population. *Salmonella* may also be found in the feces of some pets, such as hamsters and reptiles. This is of concern for child care centers as they may keep these animals in their facilities for children's enjoyment. Children and adults should always wash their hands after handling a reptile or any other animal to eliminate the risk of illness from *Salmonella*. *Salmonella* are transmitted through the fecal matter of people or animals, and are usually transmitted to humans by eating foods that have been contaminated with fecal matter via cross-contamination. As few as 15 to 20 cells, depending on the age and health of the host and strain of bacteria are necessary to cause illness (FDA 2004).

It is estimated that approximately 40,000 cases of salmonellosis are reported each year in the U.S. (FDA 2004). Infants have the highest risk of contracting salmonellosis, and the second highest risk group are children under the age of 10 (CDC 2004). Most people infected with salmonellosis develop nausea, diarrhea, fever, and abdominal cramps and normally recover without treatment, with symptoms subsiding within 5 to 7 days depending on host factors, such as age and susceptibility, ingested dose and strain characteristics. In some patients, such as children and infants, prolonged diarrhea is dangerous, as the body can be depleted of fluids and salts that are vital for the proper functioning of organs and tissues. The resulting shock to the body can be lethal to infants and there is a possibility that the bacteria can spread from the intestinal tract to the bloodstream, leading to infections in other parts of the body. If this occurs, a person must be treated immediately for the infection because at this stage it may cause death (CDC-DBMD 2004).

In 2002, the Ohio Department of Health reported that 2 children were infected with *Salmonella* from consuming raw milk from a combination dairy-restaurant-petting-zoo. Further investigation revealed that 62 individuals were case patients, i.e., an individual infected with PFGE-matched *Salmonella* Typhimurium. Forty case patients were identified as dairy customers and were included in the case control study; 56 controls were asymptomatic. Consumption of raw milk was found to be the cause of illness (Mazurek *et al.,* 2004). In 1996, the Minnesota Department of Health detected an increase in the number of reports of *Salmonella* Enteritidis infections. Hennessy and others (1996) conducted a case-control study in order to investigate the increase in reports. Results from the study implicated a nationally distributed brand of ice cream (Schwan's) in the outbreak. An estimated 224,000 persons nationwide developed *S.* Enteritidis gastroenteritis after consuming the Schwan's ice cream. In Minnesota, where the study was based, a total of 150 confirmed cases of outbreak associated salmonellosis were identified, with the median age of the patients with outbreak associated salmonellosis being 13 years old. Cross-contamination of the ice cream premix most likely occurred via tanker trailers that previously carried unpasteurized eggs containing *S.*

Enteritidis immediately before transporting the premix. The study concluded that low-level contamination of foods by *Salmonella*, and thus extremely low infectious doses, can cause disease in humans. The highest level of product contamination found in this study was only six organisms per half-cup (65 g) serving of ice cream.

Listeria monocytogenes has been recognized as a human pathogen for more than 60 years and can cause serious invasive illnesses in humans. *L. monocytogenes* are present in many animals and birds, and it has been found in soil, water, sewage, and animal feed. Five out of every 100 people carry *Listeria monocytogenes* in their intestines. This organism persists in food processing environments causing major concern for further contamination. *Listeria* has been found in a variety of raw foods, such as meats and dairy products, as well as in processed foods that have been contaminated after processing, such as soft cheeses and deli meats (CDC 2004). This poses a risk due to the fact that these foods do not require a final kill step once reaching the home.

Listeriosis is the acute illness caused by an infection from the bacteria *Listeria monocytogenes.* Since the 1980's, listeriosis outbreaks in the United States have been linked to cole slaw, milk, Mexican-style cheese, undercooked hot dogs, undercooked chicken, and delicatessen foods. While listeriosis has decreased nearly 50% since 1989, the organism remains a major concern for food processors since this infection has a mortality rate of 25-30% in infected individuals (Mastronicolis *et al.,* 1998; Miller *et al.,* 1997). An estimated 2,500 people become seriously ill with listeriosis each year in the U.S. and of those approximately 500 die (CDC-DBMD 2004). Those at risk include pregnant women, newborns, and persons with weakened immune systems. *Listeria monocytogenes* infection rates are the highest for the very young and the very old. People become infected with *Listeria monocytogenes* by eating foods that are contaminated with the bacteria, and babies can be born with listeriosis if their mothers contract the illness during pregnancy. Symptoms from this illness include fever, muscle aches, severe headache, vomiting, and other influenza-type symptoms. Newborns infected with listeriosis have been shown to develop meningitis – inflammation of the tissue surrounding the brain and/or spinal cord (CDC-DBMD 2004). The infective dose *L.*

monocytogenes is unknown but it is believed that the number of organisms necessary to cause illness varies with the strain and susceptibility of the victim.

In 2000, the New York City Department of Health and Mental Hygiene notified the CDC of 9 patients who had been infected with *Listeria monocytogenes* over the previous two months. In order to identify risk factors for infection, a case-study was conducted, where a case was defined as a patient or a mother-infant pair from whom *L. monocytogenes* that matched the outbreak PFGE pattern was isolated from the blood. Control patients with *L. monocytogenes* infections with different PFGE patterns were taken from the same states and the same time period. Results of the case study were used to trace the product suspected of causing the infections. Thirty patients from various states in the case study were infected with the same strain of *L. monocytogenes* and trace back and environmental investigation results indicated that the outbreak was caused due to delicatessen turkey meat from a variety of locations (Olsen *et al.,* 2005). In 2001, the Los Angeles County Department of Health Services/Public Health conducted a study of an outbreak of acute febrile gastroenteritis where 16 of 44 healthy attendees of a catered party became ill with diarrheal illness. In order to determine the cause of the outbreak, a case study was performed by at risk-consumers that attended the party as well as other customers of the delicatessen that supplied the party food. Stool and leftover food samples were requested from all ill party attendees. Of the 44 responding individuals who replied to the case study, 16 met the criteria for the definition of a "presumptive case". Six of eight stool specimens yielded *Listeria monocytogenes* and leftover turkey yielded *L. monocytogenes*. The median age of the attendees who became ill was 15.5 years of age. Upon completion of the study, it was determine that illness was associated with the consumption of precooked, sliced turkey supplied by the delicatessen (Frye *et al.,* 2002).

There are special food handling behaviors associated with the consumption of potentially hazardous foods, such as hot dogs and lunch meats that have been shown to contain *L. monocytogenes*. Kendall and others (2003) studied the food handling behaviors that are important for reducing the risk of foodborne illness among pregnant women, infants and young children, elderly, and immunocompromised. Results from the study

indicated that behaviors for pregnant women and children were associated with *L. monocytogenes* and a number of other foodborne pathogens. One of the consumer food handling behaviors of special importance for infants and children associated with *L. monocytogenes* included avoiding hot dogs and lunchmeats that have not been reheated to 165°F. This is important due to the fact that a number of infants and children eat these products cold. After review of the previous studies, it is clear that ready-to-eat meats, such as hot dogs and lunchmeat, are foods that pose health risks for foodborne illness due to *Listeria monocytogenes*. Since children consume these foods, it is necessary to ensure that the proper steps are taken to keep children safe from foodborne illness due to this pathogen.

2.5.2 Potential contributions of CCC personnel and facilities to food safety

Improper personal hygiene is major food safety risk in child care facilities. A study conducted by Simmons and others (1990) surveyed the frequency of handwashing by nurses in two intensive care units. A trained secret observer monitored the frequency of handwashing at necessary times, which included before intra venous care, before and after wound care, after touching a contaminated object, such as an endotracheal tube or urinary catheter, and before performing invasive procedures. Based on questionnaires completed by the nurses, self-reported handwashing frequencies as well as whether handwashing actually took place were recorded and used to calculate the percentage of appropriate handwashing. Handwashing intervention sessions were held to increase the frequency of handwashing. Results of the study indicated that handwashing frequencies increased towards the end of the study; however, results in overall frequencies were not statistically different ($p=0.0015$). Handwashing was observed in 22.0% of all necessary times before the intervention and 29.9% was observed after the intervention. The most prevalent reasons cited by nurses for not washing their hands as often as necessary were "they were too busy" (68%), "soaps cause skin irritation" (68%), "gloves usually worn" (55%) and "don't think about it" (52%) . With this trend in healthcare workers, it is reasonable to believe handwashing practices are also insufficient in child care facilities for similar reasons. Bacteria in the diarrheal stools of infected persons can be transmitted

from person-to-person if handwashing is inadequate (CDC 2004). This is particularly important for toddlers within child care facilities who are not toilet trained. Such children typically shed the organism in their feces and may not perform adequate handwashing afterwards. Other children, as well as child care workers, attending the child care facility are at high risk for infection.

The physical environment of the food preparation areas in a child care facility may influence hygienic food preparation. This in turn plays a role in the potential transmission of disease among children. The most critical aspects of the food preparation areas are the food contact surfaces and their cleanability. Environmental microbiological studies in child care centers have shown that use of easily cleaned surfaces could help reduce environmental contamination and thus its role in the transmission of disease (Petersen and Bressler 1986). There are a number of critical factors associated with food contact surfaces, such as susceptibility to scratching, ease of cleaning, type of food preparation, etc., that must be considered when developing a food contact surface. Therefore, the type of materials used in food contact surfaces and their surface characteristics must be thoroughly tested in order to determine which material is best suited for use as a food contact surface. Several materials that are typical for use as food contact surfaces are stainless steel, plastic laminate, wood, grouted tile, etc. All of these materials have different properties which make them better or worse suited for use as food contact surfaces.

Prerequisites for kitchen surfaces are durability and cleanability. with the liberal use of stainless steel, laminated plastic-covered cabinets, vinyl floors, and polyamide wall paint (Petersen and Bressler 1986). Stainless steel is the material of choice for food contact surfaces and work surfaces because of its mechanical strength, corrosion resistance, longevity and ease of fabrication (Holah and Thorpe 1990; Kusumaningrum *et al.,* 2003). Numerous researchers have studied the survival of foodborne pathogens on stainless steel and other surfaces and their contribution to cross-contamination (Kusumaningrum *et al.,* 2003; Moore *et al.,* 2003). A study by Kusumaningrum and others (2003) indicated that pathogens, such as *Salmonella* Enteritidis, *Staphylococcus*

aureus, and *Campylobacter jejuni* are capable of surviving for hours or days after contamination on stainless steel surfaces. In addition, the presence of residual food debris, such as milk or chicken residues on the surface is an important factor in the increased survival of these pathogens on the surface. Prolonged survival presents a long-term cross-contamination hazard since the pathogens were readily transferred from the kitchen sponges to stainless steel surfaces and then to foods. Moore and others (2003) studied the transfer rate of *Salmonella* Typhimurium and *Campylobacter jejuni* from stainless steel to Romaine lettuce. Stainless steel coupons ($25cm^2$) were inoculated with a 20µl of either *S.* Typhimurium or *C. jejuni* to provide an inoculum level of ~ 10^6 CFU/$28mm^2$. The inocula were dried for up to 80 min for *C. jejuni* and 120 min for *S.* Typhimurium. Bacterial transfer rates ranged from 23 to 31% for *S.* Typhimurium on wet lettuce. For *C. jejuni* transfer rates ranged from 16 to 38% for dry lettuce and from 15 to 27% for wet lettuce. Results indicated that relatively high numbers, 3 to 4 log, of *S.* Typhimurium or *C. jejuni* may be transferred to ready-to-eat foods at least one to two hours later after surface contamination has occurred.

