COMPARISON OF REDIGEL, PETRIFILM, SPIRAL PLATE SYSTEM, ISOGRID AND STANDARD PLATE COUNT FOR THE AEROBIC PLATE COUNT ON SELECTED FOODS

by

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INTRODUCTION

One of the key analyses that food microbiologists perform on food samples is the Standard Plate Count Method (SPC). Human foodstuffs frequently serve as carriers of environmental, opportunistic and sometimes pathogenic microorganisms (Insalata et al., 1967). Therefore, the numbers of these microorganisms are commonly used as an indice for assessing quality of foodstuffs (Silliker, 1963; Speck, 1976). As a microbial procedure, the SPC method involves aseptically plating a diluted sample into a general purpose agar medium, incubating the agar plates under specified conditions, and counting the resultant colonies. The count represents the number of mixed aerobic microorganisms in food. Although it is an important and accepted procedure it has several disadvantages; it takes 48 hours to complete the test, it requires several manipulations, it utilizes extensive laboratory material and space, and involves the use of hot (42-45 C) agar which may inhibit microorganisms. To minimize those disadvantages many new and improved methods have been proposed.

The development of new systems and techniques requires that they be tested for their accuracy against the SPC method. Alternative methods through ingenious modifications of the conventional SPC procedure are designed to be not only more accurate but also easier to operate and read.

The purpose of this investigation was to compare the advantages and disadvantages of the SPC method of aerobic plate count with four commercial alternative count systems (Redigel, RCR Scientific), Petrifilm (3M), ISOGRID (QA Laboratories Ltd) and Spiral Plate System (Spiral System).

LITERATURE REVIEW

A. Standard Plate Count

Since the invention of the first microscope and subsequent observation of "animalcules" by Anthony Van Leeuwenhoek in the middle 1600's the study of microorganisms slowly developed into a major field of study (Davis et al., 1976). Early workers cultured microorganisms on pieces of potato and in liquid nutrient solutions of broths. In the late 1880's use of solid media resulted in the development of several methods for the enumeration of microorganisms by colony counts (Ayres et al., 1980). Between 1881 and 1883 Robert Koch pioneered simple methods for the isolation and maintenance of pure cultures on solid media that was chemically defined (Brock, 1961). Koch's methods involved melting gelatin in hot liquid medium which could then be cooled, inoculating with bacteria, pouring onto a clear glass plate, and allowing the mixture to solidify. After incubation and growth the isolation of a large variety of microorganisms was accomplished. That product was to be referred to as "nutrient gelatin" (Bullock, 1938).

The technique used by Koch was to make tubes of sterile gelatin containing nutrients. These tubes were melted and inoculated with a small quantity of the material under investigation that contained the microorganisms he sought to observe. The tubes were then gently shaken to evenly distribute the microorganisms throughout the media. This inoculated tube of material was then poured onto a sheet of sterilized clear glass and allowed to solidify. The solidified gelatin immobilized individual bacteria in the mixture such that after growth and multiplication the individual

bacterium would result in the formation of a visible colony. These plates were covered with another sterile glass plate to protect them from contamination and drying out. The plates were also placed in an environment that provided the proper temperature for bacterial growth. Under these conditions the microorganisms would grow, utilize the media for nutrients, and develop into distinct and visible colonies within the layer of the gelatin (Conn and Conn, 1923; Koneman et al., 1983).

The essential component of the media was a 1% meat extract which served as nutrient for the microorganisms. Later, other nutrients and components were added to the medium such as various sugars, alcohols, dyes, serum, blood, salts and etc., to either enhance the nutritive value of the medium or to select for a particular biochemical property of the organism under investigation. The major disadvantages of gelatin as the solidifing agent were its low melting point of 23 C and the liquification of the gelatin by several bacteria. Optimal temperature for the incubation of most common pathogens is 37 C, thus gelatin was not a suitable solidification material for plating of these organisms.

The discovery of agar as a successful solidification material for media was made by Frau Hesse, the wife of Walter Hesse, one of Koch's previous coworkers (Bullock, 1938). Agar, obtained from red kelp, was first used in jelly making. Its application to microbiology came from its unique virtue of melting at a relatively high temperature (90-100 C) and of staying in a liquid state to ca. 42 C before solidifying into a fairly transparent material. Once solidified, agar will remain solid until heated to ca. 90-100 C. Thus, growth of a variety of organisms in the agar is

possible over a much larger and higher temperature range than in gelatin (Bullock, 1938).

Several other problems in culturing microorganisms were overcome in the next few years. The most important of these was the problem of growing bacteria on sterile glass plates, keeping plates sterile during growth, and storing the plates during growth. This problem was alleviated by an assistant of Koch's, R.J. Petri, in 1887 with his invention of the glass dish cover (Brock, 1961). This "Petri" dish was a round shallow glass dish that actually consisted of two separate glass dishes, one of which would serve as the bottom and the second, slightly larger dish, as the top or cover (Brock, 1961). This new Petri dish allowed for better examination of the contents of the plate without risk of contamination to either the plate or the observer, as well as sterile incubation of the plates containing bacteria. Petri dishes also reduced the problem of space during incubation, since they could be stacked and maintained in a smaller space. All of the procedures developed by Koch and his associates were critically analyzed by Breed and Dotterrer before adoption. Those procedures are still in use today (Breed and Dotterrer, 1916).

The standard plate count (SPC) method is presently recognized as the standard method for enumerating total bacterial count in foods. Standard plate counts are based upon the principal that each viable organism in a food sample will produce a single colony. That provides enumeration of viable microorganisms per milliliter or gram of the food sample and assumes that the bacterial suspension is homogeneous and that no aggregates of bacteria are present (Marth, 1978).

Naturally occurring microbial populations are generally large therefore, it is necessary that a sample be diluted before plating. Once diluted, known volumes of the sample are dispensed into sterile Petri dishes after which the melted, tempered nutrient agar is poured into the dishes, swirled to mix, and allowed to solidify. The plates are incubated at 25 to 37 C for 24 to 48 hours, depending upon the organism(s) desired. This allows viable cells to multiply and form visible colonies. The plates are inspected and those having between 25 and 250 colonies are counted and reported as colony forming units (CFU's) (APHA, 1985). This number is multiplied by the dilution factor to obtain the bacterial density of the original sample. The surface plate method involves dispensing and spreading a known volume of the diluent onto the surface of a pre-poured, solidified agar plate after which the plate is incubated and counted.

Liquid foods are readily diluted but alternate methods must be employed for nonliquid foods. These methods included shaking, blending or massaging the sample with diluent to release the microorganisms. The most commonly used method involves blending the sample with a specified amount of sterile buffer using an instrument such as the Waring Blender or an Osterizer. Although this method does disperse the organisms quite well it has several drawbacks. It functions by the cutting action of high speed blades which in turn create aerosols and heat, possibly injuring the microorganisms during the blending process. This method necessitates cleaning, washing and resterilizing the jars for new samples. Large numbers of jars create a demand for laboratory space. A relativley new blending method is the "Stomacher", introduced in 1972, it is particularly suitable

for preparing suspensions from foods, fabrics, swabs and other soft materials. The "Stomacher" utilizes sterile sample bags which enclose the sample and diluent in a flexible, sterile, high quality polyethylene bags that prevent contact between the machine and the sample. This allows samples to be prepared one after another without contamination to the machine itself (Sharpe and Jackson, 1972; Sharpe et al., 1972; Sharpe and Dudas, 1978). Advantages of the "Stomacher" included less damage to the microorganisms by high speed blades and an increase in temperature of less than 1 C/minute from the ambient (Sharpe and Harshman, 1976; Schiemann, 1977). It is space, time and labor saving compared to blending (Emswiler et al., 1977; Andrews et al., 1978; Deibel and Banwart, 1982).

Several common diluents used including distilled or demineralized water (buffered or unbuffered), skim milk, peptone, tryptone solutions, Ringer solutions and sodium citrate (Anon, 1968; Huhtanen et al., 1975; Keller et al., 1973; Straka and Stokes, 1957). Some of the commonly used diluents proved to be damaging or fatal to microorganisms, i.e., saline or distilled water (Butterfield, 1932). A standard formula phosphate-buffered dilution water is considered a suitable diluent for most foods (APHA, 1953, 1985). A formulated Butterfield's buffer is also suitable for most foods under Association of Official Analytical Chemists specifications (AOAC, 1980). Even with specific formulation, diluents are considered detrimental to most organisms, therefore, time lapse between dilution and actual plating should not exceed three minutes (Hartman and Huntsberger, 1961).

