EVALUATION OF THE 3M PETRIFILM ™ METHODS FOR DETERMINING THE BACTERIOLOGICAL QUALITY OF NATURALLY CONTAMINATED ICE CREAM MIXES, PASTEURIZED WHOLE EGG AND NOVELTIES

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ABSTRACT

Petrifilms standard method (PSM) and coliform (PVRBA) were compared to the standard method (SPC) and VRBA for enumerating total bacterial counts and coliforms for a total of 400 samples (20 - 30 per week) of ice cream mixes (10,14 % fat) custard mixes, pasteurized whole, and white eggs, 18, 24 oz cheese cakes, weight watchers (minis), novelties (ice cream sandwiches), cookies and ice cream pellets. Petrifilms SM and PVRBA were found to be highly correlated to the standard methods with correlation coefficients of 0.9341, 0.992, 0.998, 0.998, 0.999, 0.994, 0.996, 0.896, 0.995, slopes of 0.961, 0.969, 0.987, 0.992, 1.002, 0.963, 0.950, 0.929, 1.00 and intercepts of 0.066, 0.073, 0.066, 0.047, 0.009, 0.141, 0.140, 0.175, 0.003 for ice cream mix, ice cream sandwich, custard mixes, pasteurized whole eggs, pasteurized white eggs, 18 and 24 oz cheese cakes, minis (Weight watcher), cookies and ice cream pellets, respectively. The slopes and intercepts of the two compared methods appeared not significantly different from 1.0 and 0.0 respectively. Most of the products used did not contain countable coliforms. 3 out of 50 ice cream pellets, 4 out of 50 the minis were found to be positive for coliforms by both methods (VRBA and PVRBA). There was very good correlation between the two methods. The correlation coefficient, were 0.992 and 0.999, the slopes were 1.00 and 0.996 and the intercepts were 0.00, 0.004 for the minis and the pellets respectively. When all 400 samples were grouped for analysis, there were no significant differences between the two methods (SPC/ PSM and VRBA/PVRBA), the correlation coefficients were 0.991 and 0.999, the slopes were 0.973 and 0.996 and the intercepts were 0.08 and 0.004. The mean log counts were not statistically different 2.510 ± 1.188 / 2.531± 1.167 and 1.948 ± 0.620 / 1.946 ± 0.618, respectively. So, the petrifilm system can be effectively used in enumerating the micro flora in a wide variety of dairy product.

INTRODUCTION

Conventional and unconventional methods are widely used for counting pathogen and groups of microorganisms, such as coliform, yeast and molds and aerobic microorganisms in foods. But the needs for alternative methods in food microbiology that facilitate laboratory tasks and dramatically reduce the time, space and labor needed to obtain results are increasing.

The microbial quality of manufactured milk products is routinely assessed by plate count [the standard plate count (SPC)], which is used to estimate microbial population in dairy products, (**Messer *et al.*, 1989**). The coliforms test using violet red bile agar (VRBA) is used to evaluate the efficacy of production procedure and designed to minimize microbial contamination in processed dairy product and to detect recontamination of milk, cream and processed dairy products, (**Hartman and LaGrange, 1985**). The presence of high bacterial count or coliforms in dairy products is an indication of poor sanitation procedure and improper handling practices.

Petrifilm developed by 3M companies reduces the time and effort to enumerate bacteria as compared to the conventional poured methods. Colony counts can be taken on a small and sample ready plates without the need for the preparation of culture media, the square surface (20 cm^2) and the color marker (2,3,5-triphenyltetrazolium chloride indicator dye) highlight the colonies (**Linton *et al.*, 1997**). The different methods of petrifilm products focus on the detection and enumeration of some of the more common microorganisms needed to be detected in routine microbiological food control, such as aerobic bacteria, coliforms and Escherichia coli (**Bloch *et al.*, 1996; Smith *et al.*, 1989 and Priego *et al.*, 2000**). The petrifilm method has been collaboratively studied and is an approved official first action AOAC method for use with raw and pasteurized milk (**Ginn *et al.*, 1986**). Also American Public Health Association (APHA) has proved this method for inclusion in Standard Methods for the examination of the dairy products as an alternative microbiological method.

This study was undertaken to evaluate the efficacy of petrifilm as acceptable methods for the enumeration of total aerobic colony forming units and coliforms in ice cream mixes (10, 14 % fat), ice cream sandwiches, custard mixes, pasteurized whole and white eggs, 18, 24 oz cheese cakes, weight watchers cheese cakes (minis), cookies and ice cream pellets.

MATERIAL AND METHODS

A total of 400 samples (20 - 30 per week) of ice cream mixes, (10, 14 % fat) custard mix, pasteurized whole, and white eggs, 18, 24 oz cheese cake, weigh watchers (minis), novelties (ice cream sandwiches), cookies and ice cream pellets were received from local manufacturer and stored at 4° C immediately upon arrival. Testing was done within 24 hours of receipt.