The effect of various types of food contact surfaces, on the survival and persistence of various foodborne pathogens has been studied. A study conducted by Tebbutt (1991) compared five types of surfaces, formica, stainless steel, marble, polypropylene, and wood to assess the cleaning efficiency of food contact surfaces. A two-stage cleaning process was utilized in the study, using a detergent solution and a hypochlorite solution of 200 ppm. After spraying with the detergent solution, surfaces were wiped in a uniform manner with a 3-ply paper. After wiping, the surfaces were then sprayed with the hypochlorite solution and wiped with a separate 3-ply paper. Agar contact plates were utilized as the method of recovery and growth of total viable bacterial counts (APC) were classified as scanty (25 or fewer colonies), light (up to 75 colonies), moderate (up to 200 colonies) and heavy (confluent or almost confluent growth). Results from the study indicated that the wood and polypropylene surfaces were particularly difficult to clean. Forty of 47 wood surfaces remained heavily contaminated after cleaning and 39 of 72 polypropylene pads still had moderate numbers of bacteria on

them. Formica, stainless steel, and marble surfaces were more easily cleaned (Tebutt 1991). DeCesare and others (2003) studied the survival and persistence of *Campylobacter jejuni* and five strains of *Salmonella* species under various organic loads on 5 cm^2 samples of stainless steel, formica laminate, and glazed ceramic tile food contact surfaces. Surfaces were individually inoculated with an initial population of approximately 10^6 CFU/5 cm^2 of each type of organism suspended in trypticase soy broth or phosphate-buffered saline. Results were expressed as the time to achieve a 3 log reduction of *C. jejuni* and *Salmonella* cells per ml of phosphate-buffered saline (PBS) or trypticase soy broth (TSB). The overall time to achieve a 3-log reduction for *Campylobacter*, was the greatest for formica followed by stainless steel and ceramic tile, with averages of 207 ± 22 minutes, 169 ± 12, and 136 ± 13 minutes. For *Salmonella*, the same trend was seen with average times of 2,805 ± 731 minutes, 1,021 ± 282 minutes, and 344 ± 76 minutes for the same respective surfaces. The reduction times were influenced by the amount of organic load that was present on the surface which was simulated by using TSB (high organic load conditions) or PBS (low organic load conditions). The type of surface and the amount of organic load markedly influenced the amount of bacterial contamination remaining on the surface.

The bacterial retention of plastic and wood cutting boards was studied by (Ak *et al.,* 1994). Cutting board test surfaces (25 cm^2) were inoculated with approximately 10^8 CFU/25 cm^2 of *E. coli* O157:H7. After inoculation was completed, boards were washed in 300 ml of hot water (116°F) with 2 ml of commercial dish detergent by scrubbing with a new sponge. After washing, boards were rinsed in 116°F water. Recovery of bacteria was achieved by surface plating by pressing board samples on blood agar. For new wooden boards inoculated with *E. coli* O157:H7, after 1 hour a dramatic reduction in CFUs was observed, with counts decreasing from approximately 10^8 CFU to 10^5 CFU. In comparison, CFUs on new plastic cutting boards seemed to remain constant with no reduction in numbers during the first hour. Results from this study indicate that with reasonable cleaning effort, wooden cutting boards can be safely used in home kitchens and are unlikely to create undue risks of cross-contamination to foods.

2.5.3 Surveys of microbiological quality of surfaces in CCC

Since the 1980's, a few microbiological studies have been conducted in order to assess the sanitation in child care centers. Petersen and Bressler (1986) studied the design and construction of the day care environment by conducting an environmental microbiological survey of commonly touched surfaces within child care facilities. Surfaces included floors, tables and chairs, cots and cribs, diaper changing areas, and toilet areas. Prevalence rates of fecal coliforms were determined for all surfaces. For fecal coliforms prevalence was defined as the percentage of total samples in which the microorganisms were detected. The mean prevalence rates of fecal coliforms from surface samples were 22.5% (264 of 2,082 samples). Results from the study illustrate that fecal contamination is present on environmental surfaces within child care facilities.

Weniger and others (1983) determined the prevalence of fecal coliforms on environmental surfaces in two day care centers. Approximately 70 children were enrolled full-time and 15 children were enrolled part-time at each center. Ages of all children at each center ranged from infancy to 5 years of age. Staff members at each center received no prior notice of sampling days and times. Therefore, no changes in the cleaning and sanitation routines were applied. Surface samples, using RODAC plates, were taken during the late afternoon before closing. Results from the study indicated that environmental surfaces within child care centers may be contaminated with fecal coliforms. Of the total 398 surfaces sampled in the study, 17 (4.3%) were positive for fecal coliforms. Positive samples were obtained from toilets, diapering items, floors, furniture, and refrigerator handles.

The prevalence of rotaviruses was studied by Keswick and others (1983) on environmental surfaces and teacher's hands in child care facilities. Rotavirus is the most common cause of diarrhea in children worldwide and the leading cause of diarrhea in children less than 2 years old in day care centers (Keswick *et al.,* 1983; Parashar *et al.,* 1998). Samples from 25 areas or objects in an adjacent toilet and kitchen area were collected over a three day period in a single child care facility where 6 to 18-month old

children were enrolled. Results from the study indicated that 4 out of 25 total samples (16%) were positive for rotaviruses by fluorescence assays (FA). However, no fecal contamination was obviously present on any of the surfaces. The positive surfaces included a refrigerator door handle, a diaper pail lid, and sink, as well as the hand of a teacher who frequently diapered the children. The diaper changing counter was sampled a total of three times and was found negative each time.

Similar studies have been performed to evaluate the microbiological quality of food contact surfaces in schools and assisted-living facilities. Henroid and others (2004) conducted a microbiological evaluation of 40 school foodservice operations assessing the effectiveness of cleaning and sanitation programs of five food contact surfaces in Iowa schools. The surfaces evaluated included food preparation tables, cooking equipment, and serving trays which could cross-contaminate foods. For this study, microbial count limits were based partly on standards set by the U.S. Food Code for cleaned and sanitized equipment. The limits for acceptance were: APC < 1.3 $\log_{10}$ CFU, *Enterobacteriaceae* count < 1.0 $\log_{10}$ CFU, and *Staphylococcus aureus* < 1.0 $\log_{10}$ CFU per sample. Mean aerobic plate counts for all surfaces sampled (food preparation counter, mixing bowl or steam-jacketed kettle, handwashing sink handle, refrigerator or freezer handle, and meal tray) were high and ranged from 1.33 to 4.65 $\log_{10}$ CFU/cm^2. Mean ranges for the *Enterobacteriaceae* count were 0.16 to 1.22 $\log_{10}$ CFU/cm^2 and for the *S. aureus* count, mean counts ranged from 0.16 to 3.07 $\log_{10}$ CFU/cm^2. The handwashing sink had the highest mean bacterial counts followed by the refrigerator/freezer handle. For APC of the food contact surfaces, 36 of 40 (90%) school kitchens had an acceptable number on at least one of the food contact surfaces. For *Enterobacteriaceae* and *S. aureus*, all 40 schools achieved acceptable limits for the food preparation table and the meal trays sampled. Results of the study indicated that microbial standards for surface sanitation are achievable in schools.

In a similar study, Sneed and others (2004) studied 40 assisted-living facilities in Iowa to assess the microbiological quality of food contact surfaces (food preparation tables, cutting boards, etc.) and a surface that could cross-contaminate food (refrigerator

handles) to determine the effectiveness of the cleaning and sanitation programs within the facilities. As in the previous study, limits were set for the APC, *Enterobacteriaceae,* and *Staphylococcus aureus.* Mean aerobic plate counts for all surfaces sampled (work table/counter, cutting boards, mixing bowl/equipment, and refrigerator/freezer handle) ranged from 1.51 to 3.25 $\log_{10}$ CFU/cm^2. Mean ranges for the *Enterobacteriaceae* count were 1.34 to 2.55 $\log_{10}$ CFU/cm^2 and for *S. aureus*, mean counts ranged from 0.50 to 2.79 $\log_{10}$ CFU/cm^2. The cutting board sample had the highest mean APC, while the mixing bowl/equipment had the highest *Enterobacteriaceae* and *S. aureus* mean bacterial counts. For APC, the majority of facilities exceeded the set standards for cutting boards (72.5%) and mixing bowl/equipment (70%). For *Enterobacteriaceae* 33 facilities met the set standard for all five samples and for *S. aureus* 17 facilities achieved acceptable limits for all five samples. Results from this study indicate that cross-contamination from these surfaces is a risk for foodborne illness within these facilities.

3 MATERIALS AND METHODS

3.1 Sampling Sites and Surface Selections

A microbiological survey was conducted in six (6) Knoxville area child care facilities. The facilities represented three large (> 100 children) and three small (< 50 children) centers. Each child care facility was tested twice monthly over the course of an eight (8) month period for a total of 16 sampling periods per center. Four areas, one food service area, one diaper area, and two food preparation areas, within the child care facilities were sampled. The food service area and the food preparation areas consisted of three food contact surfaces, two food preparation surfaces (i.e. kitchen counters) and one food service surface (i.e. children's eating table), while the diaper changing area consisted of one non-food contact surface (i.e. diaper changing surface). All surfaces were tested three times daily, pre-opening, during lunch, and following final clean-up of the day, to monitor the microbiological quality of each surface throughout the day.

Surfaces of each area varied with surface types including: plastic laminate for food service surfaces, stainless steel, grouted tile, plastic laminate, and wooden laminate for food preparation surfaces, and acrylic solid surface and plastic padding for diaper changing surfaces.

3.2 Sample Preparation for Microbiological Analysis

Microbiological analysis of surfaces was done by swabbing a 50cm^2 area using a sterile stainless steel template. Sampling was performed by swabbing the area horizontally, from one side of the template to the other, and repeating vertically, and then horizontally again using 3M™ Quick Swabs (3M™ Microbiology; St. Paul, MN) following manufacturers instructions. Samples were transported to the laboratory and then plated onto Aerobic Count (AC) Plates (Petrifilm, 3M™ Microbiology) and *E. coli*/Coliform Count Plates (Petrifilm, 3M™ Microbiology) following manufacturer's instructions. Plates were incubated with the clear side up in stacks of up to 20 at 32°C for 48 hours. After incubation the plates were counted on a standard colony counter as per

manufacturers instructions and the results were recorded number of colony forming units (CFU) per 50 cm^2.

3.3 Rapid Sanitation Assays

All rapid sanitation assays were done using a sterile, stainless steel template with a 50 cm^2 area exposed. Sampling was performed by swabbing the 50 cm^2 area horizontally, from one side of the template to the other, and repeating vertically, and then horizontally again using AccuPoint ATP Surface Samplers with the AccuPoint ATP Sanitation Monitoring System (Neogen®, Inc.; Lansing, MI), Pro-tect® hygiene surface swabs (Biotrace, Neogen®, Inc.; Cincinnati, OH), and SpotCheck™ glucose swabs (Weber Scientific, Hygiena LLC; Camarillo, CA) following manufacturer's instructions for each swab. Rapid method results were read within 5 seconds to 10 minutes after sampling depending on manufacturer's instructions.

4 MICROBIOLOGICAL ANALYSIS OF FOOD CONTACT SURFACES IN CHILD CARE CENTERS

4.1 Abstract

According to the CDC, foodborne illness causes approximately 5,000 deaths each year and approximately one-third of those are children (CDC 2004). Since child care centers are an important part of society, there is a need to survey and assess sanitation of potential food contact and non-food contact surfaces within these facilities. A study of six child care centers in Knoxville, TN was conducted to assess the microbiological quality of three food contact surfaces, (food service surfaces and food preparation surfaces) and one non-food contact surface (diaper changing surfaces) to determine the effectiveness of cleaning and sanitization procedures within the facilities. Bacterial counts, aerobic plate counts, and *E. coli*/coliform counts of a 50 cm^2 area on all surfaces were determined using standard microbiological swabbing methods. Analysis was performed on all surfaces for a total of 1,149 samples. Samples were taken three times a day, pre-opening, lunch time, and following final clean-up, twice per month for eight months in each child care center (n = 288).

Mean log APC counts over the survey period were 3.04, 3.93, 3.08, 4.50, 3.45 and 4.16 $\log_{10}$ CFU/50 cm^2 for centers 1 through 6, respectively. Mean coliform counts were 1.35, 17.96, 5.59, 87.55, 10.22, and 20.43 CFU/50 cm^2 for the same respective centers. There was a significant difference between counts for center size based on mean log APC with large centers having lower mean log APC (3.55 $\log_{10}$ CFU/50 cm^2) than small centers (3.81 $\log_{10}$ CFU/50 cm^2). As for coliform counts, large centers had higher mean coliform counts (35.62 CFU/50 cm^2) than small centers (10.72 CFU/50 cm^2). However, there was no significant difference between counts for center size based on coliform counts. Coliforms were detected on 283 of 1,149 (24.7%) samples with counts ranging from 1 to 2,000 CFU/50 cm^2 while *E. coli* was detected on 18 of 1,149 (1.6%) samples with counts ranging from 1 to 35 CFU/50 cm^2.