The method by which dilutions are mixed is generally by rapid inversion of the sample container 25 times in 7 seconds over a 1 foot arc

(APHA, 1985; AOAC, 1980). Another method is to blend a 50 gram sample in 450 mls of diluent for 2 minutes (AOAC, 1980). Previous studies report that vigorous shaking of dilutions resulted in a 25% increase in counts due to the disintegration of bacterial clumps (Wilson, et al., 1935).

Inoculation and incubation of Aerobic Plate Count procedures differ for various foods depending upon the nature of the food and the specific organism or microbial flora to be enumerated (Babel et al., 1955; Hartman and Huntsberger, 1961; Randolph et al., 1973). The final count depends upon several factors including composition of nutrient media, time and temperature of incubation, aerobic or anaerobic inoculation and incubation, skill of technician and skill of the observer who counts colonies. Inaccurate counts may also occur when plates are poured with agar that is too hot (Huhtanen et al., 1975). The recommended agar temperature is 45 C $\pm$ 1 C (APHA, 1985). Lowered counts may occur from agar that cools too slowly in plastic Petri dishes, as compared to glass, or stacking plates before cooling is complete (Koburger, 1980).

The commonly accepted medium for bacterial counts is Standard Plate Count Agar which contains tryptone, glucose and yeast extract. This agar was officially recommended for use by the American Public Health Association (1953, 1985) and by the Association of Official Analytical Chemists (1980). This agar serves as a replacement for a previous plate count agar developed in the 1930's with yeast extract replacing beef extract in the formulation. Commercial companies supplying SPC agar included DIFCO, BBL, OXOID and GIBCO. The effect of various types of bacteriological peptone in the plating medium upon the enumeration of

pasteurization-resistant bacteria in milk was studied by Thomas et al., (1966). They concluded that bacteriological peptone (recommended for standard plate count) was adequate for raw milk but unsatisfactory for the determination of maximum viable bacteria in pasteurized milk (Donnelly et al., 1960; Harrigan and McCance, 1976). The heat injured bacteria appeared to be more fastidious in their nutritional requirements than the unheated control organisms (Thomas et al., 1966; Wilson et al., 1935).

The range of CFU's required to obtain a reliable count with a minimum error has been a debatable issue. The most widely accepted range was 30 to 300 colonies based upon the work of Breed and Dotterrer (1916), and Snyder (1947). However, Postgate (1969), regarded the range of 200 to 300 colonies more reliable because of a lower percentage standard error in that range. Later Tomasiewicz et al. (1980) examined the counting range and developed a statistical method to derive a mean-squared-error function based on the variance function and the square of the bias. The conclusion was that the optimal counting range was between 25 and 250 colonies and the minimum error occurred at a count of 110 colonies. Presently, APHA (1985) recommends this range for use in standard plate counts.

Incubation temperture varies with the organism(s) desired. APHA (1985) generally recommends incubation at a temperature of 32 ± 1 C for aerobic plate counts while AOAC (1980), recommends 35 ± 1 C for aerobic plate counts. Actual incubation time recommended for aerobic plate counts is 48 ± 3 hours (APHA, 1985; AOAC, 1980).

B. Redigel

Redigel (RCR Scientific, Goshen Ind.), is a new modification of the Standard Plate Count method. The Redigel Method primarily differs from the Standard Plate Count Method in the usage of different gelling agents in the two media. SPC uses agar as the gellation agent while Redigel uses a low methoxyl pectin combined with calcium ions to form a calcium pectate gel. The SPC method requires heated agar while the Redigel method allows total temperature independence in the gelling procedure, thus eliminating problems with damage, or lethal effects, to microorganisms. The nutrients for both and Redigel plate count agar and for SPC are the same.

The Redigel is provided in units that include liquid media containing nutrients and the gelling agent, and petri dishes containing a thin layer containing a "hardener" that causes the gelling agent to solidify at room termperature. The gelling agent is a low methoxyl pectin which is sensitive to metallic ions. The "hardener" agent is calcium chloride, which diffuses up through the Redigel liquid in the dish and forms a calcium pectate gel that appears similar to an agar gel. The Redigel plates may also be poured, allowed to harden and used as streak plates as well as the usual method of pour plates. The pour plate method for Redigel differs only in the sequence of steps. With SPC the inoculum is added to the plate prior to adding the melted agar and mixing, but with Redigel the liquid nutrients are added first and the inoculum is added second before mixing. The time required for solidification (hardening) to occur is approximatley the same for Redigel and SPC (ca. 30 minutes). Incubation of the two is similar as

are the operational techniques employed in the sampling and plating process. The entire system comes prepackaged and no sterilization step is needed by the user prior to use.

Advantages of the Redigel system include: no requirement for heat tempered agar, savings in preparation time, ease of field usage, no specialized training for use, ease of transport and consistency of nutrients in the agar. Redigel is presently undergoing AOAC collaborative studies and approval is expected in January, 1988 (J. Roth, personal communication). Previous studies have shown that the Redigel method is comparable to the Standard Plate Count Method with high correlation coefficients (0.99) for both arithmetic and log base ten values (Fung and Chain, 1987).

C. Petrifilm

Petrifilm is a modification of the standard plate agar method. The Petrifilm System (3M Company, Minneapolis, MN) is a dual-layer film system, rehydratable and designed for field studies as an alternative to the Standard Plate Count method. The nutrients, which are similar to those of the standard plate count agar, are imbedded in the film assemblage. Also, a cold water- soluble gelling substance is coated on the film. The plates are used by inoculating their surface with diluted samples (typically 1 ml) followed by incubation at the desired temperature and desired time before counting of the viable colonies. Sterilizing and pouring are eliminated with this system. The elimination of agar-poured plates to save time, money and storage space is the major advantage of the Petrifilm System. This method

has been successfully utilized to obtain aerobic plate counts in milk and fresh ground beef (Ginn et al., 1986; Smith et al., 1986). Petrifilm was also tested against the conventional plate count method for aerobic plate counts of several sea foods with five replicates each, and found to provide comparable date with the only differences being in materials, time and incubation space (Fung et al., 1986). Other tests have been conducted comparing the Petrifilm method against standard methods for the evaluation of coliform counts in milk samples and for the detection of contamination on various surfaces. Results of these studies showed that Petrifilm data were highly correlated with that obtained from the Standard Plate Count method on the same samples (Ginn et al., 1986; Nelson et al., 1984; McGoldrick et al., 1986).

D. Most Probable Number-MPN

The Most Probable Number (MPN) technique is a means to estimate the density of viable organisms in a given sample. The MPN technique is based on probability statistics and results from any type of MPN Analysis are directly related to the frequency of occurrence of a given sample. This estimate is obtained by dilution of the sample in such a manner that the more dilute sample will result in fewer positive tubes, which are indicated by the presence of gas or microbial growth. Usually several tubes per dilution are examined. As such, the MPN method is an indirect procedure or estimate as contrasted to direct plating techniques. It can be said that the MPN technique utilizes a "Multiple dilution to extinction" approach in estimating populations of microorganisms (Koch, 1982). This is especially

effective in situations where particular foods may have extremely low densities of microorganisms, or where particular foods may complicate other enumeration methods. Values listed in the MPN tables are based on the assumption that the microorganisms sought are homogeneously distributed throughout the sample and the homogenate (APHA, 1985). Most MPN tables include the 95% and 99% confidence limits.

The MPN method has also been used in the enumeration of and determination of the number of specific types of microorganisms in a sample. Media selective for the specific microorganisms may be used in this case. Prior to the development of the membrane filter, the MPN technique was used for the determination of coliform numbers in water samples (Kreig, 1984). This was later modified to incorporate the use of EC broth as an additional confirmatory broth for samples that gave a false result in the standard MPN method (Evans et al., 1981; Dutka, 1973). Fung and Kraft (1969, 1968) miniaturized the entire MPN process for viable cell count milk and bacterial cultures by use of the microtiter system (Fung and LaGrange, 1969; Fung et al., 1976).

E. Membrane Filter Method

One drawback of the Standard Plate Count Method is the inability of the operator to satisfactorily monitor samples with very low microbial loads. For this the Membrane Filtration technique was developed (Frazier and Gneiser, 1968). Microbial cells can be collected on the surface of the membrane and stained for counting under the microscope or the filter may be placed on the surface of a solid medium and incubated allowing viable

cells to form colonies and be counted. Pore size of the membrane filter is selected according to the microorganism to be counted. Generally membranes utilized have a pore size of 0.2 to 2.0 microns in diameter (Barber et al., 1954).

The major advantage of membrane filters is that bacteria concentrated on the surface from a relatively large volume of liquid. Problems may arise from the filtration of some foods that contain fat globules, but this may be avoided by treating the food prior to filtration with an appropriate enzyme (Merrill, 1963; Kirkman and Hartman, 1962).