Aerobic Plate Count:

A aseptically 11 grams of the samples were weighted into sterile wide mouthed containers; such as (stomacher bags and whirl pack bag), 99 ml of IDF phosphate buffer (0.0425 g/L of KH_2PO_4) were added. The sample and buffer were stirred to make homogenous fluid resulting in 1:10 dilution sample.

Petrifilms were placed on level surface left top up with pipette perpendicular to petrifilm plate, 1 ml portion of each dilution was plated on Standard Plate Count Agar (SPC, Difco), and into the center of bottom film of the petrifilm ^{TM} Standard Method (PSM, 3M health care, St. Paul, MN), carefully top film rolled to avoid entrapping air bubbles, avoiding the top to be dropped. With flat spreader on top film over inoculum gently pressure was applied to distribute inoculum over circular area before gel is formed, avoiding twisting or sliding the spreader. We waited minimum of one minute for gel to solidify then incubated at 35 ± 1° C for 48 hours ± 2 hours in the horizontal position without inverting. As many as 10 plates can be stacked on top of each other. All colonies were counted regardless of color. Results expressed in $\log_{10}$ CFU/g.

Coliforms count:

The same previous 1:10 dilution was used. A 1 ml portion of each dilution was plated on standard Violet Red Bile Agar (VRBA) which incubated at 30 - 32° C for 24 hours and examined for identification of red colonies, confirmation was made by observation of gas production at 30° C in brilliant green bile broth 2% (Difco) after 24 hours and 48 hours incubation. Petrifilm coliforms (PVRBA) were plated following manufacturer's direction. The plates were incubated at 30 - 32° C for 24 hours. Any gas produced by coliforms fermenting lactose is trapped around the colony by the film, differentiating them from other non-lactose fermenting gram negative colonies. Results expressed in $\log_{10}$ CFU/g.

Statistical analysis:

Bacterial counts were converted to $\log_{10}$ counts to correct the approximate normality and to more meet the assumption of normal distributed data. Regression analysis is a system frequently used to describe relationships between two methods. Mean of $\log_{10}$ colony forming units (mcfu/ml) were calculated for SPC/PSM and VRBA/PVRBA. The data are plotted and the best fitting straight line is fitted to the data. If the two methods are exactly the same, then this straight line has a slope of 1.0, an intercept of 0, and a correlation coefficient of 1.0. Actual data varies from this ideal; therefore with a data set, we test whether the calculated values of the slope, intercept and correlation coefficient are statistically different from the ideal values of 1.0, 0.0, and 1.0, respectively, 95 % of the time, the line will be between the

95 % confidence limits. All the statistical data processing was performed using statistical electronic software.

RESULTS AND DISCUSSION

Table (1): Statistical analysis of total bacterial counts using standard plate count (SPC) and petrifilm standard method (PSM).

Type	Size of samples	Mean log_{10} CFU/g ± S.D. (SPC)	Mean log_{10} CFU/g ± S.D. (PSM)	Mean log difference	Correlation coefficient	Slope	Intercept
Ice cream mixes	50	2.172 ± 0.851	2.220 ± 0.820	0.048	0.934	0.961	0.006
Ice cream sandwiches	60	1.949 ± 0.557	1.964 ± 0.544	0.015	0.992	0.969	0.073
Custard mixes	30	2.571 ± 1.604	2.702 ± 1.548	0.131	0.998	0.987	0.066
Pasteurized whole eggs	20	2.713 ± 0.989	2.894 ± 0.832	0.181	0.998	0.992	0.047
Pasteurized white eggs	40	3.470 ± 1.407	3.660 ± 1.226	0.190	0.999	1.002	0.009
18 & 24 oz cheese akes	50	3.608 ± 0.283	3.616 ± 0.274	0.008	0.994	0.963	0.141
Minis (WW)	50	2.420 ± 0.560	2.447 ± 0.531	0.027	0.996	0.950	0.140
Cookies	50	0.631 ± 0.702	0.789 ± 0.730	0.158	0.896	0.929	0.175
Ice cream pellets	50	3.556 ± 0.337	3.567 ± 0.338	0.011	0.995	1.00	0.003
Total	400	2.510 ± 1.188	2.531 ± 1.167	0.021	0.991	0.973	0.088

SD: Standard deviation

CFU: Colony forming unit

Table (2): Statistical comparison of total coliform count using standard violet red bile agar (VRBA) and petrifilm coliforms (PVRBA).