These findings demonstrate that microbial contamination is present on food contact surfaces of child care centers. Due to the high risk of foodborne illness associated with children, the possibility of cross-contamination from food contact or non-food contact surfaces to foods is an aspect of food safety that requires more attention. Emphasis on training and the development of modified standard sanitation operating procedures (SSOPs) for child care centers is needed to reduce potential hazards.

4.2 Introduction

Safe food handling in child care centers is important because children are one of the highest risk groups for foodborne illness (Gerba *et al.,* 1996). Food handling practices within these facilities have a great impact on food safety; however, little research has been done to assess the facilities or their practices. Petersen and Bressler (1986) suggested that the food preparation area of a child care center may play a role in the transmission of disease among children. Effectively cleaned and sanitized food contact surfaces could help reduce environmental contamination and thus its role in the transmission of disease in child care centers.

Food contact surfaces are a major concern for food service facilities in controlling the spread of foodborne pathogens. Food service areas are considered critical to health, and therefore the microbiological quality of these surfaces as well as non-food service surfaces in child care facilities must be surveyed and assessed. Although many cases of foodborne illness have been attributed to inadequate cooking, temperature abuse, and the use of contaminated raw ingredients, cross-contamination between raw and cooked foods via food contact surfaces has also been identified as a significant risk factor (DeCesare *et al.,* 2003). Survey and surveillance data indicate that 25% of people do not clean and sanitize surfaces after cutting raw meat or poultry (National Center for Health Statistics 1995). Without adequate cleaning and sanitizing, residues of raw meat, poultry exudates, and other foods may remain on kitchen surfaces, serving as sources of microbial contamination for raw vegetables or other ready-to-eat (RTE) foods (DeCesare *et al.,* 2003).

Studies have shown that both the type of contact surface and the level of organic matter can influence the survival of foodborne pathogens on food contact surfaces. Frank and Chmielewski (1997) conducted a study to evaluate the effectiveness of chlorine on the sanitation of stainless steel and other domestic food preparation surfaces. Results from the study indicated that sodium hypochlorite solution was most effective on smooth stainless steel and polycarbonate surfaces reducing cell populations to less than 1.0 log CFU/cm^2. Cell populations remained greater than 1.0 log CFU/cm^2 on abraded stainless steel and mineral resin surfaces. Mafu and others (1990) studied the efficiency of sodium hypochlorite against *Listeria monocytogenes* on stainless steel, glass, polypropylene and rubber surfaces. For stainless steel and glass a concentration of 200 ppm, the manufacturer's recommended concentration, was effective for inactivation of *L. monocytogenes.* However, for polypropylene and rubber a concentration of 800 ppm was required. These results indicate that certain types of food contact surfaces may be better suited for use as food contact surfaces within child care centers.

Although numerous investigators have reported the survival of various enteric viruses on food preparation surfaces, there has been few attempts to evaluate the persistence of foodborne bacterial pathogens on those same surfaces (DeCesare *et al.,* 2003). The objective of this study was to survey the microbiological quality of food and non-food contact surfaces in child care centers to determine the sanitation quality of these surfaces.

4.3 Materials and Methods

4.3.1 Sampling sites and surface selections

A microbiological survey was conducted in six (6) Knoxville area child care facilities. The facilities represented three large (> 100 children) and three small (< 50 children) centers. Each child care facility was tested twice monthly over the course of an eight (8) month period for a total of 16 sampling periods per center. Four areas, one food service area, one diaper area, and two food preparation areas, within the child care

facilities were sampled. The food service area and the food preparation areas consisted of three food contact surfaces, two food preparation surfaces (i.e. kitchen counters/cutting boards) and one food service surface (i.e. children's eating table), while the diaper area consisted of one non-food contact surface (i.e. diaper changing surface). All surfaces were tested three times daily, pre-opening, during lunch, and following final clean-up of the day, to monitor the microbiological quality of each surface throughout the day. Surface types of each area within each center varied and included plastic laminate for food service surfaces, stainless steel, grouted tile, plastic laminate, and wooden laminate for food preparation surfaces, and acrylic solid surface and plastic padding for diaper changing surfaces (Table 4.1).

Table 4.1: Sampling area surface types by center

	Surface Types (Area Number)			
Center #	**Food Service (No. 1)**	**Diaper Area (No. 2)**	**Food Prep No. 1 (No. 3)**	**Food Prep No. 2 (No. 4)**
1 (Large)	Plastic Laminate	Solid-Surface	Stainless Steel	Stainless Steel
2 (Small)	Plastic Laminate	Plastic Pad	Grouted Tile	Grouted Tile
3 (Small)	Plastic Laminate	Plastic Pad	Plastic Laminate	Wood Laminate
4 (Small)	Plastic Laminate	Plastic Pad	Plastic Laminate	Plastic Laminate
5 (Large)	Plastic Laminate	Solid-Surface	Plastic Laminate	Plastic Laminate
6 (Large)	Plastic Laminate	Plastic Pad	Stainless Steel	Stainless Steel

4.3.2 Sample preparation for microbiological analysis

Sampling was done using a sterile stainless steel template, which exposed a surface area of 50 cm^2. Flat food contact surfaces and non-food contact surfaces were swabbed using 3M™ Quick Swabs (3M™ Microbiology; St. Paul, MN). Sampling was performed by swabbing the area horizontally, from one side of the template to the other, and repeating vertically, and then horizontally again using 3M™ Quick Swabs following manufacturer's instructions to determine the aerobic plate count (APC). Swabs contained 1 ml of Letheen broth which aided in the neutralization of possible sanitizers on surfaces. After sampling, the swabs were marked with an identification code and placed in food storage bags in an insulated tote bag and transported to the Food Microbiology Laboratory at The University of Tennessee. The total transport time was less than 30 minutes and samples were stored at 4°C until testing. All samples were analyzed within 1 hour of arrival at the laboratory. Using Aerobic Count (AC) Petrifilm Plates (3M™ Microbiology; St. Paul, MN), the samples were plated and incubated according to manufacturers instructions. Swab samples were vigorously shaken and 1 ml of broth was plated onto the Aerobic Count (AC) Petrifilm Plates. The plates were incubated with the clear side up in stacks of up to 20 at 32°C for 48 hr. After incubation all red colonies, regardless of size or intensity, on the plates were counted on a standard colony counter.

Sampling procedures for *E. coli*/Coliform Counts were done in a similar manner to that of the aerobic plate count. After surface swabbing was completed, samples were plated and incubated according to manufacturers instructions. Swab samples were vigorously shaken and 1 ml of broth was plated onto *E. coli*/Coliform Count Petrifilm Plates (3M™ Microbiology; St. Paul, MN). The plates were incubated with the clear side up in stacks of up to 20 at 32°C for 48 hr. For this method, all red colonies on the plate surrounded by one or more gas bubbles were counted as coliforms; whereas, all blue colonies were counted as *E. coli* colonies. Results for all counts were reported as the number of colony forming units per 50 cm^2.

4.3.3 Statistical analysis

Tukey's multiple range test and ranking analyses were performed to determine significant differences ($P<0.05$) between centers, center sizes (small vs. large centers), area, surfaces, and time of day. Least squares means were analyzed using Statistical Analysis System (SAS Institute, Cary, NC).

4.4 Results

Results for various microbiological analyses were expressed as the mean number of total bacteria (APC) and coliforms per 50 cm^2 area and by prevalence rates of the presence of coliforms and *E. coli*, i.e., the percentage of total samples in which coliforms or *E. coli* were detected, per 50 cm^2 area.

Mean log APC counts over the survey period were 3.04, 3.93, 3.08, 4.50, 3.45 and 4.16 $\log_{10}$ CFU/50 cm^2 for centers 1 through 6, respectively (Table 4.2). Mean coliform counts were 1.4, 18.0, 5.6, 87.6, 10.2, and 20.4 CFU/50 cm^2 for the same respective centers (Table 4.2). Centers 1, 3 and 5 were similar and had significantly lower ($p < 0.05$) mean log APC counts and mean coliform counts than centers 2, 4 and 6. Center 4 had both the highest mean log APC and coliform counts of other centers.

Mean bacterial counts by center size per 50 cm^2, small (< 50 children) versus large (> 100 children) are shown in Table 4.3. Large centers had a significantly lower mean ($p < 0.05$) log APC (3.55 $\log_{10}$ CFU/50 cm^2) than small centers (3.81 $\log_{10}$ CFU/50 cm^2). As for coliform counts, large centers had higher mean coliform counts (35.6 CFU/50 cm^2) than small centers (10.7 CFU/50 cm^2). However, there was no significant difference between counts for center size based on coliform counts.

Table 4.2: Mean bacterial counts by child care center for all surfaces

Center	Mean Aerobic Plate Count (log_{10} CFU/50cm^2) *	Mean Coliform Count (CFU/50cm^2)
1	3.04^{C}	1.4^{D}
2	3.93^{B}	18.0^{C}
3	3.08^{C}	5.6^{D}
4	4.50^{A}	87.6^{A}
5	3.45^{C}	10.2^{D}
6	4.16^{AB}	20.4^{B}

* log_{10} CFU = number of colony-forming units in logs; Letter groupings within columns indicate significance difference at P<0.05; Mean separation results for log APC derived from Least Squares Means test. Mean separation results for coliforms derived from non-parametric ranking analysis.

Table 4.3: Mean bacterial counts by child care center size for all surfaces

Center Size†	Mean Aerobic Plate Count (log_{10} CFU/50cm^2) *	Mean Coliform Count (CFU/50cm^2)
Small	3.81^{A}	10.7
Large	3.55^{B}	35.6

* log_{10} CFU = number of colony-forming units in logs; † Center Size: large centers (> 100 children) = 1, 5, and 6 and small centers (< 50 children) = 2, 3, and 4; Letter groupings within columns indicate significance difference at P<0.05; Mean separation results for log APC derived from Least Squares Means test. Mean separation results for coliforms derived from non-parametric ranking analysis.

Mean bacterial counts by area per 50 cm^2 within centers are shown in Table 4.4. Mean log APC counts were 3.78, 3.63, 3.54 and 3.78 log_{10} CFU/50 cm^2 for food service, diaper changing, food preparation 1, and food preparation 2, respectively. Mean coliform counts by area were 23.2, 5.2, 18.0, and 45.6 CFU/50 cm^2 for the same respective areas. There was no significant difference between areas for mean log APC counts or mean coliform counts. For mean log APC counts, least squares means were generated in order to determine if center size had an effect on mean log APC counts of areas within centers.

Mean log APC counts by area for small centers were 3.86, 3.51, 3.73, and 4.15 log_{10} CFU/50 cm^2 for areas 1 through 4, respectively (Table 4.5). For areas within larger centers, mean log APC counts were 3.70, 3.76, 3.34, and 3.42 log_{10} CFU for the same respective areas. The diaper changing area and food preparation area 1 were similar but were significantly different ($p < 0.05$) than the food preparation area 2 within small centers. Food preparation area 2 had the highest mean log APC counts within small centers. In comparison to small centers, the diaper changing area and food preparation area 1 in large centers were significantly different ($p < 0.05$) from each other but were similar to the food service area and food preparation area 2. The diaper changing area had the highest mean log APC counts within large centers.

In order to determine if significant differences exist between surface types, plastic laminate, acrylic solid surface, wood laminate, stainless steel, plastic padding, and grouted tile within each center least squares means of log APC and mean coliform counts were analyzed. Table 4.6 shows mean log APC and mean coliform counts by surface type. Mean log APC counts were 4.05, 3.90, 3.81, 3.39, 3.08, and 3.07 (log_{10} CFU/50 cm^2) for grouted tile, plastic padding, plastic laminate, stainless steel, acrylic, and wooden laminate, respectively. Significant differences ($p < 0.05$) were found between surface types with plastic laminate, plastic padding, and grouted tile being similar, but significantly different from acrylic, wood laminate, and stainless steel surfaces.