Microorganisms on membrane filters have also been grown on agar containing vital stain to help observe the colonies formed. This method was determined to be statistically valid when compared to the Standard Plate Count Method (Winter et al., 1971).

F. Hydrophobic Grid-Membrane Filters-ISOGRID

Hydrophobic Grid-Membrane filters (HGMF) consist of a conventional membrane filter divided into square grids by hydrophobic materials, forming growth compartments. These were introduced by Sharpe and Michaud in 1974. The HGMF is essentially a membrane filter which has been divided into growth compartments by the application of a hydrophobic grid. These compartments are termed as "growth units" rather than "colony forming units" (Sharpe and Michaud, 1975). The count on the HGMF is determined by a most probably number calculation, and is considered more precise than the traditional pour or spread plate (Sharpe and Michaud, 1974).

Another advantage of the HGMF is its large operating range (three log

cycles) which can reduce or eliminate the normal requirement of multiple dilutions.

One such HGMF is the ISOGRID System, manufactured by QA Laboratories Ltd, Toronto, Canada. This particular system has a grid pattern consisting of 1600 growth compartments which gives an effective MPN counting range of three log cycles (Entis, 1986b). QA has also developed an optional ISOGRID Line Counter that simplifies counting and automatically calculates the MPN for the count (Fruin and Clark, 1977; Entis, 1986a; Fruin and Gutheriz, 1977).

The procedure consists of weighing, blending and enzyme treating samples before passing through the prefilter which traps food particles larger than 5 microns, then through the HGMF which captures microorganisms within one of the 1600 growth cells. The inoculated HGMF (equivalent to 1600 MPN tubes), is placed on selective agar, incubated under the desired conditions, and counted on the ISOGRID line counter. This system has been successfully utilized for several viable cell counts on various foods (Entis, 1986a). Then enumeration of bacteria with the HGMF system is comparable to the counts obtained with conventional membrane filters and pour or spread plates (Sharpe and Michaud, 1975). It was demonstrated by Brodsky et al. (1982) that HGMF produced aerobic plate counts in foods that were equivalent to or greater than counts utilizing the traditional methods. The results of Brodsky et al. (1982) indicated that the automated HGMF system was an effective alternative to the conventional MPN and spread techniques for the enumeration and isolation of microorganisms on selective media. Other applications have included the method developed by Lin et al. (1984) and Lin

and Fung (1985) using trypan blue agar for the enumeration of yeast and mold and the development of an enzyme- linked immunosorbent assay for the direct enumeration of confirmed Staphylococcus aureus by Peterkin and Sharpe (1984).

The most recent development is the MI-100 HGMF Interpreter System which employs a video image analysis system to enumerate and identify microorganisms in food, beverages, water or cosmetics. The Interpreter was developed to analyse samples that have been inoculated onto the ISOGRID HGMF membrane filter. The entire process using the Interpreter system requires only three seconds to verify the Petri dish contains an HGMF, ensure the whole grid is visible, examine all 1600 cells and determine how many cells contain the target organism.

Overall there are several advantages of the HGMF system for the enumeration of microorganisms over the Standard Plate Count Method (Brodsky et al., 1982) including;

1. No temperature stress to microorganisms from molten agar.
2. Availability of rapid results from a sample.
3. The removal of soluble materials from water that could interfere with growth.
4. A counting precision that is greater than that provided by a conventional MPN.
5. Allowing for resuscitation of stressed or injured cells on a repair medium thus increasing their recovery.
6. The ability of the HGMF to detect very low numbers of microorganisms by the filtration of large volumes.

G. Spiral Plate System

The Spiral Plate System (Spiral System, Inc. Bethesda, MD.) is a semi-automated plating technique that greatly reduces manpower and material costs generally associated with Standard Pour Count method. The system contrasts with spread or pour plate procedures in that no serial dilutions are necessary. The Spiral Plate System is a specialized dispenser which distributes approximately 50 µmls of the sample on the surface of a rotating 10-cm agar plate from the center of the plate to the outer edge in an ever-decreasing amount. The sample is deposited in the form of an Archimedes spiral in such a manner that the volume of any portion of the plate is definite and always the same.

The original concept of adding inoculum to agar plates in the form of an Archimedes spiral was introduced by Campbell in 1971. Further development of the concept was done by Gilchrist et al. (1973), and later a semi-automated system known as the Spiral Plate System was developed. This system was adopted as an official first action for foods and cosmetics by AOAC in 1977 (Gilchrist et al., 1977). The method is currently listed as an alternative microbiological method in the 15th edition of Standard Methods for the Examination of Dairy Products (APHA, 1985). The present system consists of a spiral plater, a laser bacteria colony counter and a CASBA^{TM} data processor.

The major contrast between the Spiral Plate System and standard procedures is the lack of serial dilutions since the concentration range on a single plate is 10^4:1. As the spiral plater deposits the liquid sample from center to outer edge the amount deposited is increasingly smaller so that the

final dilution at the outer edge is the equivalent of a 10^4 dilution (Tilton and Ryan, 1978; Trinel et al., 1983; Trotman, 1971). After incubation the colonies appear on the lines of the spiral, and the bacterial density is determined by counting the colonies, using a specialized colony counter, on a countable portion of the plate and dividing the number by the volume of the sample plated in the area(s) counted. There is also a laser counter that may be used to count the colonies. An electronically guided laser beam scans the plate in a spiral from the outer edge toward the center of the plate. Any interference in the transmission of the light beam, caused by a colony, is detected and used to calculate the number of colonies in an annular area of the plate or over the whole plate. The laser counter is designed to enable a preset number of colonies to be counted. If the plate contains less than this number then the laser will count all the colonies and the plate count is presented as a digital readout. This count is divided by the inoculum volume to give the count in colonies per ml and total count is calculated. However, if the plate contains more than the preset number of colonies the counter stops and displays the area in which the preset number was counted. The colony count per ml is then determined by reading this area from a calibration curve which is prepared by plotting the areas in which the preset number were calculated against surface drop counts of the same samples. The Laser Bacteria Colony Counter can also be used with conventional pour plates if they are of a transparent type of agar. Limits of estimation with the spiral plate method are 10^3 to 10^6 CFU/ml of suspension (Gilchrist et al., 1973) on a 9 cm Petri plate. The upper limit can be extended by greater dilution of the sample, but the lower limit is fixed due to the specific amount of inoculum which is applied. The range can be

extended by increasing the inoculum size or the plate dimensions to a range of 10^2 to 10^6 CFU/ml or g. In a study done by Jarvis et al. (1977) it was found that counts would be slightly higher on the spiral plate system when used with low levels of contamination (i.e. 10^3 to 10^4 CFU/ml or g).

Comparable results were produced in comparisons between the Spiral Plate System technique and the Standard Plate Count for microorganisms in pure cultures (Gilchrist et al., 1973), milk (Donnelly, 1976), seafood, spices and several cooked meats (Gilchrist et al., 1977). Also Jarvis et al. (1977) did comparative work using samples of four types of food and comparing pour plate, surface spread plate, drop count and spiral plate count methods. It was found that there were significant correlations between the various methods, with no real difference between the results from the individual samples. Later Hedges et al. (1978) stated that the overall precision of the spiral plate method would generally exceed that of the surface drop and agar droplet method. The spiral plate technique was found capable of replacing the surface drop count method in the routine monitoring of many foods and produce reliable results from the standpoint of practical microbiology. Since that time the system has been used extensively in the U.S.A. for a variety of foods (Schalkowsky, 1986).

There are several advantages of the Spiral Plate System one of which is that minimal training in the technique was required in order to obtain reproducible and accurate results. Single plates may be inoculated in less than 25 seconds, and with an experienced operator 70 plates per hour could be processed. This is twice the capacity of the surface drop technique and 6 times that of the pour plate method (Kramer et al., 1979).

One of the minor problems of the system is the occasional blockage of the inoculation stylus by large suspended food particles. This is alleviated by flushing out the obstruction using a syringe and sterile water. The plates used with this method must be of uniform depth and prepared on a level surface or the results will be discrepant. The system requires several adjustments in the set up and monitoring of accuracy, but once adjusted requires only minor re-adjustment during usage. The problem with particles can also be alleviated with the use of an improved Stomacher 400 bag which has a filter in the bag that prevents solid particles from coming into the spiral plating system (Konuma et al., 1982).

H. Microbial Standards

A standard is defined in the Websters New International Dictionary, 1934, Unabridged 2nd edition, as the following;

> "That which is established by authority, custom, or general consent, as a model or example; criterion; test in general, a definite level, degree, material, character, quality, or the like, viewed as that which is proper and adequate for a given purpose."

