

Katia Leani O. Souza², ADRIANA R. TASSINARI¹; Maria Teresa Destro²; Bernadette D. G. M. Franco²; Mariza Landgraf²

¹ 3M do Brasil Ltda

² Faculty of Pharmaceutical Sciences, University of Sao Paulo, Brazil

ABSTRACT

Traditional methodologies used for enumeration of psychrotrophic microorganisms in food are time consuming, usually taking up to 10 days. There are very few studies suggesting the reduction of this time by increasing incubation temperatures, however, one of these suggestions, found at American Public Health Association [APHA], uses the combination of 25 h at 21°C for milk and cream. 3M™ Petrifilm™ Aerobic Count [AC] Plates have been designed to be used in the enumeration of mesophilic microorganisms, but to date, there has been little published data for enumeration of psychrotrophs.

This study was initiated to evaluate the efficacy of incubation for 25h at 21°C for the enumeration of psychrotrophs in naturally contaminated dairy product samples, and to verify the usefulness of 3M Petrifilm AC plate under these conditions.

Seventy-four dairy product samples (cheeses, raw and pasteurized milk) were simultaneously analyzed using three methods: APHA recommendations for psychrotrophs - Plate Count Agar [PCA] at 7°C/10d; PCA at 21°C/25h and 3M Petrifilm AC plates at 21°C/25h. The results were compared, submitted to Kruskal-Wallis test (Minitab® Release 14.20 Statistical Software) and linear regression. For statistical purposes, only samples showing <1 log CFU/ml by all three methods were considered equal to 1 log.

Results analyzed using Kruskal-Wallis test and linear regression showed that counts between traditional media (PCA) using both incubation conditions were not statistically different ($p > 0.05$; $R^2 = 94.5\%$). The R^2 for the comparison between 3M Petrifilm AC plates and PCA at 21°C/25h and 7°C/10d were 95.9% and 92%, respectively; and the p-value was > 0.05 for both.

3M Petrifilm AC plates show promise as an alternative method to the traditional enumeration of psychrotrophs in dairy products, when 21°C/25h is used. These findings would help the dairy industry obtain results in a more timely and easy manner. Additional studies to verify these conclusions and application are recommended.

INTRODUCTION

In Brazil, like other countries, the milk in bulk must be immediately refrigerated after milking in the farm at temperature below 7°C. The transportation must be refrigerated and the product can't arrive at the plant with the temperature above 10°C (Brasil, 2002).

The maintenance of milk in cold chain makes with the only microorganisms capable of multiplying themselves are the psychrotrophs. There are many species of psychrotrophic microorganisms, but the most common in the raw milk are Gram negative rods (*Pseudomonas*, *Acinetobacter*, *Alcaligenes*), besides coliforms and Gram positive microorganisms like *Bacillus*, *Micrococcus* and *Enterococcus* (Cousin, 1982).

The storage of large volumes of raw milk in the dairy industry at temperatures between 1 and 6°C favors psychrotrophic bacteria development and may result in undesirable changes in the milk itself and milk products. The bacteria presence indicates that unsatisfactory conditions occur during the whole process of handling the milk from milking to the arrival at the dairy industry.

The group of microorganisms called psychrotrophic even in temperature below 10°C finds a favorable environment for its multiplication. The term psychrotrophic microorganisms in the dairy scenery comprises different genus that are able to present a considerable growth in the milk and dairy products, even refrigerated at temperatures from 2°C to 10°C, independently of its excellent growth temperature.

This group, besides substantial growth is able to produce enzymes that can cause negative alteration in the milk properties. These enzymes, lipases e proteases, are not easily destroyed by the thermal processes where milk is submitted. For being considered thermo-resistant, even in low concentrations, the actions of these enzymes change the flavor either the milk or dairy products storage under refrigeration (Bishop, White, 1986).

Examples of the time and temperature of incubation for psychrotrophic traditional plate counts are 10 days at 7°C for pour plates or 7 to 8 days at 7°C for spread plates and 16 h at 17°C followed by 3 days at 7°C, according to American Public Health Association [APHA].

Traditional methodologies used for enumeration of psychrotrophic microorganisms in food are time consuming, usually taking up to 10 days. There are very few studies suggesting the reduction of this time by increasing incubation temperatures, however, there are some suggestions using the combination 21°C/25 h for milk and cream, 18°C/45 h for milk and 25°C/24 h for meat. However, these time/temperature combinations have not been validated, so the incubation at 7°C for 10 days is still considered standard (APHA, 1992; Cousin, Jay, Vasavada, 2001).

3M™ Petrifilm™ Aerobic Count Plates have been designed to be used in the enumeration of mesophilic microorganisms, but to date, there has been little published data for enumeration of psychrotrophs.

Bailey, Cox (1987) showed that Petrifilm plates presented equivalent results with traditional method for enumerating psychrotrophic microorganisms in poultry. Specific studies using milk and dairy products have not been found.