Table 4.4: Mean bacterial counts in child care centers by sampling area

	Mean Aerobic Plate Count (log_{10} CFU/50cm^2) *	Coliform Count (CFU/50cm^2)
Area	**Mean**	**Mean**
Food Service	3.78	23.2
Diaper Changing	3.63	5.2
Food Preparation 1	3.54	18.0
Food Preparation 2	3.78	45.6

* log_{10} CFU = number of colony-forming units in logs; Letter groupings within columns indicate significance difference at P<0.05; Mean separation results for log APC derived from Least Squares Means test. Mean separation results for coliforms derived from non-parametric ranking analysis.

Table 4.5: Mean bacterial counts in child care centers by center size and area within centers

Center Size†	Area	Mean Aerobic Plate Count (log_{10} CFU/50cm^2) *
Small	**Food Service**	3.86^{AB}
	Diaper Changing	3.51^{B}
	Food Preparation 1	3.73^{B}
	Food Preparation 2	4.15^{A}
Large	**Food Service**	3.70^{AB}
	Diaper Changing	3.76^{A}
	Food Preparation 1	3.34^{B}
	Food Preparation 2	3.42^{AB}

* log_{10} CFU = number of colony-forming units in logs; † Center Size: large centers (> 100 children) = 1, 5, and 6 and small centers (< 50 children) = 2, 3, and 4; Letter groupings within center size columns indicate significance difference at P<0.05; Mean separation results for log APC derived from Least Squares Means test.

Table 4.6: Mean bacterial counts in child care centers by surface type

Surface/(n)†	Mean Aerobic Plate Count (log_{10} CFU/50cm^2) *	Mean Coliform Count (CFU/50cm^2)
Plastic Laminate (480)	3.81^A	37.5
Acrylic (96)	3.08^B	2.6
Wood Laminate (95)	3.07^B	9.1
Stainless Steel (192)	3.39^B	10.7
Plastic Padding (143)	3.90^A	7.5
Grouted Tile (143)	4.05^A	16.6

* log_{10} CFU = number of colony-forming units in logs; † (n) =total number of samples per surface type; Letter groupings within columns indicate significance difference at P<0.05; Mean separation results for log APC derived from Least Squares Means test. Mean separation results for coliforms derived from non-parametric ranking analysis.

Grouted tile had higher mean log APC counts than all other surfaces. Mean coliform counts were 37.5, 16.6, 10.7, 9.1, 7.5, and 2.6 CFU/50 cm^2 for plastic laminate, grouted tile, stainless steel, wood laminate, plastic padding, and acrylic, respectively. Non-parametric ranking analysis indicate that plastic laminate surfaces have higher coliform counts than all other surfaces.

Based on surface type samples, coliforms were detected in 283 of 1,149 (24.7%) samples and ranged from 1 to the maximum detectable level of 2,000 CFU/50 cm^2 (Table 4.7). Plastic laminate and plastic padding surfaces had the highest percentage of positive coliforms compared to other surfaces.

E. coli was detected in 18 of 1,149 (1.6%) samples and ranged from 1 to 35 CFU/50 cm^2 (Table 4.8). Plastic padding and grouted tile surfaces had the highest percentage of *E. coli* counts compared to other surfaces. Statistical analysis using the Fisher's Exact test indicated that there was a significant association between the presence/absence of *E. coli* and the type of surface tested (exact p=0.0015).

Table 4.7: Frequency of coliform positive samples on surfaces in child care centers by surface type

Surface	Coliform count/(n)*	% positive	Range‡
Plastic Laminate	137/480	28.5	1 – 2000
Acrylic	13/96	13.5	1 – 195
Wood Laminate	20/95	21.1	1 – 600
Stainless Steel	35/192	18.2	1 – 573
Plastic Padding	44/143	30.8	1 – 398
Grouted Tile	34/143	23.8	1 – 2000

* Coliform counts CFU per 50 cm^2/ number of coliform positive samples per total number of samples; ‡Range of coliform CFU's or actual count for area; Statistical analysis for % coliform by surface type derived from Chi-Square test ($p < 0.0018$).

Table 4.8: Frequency of *E. coli* positive samples on surfaces in child care centers by surface type

Surface	*E. coli* count/(n)*	% positive	Range‡
Plastic Laminate	1/480	0.2	7 – 27
Acrylic	1/96	1.0	3
Wood Laminate	1/95	1.1	35
Stainless Steel	2/192	1.0	2
Plastic Padding	8/143	5.6	1 – 12
Grouted Tile	5/143	3.5	1

* *E. coli* counts CFU per 50 cm^2/ number of *E. coli* positive samples per total number of samples

‡ Range of *E. coli* CFU's or actual count for area; Statistical analysis for % *E. coli* by surface type derived from Fisher's Exact Test (exact $p=0.0015$).

Mean log APC counts by time were 3.71, 3.88 and 3.46 $\log_{10}$ CFU/50 cm^2 for pre-opening, lunch time, and final clean-up, respectively (Table 4.9). Mean coliform counts were 12.3, 40.8, and 16.0 CFU/50 cm^2 for the same respective times (Table 4.9). For mean log APC counts, pre-opening and lunch time were significantly higher than final clean-up. For mean coliform counts, pre-opening and final clean-up were significantly lower than lunch time.

Tables 4.10 and 4.11 show the frequency of coliform and *E. coli* positive samples by time per area. Coliforms were detected in 283 of 1,149 (24.6%) samples and ranged from 1 to the maximum detectable level of 2,000 CFU/50 cm^2. 47.7% of the positive samples were detected in the food preparation areas and 26.3% in the food service area. The diaper changing area had the lowest percentage (24.3%). *E. coli* was detected in 18 of 1,149 (1.6%) samples and ranged from 1 to 35 CFU/50 cm^2. 50% of the positive samples were from the diaper changing area and 44% from the food preparation areas.

Table 4.9: Mean bacterial counts on surfaces in child care centers by time of sampling

Time	Mean Aerobic Plate Count ($\log_{10}$ CFU/50cm^2) *	Mean Coliform Count (CFU/50cm^2)
Pre-opening	3.71^A	12.3^B
Lunch	3.88^A	40.8^A
Final Clean-up	3.46^B	16.0^B

* $\log_{10}$ CFU = number of colony-forming units in logs; Letter groupings within columns indicate significance difference at $P<0.05$; Mean separation results for log APC derived from Least Squares Means test. Mean separation results for coliforms derived from non-parametric ranking analysis.

Table 4.10: Frequency of coliform positive samples in child care centers by sampling time and area sampled

	Area							
	Food Service (n = 289)[†]		Diaper Area (n = 288)		Food Preparation 1 (n = 289)		Food Preparation 2 (n = 286)	
Time	POS[‡]	Range[*]	POS	Range	POS	Range	POS	Range
Pre-opening	16	1-133	19	1-398	24	1-980	27	1-970
Lunch	36	1-2000	31	1-78	23	1-1260	34	1-1980
Final Clean-up	24	1-354	20	1-53	14	1-560	15	1-1030

[†] n = total number of samples taken for each area; [‡] Number of positive samples for coliforms;* Range of coliforms CFUs or actual counts for area.

Table 4.11: Frequency of *E. coli* positive samples in child care centers by sampling time and area sampled

	Area							
	Food Service (n = 289)[†]		Diaper Area (n = 288)		Food Preparation 1 (n = 289)		Food Preparation 2 (n = 286)	
Time	POS[‡]	Range[*]	POS	Range	POS	Range	POS	Range
Pre-opening	0	-	2	1-10	1	27	2	1-35
Lunch	0	-	3	1	0	-	4	1-2
Final Clean-up	1	7	4	1-12	1	1	0	-

[†] n = total number of samples taken for each area; [‡] Number of positive samples for *E. coli*;* Range of *E. coli* CFU's or actual count for area.

4.5 Discussion

Environmental microbiological surveys have been widely used as a means of assessing the presence of bacterial contamination on food contact surfaces within food processing and retail facilities. Even though these surveys have some limitations in terms of receiving results in a timely manner, they do allow for a quantitative assessment of the number of bacteria present on a surface. Results from this survey indicate that microbial contamination is present on food contact and non-food contact surfaces in child care facilities and possibly result from inadequate cleaning and sanitizing practices as well as inadequate personal hygiene by facility personnel. As indicated by the results, there were a number of factors, such as center size, area within the facilities, surface type, and time of day that influenced the bacterial contamination present on food contact and non-food contact surfaces within these facilities.

Significant differences between centers and center size may be due to the design and construction of facilities which may in turn affect the availability of handwashing facilities and the separation of various age groups of children within the centers. The various facility design characteristics of each center in this study are shown in Table 4.12.

Table 4.12: Facility design characteristics of child care centers

	Center # †					
Facility Design	**1**	**2**	**3**	**4**	**5**	**6**
Handwashing	Y	Y	N	Y	Y	Y
Age Grp. Sep.[a]	Y	Y	Y/N	Y	Y	Y
Bathroom in Room	Y	Y	N	N	Y	N
Cafeteria	Y	N	N	N	N	N
Mult. Duties[b]	N	Y	Y	N	N	Y

† Center sizes: small centers (<50 children) = 2, 3, and 4; large centers (>100 children) = 1, 5, and 6

[a] Children within center separated by age group; [b] Staff with multiple duties, i.e. diaper changing, food preparation, etc.

All centers except center 3 had easily accessible handwashing facilities in the classrooms. Allwood and others (2004) investigated the availability of handwashing facilities in 123 retail food establishments (RFEs) and determined that inaccessibility of handwashing facilities within the RFE's had an impact on the frequency of handwashing. Only 68 (55%) of RFE's in the study were fully equipped with proper and accessible handwashing facilities according to the Minnesota food code. Petersen and Bressler (1986) performed an environmental microbiological study in child day care centers where hands of care givers within the centers were sampled. The authors concluded that hands comprise a major focus for fecal contamination and that handwashing facilities should be readily available to staff and children. This is particularly necessary in diaper changing areas and food preparation areas. While center 3 had no easily accessible handwashing facilities, it had the second lowest mean log APC counts (3.08 $\log_{10}$ CFU/50 cm^2) and mean coliform counts (5.6 CFU/50 cm^2) throughout the study. Therefore accessibility of handwashing facilities was not the only factor in the amount of bacterial contamination found on food contact surfaces within the facilities.

Another factor that may have affected the amount of bacterial contamination present in centers was the separation of children by age group within centers. All child care centers in this study had separate rooms for each age group, i.e., infants, toddlers, and young children. Petersen and Bressler (1986) found that the amount of fecal coliforms in surface, air and hand samples was related to the age of children. This was due to the fact that fecal coliforms are generally associated with diaper changing and as children become toilet trained this factor lessens. With children being separated by age group, diaper changing areas can be restricted to locations designated for infants. Toddler rooms can also have restricted, designated diaper changing areas as well. However, they are also equipped with training toilets and junior size toilets to begin the toilet training process. In the present study, centers 1, 2 and 5 were equipped with bathrooms designated for toilet training, while center 3, 4, and 6 were not. Based on significant difference results for log APC and coiforms, center 1 and center 5 were similar having significantly lower ($p < 0.05$) counts than center 2. Therefore, whether or not classrooms

were equipped with toilet training facilities did not seem to have an effect on the amount of bacterial contamination present.

In the present study only one center was equipped with a designated cafeteria (Center 1). The dual usage of rooms as classrooms and food service areas in child care centers may have an effect on mean log APC counts. Center 1 had the lowest mean log APC counts of all centers. Thompson (1994) studied the control and transmission of infectious diarrhea in child care settings. The author concluded that the design and construction of child care centers were of importance and indicated that the separation of diaper changing and toilet areas from food handling and eating areas is necessary. The low mean log APC and coliform counts in Center 1 could possibly be attributed to the separation of the cafeteria from all other areas in the center. Food preparation areas within all centers were centrally located, except for center 4 where the kitchen was located in a basement. After preparation, meals were transported and distributed to the classrooms. Center 4 had the highest mean log APC counts (4.50 $\log_{10}$ CFU/50 cm^2) out of all centers. This difference in location of the food preparation area may have had an effect on mean log APC counts in this study.