Utilizing the definition for standard and applying it to the area of food microbiology there are several factors to be taken into consideration for the establishment of such a lower standard. First, it is understood that foods with lower numbers of microorganisms are generally considered to be safer for consumption and also have a longer storage life. Thus, there is a direct correlation between microbial load and these two factors. Standards adopted

for foods must take these factors into consideration and establish limits for microbial loads in different foods. The standard must be reasonable and attainable by the manufacturers and their practices. The standard should serve to present a criterion as to acceptability of nonacceptability of a food for consumption or further processing. As such a standard must be first established by a series of tests as to the microbial load of foods at what would be considered unsafe or spoilage levels (AOAC, 1980).

The techniques for establishment of standards must be uniform. Lack of such uniformity will lead only to discrepant results and a nonenforceable standards for the foods. Reproducible methods of analysis are then of the utmost importance to the microbiologist. Each food and its source must be viewed independently to set such standards. There are some foods for which there is little need for extensive standards because of their source or intrinsic properties. Other foods, particularly those of animal origin or products must be viewed with greater scrutiny since these are primary vehicles for organisms that cause food-borne illness.

Standard Plate Counts (aerobic plate counts), serve to help establish these standards. The process of food deterioration continues, as well as the food quality, by microbial activity on the food and ceases only upon proper processing or consumption. These total counts reflect the condition of the food in terms of handling, decomposition, freshness and sanitary treatment. Low counts however do not always reflect safety and some foods must be further tested for the presence of specific pathogens. An example of this is the study done by Montford and Thatcher (1961) on frozen egg preparations. In this study Salmonella isolates were obtained from low count foods (less

than 5000 CFU/ml or g) while several high count foods were Salmonella free (greater than 5000 CFU/ml or g). Yet other foods may harbor low numbers of toxin producing organisms while maintaining a low aerobic count. Still other foods are expected to have high microbial loads as the microoganisms is an essential part of the food product itself as example; yogurt, sauerkraut, some sausages, and other fermented or related foods. Because of these and other factors it must be noted that each food will have a different standard of acceptability. This is further demonstrated in Table 1 which shows the bacterial levels at odor and slime points for protein foods (Elliot and Michener, 1961; Thiebaud and Fung, 1986). This table also exemplifies the fact that the spoilage point level varies with different classes of foods. Microbial standards in foods is a point of intense debate among food microbiologists. Some recommend standards that are listed in Microorganisms in Foods. 2. Sampling for Microbiological Analyses: Principles and Specific Applications (ICMSF, 1974).

Table 1. Bacterial Level at Odor or Slime Point for Protein Foods[a]

Logarithm of no. of cell/cm^2		
Food	Odor	Slime
Poultry Meat	6.5	-
	8	8-9
	7	7.5
	6.9	-
	5.2	-
	7-8[b]	
Beef	-	7.7-8
	-	7.7
	-	7.5
	-	6.5
	-	7-8
	7.7-8	-
	8.7[b]	-
	6.3-7	8
	8[bc]	-
	7	7.8
Processed meats	-	7-8[b]
Frankfurters	8-8.5	8.5
Wiltshire bacon	-	6.7-8
Wiltshire bacon	-	7
Fish	6-6.6[b]	-
Haddock	6[b]	-
Fish	7-8.5	-
Fish	6.5[b]	-
Oysters	4-5.7[b]	-
Crabmeat	8[b]	-
Shell eggs	7[b]	-
Frozen eggs	6.7[b]	-
Liquid eggs	7[b]	-
Chicken pies	5[d]	-

[a]For original source of these data, see Elliot and Michener (1963), adopted by Thiebaud and Fung (1986).
[b]per gram
[c]Yeast
[d]Unacceptable flavor

I. Errors

The process of microbial sampling is not without areas of possible error. These errors can be lessened somewhat by the proper application and use of instruments or equipment to best fit the job. Some areas of possible errors are as follows:

1. Pipettes: Wilson et al. (1935), found that pipette errors of up to 9% are introduced when a portion of a sample or dilution is transferred, due to bacterial adherence to the inner wall surface. The error is reducible by repeated wettings of the inside of the pipette with the sample prior to transferral (Wilson et al., 1935).

   Several errors may be involved when serial dilutions are carried out with in accurate measuring devices or lack of care, such as the use of 1 ml pipettes to transfer 9 ml volumes of diluent (Felland and Nadig, 1965, Marth, 1965). The effect in errors is presented in Table 2 (Jennison and Wadsworth, 1940, adopted by Thiebaud and Fung, 1986).

2. Dilutions: There are specific methods for the mixing of dilutions. Mixing the sample by rapidly inverting the sample container 25 times in 7 seconds over a one foot arc, is recommended in Standard Methods for the Examination of Dairy Products (APHA, 1985). Vigorous shaking was found to increase counts by as much as 25% in a study by Wilson et al. (1935). AOAC recommends either blending or shaking (according to standards) to mix the sample (AOAC, 1980). The most recent mixing method is by use of the Stomacher machine, in which a sterile bag containing sample and diluent is massaged.

3. Counts: There are several factors that may effect the number of colonies counted on a plate (Fowler et al., 1978). First is the human element of error, where the count depends upon the skill of the observer (Peeler et al., 1982). Next, is the composition of the nutrient medium and the temperature at which the agar is poured. Counts may be inaccurate when plates are poured with an agar whose temperature is too hot (Huhtanen et al., 1975). The recommended agar temperature is 45 ± 1 C (APHA, 1985; AOAC, 1980). Plates that are stacked too high or cooled too slowly will also exhibit lowered counts (Koburger, 1980).

4. Incubation and Inoculation: Samples should be prepared and inoculated within 3 to 5 minutes of dilution (Hartman and Huntsberger, 1961).Pipettes should accurately deliver the required volume of inoculum and should never be used to deliver less than 10% of their total volume (AOAC, 1980). Once inoculum and agar are in the petri dish mixing must be complete enough to ensure a uniformity of colony distribution. Incubation time is dependent upon the organism(s) to be enumerated and recommended temperatures should not be deviated from more than ± 1 Celsius from the standard temperature (AOAC, 1980; APHA, 1985).

Table 2: Error of the Serial Dilution Procedures as a Function of the Accuracy of Pipetting and Diluent Volume[a]

Diluting Volume ml	Pipette S.D. of the volume ml	Diluent volume ml	S.D. of the diluent volume ml	Error arising from the dilution expressed in % S.D. at dil. levels			
				10^2	10^4	10^6	10^8
1	±0.01	9	±0.1	±2.8	±5.7	±8.5	±11.3
1	±0.01	99	±1	±1.4	±2.8	±4.2	±5.7
1	±0.3	9	±0.3	±8.4	±17.1	±25.5	±33.9
1	±0.03	99	±3	±4.2	±8.4	±12.6	±17.1

[a] For original source of these data, see Jennison and Wadsworth (1940), adopted by Thiebaud and Fung (1986).

MATERIALS AND METHODS

A. Food Tested:

Seven different foods, representative of five different food groups (skinless chicken breast, fresh ground beef, fresh ground pork, packaged whole shelled pecans, raw milk, thyme and whole wheat flour), were used in this study. Twenty samples of each food were obtained from local sources.

B. Methods and Procedures:

Sample preparation required a 10 gram sample, of each selected food, that was divided into two 5 gram samples. One of the 5 gram samples was added to 45 ml of sterile phosphate buffer in a stomacher bag and massaged in the stomacher for one minute. This was used for viable cell count evaluation using the SPC, Redigel, Petrifilm™ and Spiral Plate systems. The second 5 gram sample was added to 45 ml of sterile peptone/tween 80 diluent in a stomacher bag and massaged for one minute. Dilutions were made to ensure bacterial counts within the range of 25 to 250 CFU's/plate on SPC. Duplicate plates were done for each sample at each dilution on all systems. All plates for all systems for one sample were inoculated within the same 5 minute time span. Following inoculation all plates were incubated together at 32 ± 1°C for 48 ± 3 hours, at which time counts were made.

Duplicate environmental control plates were exposed for 15 minutes each AM and PM on days that samples were processed. This was to verify

that the results were not affected by environmental contamination. SPC was used for the plating media.

1. Standard Plate Count:

   One ml of the appropriate dilution was added to duplicate plates after which approximately 20 ml of melted and tempered (42-45°C) SPC agar (Difco Bacto Plate Count Agar-Standard Methods Agar) was added. The plates were then mixed by swirling 15 times in each direction. After solidification (ca 30 min) the plates were inverted and incubated for 48±3 hours at 32±1°C. Counts were done on plates containing between 25 and 250 CFU's using a Fisher Accu-lite colony counter. (APHA, 1985).

