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Note

Comparison between Colifast® Milk and the standard method for the detection of coliforms in pasteurised milk

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Abstract – Colifast® Milk is a rapid screening test for the detection of total coliforms in milk based upon the measurement of change in fluorescence during an incubation period, due to the targeted β-D-galactosidase activity on 4-methyl-umbelliferone-β-D-galactoside contained in a selective growth medium. In this work, 800 samples of homogenised pasteurised milk, with different fat content (1.5 and 3.5%) and contaminated with various concentrations of coliforms (from 0.03 to > 10000 CFU·mL^{-1}), were analysed in order to compare the results obtained by Colifast® Milk with those by the standard method. The effect of the incubation temperature (30 and 39 °C) was also investigated. For the totality of samples the correspondence between coliform counts obtained by the two methods was 64% (r^2 = 0.743; P = 0.88). The performance of Colifast® Milk was notably affected by the level of contamination, since for samples with coliforms > 10 CFU·mL^{-1} the correspondence between the two methods achieved 86% (r^2 = 0.837; P = 0.94) whereas for samples with coliforms ≤ 10 CFU·mL^{-1} it fell to 42% (r^2 = 0.073; P = 0.33). Fat content also influenced the response of the Colifast® system since the correspondence decreased from 80% (r^2 = 0.767; P = 0.91) for semi-skimmed milk to 48% (r^2 = 0.724; P = 0.63) for whole milk. Incubation at 30 °C improved the recovery of coliforms by Colifast® Milk as the correspondence between values obtained with the two methods reached 72% (r^2 = 0.760; P = 0.89) if compared with 56% (r^2 = 0.735; P = 0.87) when the incubation temperature was 39 °C. Under these operating conditions the sensitivity showed by the fluorometric method appeared to be not sufficient for the detection of coliforms in pasteurised milk.

Colifast® Milk / coliform / method / milk

Résumé – Comparaison entre le Colifast® Milk et la méthode standard pour la détection des coliformes dans le lait pasteurisé. Colifast® Milk est un test de culture rapide pour la détermination des coliformes totaux dans le lait, basé sur la mesure du changement de fluorescence, pendant une période d'incubation, dû à l'activité spécifique de la β-D-galactosidase sur le 4-methylumbelliferone-β-D-galactoside contenu dans un milieu sélectif de croissance. Dans ce

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travail, 800 échantillons de lait homogénéisé pasteurisé, à différentes teneurs en matière grasse (1,5 et 3,5 %) et contaminés avec différentes concentrations de coliformes (de 0,03 à > 10000 UFC·mL^{-1}), ont été analysés pour comparer les résultats obtenus par le système Colifast® avec ceux obtenus par la méthode standard. L'effet de la température d'incubation (30 et 39 °C) a été aussi étudié. Sur l'ensemble des résultats, la correspondance entre les comptages de coliformes obtenus par les deux méthodes était de 64 % (r^2 = 0,743 ; P = 0,88). La performance du Colifast® Milk est considérablement influencée par le niveau de contamination en coliformes puisque pour les échantillons contenant plus de 10 UFC·mL^{-1} la correspondance entre les deux méthodes atteignait 86 % (r^2 = 0,837 ; P = 0,94), alors que pour ceux avec un taux ≤ 10 UFC·mL^{-1} elle tombait à 42 % (r^2 = 0,073 ; P = 0,33). De plus, la quantité de matière grasse a influencé la réponse du système Colifast®, la correspondance étant de 80 % (r^2 = 0,767 ; P = 0,91) pour le lait demi-écrémé et seulement de 48 % (r^2 = 0,724 ; P = 0,63) pour le lait entier. L'incubation à 30 °C a amélioré la récupération des coliformes par Colifast® Milk car la correspondance entre les valeurs obtenues avec les deux méthodes atteignait 72 % (r^2 = 0,760 ; P = 0,89) au lieu de 56 % (r^2 = 0,735 ; P = 0,87) pour une température d'incubation de 39 °C. Dans ce procédé analytique, la sensibilité de la méthode fluorométrique ne paraît pas suffisante pour la détection des coliformes dans le lait pasteurisé.

Colifast® Milk / coliforme / méthode / lait

1. INTRODUCTION

Ubiquity and sensitivity to heating makes coliforms precious marker organisms to assess the level of hygienic conditions and the efficacy of pasteurisation in dairy production. The presence of coliforms in pasteurised milk can be substantially originated from two events: an insufficient heat treatment, that frequently relies on a low microbiological quality of raw milk, principally in unfavourable geographical areas [16, 17]; or a contamination of pasteurised product during packaging despite the surveillance programs of hygiene control throughout the process [1–3].