Staff, i.e., teachers and directors, at some centers (2 and 3) had to perform multiple duties, such as diaper changing and food preparation. There is an increased risk of transmission of infectious diarrhea where staff combine diaper changing and food preparation duties (Ekanem *et al.,* 1983; Thompson 1994). Center 2 had the second highest mean log APC counts (3.93 $\log_{10}$ CFU/50 cm^2) and center 3 had the lowest mean log APC counts (3.08 $\log_{10}$ CFU/50 cm^2). However, center 3 was similar to centers 1 and 5 whose staff did not have multiple duties. Therefore, the factor of multiple duties for staff may have had little effect on the mean log APC counts found in this study.

In addition to facility design and construction, the association of improper cleaning and sanitizing and handwashing procedures within these facilities may be linked to various demographic variables, such as gender, ethnicity, age, education, and environmental setting, i.e., metropolitan versus rural.

The various demographic characteristics of individuals working in child care centers in this study are shown in Table 4.13. Klontz and others (1995) studied the prevalence of food preparation behaviors associated with increased risk of foodborne illness by various demographic characteristics. The study indicated that one-fourth (423 out of 1,620 respondents) do not clean and sanitize cutting boards after cutting raw meat or chicken. Males and individuals between the ages of 18 and 39 are more likely to use an unwashed cutting board ($p< 0.01$) as compared to females and individuals over 40 years of age. There was no significant difference between education, having less than a high school education versus more, and race, white versus non-white, demographics. Patil and others (2005) conducted a meta-analysis to study consumer food safety knowledge and practices based on demographic differences. Demographic characteristics included gender, ethnicity (Caucasian, Hispanic, African American, Asian, and others), age (young, 18-29, mid-age, 30 to 54, senior), education (less than high school, high school, more than high school), and metropolitan (metro, greater than 1,000,000) versus nonmetropolitan (nonmetro, <1,000,000).

Table 4.13: Demographic characteristics of individuals working in child care centers

	Center # [†]					
Demographic Characteristics	**1**	**2**	**3**	**4**	**5**	**6**
Gender	Female	Female	Female	Female	Female/Male	Female/Male
Ethnicity[a]	W/AA	W/AA	W	W	AA/W	AA/W
Age[b]	All	All	All	Mid-age (30 - 54)	All	Young (18-29) Mid-age (30- 54)
Education[c]	> HS	= HS > HS	= HS	> HS	> HS	= HS > HS
Metropolitan vs Rural	Metro.	Metro.	Rural	Rural	Metro.	Rural

[†] Center Sizes: Small centers (< 50 children) = 2, 3, and 4; Large centers (> 100 children) = 1, 5, and 6;
[a] Ethnicity: AA= African American, W= White; [b] Age: Young = 18-29, Mid-age = 30-54, Senior = 55 +;
[c] Education: < High school, = High school, > High School.

Results from the study indicated that significant differences ($p < 0.05$) among various demographic categories were evident. For good hygiene practices, that included washing hands before and after handling raw meat and poultry, before and after food preparation, and after bathroom use, females (71.4%) were more likely to use good hygiene practices than males (58.1%). Significant differences between Caucasians and Hispanics, and African Americans and Asians were not significantly different. 72.0% of African Americans reported using good hygiene practices, followed by Asians (70.6%), Hispanics (65.9%), and Caucasians (65.7%). For the age demographic, 70.3% of seniors (55 +) reported using good hygiene practices as opposed to 63.1% of young people (18 – 29). For the prevention of cross-contamination, which included washing cutting boards before reuse and washing counter tops with soap and water, results for the various demographics were also generated. 81.7% of females use techniques that prevent cross-contamination as opposed to 63.9% of males. For ethnicity, 83.0%, 80.0%, 80.0%, and 74.0%, for African American, Caucasian, Hispanic, and Asian respectively, report using cross-contamination prevention techniques. Again, as seen for good hygiene practices, seniors (82.4%) have the highest percentage of using cross-contamination prevention techniques, with young age group individuals with the lowest percentage. Metropolitan status had the highest percentage, 81.0% of prevention technique usage; however, non metropolitan had a percentage of 80.0%. As compared to the demographic studies previously discussed, interpretation of educational demographics in this study differed.

Due to the majority of the staff at all centers being female, a mixture of either African American and/or White, and a mixture of various age groups, the impact of gender, ethnicity, and age on food safety could not be sufficiently analyzed.

Varying degrees of education received by facility staff may affect the level of sanitation within centers. Continuing education and training of child care center staff is necessary to update knowledge on food safety and to encourage the use of appropriate preventative measures during food preparation and food service. It is necessary for directors within these facilities to be able to properly describe and demonstrate proper

cleaning and sanitizing and handwashing procedures to all staff within the facility in order to ensure that appropriate procedures are taking place at all times. If directors are not able to properly demonstrate and describe handwashing and sanitizing procedures, then staff at child care centers will not have the knowledge to be able to perform these procedures correctly due to improper training. Allwood and others (2004) surveyed the effect of handwashing training, and the ability of the person-in-charge (PIC) to demonstrate proper handwashing techniques to staff in retail food establishments (RFE). Of 123 RFE's, handwashing training ranged from no formal training in 14% of establishments to six different methods in one establishment. RFE's that used one method of training, such as demonstration and explanation, employees were two to three times more likely to demonstrate proper technique than were employees who received no formal training. However, the PIC's ability to demonstrate proper technique was a major factor in the effectiveness of all training methods. In the study, 60 to 80% of employees were able to demonstrate proper handwashing techniques when they received training from a PIC who demonstrated handwashing techniques correctly; however, 30% of workers could not demonstrate proper techniques due to improper training by PIC's.

Based on the literature as well as bacterial contamination trends seen within each center in this study, there is an indication that demographics may have an effect on the amount of bacterial contamination found in child care centers.

The mean log APC and coliform counts for all areas within centers were not significantly different. There was an expectation that the diaper changing areas would have higher bacterial counts than other areas. However this was not the case. The mean coliform counts for diapering areas were notably lower with an overall mean count of 5.18 CFU/50 cm^2. The lower levels of bacterial counts found in the diapering area might be attributed to the heavy use of sanitizers in this area. Diapering areas may be more regularly sanitized due to common knowledge of the health risk associated with these areas, whereas other areas are not as frequently sanitized. Using RODAC plates, Petersen and Bressler (1986) had similar findings of lower mean numbers of coliforms on commonly touched surfaces, such as diaper changing areas and tables, in seven child care

centers. Results of their study indicated that mean counts of coliforms were comparable; however, the diaper changing areas were noticeably lower (77 CFU/ RODAC plate) than the overall mean of 240 CFU per RODAC plate as compared to all other surfaces.

Within small centers, the diaper changing area had the lowest mean log APC counts (3.51 $\log_{10}$ CFU/50 cm^2) (Table 4.5). However, for larger centers the diaper changing area had the highest mean log APC counts (3.76 $\log_{10}$ CFU/50 cm^2). This may be due to the fact that there is a greater chance of contamination due to larger numbers of children. Attendance at larger child care centers has been shown to be a significant risk factor for the transmission of bacterial pathogens such as *E. coli* (Belongia *et al.,* 1993).

The individual areas, i.e., food service, diaper changing, and food preparation, sampled during the study were made of materials including plastic laminate, acrylic solid surface, wood laminate, stainless steel, plastic padding, and grouted tile (Table 4.1). Prerequisites for food preparation surfaces emphasize durability and cleanability, with the liberal use of stainless steel and plastic laminate counter tops. Food service areas should consist of easily cleanable tile and plastic laminate surfaces. Surfaces of diaper changing areas should be covered with plastic laminate, easily cleaned, and light color so that soil can be detected (Petersen and Bressler 1986).

Mean log APC counts for all surfaces were lowest for acrylic solid surface, wooden laminate, and stainless steel (Table 4.6). Plastic laminate, plastic padding, and grouted tile had significantly higher mean log APC counts. Higher coliform counts were found on plastic laminate followed by grouted tile and plastic padding. The lowest coliform counts were for stainless steel and acrylic. Stainless steel has been reported to be the ideal material for food processing since it is chemically and physiologically stable at various food processing temperatures, easy to clean, and has a high resistance to corrosion (Lomander *et al.,* 2004; Zottola and Sasahara 1994). It was expected that stainless steel would have a low bacterial count due to its ease of cleaning. Additionally, it was expected that grouted tile would have a high bacterial count due to the difficulty of cleaning the crevices between tiles making the removal of bacteria more difficult.

Stainless steel had high APC counts and coliform counts (DuPont 2005; Lomander *et al.,* 2004). Grouted tile had both high APC and coliform counts confirming it to be difficult to clean and sanitize. One point that should be mentioned is that the total number of samples varied by surface type. The total number of samples per surface varied due to the fact that there were a larger number of centers containing stainless steel surfaces as opposed to only one center containing acrylic surfaces. In addition, certain types of surfaces such as grouted tile were only in a single center. Therefore, the level of contamination could have been due as much to center as surface.

Results of prevalence rates of coliforms were somewhat similar to mean coliform counts for each surface (Table 4.7). Plastic padding had the highest percentage of coliform positive samples by surface type followed by plastic laminate and grouted tile. The lowest prevalence rates were seen with acrylic and stainless steel surfaces. Results of prevalence rates of *E. coli* were comparable for plastic laminate, acrylic, stainless steel, and wood laminate surfaces (Table 4.8). Based on the percentage of *E. coli* positive samples, it was seen that plastic laminate had the lowest percentage of *E. coli* positive samples, followed by acrylic and stainless steel. Plastic padding and grouted tile had the highest percentage of *E. coli* positive samples. There is a noticeable disparity in the number of *E. coli* positive samples between all other surfaces and plastic padding and grouted tile. Again, this could have been due as much to center as surface type. Information available for acrylic, plastic laminate, and stainless steel indicates that these surfaces are easily cleanable, and non-porous (DuPont 2005; Lomander *et al.,* 2004). These factors affect the level of bacterial contamination on the surface. All three surfaces had the lowest percentage of *E. coli* positive samples. It is reasonable to conclude that the plastic padding had a higher percentage of *E. coli* positive samples due to the fact that the surface was located in a diaper changing area. The higher percentage of *E. coli* positive samples on the grouted tile can again possibly be attributed to the difficulty in cleaning the surface. Therefore, food contact surfaces in child care centers should be free of cracks or crevices therefore making them easy to clean.

One concern that child care center staff may have after learning of the prevalence rates of *E. coli* within the centers is whether these findings indicate the presence of pathogenic *E. coli* O157:H7. For the detection of *E. coli*, the Petrifilm™ method was employed. *E. coli*/coliform Petrifilm™ incorporates the substrate 5-bromo-4-chloro-3-indoyl- β- D-glucuronide (BCIG is a chromogenic GUD substrate). Presumptive *E. coli* colonies may be identified through production of β –glucuronidase (GUD). GUD is commonly produced by *E. coli* and has been utilized as a differential characteristic in coliform recovery media containing various β-D-glucuronic acid substrates, such as 4-methylumbellifery- β- D-glucuronic acid (MUG is a fluorogenic substrate) and BCIG. On Petrifilm, *E. coli* is distinguished from other colonies by the formation of blue colonies. Reports indicate that 92-99% of *E. coli* isolates produce GUD. The pathogenic serotype (type II) of *E. coli*, *E. coli* O157:H7, differs metabolically from other strains of *E. coli* in that they are slow or non-fermenters of sorbitol and lack the enzyme β –glucuronidase indicating that these colonies are not *E. coli* O157:H7 (Kornacki and Johnson 2001). Therefore, *E. coli* on *E. coli*/coliform Petrifilm™ are not serotype O157:H7.