Type	Size of samples	Mean log_{10} CFU/g ± S.D. (VRBA)	Mean log_{10} CFU/g ± S.D. (PVRBA)	Mean log difference	Correlation coefficient	Slope	Intercept
Ice cream mixes	50	0	0	0	0	0	0
Ice cream sandwiches	60	0	0	0	0	0	0
Custard mixes	30	0	0	0	0	0	0
Pasteurized whole eggs	20	0	0	0	0	0	0
Pasteurized white eggs	40	0	0	0	0	0	0
18 & 24 oz cheese akes	50	0	0	0	0	0	0
Minis (WW)	50 (4 +ve)	2 ± 0.480	2.041 ± 0.502	0.027	0.992	1.00	0
Cookies	50	0	0	0	0	0	0
Ice cream pellets	50 (3+ve)	2.301 ± 0.208	2.370 ± 0.338	0.011	0.999	0.996	0.004
Total	400	1.948 ± 0.620	1.946 ± 0.618	- 0.002	0.999	0.997	0.005

SD: Standard deviation

CFU: Colony forming unit

Results of the statistical analysis for a total of 400 samples tested are summarized in Tables 1 and 2 and Figures 1 and 2. Petrifilm-SM gave a correlation coefficient with the standard plate count of 0.9341, 0.992, 0.998, 0.998, 0.999, 0.994, 0.996, 0.896 and 0.995 for ice cream mixes, ice cream sandwiches, custard mixes, pasteurized whole eggs, pasteurized white eggs, 18 & 24 oz cheese cakes, minis (weight watchers), cookies, and ice cream respectively. This meant that they were highly correlated. But proper comparison of microbial enumeration methods require not only correlation value evaluation, but it is imperative that one consider the validity of the line of best fit which better represent the true value of the obtained data. This can be accomplished by evaluating the slope and the intercept of such a line (Figure 1), and by reviewing the mean log difference. If the mean log difference is unacceptably large, and if the slope and the intercept are much removed from 1.00 and 0.00 respectively, the correlation coefficient is of a little value.

Table (1) lists the slopes and the intercepts of the two compared methods which appeared that they were not significantly different from 1.0 and 0.0 respectively. Slopes were 0.961, 0.969, 0.987, 0.992, 1.002, 0.963, 0.950, 0.929, 1.00, while intercepts were 0.066, 0.073, 0.066, 0.047, 0.009, 0.141, 0.140, 0.175, 0.003 for the ice cream mixes, ice cream sandwiches, custard mixes, pasteurized whole eggs, pasteurized white eggs, 18 & 24 oz cheese cakes, minis (weight watcher), cookies and ice cream pellets respectively. Most of the samples had very low bacterial counts and thus low dilutions were made. It was very easy to count the colonies on PSM because the 2,3,5-triphenyltetrazolium stains the colonies red and white background. Type of the products had no effect on the recovery of bacteria on the petrifilm method as compared to the standard method.

The two methods for enumerating coliforms were compared statistically (see Table 2). Most of the products used did not contain countable coliforms. 3 out of 50 ice cream pellets, 4 out of 50 minis were found to be positive for coliforms by both methods (VRBA and PVRBA). Colonies were confirmed by the brilliant green bile broth 2%. There was very high correlation between the two methods. The correlation coefficients were 0.992 and 0.999, the slopes were 1.00 and 0.996 and the intercepts were 0.00 and 0.004 for the minis and pellets respectively. When all 400 samples were grouped for analysis, there were no significant differences between the two methods (SPC/PSM and VRBA/PVRBA). The correlation coefficients were 0.991 and 0.999, the slopes were 0.973 and 0.996 and the intercepts were 0.08 and 0.004 respectively. The mean log counts were not statistically different 2.510 ± 1.188, 2.531 ± 1.167 and 1.94 ± 0.62, 1.94 ± 0.61 respectively (See Tables 1,2 and Figures 1,2).

Our results were inagreement with **McAllister *et al.*, (1987)** who found that there were no significant differences between petrifilm versus the standard method, the correlation coefficient was 0.98, the slope was 1.01 and the intercept was 0.02, and the mean log counts were not significantly different in enumerating bacteria in processed fluid milk samples.

Smith *et al.*, (1989) compared the petrifilm methods to the standard method in frozen dessert products and they found that the frozen dessert mixes did not contain countable coliforms, therefore, each sample inoculated with naturally occurring coliforms which had been isolated from raw milk. The authors found that the standard VRBA produced significantly higher counts than the PVRBA, and this was not inagreement with that we found in our study as both methods VRBA /PVRBA yield almost the same CFU counts 1.948 ± 0.620 and 1.946 ± 0.618 respectively. This might be because there is only few samples are containing coliforms.

Jordano and Medina (1999) detected the presence of coliforms in 66.69 % of the food studied (pasteurized milk, raw minced meat, fresh straw berries and a frozen green beans) and counts taken with petrifilm coliform count were higher than those achieved with VRBA with a close correlation between the two methods.