OBJECTIVE

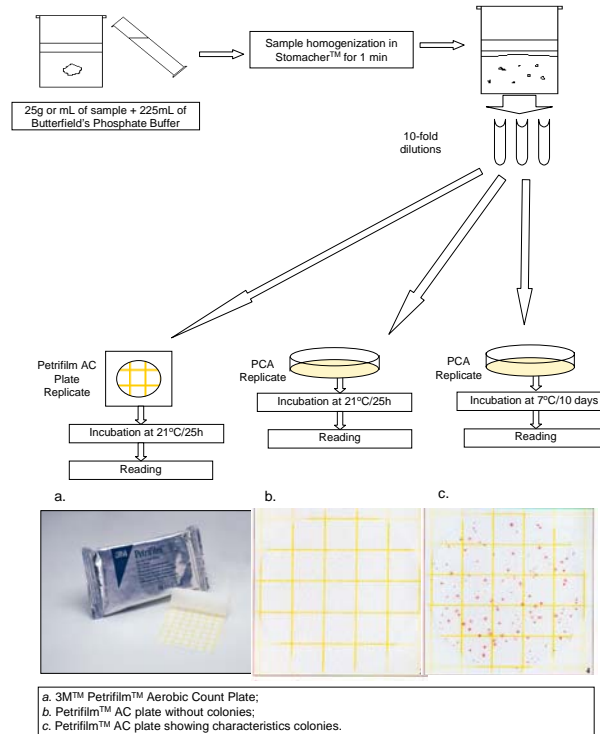
To evaluate the efficacy of incubation for 25h at 21°C for the enumeration of psychrotrophs in naturally contaminated dairy product samples, and to verify the usefulness of 3M Petrifilm AC plate under these conditions.

MATERIALS AND METHODS

Amongst the 74 food samples examined, 68 samples were considered in this study:

Cheese – 17
Pasteurized Milk – 23
Raw Milk – 28

These samples were bought in bakeries, supermarkets, farms in the Sao Paulo and Cunha cities, Sao Paulo State.



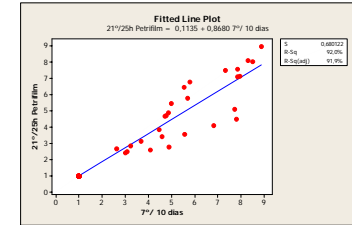
REFERENCES

1. APHA. Standard Methods for the Examination of Dairy Products. 16. ed. Washington: APHA, 1992:546p.
2. Bailey, J. S., Cox, N. A. Evaluation of the Petrifilm SM and VRB dry media culture plates for determining microbial quality of poultry. J. Food Prot. 50:643-644, 1987.
3. Bishop, J. R., White, C. H. Assessment of dairy product quality and potential shelf-life – a review. J. Food Prot. 49:739-753, 1986.
4. Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa 51. 18 de setembro de 2002.
5. Cousin, M. A. Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. J. Food Prot., 45:172-207, 1982.
6. Cousin, M. A., Jay, J. M., Vasavada, P. C. Psychrotrophic microorganisms. In: Downes, F. P., Ito, K. ed. Compendium of Methods for the Microbiological Examination of Foods. 4. ed. APHA, 2001.
7. International Dairy Federation. Milk and milk products – general guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbial examination. IDF122, 2nd ed. 2001.

RESULTS AND DISCUSSION

A total of 68 samples were submitted to the statistical analysis. The results were compared, submitted to Kruskal-Wallis test (Minitab® Release 14.20 Statistical Software) and linear regression. For statistical purposes, only samples showing <1 log CFU/ml by all three methods were considered equal to 1 log.

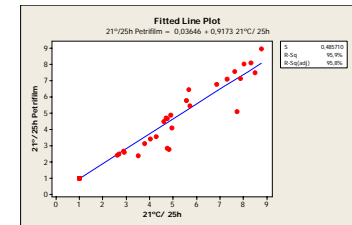
FIGURE: LINEAR REGRESSION OF 3M PETRIFILM AC PLATE (21°C/25h) AND PCA (7°C/10 DAYS)



Results analyzed using Kruskal-Wallis test and Pearson Correlation for this comparison showed that counts were not statistically different (p-value = 0,5; Correlation = 0.959, p-value = 0,00).

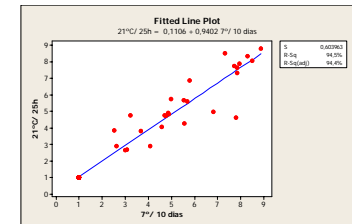
The comparison between 3M Petrifilm AC plates and PCA at 21°C/25h showed that counts were not statistically different also (p-value = 0,715; Correlation = 0.979, p-value = 0,00), as shown in the Figure below.

FIGURE: LINEAR REGRESSION OF 3M PETRIFILM AC PLATE (21°C/25h) AND PCA (21°C/25h)



The comparison between PCA at 7°C/10 days and PCA at 21°C/25h showed the same results as the previous one (p-value = 0,758; Correlation = 0.972, p-value = 0,00), as shown in the Figure below.

FIGURE: LINEAR REGRESSION OF PCA (7°C/10 DAYS) AND PCA (21°C/25h)



The use of Petrifilm plates as a rapid method makes easy the work in the lab, besides resulting in reliable data due to eliminating the mistakes from medium preparation and material sterilization.

CONCLUSION

3M Petrifilm AC plates show promise as an alternative method to the traditional enumeration of psychrotrophs in dairy products, when 21°C/25h is used. These findings would help the dairy industry obtain results in a more timely and easy manner. Additional studies to verify these conclusions and application are recommended.