Mean log APC counts by sampling time showed that pre-opening and lunch time were significantly higher ($p < 0.05$) than following final clean-up of the day. It was expected that lunch time would have the highest mean log APC counts. This finding can be attributed to the fact that during this time, there is increased traffic and activity within the centers. The final clean-up of the day had the lowest mean log APC counts and this can be attributed to the decreased traffic and activity after children and staff have left for the day. No significant difference between sampling times was found for coliform counts. The increase in APC counts from the end of the day to the start of the next day indicates surfaces are being contaminated after the centers are cleaned and sanitized. This could be explained for some centers by the fact that they are used for other purposes in the evening such as churches of community centers. Also, after final clean-up of the day by facility staff, after-hours janitorial staff could possibly be placing chairs or equipment on tables

in order to clean and sanitize floors. Where no such use could be identified, contamination via air or pests is possible.

Frequency of coliform positive samples by time within area reflected the overall coliform results seen for the areas. The lowest number of positive coliform samples for the food preparation areas was following clean-up. For the food service area there were lower numbers of positive coliform samples at the pre-opening and for the diaper changing area the pre-opening and following final clean-up samples were approximately equal. For *E. coli*, the positive samples that occurred in the diaper changing area were spread throughout the day. The next most prevalent *E. coli* site was at lunch on one of the food preparation areas. It must be noted that there were a maximum of 2 *E. coli* detected at the latter site. Results for *E. coli* indicate that only the diaper changing area may be a site that needs to be better sanitized immediately following use.

4.6 Conclusions

These findings demonstrate that microbial contamination is present on food contact surfaces and non-food contact surfaces of child care facilities. Results of this study indicate that there are a number of factors such as center size, area within the facility, surface type, and time of day that affect the amount of bacterial contamination that may be present within the facilities. Due to the high risk of foodborne illness associated with children, the possibility of cross-contamination from food contact or non-food contact surfaces to foods is an aspect of food safety that requires more attention. Effective cleaning and sanitizing of food contact and non-food contact surfaces in child care facilities is critical if cross-contamination is to be reduced. One of the ultimate challenges is to raise the level of awareness of child care facility staff about the risks associated with ineffective cleaning and sanitizing of these surfaces. More emphasis on training and the development of standard sanitation operating procedures (SSOP's) is needed to reduce potential hazards.

5 ASSESSMENT OF RAPID SANITIATION ASSAY USAGE ON FOOD CONTACT SURFACES IN CHILD CARE CENTERS

5.1 Abstract

Young children are one of the most susceptible groups to foodborne illness (Gerba *et al.,* 1996). Therefore, the results of cleaning and sanitization practices of child care facility food preparation and food service surfaces must be obtained in timely manner in order to allow for immediate remedial action to be taken. The objective of this study was to evaluate the sanitation of food preparation and service areas in child care facilities using three rapid sanitation assays and compare them to standard microbiological evaluation methods. A study of six child care centers in Knoxville, TN was conducted to assess the applicability of using three rapid sanitation assays on food contact (food service and food preparation) and non-food contact (diaper changing) surfaces to determine the effectiveness of cleaning and sanitization procedures within the centers. Samples were taken three times a day (pre-opening, lunch time, and following final clean-up) twice per month for eight months in each center. A 50 cm^2 area was swabbed using methods designed to detect ATP bioluminescence, protein or glucose. In addition, aerobic plate counts (APC) were done for each area.

Microbial data and ATP, protein and glucose results were available from 1,129 samples. Correlations between rapid sanitation assays and microbiological analysis (APC) were determined using Spearman's rho analysis. Correlations ($p < 0.01$) were found, using Spearman's rho analysis between the APC and the ATP ($r = 0.26$) and protein assays ($r = 0.16$). Results were also reported as percent agreement, i.e., the number of times the rapid sanitation assays and APC were in agreement of a "clean" or "dirty" surface or area. Surfaces and areas with less than 1,000 CFU/50 cm^2 and less than 300 RLU or a negative protein or glucose reaction were considered "clean" and greater than 1,000 CFU/50 cm^2 and greater than 300 RLU or a positive protein or glucose reaction were considered "dirty". The overall percent agreement between APC and the ATP bioluminescence was 24.3%, between APC and the protein assay was 68.7%, and

between APC and the glucose assay was 81.9%. Results for the ATP bioluminescence assay were also expressed as frequency range percentages of sample relative light units (RLUs). Manufacturer's pre-set limits indicate that less than 200 RLU detected was "clean", 200 to 300 RLU was marginal, and greater than 301 RLU was "dirty". Frequency range percentages for ATP indicate that 85.0% of samples were "dirty", with the greatest percentage (34.7%) between 1,000 and 5,000 RLU. However, 16.2% of samples were "clean" with RLU counts less than 200.

Although, not highly reliable indicators of microbial contamination, these findings indicate that rapid assays may be useful for monitoring the sanitation of food contact surfaces in child care centers. These tests could assist in improving sanitation and preventing cross-contamination by detecting unsanitary surfaces with food residues remaining on the surfaces.

5.2 Introduction

Preventative risk-based food safety management systems, such as HACCP, require that hygiene monitoring provide results rapidly and in time for remedial action so as to be able to regain control of a process and/or product (Griffith *et al.,* 1997; Moore and Griffith 2002). Therefore, it may be necessary to implement rapid hygiene monitoring systems that allow for results to be obtained in a shorter time than conventional microbiological methods such as swabbing/plating or agar contact methods which require incubation for 24-48 hours (Davidson *et al.,* 1999).

Rapid hygiene monitoring instruments and kits that utilize ATP bioluminescence are available to the food industry. These systems can provide a real-time estimate of total surface contamination resulting in an indication of overall cleaning efficacy (Griffiths 1997; Moore and Griffith 2002). In the ATP bioluminescence test, ATP in the sample drives the production of oxyluciferin and light from luciferin via the enzyme luciferase (Larson *et al.,* 2003). The quantity of light is expressed in relative light units (RLUs) and directly correlates with the amount of ATP present and thus the biological load on the

sampled area (Deshpande 2001). While some have argued that the main concern should be the level of bacterial contamination on a surface, organic matter as food soil remaining on a surface can serve as a source of nutrients for microorganisms thus supporting their growth (Davidson *et al.,* 1999). The ATP bioluminescence therefore provides an estimation of total cleanliness of the surface, not only detecting bacterial contamination but also organic materials remaining on the surface as well.

Several studies have compared the ATP bioluminescence method to traditional microbiological methods. Some studies have reported a positive correlation between the two methods while others have found the opposite (Illsley *et al.,* 2000; Larson *et al.,* 2003; Tebbutt 1999). Larson and others (2003) compared conventional swabbing methods for the detection of microorganisms to ATP bioluminescence on 225 kitchen table surfaces in homes. No significant correlation between ATP and colony forming units (CFUs) from total plate counts ($r = 0.004$, $P = 0.58$) were found. Researchers in the study concluded that ATP bioluminescence is not a reliable substitute for conventional swabbing methods when the amount of microbial contamination on a surface is desired. Tebbutt (1999) compared conventional swabbing methods for the detection of microorganisms to ATP bioluminescence on 139 cutting boards used in hotel kitchens. A positive correlation was found between the bacterial count and the amount of ATP detected ($r = 0.58$). Due to the low correlation coefficient, researchers concluded that ATP bioluminescence is not fully reliable for highlighting the bacterial risk associated with food contact surfaces but the method did allow for on the spot-remedial action to be taken. Illsley and others (2000) compared standard surface swabbing techniques to ATP bioluminescence to determine the adequacy of the ATP methods for evaluating sanitation in a baking facility. The standard swabbing techniques and the ATP bioluminescence method were compared as to the percent of times both methods agreed to pass or fail a surface. Based on percent agreement (81.6% to 83.3%), the study concluded that there was a good correlation between the results of the two methods (Illsley *et al.,* 2000).

In addition to ATP bioluminescence assays, there are also kits that detect either protein or glucose and utilize color indicators to indicate residues. These rapid detection

methods are designed to be presence/absence tests that detect food residues containing proteins or glucose that may remain on a surface following cleaning. Tebbutt (1999) compared conventional swabbing methods for the detection of microorganisms to protein detection methods on 139 cutting boards used in hotel kitchens. A positive correlation was found between bacterial counts and the amount of protein detected ($r = 0.67$). As with ATP, the researchers concluded that the presence or absence of protein could not always be relied upon as an indicator of microbial contamination on surfaces. Moore and Griffith (2001) conducted a study to compare a rapid protein detection method to traditional agar-based methods for standard plate counts. There was a 68.9% agreement (28.9% passing tests and 40% failing tests) between results of the two methods.

The objective of this study was to assess three rapid sanitation assays to evaluate their applicability for usage in child care centers to determine the sanitation quality of food contact and non-food contact surfaces. The ultimate goal was to determine if one of the rapid sanitation assays could be used by centers to monitor sanitation.

5.3 Materials and Methods

5.3.1 Sampling sites and surface selections

The study was conducted in six (6) Knoxville, TN area child care centers. The centers represented three large (> 100 children) and three small (< 50 children) centers. Each child care center was tested twice monthly over the course of an eight (8) month period for a total of 16 sampling periods per center. Four areas, one food service area, one diaper changing area, and two food preparation areas, within the child care centers were sampled. The food service area and the food preparation areas consisted of three food contact surfaces, two food preparation surfaces (i.e. kitchen counters/cutting boards) and one food service surface (i.e. children's eating table), while the diaper changing area consisted of one non-food contact surface (i.e. diaper changing surface). All surfaces were tested three times daily(pre-opening, during lunch, and following final clean-up of the day) to monitor the microbiological quality of each surface throughout the day.

Table 5.1: Sampling area surface types by center

	Surface Types (Area Number)			
Center #	**Food Service (No. 1)**	**Diaper Area (No. 2)**	**Food Prep No. 1 (No. 3)**	**Food Prep No. 2 (No. 4)**
1 (Large)	Plastic Laminate	Solid-Surface	Stainless Steel	Stainless Steel
2 (Small)	Plastic Laminate	Plastic Pad	Grouted Tile	Grouted Tile
3 (Small)	Plastic Laminate	Plastic Pad	Plastic Laminate	Wood Laminate
4 (Small)	Plastic Laminate	Plastic Pad	Plastic Laminate	Plastic Laminate
5 (Large)	Plastic Laminate	Solid-Surface	Plastic Laminate	Plastic Laminate
6 (Large)	Plastic Laminate	Plastic Pad	Stainless Steel	Stainless Steel

Surfaces of each area within each center varied with surface types including: plastic laminate for food service surfaces, stainless steel grouted tile, plastic laminate, and wood laminate for food preparation surfaces, and acrylic solid surface and plastic padding for diaper changing surfaces. Table5.1 shows all surface types by area by center.

5.3.2 Sample preparation for rapid sanitation assays

Three rapid sanitation assays were used to monitor sanitation of surfaces within child care centers, ATP bioluminescence assay, protein assay, and glucose assay. For ATP detection, a bioluminescence assay, the AccuPoint ATP Sanitation Monitoring System (Neogen®, Inc.; Lansing, MI), was utilized and a 50 cm^2 area was swabbed using AccuPoint ATP Surface Samplers. Sampling was performed by swabbing the area horizontally, from one side of the template to the other, and repeating vertically, and then horizontally again using the AccuPoint ATP Surface Samplers. After sampling the swab was placed back into the cartridge to the first stop and then placed into the reader. The swab sampling pad was then pressed completely into the cartridge where it then mixed with the luciferase at the bottom of the cartridge. The reader's door was then closed and

results, reported in relative light units (RLU), were reported within 10 seconds and recorded. Frequency ranges of ATP counts for areas in the six centers were calculated.

For protein detection, Pro-tect® hygiene surface swabs (Biotrace, Neogen®, Inc.; Cincinnati, OH) were utilized. Sampling was performed by swabbing a 50 cm^2 area horizontally, from one side of a sterile template to the other, and repeating vertically, and then horizontally again using the protein detection swab. After sampling, to activate the colorimetric reaction, the swab was inserted back into the tube and pressed to immerse the tip into the solution at the bottom of the tube. Results were compared to the Pro-tect label and reported in 10 minutes and then recorded.