Priego *et al.*, (2000) studied six batches of different food like egg, green bean, fresh sausage, raw minced meat and raw milk, a close correlation was found between petrifilms and standard method in counting coliforms (correlation coefficient = 0.860) and greater sensitivity as 93.33 % of the samples displayed higher count on petrifilm.

The petrifilm TM standard method and the PVRB are more advantageous to use than the standard methodology in the quality control laboratories. There is no media preparation required for the petrifilm, the incorporation of the indicator dye into the plate, and the formation of a gas bubble adjacent to a coliform colony on the PVRB plate aid in the identification of valid microbial colonies. The plates can be stacked and incubated in considerably less space than the standard petri dishes, less labor, more convenient and accurate.

So, our conclusion is that the petrifilm system can be effectively used in enumerating the microflora in a wide variety of dairy product and approved to be successful method in monitoring their quality.

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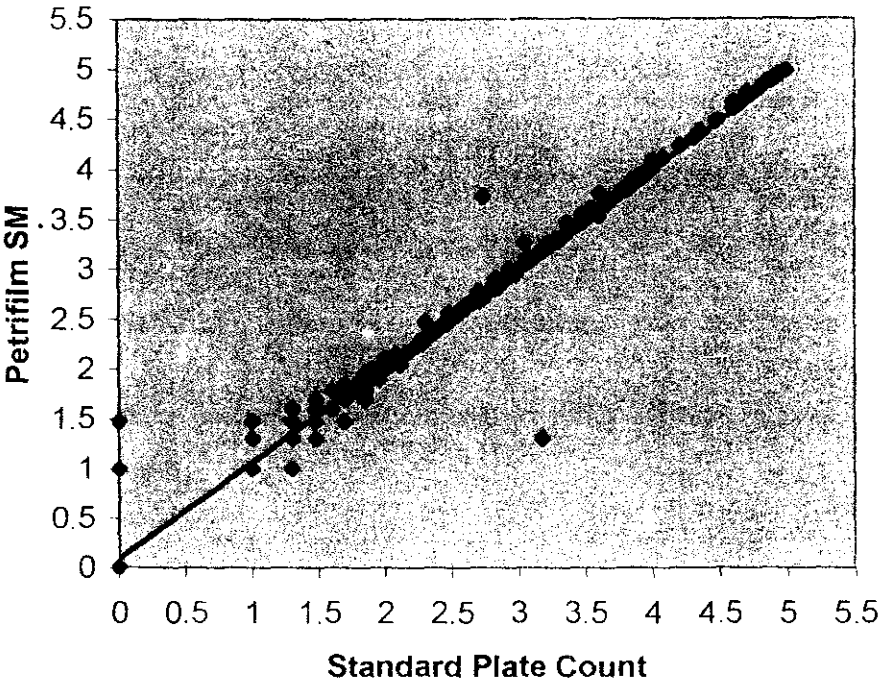

Fig. (1): All samples: Regression line and the relationship between the standard plate count and the PSM. (Petrifilm method plotted against the standard Plate count (in log_{10} cfu/g)

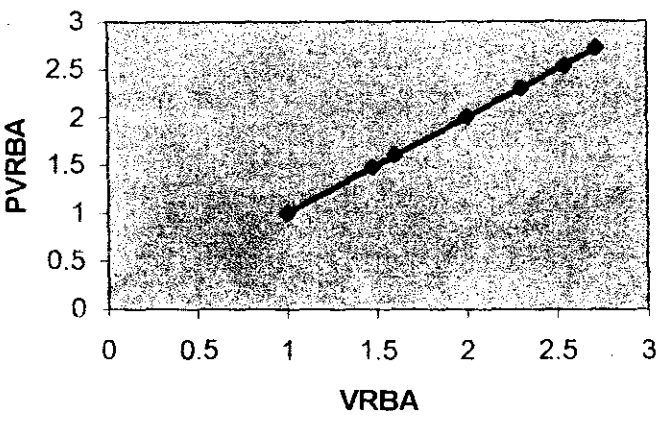

Fig. (2): Relationship and the regression between VRBA and petrifilm VRBA log_{10} CFU.

الملخص العربي

تقييم طريقة البتريفيلم لمعرفة الجودة البكتريولوجية لبعض منتجات الألبان والبيض

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تمت المقارنة بين طريقة البتريفيلم والطريقة القياسية في تقيم العدد الكلى للبكتريا ومجموعة الكوليفورم لعدد ٤٠٠عينة من الآيس كريم و الكاستر و البيض و كيكة الجبنة و غيرها و أثبتت النتائج أنه لا يوجد فرق واضح بين الطريقتين في العد ولذلك يمكن استخدام طريقة البتريفيلم على نطاق واسع في عد البكتريا المختلفة في منتجات الألبان.