2. Redigel:

   Redigel standard plate count media comes in presterilized and premeasured tubes which are at ambient temperature. To inoculate, the tube of Redigel is first added to each of the specially pretreated Redigel plates. Next, 1 ml of the same dilution used for SPC method was added to each plate. The plates were then mixed by swirling 15 times in each direction. After solidification (ca 30 min) the plates were inverted and incubated with the SPC plates for the designated time and temperature. Counts were performed the same as on the SPC plates. (APHA, 1985).

3. Petrifilm:

   One ml of the same dilution as was used for the SPC and Redigel

methods was used for inoculation of the Petrifilm. The top film was lifted up and 1 ml of the dilution was placed in the center of the "plate". Next the top of the film was gently rolled down and a press plate (provided with the Petrifilm) was used to distribute the inoculum into a circular shape. Petrifilm plates were also incubated with the SPC and Redigel plates for the designated time and temperature. Counts were made manually. (Ginn et al., 1986)

4. ISOGRID:

One ml to 10 ml of the original homogenate, or appropriate dilution was passed through the Isogrid system and captured on the hydrophobic grid membrane filter (using vacuum). The filter was then aseptically removed using sterile forceps and placed, grid-side-up, on the surface of a pre-poured and solidified plate of TSA/FG agar (Difco Bacto Tryptic Soy Agar with Fast Green FCF Food Green 3 from Sigma Chemical and Co.) The plate was then inverted and incubated along with plates from the other systems at the designated time and temperature. Although it has been reported that a single HGMF filtration can be used in place of duplicate pour plates without loss of precision (Brodsky et al., 1982) duplicated plates were used with this system also. Counts were determined manually on the ISOGRID Line Counter (QA Labortories Ltd.) and the MPN was calculated by depressing the MPN key on the counter. The MPN could also be calculated with the formula;

$$MPN = 1600 \times \log_e (1600/1600\text{-score})$$

The MPN is multiplied by the overall dilution factor to obtain the total bacterial count/g or ml. (Entis, 1986a).

5. Spiral Plate System:

A sample of the original homogenate or appropriate dilution was used with the automatic diluting system. The system automatically deposits 49.5 µl in an Archimedes spiral at a decreasing rate from the center to the edge, thereby fixing the volume-area relationship for any portion of the plate. This is done on a previously poured, solidified and dried SPC plate. (The Spiral Plate System used was the Model D) The plates were inverted and incubated with the other plates from other systems for the designated time and temperature. After incubation the spiral plate was centered over the grid of the colony viewer (Spiral Plate model MV) which consists of a circle divided into 5 areas by 4 concentric circles equidistant along diameter and 4 to center and into eight 45° octants. Any octant sector was chosen and colonies counted from outer edge toward center until 20 colonies had been counted. Counting continued until all remaining colonies, in the segment which contained the 20th colony, had been counted. The opposite segment was also counted and the counts added together. For plates with less than 20 colonies in an octant, all colonies on the plate were counted and designated as a total count (T). The number of colonies counted were divided by the corresponding volume of sectors counted to obtain bacterial count/ml or g (Gilchrist et al., 1977).

C. Cost Analysis

Calculation of cost and time did not include actual time involved in plate counts. Time and cost of each systems basic plate was calculated from material, media and labor costs. For this comparison the prices of already prepared Standard Plate Count agar plates and Trypticase Soy with Fast Green plates from a commercial manufacturer were used. Labor costs were determined using a stopwatch during time needed for dilution, filtration, inoculation and any necessary mixing of the sample with agar during the plating process. Labor was calculated at an average cost of $12.50 per hour.

Costs were calculated per plate thus it must be taken into consideration that ISOGRID and Spiral Plate System do not advocate the need for duplicate plates or multiple dilutions. This should create a larger savings per sample with the use of less plates.

RESULTS AND DISCUSSION

A. Correlations of Each Food:

A total of seven foods, (chicken breast, ground beef, ground pork, pecans, raw milk, thyme and whole wheat flour) with twenty samples of each, were evaluated. The resultant data were tested statistically both with the arithmetic values and the log base ten values. The data and the values obtained after statistical evaluation are given on the following tables; tables 3-9 represent the original aerobic counts, expressed as log base ten values, for each food evaluated, and tables 3a-9a give the Pearson correlation coefficients for the 5 systems tested using both the original arithmetic values and the logarithmic variables.

Tables 3 and 3a represent the data and results on the chicken breast samples that were evaluated. In the correlation of the original variables and the logarithmic variables the Petrifilm was correlated most highly with the Standard Plate Count (SPC) method with correlation coefficients of 0.99963 and 0.99977 respectively. This was only slightly higher than the correlations between SPC and Redigel at 0.99855 and 0.99962 respectively. The other two systems also correlated quite highly with values of 0.97017 and 0.90479 for the Spiral Plate System (Spiral P.) followed by the ISOGRID with correlation values of 0.96992 and 0.90457. Between the systems themselves (excluding SPC) Redigel and Petrifilm had high correlations with each other while the ISOGRID and Spiral Plate System had high degree of correlation with each other. Overall the values expressed showed a high degree of agreement between and among the five

systems on this particular food with both the arithmetic and logarithmic variables.

The results and data on the ground beef samples that were evaluated are shown on tables 4 and 4a. In the correlation of the original variables and the logarithmic variables the Redigel was correlated most highly with the Standard Plate Count (SPC) method with correlation coefficients of 0.99950 and 0.99994 respectively. This was slightly higher than the correlation between SPC and Petrifilm at 0.99912 and 0.99986 respectively. The other two systems also correlated quite highly with values of 0.99627 and 0.99968 for the Spiral Plate System followed by correlation values of 0.99561 and 0.99961 for the ISOGRID. In statistical evaluation between the systems themselves (excluding SPC) Redigel and Petrifilm had a high degree of correlation as did the Spiral Plate System and ISOGRID. Statistical evaluation showed a high degree of agreement between and among the five systems, using both arithmetic and logarithmic variables, on this particular food.

Tables 5 and 5a represent the results and data obtained in the evaluation of ground pork samples. In comparing the correlation of the original variables and the logarithmic variables Petrifilm had the highest correlation on the original variables while Redigel had the highest correlation on the logarithmic variables, with original and logarithmic values for Petrifilm of 0.9998 and 0.9990, respectively, and for Redigel of 0.99991 and 0.99998 respectively. The values for the other two systems were somewhat lower with both systems expressing a correlation of 0.99996 on the original variables and Spiral Plate System correlating at

0.99987 followed by ISOGRID at 0.99984 on the logarithmic variables. Between the systems themselves (Excluding SPC) the ISOGRID, Petrifilm and Spiral Plate System had the highest degree of agreement with the Redigel correlation being only slightly lower both using the original variable and the logarithmic variables. Agreement among and between all five systems was high, using both sets of variables, on this set of food samples.

Statistical analysis for the pecan samples that were evaluated are shown on table 6 and 6a. In the correlation of the original variables and the logarithmic variables Redigel was most highly correlated with the SPC method having correlation coefficients of 0.99935 and 0.99720 respectively. This was very close to the correlations between SPC and Petrifilm and 0.99849 and 0.99490 followed by ISOGRID at 0.99823 and 0.99503 and Spiral Plate System at 0.99793 and 0.99369 respectively. The greatest agreement among and between the systems themselves (excluding SPC) was between Redigel, Petrifilm and Spiral Plate System with ISOGRID correlating very closely. There was expressed a very high degree of agreement between and among all five of the systems on this set of food samples.

Tables 7 and 7a represent the data and results on the raw milk samples that were evaluated. In the correlation of original variables the Spiral Plate System and ISOGRID were most highly correlated at 0.99985 followed by Petrifilm at 0.99983 and Redigel at 0.99966 as compared to Standard Plate Count method. In the correlation of the logarithmic variables with SPC and the other four systems Petrifilm, Spiral Plate

System and ISOGRID all correlated at 0.99995 followed closely by the correlation with Redigel at 0.99989. The greatest degree of agreement between and among the five systems was between ISOGRID and Spiral Plate System, followed by Redigel and ISOGRID and by Petrifilm and Spiral Plate System. Correlation coefficients between and among the five systems tested with this set of food samples shows a high degree of agreement.

The resultant data for the thyme samples that were evaluated are given on tables 8 and 8a. In the correlation of the original variables and the logarithmic variables Redigel was most highly correlated with the Standard Plate Count (SPC) method having correlation values of 0.99705 and 0.99747 respectively. This was slightly higher than the correlation values for Petrifilm at 0.99298 and 0.99447, respectively, for the original variables and the logarithmic variables. The Spiral Plate System correlated higher for both variables than did ISOGRID with the correlations for Spiral Plate System at 0.98788 and 0.98636 respectively compared to ISOGRID with correlations of 0.98508 and 0.97976 respectively. The degree of agreement between the systems (excluding SPC) was greatest between Redigel and Petrifilm and between Spiral Plate System and ISOGRID. The correlations were all 0.9+ among and between all five systems showing a high degree of agreement on this particular set of food samples.