Standard methods for the detection of coliforms [9–11, 18] need 24–48 h to get the results; meanwhile, the milk packs are already sold and consumed. Since 1990 a remarkable development of alternative methods for the detection of coliforms in foods has occurred [5, 7, 9, 13, 14], having the purpose of shortening the time of the analytical response. Some rapid techniques exploit the enzymatic activity of coliforms on fluorogenic substrates, such as 4-methylumbelliferyl-β-D-galactoside (MU-Gal) and 4-methylumbelliferyl-β-D-glucoronoside (MU-Glu), monitoring the change of fluorescence in samples incubated under standard conditions in a selective medium. Some authors [8, 12, 13] have observed a good correlation between the concentration of free 4-methylumbelliferone (MU) following the hydrolysis of the fluorogenic substance and the coliform counts detected by standard methods. The Colifast® method, proposed by Colifast® Systems ASA [6, 15], applies this principle to estimate the number of coliforms in water and milk samples within a few hours.

The aim of this work was to verify the reliability of the Colifast® Milk method for the enumeration of the coliforms in pasteurised milk, comparing the analytical results of the rapid method with those obtained by the reference method (IDF Standard 73B: 1998) [11].

2. MATERIALS AND METHODS

2.1. Milk samples

1 L samples of homogenised pasteurised milk were contaminated or not with various volumes (0.1, 1, 10, 100 mL) of raw milk in order to obtain different counts of coliforms. Particularly, 200 samples of whole milk (3.5% fat) with a low level of coliforms (≤ 10 CFU·mL^{-1}), 200 samples

of semi-skimmed milk (1.5% fat) with a low level of coliforms (≤ 10 CFU·mL^{-1}), 200 samples of whole milk (3.5% fat) with a high level of coliforms (>10 CFU·mL^{-1}) and 200 samples of semi-skimmed milk (1.5% fat) with a high level of coliforms (>10 CFU·mL^{-1}) were examined.

2.2. Enumeration of coliforms

Each sample was tested with Colifast® Milk [6, 15] and the standard method [11] in order to evaluate the performance of the rapid system. According to the standard method a Most Probable Number (MPN) technique was used for milks with a presumptive low concentration of coliforms (≤ 10 CFU·mL^{-1}), whereas the plate count technique (VRBLA) was used for milks with a higher concentration of coliforms (>10 CFU·mL^{-1}). The enumeration of coliforms was contemporarily carried out by the Colifast® MicroDetector following the instructions of the manufacturer. The system utilises the fluorogenic detection of coliforms based upon the activity of the β-D-galactosidase hydrolysing the 4-methylumbelliferyl-β-D-galactoside present in the growth selective medium. A 30 mL milk sample was added to 10 mL of Colifast® Milk Medium and incubated at 39 °C (for 400 samples), as suggested by the supplier, or at 30 °C (for the other 400 samples), considering the temperature used in the standard method and the high incidence of psychrotrophic species in raw milk. After 1, 3, 7, 8 and 9 h of incubation, a 3 mL subsample was mixed with 100 μL of Colifast® Media Developer and the fluorescence originated was measured in a Colifast® Micro-Detector. The results are given in a presence/absence (P/A) format; the manufacturer emphasises that the system gives a response in a maximum of 9 h for 1 CFU·mL^{-1}. The calibration procedure was executed daily before operating, for each type of milk, according to the recommendations of the supplier. All materials employed were furnished by Colifast Systems ASA [6].

2.3. Statistical analysis

The performance of the rapid method was evaluated as per cent correspondence, comparing the number of samples with a positive result with the Colifast® Milk test with respect to that obtained with the standard method, for each type of milk incubated at different temperatures or levels of contamination. The values of coliforms detected in positive samples by both methods were submitted to regression analysis [4]. The influence of some factors such as coliform counts, fat content and temperature of incubation were investigated. The Fischer's test was used to estimate the level of significance. The Systat software version 5.0 (Systat Inc., Evareston, USA) was used for data elaboration.

3. RESULTS AND DISCUSSION

Samples were grouped by levels of contamination because Colifast® Milk gives results expressed as a range of values depending on the incubation time necessary to achieve a fixed detection limit of fluorescence [6]. For each level of coliform counts, the distribution of the number of samples obtained with Colifast® Milk compared with the number of samples investigated with the standard method is reported in Table I. In general for semi-skimmed milk incubated at 39 °C with Colifast® Milk, the correspondence between the two methods reached 58% (104 positive samples revealed by Colifast® Milk in comparison with 179 determined with the standard method). Particularly the correspondence decreased from 80%, for heavily contaminated samples (> 10000 CFU·mL^{-1}), to 20%, with milk showing coliform counts ≤ 10 CFU·mL^{-1} (only 16 positive samples with Colifast® Milk compared with 79 positive samples ascertained with the standard method). In the case of whole milk incubated at 39 °C with Colifast® Milk, the correspondence was 45% (83 positive samples determined by the rapid method in comparison with 184 positive

Table I. Comparison between standard method and Colifast® Milk method: distribution of the number of samples for each level of coliforms in milk with different fat content and incubated at two temperatures.