SpotCheck™ glucose swabs (Weber Scientific, Hygiena LLC; Camarillo, CA) were utilized for the detection of simple sugars, i.e. glucose remaining on the surface. Sampling was performed by swabbing a 50 cm^2 area horizontally, from one side of a sterile template to the other, and repeating vertically, and then horizontally again using the detection swab. The swab was then placed back into the tube and the snap valve was broken and the bulb was squeezed to expel the liquid so that it bathes the swab tip. The device was then shaken to release the product residue from the swab to wait for the colorimetric reaction to take place. Results were reported after 1 minute and then recorded.

In order to determine if correlations exist between the rapid sanitation assays and bacterial counts, microbiological analysis, using Aerobic Count (AC) Petrifilm plates (3M Microbiology, St. Paul, MN), was performed in adjacent 50 cm^2 areas to determine aerobic plate counts.

5.3.3 Statistical analysis

Correlations between rapid sanitation assays and standard microbiological analysis were determined using Spearman's Rho analysis ($P<0.01$). Frequencies were calculated for all rapid methods to determine the percentage of positive and negative samples within a specified range of APC.

5.4 Results

Correlations were determined between the rapid assays and standard microbiological analysis (APC) (Table 5.2). Correlations ($p < 0.01$) were found between the APC and the ATP ($r = 0.26$) and protein (Pro-tect®) assay ($r = 0.16$). There was no correlation between the APC and glucose (SpotCheck™) assay.

In order to determine the efficacy of the rapid sanitation assays surveyed, results were reported as the percentage of times the rapid assays and APC agreed a test surface or area was "clean" or "dirty" For APC, acceptable limits for child care centers were established based on standards defined for cleaned and sanitized food service equipment by the U.S. Public Health Service (USPHS) (U.S. Public Health Service/Food and Drug Administration (US PHS/FDA) 1997). Limits of < 1.3 $\log_{10}$ (20 CFU/cm^2) are considered "clean" by the USPHS which is equal to 1,000 CFU/50 cm^2. For the ATP bioluminescence assay, the manufacturer's recommendations are that that a surface with < 200 RLU is "clean", 200 to 300 RLU is marginal, and > 301 RLU is "dirty." Therefore, the limits used in the present study were <300 RLU was considered clean and >301 RLU was defined as "dirty." For protein and glucose assays, a negative color reaction was considered "clean" and a positive reaction as "dirty.".An example of how results between rapid methods (using the ATP assay) and APC were compared is shown in Table 5.3.

Table 5.2: Correlations between APC and rapid sanitation assays

	APC	**ATP**	**Pro-tect®**	**SpotCheck™**
APC	-	.26*	.16*	-.06
ATP	.26*	-	.06	-.03
Pro-tect®	.16*	.06	-	-.08
SpotCheck™	-.06	-.03	-.08	-

* Correlation at $p < 0.01$; Correlation results derived from Spearman's rho analysis.

Table 5.3: Example table of comparison results between APC and ATP assay according to "clean" versus "dirty"

	ATP Assay RLU‡	
APC (CFU 50/cm^2)	**≤ 300**	**> 301**
≤ 1,000	Agree (clean)*	Disagree (false-positive)
> 1,001	Disagree (false-negative)	Agree (dirty)*
% Agreement	%	

‡ Relative light units = RLU, range: clean = 0 to 300 and dirty = > 300; * Number of samples that were "clean" or "dirty" by both methods.

Agreement for a "clean" surface was defined as an APC ≤ 1,000 CFU/50 cm^2 and an RLU ≤ 300 or a negative protein or glucose colorimetric reaction. A "dirty" surface was defined as an APC > 1,001 CFU/50 cm^2 and RLU > 301 RLU or a positive protein or glucose color reaction. The number of times in which the APC agreed with the ATP assay is indicated by an asterisk. Disagreement could be classified as either a false-positive or false-negative. False-positive result was when the APC was ≤ 1,000 CFU/50 cm^2 and the rapid assays indicated an RLU > 301 or a positive protein or glucose color reaction. A false-negative result was when the APC was > 1,001 CFU/50 cm^2 and an RLU of < 300 or a negative result from the protein or glucose color reactions occurred.

Microbial data, ATP, protein and glucose readings were available from 1,129 samples. The overall percent agreement between APC and the ATP bioluminescence assay was 24.3% (Table 5.4). The greatest disagreement was in the "false-positive" category, i.e., 847 samples were "dirty" according to the ATP bioluminescence assay but were "clean" according to APC.

Table 5.4: Comparison of the ATP bioluminescence assay and aerobic plate counts on surfaces from child care centers

	ATP Assay, RLU‡	
APC (CFU 50/cm^2)	**≤ 300**	**> 301**
≤ 1,000	246*	847
> 1,001	7	28*
% Agreement*	24.3%	

‡ Relative light units range: clean ≤ 300 and dirty > 301; *Samples that were "clean" or "dirty" by both methods.

The overall percent agreement by surface type between APC and the ATP bioluminescence assay was 32.3% for acrylic, followed by 31.3%, 27.9%, 24.3%, 21.4%, and 14.8% for grouted tile, plastic padding, stainless steel, plastic laminate, and wooden laminate respectively (Table 5.5). Percent disagreement between both methods by surface type was 85.2% for wooden laminate followed by 78.6%, 75.7%, 72.1%, 68.8%, and 67.7% for plastic laminate, stainless steel, plastic padding, grouted tile, and acrylic respectively. The greatest disagreement for all surface types was the false-positive, i.e., a larger number of samples for each surface were "dirty" according to the ATP bioluminescence assay but were "clean" according to APC, with percentages being 84.2%, 77.9%, 75.7%, 71.4%, 68.8%, 65.6% for the same respective surfaces.

Based on sampled area, the overall percent agreement between APC and the ATP bioluminescence assay was 25.7%, 25.1%, 24.2%, and 21.5% for the diaper changing, food preparation 1, food preparation 2, and food service, respectively (Table 5.6). As with surface samples, the greatest disagreement for all areas was the false-positive, with percentages being 72.9%, 73.5%, 75.4%, and 78.5% for the same respective areas.

Table 5.5: Comparison between the ATP assay and aerobic plate counts of various surface types in child care centers

		ATP Assay RLU[‡]		
Surface/(n)[†]	**APC (CFU/50 cm^2)**	**≤ 300**	**> 301**	**% [a]**
Plastic Laminate (467)	**≤ 1,000**	84*	364	21.4
	> 1,001	3	16*	
Acrylic (93)	**≤ 1,000**	29*	61	32.3
	> 1,001	2	1*	
Wood Laminate (95)	**≤ 1,000**	14*	80	14.8
	> 1,001	1	0*	
Stainless Steel (189)	**≤ 1,000**	43*	143	24.3
	> 1,001	0	3*	
Plastic Padding (140)	**≤ 1,000**	33*	100	27.9
	> 1,001	1	6*	
Grouted Tile (144)	**≤ 1,000**	43*	99	31.3
	> 1,001	0	2*	

[†] n= total number of samples per surface type; [‡] Relative light units range: "clean" ≤ 300 and "dirty" > 300; [a] Total percentage of surface samples in agreement by both methods; * Number of samples that were "clean" or "dirty" by both methods by surface type.

Table 5.6: Comparison between the ATP assay and aerobic plate counts of various areas in child care centers

		ATP Assay RLU[‡]		
Surface/(n)[†]	**APC (CFU/50 cm^2)**	**≤ 300**	**>301**	**% [a]**
Food Service (284)	**≤ 1,000**	54*	223	21.5
	> 1,001	0	7*	
Diaper Changing (280)	**≤ 1,000**	65*	204	25.7
	> 1,001	3	7*	
Food Prep 1 (283)	**≤ 1,000**	64*	208	25.1
	> 1,001	1	7*	
Food Prep 2 (281)	**≤ 1,000**	62*	212	24.2
	> 1,001	1	6*	

[†] n= total number of samples per area; [‡] Relative light units range: "clean" ≤ 300 and "dirty" > 300; [a] Total percentage of surface samples in agreement by both methods; * Number of samples that were "clean" or "dirty" by both methods by area.

The overall percent agreement between APC and the protein and glucose assay was 68.7% for protein and 81.9% for glucose (Table 5.7). The greatest disagreement between methods was the false-positive, i.e., 312 samples were "dirty" according to the protein assay and 177 were "dirty" according to the glucose assay but were "clean" according to APC.

The overall percent agreement between APC and the protein assay by surface type sampled was 81.1% for wooden laminate, followed by 73.6%, 73.1%, 70.4%, 67.4%, and 55.6% for plastic padding, acrylic, plastic laminate, grouted tile, and stainless steel, respectively (Table 5.8). For the glucose assay, overall percent agreement by surface type was 93.7% for wooden laminate, followed by 93.5%, 94.3%, 89.6%, 80.1%, and 60.3% for acrylic, plastic padding, grouted tile, plastic laminate, and stainless steel, respectively. For both the protein and glucose assay, percent disagreement varied by surface type. Disagreement for the majority of surfaces was classified as a false-positive; however, for acrylic surfaces false-negative results were higher for the protein assay and an equal number of false-negative and false-positive results for the glucose assay, and for plastic padding false-negative results for the glucose assay were higher.

Table 5.7: Comparison of protein and glucose assays and aerobic plate counts on surfaces from child care centers

	Protein[a]		**Glucose[b]**	
APC (CFU/50 cm^2)	**Negative**	**Positive**	**Negative**	**Positive**
≤ 1,000	764 *	312	920*	177
> 1,001	41	12*	27	5*
% Agreement	68.7%		81.9%	

[a,b]negative = "clean" no protein or glucose residue present and positive = "dirty" protein or glucose residues present; * Number of samples that were in agreement by both methods.

Table 5.8: Comparison of protein and glucose assays and aerobic plate counts on various surface types in child care centers

		Protein			Glucose		
Surface/(n)[†]	**APC (CFU/50 cm^2)**	**NEG**	**POS**	**%[a]**	**NEG**	**POS**	**%**
Plastic Laminate (467)	**≤ 1,000**	326*	127	70.4	372*	75	80.1
	> 1,001	7	3*		13	2*	
Acrylic (93)	**≤ 1,000**	68*	9	73.1	87*	3	93.5
	> 1,001	16	0*		3	0*	
Wood Laminate (95)	**≤ 1,000**	77*	19	81.1	89*	6	93.7
	> 1,001	0	0*		1	0*	
Stainless Steel (189)	**≤ 1,000**	101*	77	55.6	112*	77	60.3
	> 1,001	10	4*		3	2*	
Plastic Padding (140)	**≤ 1,000**	103*	38	73.6	132*	2	94.3
	> 1,001	0	0*		6	0*	
Grouted Tile (144)	**≤ 1,000**	89*	42	67.4	128*	14	89.6
	> 1,001	5	8*		1	1*	

[†] n= total number of samples per area; [a] Total percentage of surface samples with specified APC with negative results for protein or glucose; * Number of samples that were in agreement by both methods by surface type.

The overall percent agreement between APC and the protein assay by area was 82.1% for diaper changing, 71.5% for food service, 67.1% for food preparation 1, and 63.0% for food preparation 2. The overall percent agreement between APC and the glucose assay was 96.4% for diaper changing, followed by 80.1%, 78.5%, and 74.2% for food preparation 2, food service, and food preparation 1, respectively (Table 5.9). For the majority of areas sampled, percent disagreement was classified as false-positive, except for the glucose assay on the diaper changing area. This area resulted in a false-negative, i.e., 10 samples were "clean" according to the glucose assay but were "dirty" according to APC.