The results and data representing the whole wheat samples that were evaluated are given on tables 9 and 9a. Table 9a shows the correlation of the original variables and logarithmic variables to be highest between the Standard Plate Count method and Redigel at correlation coefficients of 0.99903 and 0.99808 respectively. This is followed by the correlations of

ISOGRID with SPC of 0.99860 and 0.99664 respectively. Petrifilm correlated with Standard Plate Count method with values of 0.99843 and 0.99468 followed by the correlations with Spiral Plate System of 0.99829 and 0.99672, respectively, for the original variables and the logarithmic variables. The greatest degree of agreement between the systems (excluding SPC) was between Redigel and Petrifilm followed by the degree of agreement between ISOGRID and Spiral Plate System. Statistical evaluations of the original and logarithmic variable for the samples of whole wheat flour showed a high degree of agreement between and among all five of the systems tested.

Correlation coefficients of all seven foods tested with the five systems were 0.9+. This showed that the systems were all quite comparable in the enumeration of aerobic microorganisms in the foods tested. Previous studies, however, have shown that this is not always the case. Depending upon the food sampled each system has certain advantages and disadvantages associated with aerobic plate counts.

B. Advantages and Disadvantages with Each System:

1. Standard Plate Count:

This has been the "standard" method for many years. There are problems associated with this particular system, however. The use of hot agar creates the possibility of damage or death to the microorganisms (Huhtanen et al., 1975) or lowered counts from slow cooling agar (Koburger, 1980). Space requirements for this method are large and preparation fairly extensive. This method is however the most widely used,

accepted and best known.

2. Redigel:

Redigel is similar to Standard Plate Count in application and incubation without the requirement of preparation time or melting and heat tempering of agar. In formulation Redigel is consistent and human errors in formulation or preparation are minimized. Previous studies using Redigel have shown higher aerobic counts on Redigel versus SPC on samples with possible high psychrotroph population such as selected raw seafoods (personal communication J. Roth RCR Scientific and D.Y.C. Fung, 1987). The system has minor differences such as smaller colonies with some samples (ie. raw milk) than on Standard Plate Count media.

3. Petrifilm:

The film assembly of the Petrifilm is extremely easy to use. Counting of the resultant colonies is facilitated by slow acting TTC (triphenyltetrazolium chloride) in the media formulation. The TTC does not appear to have an inhibitory effect on microorganism counts. Studies with Petrifilm and SPC have shown higher aerobic plate counts on Petrifilm from samples of selected possible high psychrotroph seafoods (personal communication D.Y.C. Fung, 1987). Petrifilm requires approximately one-tenth the space for storage and incubation as does SPC. However, liquid inoculum must be carefully spread to avoid partial loss of inoculum sample from between sheets of the film assembly during pressing with the "spreader plate" provided.

4. ISOGRID:

This system also requires the use of only one plate. The MPN replication by the hydrophobic grid membrane filter gives a high degree of accuracy (Sharpe and Michaud, 1974; Ligugnana, 1982). However, the filtration of particulate matter is difficult and certain foods do require enzyme pretreatment before filtration (Brock, 1983; Brodsky et al., 1982). The system requires preparation of agar plates, sterilization of filtration units, purchase of special equipment and special training.

5. Spiral Plate System:

Resultant plates from use of the Spiral Plate System are easy to count and may also be counted with a special laser counter. Since the plater automatically dilutes the sample only one plate rather than two are needed per dilution (Jarvis et al., 1977; Anon, 1977; Gilchrist et al., 1977; Peeler et al., 1977). Drawbacks of the system include problems with plating samples with particulate matter or high starch content, the requirement of uniform depth, bubble free and moisture free plates. Special equipment must be purchased to use this system.

All systems evaluated performed quite well. However, some foods were more easily evaluated with particular systems. An example of this was the evaluation of meats, thyme and whole wheat flour. These foods were easily sampled on all systems except ISODRID and Spiral Plate System. Extra time for enzyme treatment and/or clogging of filters or

stylus by particulate matter made these two systems slower and more tedious. Redigel colonies appeared smaller and more difficult to count for milk samples. Petrifilm performed well on all samples, however plates close to the 250 CFU's maximum were more difficult to count than with other systems due to the smaller growth area.

Ease of preparation, use and counting favored Redegel, Petrifilm and Spiral Plate System over the other two systems. All systems were statistically valid and final choice of a particular system should be based on samples to be tested as well as personal preferences.

C. Cost Analysis

Table 10 consists of cost analysis of the five systems. Descending order of cost per viable cell count was: Standard Plate Count, Redigel, Petrifilm, ISOGRID and Spiral Plate System. It should be noted that Spiral Plate System and ISOGRID require only one plate compared with multiple plates required by other systems. However, Spiral Plate System and ISOGRID do require the purchase of initial equipment and supplies.

Table 3. Comparative Analysis of Chicken Breast (Plated on Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID) By Pearson Correlation Coefficient

Correlations of Original Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99855	0.99963	0.97017	0.96992
Redigel	0.99855	1.00000	0.99916	0.96917	0.96875
Petrifilm	0.99963	0.99916	1.00000	0.97089	0.97056
Spiral P.	0.97017	0.96917	0.97089	1.00000	0.99988
ISOGRID	0.96992	0.96875	0.97056	0.99988	1.00000

Correlations of Logarithmic Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99962	0.99977	0.90479	0.90457
Redigel	0.99962	1.00000	0.99973	0.90433	0.90416
Petrifilm	0.99977	0.99973	1.00000	0.90570	0.90547
Spiral P.	0.90479	0.90433	0.90570	1.00000	0.99988
ISOGRID	0.90457	0.90416	0.90547	0.99988	1.00000

Table 3a. Comparative Aerobic Counts of Chicken Breast by Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID in Log base$_{10}$ CFU's/g

Sample #	SPC	REDIGEL	PETRIFILM	SPIRAL P.	ISOGRID
1	4.8293	4.8195	4.8357	4.8296	4.8312
2	4.7853	4.7924	4.7782	4.7803	7.7850
3	5.0934	5.0934	5.0899	5.0981	5.0999
5	4.9731	4.9800	4.9754	4.9683	4.9750
6	5.5199	5.5051	5.4914	5.5031	5.5263
7	5.4264	5.4698	5.4771	5.4586	5.4735
8	5.8573	5.8663	5.8513	5.8609	5.8663
9	4.3892	4.3802	4.3617	4.3892	4.3997
10	4.8893	4.8865	4.8779	4.8820	4.8976
11	5.5911	5.5911	5.5798	5.5911	5.5982
12	5.7482	5.7364	5.7364	5.7686	5.7694
13	5.1903	5.1761	5.1761	5.1973	5.1818
14	5.5502	5.5502	5.5563	5.5838	5.5888
15	5.3222	5.3324	5.3222	5.3493	5.3664
16	5.7853	5.7364	5.7597	5.7789	5.7903
17	6.0453	6.0550	6.0334	6.0436	6.0479
18	5.9685	5.9731	5.9638	5.9743	5.9780
19	5.8293	5.8325	5.8293	5.8370	5.8162
20	5.7853	5.7993	5.7818	5.8048	5.8162

Table 4. Comparative Analysis of Ground Beef (Plated on Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID) by Pearson Correlation Coefficient

Correlations of Original Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99950	0.99912	0.99627	0.99561
Redigel	0.99950	1.00000	0.99948	0.99593	0.99610
Petrifilm	0.99912	0.99948	1.00000	0.99636	0.99650
Spiral P.	0.99627	0.99593	0.99636	1.00000	0.99894
ISOGRID	0.99561	0.99610	0.99650	0.99894	1.00000

Correlations of Logarithmic Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99994	0.99986	0.99968	0.99961
Redigel	0.99994	1.00000	0.99993	0.99975	0.99970
Petrifilm	0.99986	0.99993	1.00000	0.99975	0.99970
Spiral P.	0.99968	0.99975	0.99975	1.00000	0.99994
ISOGRID	0.99961	0.99970	0.99970	0.99994	1.00000

Table 4a. Comparative Aerobic Counts of Ground Beef by Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID in log base$_{10}$ CFU's/g