Table 5.9: Comparison of protein and glucose assays and aerobic plate counts of various areas in child care centers

		Protein			Glucose		
Area/(n)[†]	**APC (CFU/50 cm^2)**	**NEG**	**POS**	**%**[a]	**NEG**	**POS**	**%**
Food Service (284)	**≤ 1000**	201*	73	71.5	223*	54	78.5
	> 1001	5	2*		4	0*	
Diaper Changing (280)	**≤ 1000**	225*	47	82.1	270*	2	96.4
	> 1001	5	5*		10	0*	
Food Prep 1 (283)	**≤ 1000**	184*	89	67.1	207*	66	74.2
	> 1001	3	6*		6	3*	
Food Prep 2 (281)	**≤ 1000**	175*	100	63.0	223*	52	80.1
	> 1001	6	2*		6	2*	

[†] n= total number of samples per area; [a] Total percentage of surface samples with specified APC with negative results for protein or glucose; * Number of samples that were in agreement by both methods by area.

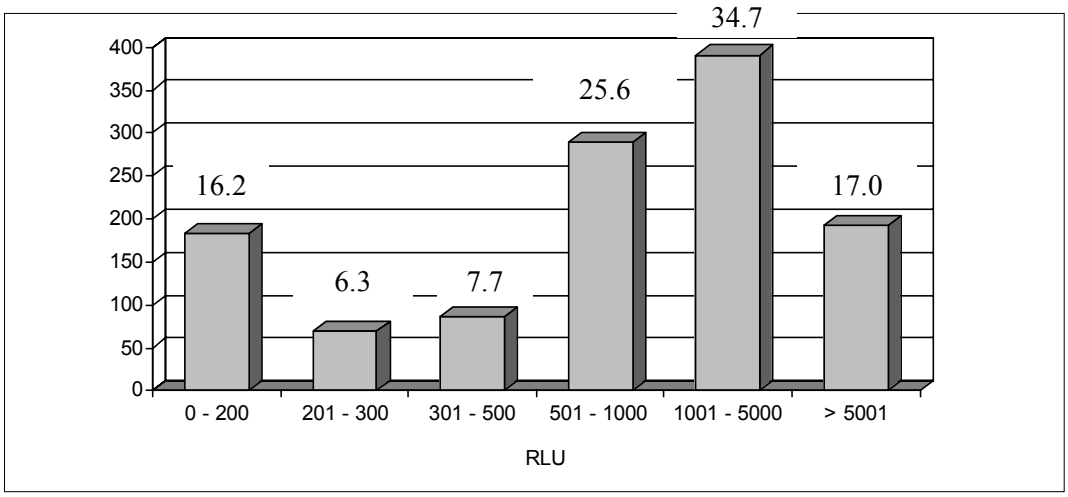

Figure 5.1: Frequencies of relative light unit (RLU) counts per 50 cm^2 area obtained with the ATP Sanitation Monitoring System on surfaces in child care centers (n =1,129). Manufacturer limits for acceptability of cleanliness of a surface are: pass (clean) = 0 to 200, marginal = 201 to 300, fail (dirty) = > 300.

Figure 5.1 shows the sample RLU count frequency range percentages per 50 cm^2 area for the AccuPoint ATP Sanitation Monitoring System. For food manufacturers, the pre-set limits of the ATP monitoring system are pass, marginal, or fail, with pass being 0 to 200, marginal between 201 to 300, and fail > 301. Of a total of 1,129 samples, the majority (85.0%) were failing, with 34.7% between 1000 and 5000. However, 16.2% of samples were passing with RLU counts less than 200.

5.5 Discussion

Environmental microbiological sampling has been widely used as a means of assessing the presence of bacterial contamination on food contact surfaces within food processing and retail facilities. However, due to the time involved in obtaining results from microbiological sampling analysis, it may be necessary to implement rapid hygiene monitoring systems that allow for results to be obtained in a shorter time than conventional microbiological methods which require incubation for 24-48 hours (Davidson *et al.,* 1999).

In contrast to the food processing and food service industries, sanitation in child care food service is generally much less defined. Rapid sanitation assays, such as ATP bioluminescence, protein, and glucose can be used to provide an on-the-spot assessment of surface contamination; however, whether contamination is bacterial or food residue is unknown. Before any type of rapid sanitation assay can be used to monitor the sanitation of food contact surfaces, it is necessary to assess its performance by comparison to traditional methods such as microbiological sampling (Griffith *et al.,* 1997). How well a new rapid sanitation assay correlates with results of traditional methods will usually determine how well it is accepted and can be implemented to monitor sanitation. The link between bacterial counts and rapid sanitation assays has been studied by various researchers to determine if rapid sanitation assays have the potential to monitor sanitation within food processing or food service facilities (Poulis *et al.,* 1993; Tebbutt 1999).

Many attempts have been made to correlate ATP levels with total bacterial count methods, with mixed success. In this study, weak correlations were found between APC and the ATP bioluminescence assay ($r = 0.26$) and protein assays ($r = 0.16$) (Table 5.2). There may be several reasons why correlation results in this study were lower than expected. ATP is present in all living cells and organic material, i.e. microorganisms, food residue, body secretions, etc., and one limitation to ATP bioluminescence is that it detects ATP found from all sources and is not able to differentiate between sources of ATP. Therefore, the total amount of ATP detected by the ATP bioluminescence method will be inclusive of both microbial ATP as well organic ATP.

A second reason why there was a low correlation between the ATP bioluminescence assay and APC counts is the limits of detection for the ATP bioluminescence assay maybe lower than the amount of microbial contamination present on the surface. Most ATP bioluminescence assays have been reported to have a limit of detection of 10^4 to 10^5 CFU (Davidson *et al.,* 1999). Various samples in this study may have had lower amounts of bacteria present on the surface than the ATP bioluminescence assay was able to detect. Due to this reason, some researchers have indicated that the ATP bioluminescence assay should not be used as an estimator of microbial load but

more as a monitor of overall sanitation. For example, Poulis and others (1993) reported a poor relationship between ATP bioluminescence and APC counts from food processing facilities. Despite weak correlations ATP results indicate that there is a risk for cross-contamination via food contact surfaces and re-cleaning of the surface is necessary since the type of ATP present, i.e. microbial or food residue is unknown. The presence of any food residues may serve as a source of nutrients for microorganisms providing an opportunity for growth.

For the ATP, protein, and glucose assays, percent agreement with the APC on whether a surface was "clean" or "dirty" was 24.3%, 68.7% and 81.9%, respectively. Results indicate that the glucose assay, SpotCheck™, had the highest percent agreement with APC of the other two assays. However, it must be noted that RLU limits on the ATP assay can be adjusted; therefore, the low percent agreement between the ATP assay and APC may be improved if RLU limits indicating a "clean" surface were adjusted to be slightly higher for child care centers than the manufacturer's limits used for food processing facilities.

Percent disagreement could be broken down into false-positive and false-negative results. The percentage of false-negatives, or cases where small amounts of ATP, protein, or glucose were present and high numbers of microorganisms were present, were very low with percentages being 0.6%, 3.6% and 2.4%, for the respective assays. However, the percentage of false-positives, or cases where high amounts of ATP, protein, or glucose were present and lower numbers of microorganisms were present, were higher with percentages being 75.1%, 27.6% and 15.7% for the same respective assays (Table 5.4 and 5.7). False-negative results indicate that there is an increased risk for cross-contamination due to the fact the assays are not detecting microbial contamination on a surface. In this study, lower percentages of false-negatives are good due to the fact that this result is potentially more serious. In this situation, it is much better to receive a false-positive indicating an unclean surface based on the ATP, protein, or glucose assay as opposed to a false-negative and these assays not detect microbial contamination present. In this study, the false-positive is much less costly since the sample is "clean" by APC

indicating that the rapid sanitation assays are detecting food residues remaining on a surface.

To determine if the rapid sanitation assays would be less or more effective on various surface types or areas within child care centers comparisons between ATP, protein and glucose assays and APC counts were made. Percent agreement of all surfaces sampled, plastic laminate, acrylic solid surface, wood laminate, stainless steel, plastic padding, and grouted tile varied for each assay with ranges between 14.7% to 32.3% for the ATP assay (Table 5.5), 55.6% to 81.1% for protein, and 60.3% to 93.7% for glucose (Table 5.8). These variations in trends in percent agreement between surface samples indicate that the effectiveness of the rapid sanitation assays may be affected by the type of surface sampled. Based on false-negative results, acrylic surfaces and plastic padding surfaces had a higher percentage of false-negatives than false-positives for the protein assay than all other surfaces. This trend may indicate that the protein assay may be less capable of detecting a "dirty" surface on acrylic and plastic padding surfaces than all other surface types. This trend is also seen for the glucose assay with the acrylic surfaces.

Percent agreement between APC and ATP bioluminescence for all areas sampled, food service, food preparation, and diaper changing within child care centers were comparable ranging from 21.5% to 25.7% (Table 5.6). This trend indicates that the ATP bioluminescence assay was not affected by the type of area sampled. However, for the protein and glucose assays, percent agreement with APC for areas sampled varied (Table 5.9). With percent agreement ranging from 63.0% to 82.1% for protein and 74.2% to 96.4% for glucose, trends indicate that these methods may be affected by the type of area sampled within child care centers. As for the false-negative results, the diaper changing area had a higher percentage of false-negative results for the glucose assay than all other areas. This result indicates that the glucose assay may not be less effective in the diaper changing areas due to the fact that this method is designed for the detection of glucose residues as opposed to microbial contamination. The glucose assay may not be capable of detecting microbial contamination present in this area.

For the ATP bioluminescence assay, frequency count range percentages (Figure 5.1) indicate that lower manufacturer's limits for "passing" or "failing" samples, despite being set for food processing facilities, are achievable by child care centers. However, as previously stated, the percentage of "passing" samples may increase if the manufacturer's pre-set limits are adjusted to be slightly higher for child care centers than those used for food processing facilities.

In this study, all rapid sanitation assays were easy to use. However, the protein assay relied on matching one of four distinct color changes with only the first grade being "clean". At times it was difficult to decipher the color changes associated with the protein assay. Despite this fact, due to the ease of usage, non-technically trained staff within the child care centers would have the ability to carry out sanitation monitoring using these methods. Training on the correct usage and interpretation of all methods should be available through University Extension and manufacturer's support. Before these rapid sanitation assays can be implemented within child care facilities the cost associated with purchasing and supply of equipment must be considered. For example, the AccuPoint ATP Sanitation Monitoring System used in this study cost approximately $1,400. This initial investment maybe too considerable for smaller facilities to manage. However, centers must decide if this initial investment is too costly in relation to failing to identify an unsanitary food contact surface.

5.6 Conclusions

This study has demonstrated the efficacy of rapid sanitation assays for usage within child care centers. Although not fully reliable for identifying the bacterial risk associated with food contact surfaces within these facilities, these methods do allow for on-the-spot remedial action to be taken in the case of an unsanitary food contact surface is found. This immediate action will aid in the reduction of cross-contamination via food contact surfaces and will reduce the risk of foodborne illness associated with it. Based on the ease of usage, the ATP assay and the SpotCheck™ assay are the easiest to read and

interpret. However, based on cost, the ATP assay may be too costly for smaller centers to purchase. The SpotCheck™ assay will be more affordable.

6 CONCLUSIONS

Microbial contamination is present on food contact and non-food contact surfaces within child care centers and therefore increases the risk of cross-contamination of foodborne pathogens. Due to this fact it is necessary to monitor the cleaning and sanitation practices within these facilities.

The incorporation of rapid sanitation assays such as ATP bioluminescence, protein assays, and glucose assays will aid in the reduction of surface contamination by allowing for on-the-spot remedial action to be taken in the case of an unsanitary food contact surface. These assays will provide information as to the overall sanitary condition of food contact and non-food contact surfaces within the child care centers, not only detecting possible sources of bacterial contamination but also detecting food residues that may act as a source of nutrients for microorganisms.

Due to the high risk of foodborne illness associated with children, the possibility of cross-contamination from food contact or non-food contact surfaces to foods is an aspect of food safety that requires more attention. These findings demonstrate that microbial contamination is present on food contact surfaces and non-food contact surfaces of child care centers. Modified SSOP's for child care centers and training in conjunction with rapid sanitation assays may prove to be beneficial tools in improving food safety within child care centers.

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