Sample #	SPC	REDIGEL	PETRIFILM	SPIRAL P.	ISOGRID
1	4.2175	4.2042	4.1614	4.2041	4.1959
2	7.4314	7.4314	7.4472	7.5072	7.5321
3	7.7520	7.7634	7.7443	7.7259	7.7536
4	7.2304	7.2672	7.2553	7.3032	7.3096
5	7.4698	7.4983	7.4914	7.4654	7.4900
6	4.3118	4.2900	4.2672	4.2900	4.3212
7	6.7520	6.7597	6.7160	6.7752	6.7871
8	6.6812	6.6767	6.6580	6.6767	6.6972
9	6.8357	6.8420	6.8293	6.8645	6.8627
10	6.9566	6.9542	6.9542	6.9559	6.9533
11	5.1206	5.1255	5.1189	5.1176	5.1189
12	7.7404	7.7443	7.7243	7.7520	7.7423
13	7.8513	7.8482	7.8420	7.8335	7.8290
14	7.1614	7.1461	7.1303	7.1239	7.1123
15	7.4843	7.4843	7.4548	7.4976	7.5045
16	4.8633	4.8692	4.8603	4.8890	4.8848
17	7.1414	7.1399	7.1367	7.1289	7.1315
18	7.2788	7.2788	7.2788	7.3149	7.3294
19	6.9890	6.9956	6.9934	6.9939	6.0011
20	7.5502	7.5441	7.5250	7.5623	7.5694

Table 5. Comparative Analysis of Ground Pork (Plated on Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID) by Pearson Correlation Coefficient

Correlations of Original Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99991	0.99998	0.99996	0.99996
Redigel	0.99991	1.00000	0.99987	0.99983	0.99983
Petrifilm	0.99998	0.99987	1.00000	0.99998	0.99994
Spiral P.	0.99996	0.99983	0.99998	1.00000	0.99998
ISOGRID	0.99996	0.99983	0.99994	0.99998	1.00000

Correlations of Logarithmic Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99998	0.99990	0.99987	0.99984
Redigel	0.99998	1.00000	0.99992	0.99988	0.99986
Petrifilm	0.99990	0.99992	1.00000	0.99993	0.99992
Spiral P.	0.99987	0.99988	0.99993	1.00000	0.99999
ISOGRID	0.99984	0.99986	0.99992	0.99999	1.00000

Table 5a. Comparative Aerobic Counts of Ground Pork by Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID in log base $_{10}$ CFU's/g

Sample #	SPC	REDIGEL	PETRIFILM	SPIRAL P.	ISOGRID
1	5.5441	5.5441	5.5315	5.5211	5.5211
2	5.7782	5.7672	5.7782	5.8041	5.8024
3	5.5119	5.5119	5.4624	5.4822	5.4800
4	5.9004	5.9031	5.8893	5.8913	5.8893
5	5.6580	5.6484	5.6484	5.6385	5.6380
6	5.5378	5.5378	5.5185	5.5521	5.5551
7	5.6628	5.6580	5.6532	5.6656	5.6693
8	5.6767	5.6767	5.6857	5.6803	5.6862
9	5.6721	5.6767	5.7033	5.6964	5.6994
10	5.6075	5.6075	5.6021	5.6048	5.6080
11	4.7160	4.7076	4.6902	4.7063	4.7097
12	4.4914	4.4914	4.4983	4.4893	4.4990
13	4.7482	4.7364	4.7404	4.7723	4.7846
14	4.5441	4.5250	4.5250	4.5192	4.5321
15	4.6075	4.6232	4.6335	4.6566	4.6670
16	8.0737	8.0810	8.0774	8.0759	8.0743
17	7.9934	7.9978	7.9978	7.0007	7.0007
18	7.3324	7.3324	7.3424	7.3636	7.3711
19	8.2683	8.2648	8.2707	8.2729	8.2731
20	7.8573	7.8663	7.8513	7.8546	7.8618

Table 6. Comparative Analysis of Pecans (Plated on Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID) by Pearson Correlation Coefficient

Correlations of Original Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99935	0.99849	0.99793	0.99823
Redigel	0.99935	1.00000	0.99918	0.99878	0.99883
Petrifilm	0.99849	0.99918	1.00000	0.99948	0.99938
Spiral P.	0.99793	0.99878	0.99948	1.00000	0.99959
ISOGRID	0.99823	0.99883	0.99938	0.99959	1.00000

Correlations of Logarithmic Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99720	0.99490	0.99369	0.99503
Redigel	0.99720	1.00000	0.99907	0.99827	0.99849
Petrifilm	0.99490	0.99907	1.00000	0.99914	0.99879
Spiral P.	0.99369	0.99827	0.99914	1.00000	0.99951
ISOGRID	0.99503	0.99849	0.99879	0.99951	1.00000

Table 6a. Comparative Aerobic Counts of Pecans by Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID in log base$_{10}$ CFU's/g

Sample #	SPC	REDIGEL	PETRIFILM	SPIRAL P.	ISOGRID
1	6.3892	6.4150	6.4232	6.4023	6.4257
2	6.5051	6.5051	6.4983	6.4893	6.5085
3	6.5315	6.5378	6.4983	6.4907	6.5340
4	6.5682	6.5798	6.5563	6.5434	6.5700
5	6.9165	6.9243	6.9085	6.9031	6.9188
6	7.1004	7.1038	7.1004	7.1016	7.1011
7	6.9777	6.9890	6.9823	6.9703	6.9814
8	7.0792	7.0755	7.0719	7.0658	7.0671
9	6.6628	6.6580	6.6435	6.6469	6.6618
10	7.0934	7.0934	7.0899	7.0848	7.1000
11	6.8663	6.8808	6.8573	6.8788	6.8800
12	7.0354	7.0394	7.0107	7.0013	7.0060
13	7.1399	7.1446	7.1399	7.1369	7.1388
14	6.7520	6.7559	6.7364	6.7360	6.7455
15	6.3010	6.3222	6.3010	6.3010	6.3160
16	7.0934	7.0917	7.0864	7.0821	7.0849
17	6.3199	6.4314	6.4393	6.4385	6.4409
18	6.7520	6.7559	6.7597	6.7593	6.7657
19	6.2788	6.3010	6.2788	6.2292	6.2430
20	6.7672	6.7634	6.7520	6.7524	6.7679

Table 7. Comparative Analysis of Raw Milk (Plated on Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID) by Pearson Correlation Coefficient

Correlations of Original Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99966	0.99983	0.99985	0.99985
Redigel	0.99966	1.00000	0.99975	0.99986	0.99991
Petrifilm	0.99983	0.99975	1.00000	0.99990	0.99975
Spiral P.	0.99985	0.99986	0.99990	1.00000	0.99993
ISOGRID	0.99985	0.99991	0.99975	0.99993	1.00000

Correlations of Logarithmic Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99989	0.99995	0.99995	0.99995
Redigel	0.99989	1.00000	0.99988	0.99988	0.99993
Petrifilm	0.99995	0.99988	1.00000	0.99993	0.99991
Spiral P.	0.99995	0.99988	0.99993	1.00000	0.99997
ISOGRID	0.99995	0.99993	0.99991	0.99997	1.00000

Table 7a. Comparative Aerobic Counts of Raw Milk by Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID in log base 10 CFU's/ml

Sample #	SPC	REDIGEL	PETRIFILM	SPIRAL P.	ISOGRID
1	4.7924	4.7924	4.7889	4.8021	5.8065
2	4.8420	4.8513	4.8357	4.8503	4.8585
3	5.1123	5.1038	5.1106	5.1148	5.1163
4	4.8357	4.8357	4.8325	4.8410	4.8460
5	4.3617	4.3522	4.3522	4.3570	4.3757
6	5.1335	5.1351	5.1255	5.1435	5.1458
7	4.6385	4.6532	4.6232	4.6385	4.6469
8	4.9777	4.9708	4.9494	4.9557	4.9666
9	4.4843	4.5119	4.4843	4.4942	4.5011
10	4.8779	4.8751	4.8808	4.8791	4.8868
11	6.0569	6.0569	6.0531	6.0633	6.0671
12	5.6128	5.6128	5.5966	5.6165	5.6160
13	5.9614	5.9708	5.9638	5.9673	5.9701
14	5.3010	5.2788	5.2788	5.3212	5.3149
15	5.8096	5.8096	5.8028	5.8228	5.8245
16	6.6232	6.6180	6.6075	6.6186	6.6355
17	6.9494	6.9518	6.9518	6.9547	6.9583
18	6.4232	6.4548	6.4065	6.4401	6.4624
19	6.8663	6.8692	6.8692	6.8811	6.8825
20	7.0434	7.0294	7.0334	7.0428	7.0477

Table 8. Comparative Analysis of Thyme (Plated on Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID) by Pearson Correlation Coefficient

Correlations of Original Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99970	0.99298	0.98788	0.98508
Redigel	0.99705	1.00000	0.99521	0.98975	0.98618
Petrifilm	0.99298	0.99521	1.00000	0.99283	0.98728
Spiral P.	0.98788	0.98975	0.99283	1.00000	0.99726
ISOGRID	0.98508	0.98618	0.98728	0.99726	1.00000

Correlations of Logarithmic Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99747	0.99447	0.98636	0.97976
Redigel	0.99747	1.00000	0.99328	0.98511	0.97866
Petrifilm	0.99447	0.99328	1.00000	0.98916	0.98127
Spiral P.	0.98636	0.98511	0.98916	1.00000	0.99685
ISOGRID	0.97976	0.97866	0.98127	0.99685	1.00000

Table 8a. Comparative Aerobic Counts of Thyme by Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID in log base$_{10}$ CFU's/g

Sample #	SPC	REDIGEL	PETRIFILM	SPIRAL P.	ISOGRID
1	7.8388	7.8420	7.8388	7.8385	7.8482
2	7.1614	7.1761	7.0792	7.0934	7.0899
3	7.4914	7.4914	7.4548	7.4548	7.4579
4	7.8062	7.8129	7.7993	7.8014	7.8028
5	7.4232	7.4150	7.3892	7.4548	7.4786
6	7.2788	7.2788	7.2672	7.3096	7.2967
7	7.5441	7.5502	7.5441	7.5250	7.5302
8	7.3424	7.3424	7.3222	7.2978	7.3032
9	7.7443	7.7559	7.7520	7.7631	7.7563
10	7.6128	7.6284	7.6232	7.6128	7.6191
11	7.5855	7.5855	7.5623	7.5866	7.5933
12	7.5185	7.4843	7.5250	7.5192	7.5079
13	7.7033	7.7033	7.6857	7.6794	7.7093
14	7.4393	7.4472	7.4393	7.4306	7.4676
15	7.7559	7.7284	7.6946	7.7020	7.7185
16	7.4472	7.4232	7.4232	7.4338	7.4579
17	7.6675	7.6721	7.6675	7.7046	7.7259
18	7.4914	7.4843	7.4548	7.5257	7.5527
19	7.2788	7.2900	7.2430	7.3314	7.3747
20	7.7520	7.7443	7.7597	7.7716	7.7825

Table 9. Comparative Analysis of Whole Wheat Flour (Plated on Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID) by Pearson Correlation Coefficient

Correlations of Original Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99903	0.99843	0.99843	0.99860
Redigel	0.99903	1.00000	0.99829	0.99770	0.99781
Petrifilm	0.99843	0.99829	1.00000	0.99711	0.99732
Spiral P.	0.99829	0.99770	0.99711	1.00000	0.99987
ISOGRID	0.99860	0.99781	0.99732	0.99987	1.00000

Correlations of Logarithmic Variables

	SPC	Redigel	Petrifilm	Spiral P.	ISOGRID
SPC	1.00000	0.99808	0.99468	0.99672	0.99664
Redigel	0.99808	1.00000	0.99649	0.99589	0.99550
Petrifilm	0.99468	0.99649	1.00000	0.99052	0.99018
Spiral P.	0.99672	0.99589	0.88052	1.00000	0.99978
ISOGRID	0.99664	0.99550	0.99018	0.99978	1.00000

Table 9a. Comparative Aerobic Counts of Whole Wheat Flour by Conventional, Redigel, Petrifilm, Spiral Plate and ISOGRID

Sample #	SPC	REDIGEL	PETRIFILM	SPIRAL P.	ISOGRID
1	4.74036	4.75587	4.73640	4.74273	4.74390
2	4.24304	4.26717	4.24304	4.26126	4.25406
3	4.02119	4.02119	3.92942	4.08279	4.08991
4	4.29003	4.29003	4.30103	4.31492	4.32118
5	4.70329	4.70329	4.68574	4.69548	4.69775
6	4.20412	4.20412	4.16137	4.19033	4.20140
7	4.53782	4.53148	4.51188	4.53212	4.53656
8	4.25527	4.27875	4.24304	4.28780	4.29115
9	4.19033	4.21748	4.19033	4.16435	4.16879
10	4.65801	4.65801	4.63347	4.65514	4.65706
11	4.55630	4.55023	4.55023	4.54962	4.55328
12	4.84510	4.83885	4.83569	4.82834	4.83251
13	4.43933	4.44716	4.44716	4.42078	4.42406
14	4.69461	4.68574	4.69020	4.71349	4.71054
15	4.31175	4.31175	4.30103	4.32325	4.34143
16	4.16137	4.20412	4.20412	4.19312	4.19451
17	4.49136	4.48430	4.49831	4.49136	4.50106
18	4.76343	4.76716	4.75967	4.76380	4.76530
19	3.97772	4.04139	4.00000	4.03543	4.04922
20	4.26717	4.26717	4.27875	4.26126	4.26717

Table 10. Total Cost Analysis Per Plate (Per Viable Cell Count[3])

System	Material & Media Cost	Labor Costs	Total Cost
Standard Plate Count	$2.06 (12.36)	.21 (1.26)	2.27 (13.62)
Redigel[1]	1.16 (6.96)	.21 (1.26)	1.37 (8.22)
Petrifilm[1]	1.16 (6.96)	.21 (1.26)	1.37 (8.22)
Spiral Plate System*	2.06 (2.06)	.21 (.21)	2.27 (2.27)
ISOGRID*[2]	3.01 (3.01)	.32 (.32)	3.33 (3.33)

Notes:
*Does not include initial cost of equipment (Spiral Plate System ranges from $11,700 to $12,500 including the plater, vacuum system and colony counter; ISOGRID ranges from $2,500 to $4,000 including the line counter, vacuum system, 12 filter heads, 3 clamps and 100 filters. Approximate costs as of 3-1-88)
1. Cost per plate is reduced by quantity purchase
2. Does not reflect possible enzyme pretreatment before filtration-cost averages 30¢ per sample for enzyme treatment
3. Assumes an average of six plates for one viable cell count at necessary dilutions.

CONCLUSIONS

In conclusion, all five of the systems evaluated were highly comparable and exhibited a high degree of accuracy and agreement. Cost per system was also quite comparable however factors such as multiple plating of single samples or need for duplicate plates must be taken into consideration when determining the most cost efficient system. All systems correlated with one another to a very high degree and as such are statistically comparable to the same degree. This is the first simultaneous comparison of four new systems against the conventional system using the same food samples in the same laboratory.

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COMPARISON OF REDIGEL, PETRIFILM, SPIRAL PLATE SYSTEM, ISOGRID AND STANDARD PLATE COUNT FOR THE AEROBIC PLATE COUNT ON SELECTED FOODS

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ABSTRACT

Presently the Standard Plate Count (SPC) method is highly recognized as the standard method for enumeration of total aerobic bacterial count in foods. Several other methods have also been developed for the enumeration of total aerobic bacterial count. Some of these methods are able to more readily monitor samples at very low or very high microbial loads.

Standard Plate Count (SPC) is based upon the principle that each viable organism in a sample will produce a single colony thus providing enumeration of viable microorganisms per milliter or gram of the food sampled. However, this method was not considered as accurate as hydrophobic grid membrane filters (ISOGRID) utilizing MPN, for the monitoring of samples with low microorganism counts. Some of the same problems occur at the opposite end of the spectrum, or samples with high counts. A system designed to compensate for such counts is the computer assisted spiral bioassay system (Spiral Plate System) which automatically dilutes a sample during plating. This allows for distribution of colonies in a sample with large microbial loads by the development of increasingly dilute sections on a single plate which may be counted manually or by a special laser counter. The Spiral Plate System also facilitates plating due to its automatic dilution during plating thus shortening time normally required in the dilution process for plating samples.

Other systems such as Petrifilm and Redigel were developed to duplicate the counts obtained with Standard Plate Count but with more ease of use and application. The Petrifilm can be utilized anyplace as it requires no liquid agar with the diluent simply being placed between the sheet assembly that contains the rehydratable media. Redigel is also easy

to utilize as it comes in premeasured sterile tubes and pretreated plates. The tubes require no melting and can be maintained at room temperature or refrigerated for longer shelf life.

This study tested the reliability of these alternate systems against the Standard Plate Count method by evaluation of seven foods (chicken breast, ground beef, ground pork, raw milk, thyme, pecans and whole wheat flour, twenty samples each) by all five systems. Standard plate count agar and nutrients were used for all of the samples. Incubation was at 35C for 48 hours.

The results indicated a 0.9+ correlation coefficient between and among all five systems studies, indicating that the alternative systems are as reliable as the Standard Plate Count method on the foods tested.