Level of coliforms by standard method (CFU·mL^{-1})		Type of milk	T (°C)	Number of samples	Number of samples at each level according to Colifast® method					
					A	B	C	D	E	F
A	> 10000	SS	39	25	20	5	0	0	0	0
B	1001–10000			26	1	19	6	0	0	0
C	101–1000			37	0	3	26	8	0	0
D	11–100			12	0	0	0	0	0	12
E	0.03–10			79	0	0	0	0	16	63
F	negative			21	0	0	0	0	0	21
A	> 10000	W	39	23	23	0	0	0	0	0
B	1001–10000			53	14	34	5	0	0	0
C	101–1000			15	0	1	2	4	0	8
D	11–100			9	0	0	0	0	0	9
E	0.03–10			84	0	0	0	0	0	84
F	negative			16	0	0	0	0	0	16
A	> 10000	SS	30	16	16	0	0	0	0	0
B	1001–10000			58	4	49	5	0	0	0
C	101–1000			17	0	1	14	2	0	0
D	11–100			9	0	0	0	6	0	3
E	0.03–10			87	0	0	0	0	84	3
F	negative			13	0	0	0	0	0	13
A	> 10000	W	30	15	13	2	0	0	0	0
B	1001–10000			50	5	32	11	2	0	0
C	101–1000			17	0	0	3	6	0	8
D	11–100			18	0	0	0	0	0	18
E	0.03–10			80	0	0	0	0	0	80
F	negative			20	0	0	0	0	0	20

SS = semi-skimmed milk; W = whole milk.

samples with the standard method). However, for samples with a high concentration of coliforms the correspondence achieved 100%, while it decreased when whole milk contained coliforms ≤ 10 CFU·mL^{-1}; in fact, at this level no positive sample was detected by Colifast® Milk whereas the standard method revealed 84 positive samples.

Considering that a temperature of 39 °C is too high to recover psychrotrophic coliforms and that the temperature of incubation for the standard method is 30 °C, another set of samples were analysed, reducing the temperature of incubation to 30 °C with Colifast® Milk. For semi-skimmed milk the correspondence between the two methods increased to 97%

(181 positive samples revealed by Colifast® Milk in comparison with 187 determined with the standard method). Also, at a level of coliforms ≤ 10 CFU·mL^{-1} the correspondence improved, reaching 97% (84 positive samples with Colifast® Milk compared with 87 positive samples ascertained with the standard method). Nevertheless, whole milk incubated at 30 °C with Colifast® Milk revealed a correspondence of 41% (74 positive samples with the rapid method in comparison with 180 positive samples determined with the standard method). Particularly, the correspondence decreased from 87% for samples showing counts > 10000 CFU·mL^{-1}, to zero for milk containing coliforms ≤ 10 CFU·mL^{-1} (no positive sample with Colifast® Milk in comparison with 80 detected with the standard method).

As Table I shows, independent of fat content, temperature of incubation or level of contamination, 288 samples resulted in a false negative with the Colifast® system, over all when low coliform counts were present in the milk. On the other hand, the percent of correspondence between the two methods achieved 100% for milk samples negative to coliforms (70 negative samples with Colifast® Milk corresponding to the same 70 negative samples with the standard method). This result excludes the presence of false positives by the Colifast® system and confirms the specificity of the Colifast® Milk Medium for the growth of coliforms.

The coefficients of determination between the values of coliforms for positive samples with both methods were calculated, considering the level of contamination, fat content and temperature of incubation. The correspondence and the coefficient of determination were good, respectively, 86% and r^2 = 0.837 (P = 0.94), when the number of coliforms present in milk was > 10 CFU·mL^{-1} (342 samples), while they became significantly poor (correspondence = 42%; r^2 = 0.073; P = 0.33) for concentrations ≤ 10 CFU·mL^{-1} (170 samples). As the sensitivity of Colifast® Milk for the enumeration of coliforms proves to be lower than the standard limit established for pasteurised milk, this system is not reliable for evaluating the conformity of the product to regulation.

The fat content, independently of the level of contamination and temperature of incubation, affected the performance of Colifast® Milk since the correspondence decreased from 80% for semi-skimmed milk to 48% for whole milk. The coefficient of determination between the values obtained with both methods was significantly higher (r^2 = 0.767; P = 0.91) for semi-skimmed milk (319 samples) than that found (r^2 = 0.724; P = 0.63) for whole milk (193 samples). Probably the fluorescent emission is quenched by a phenomenon of diffusion of the light due to fat globules. The amount of fat deriving from the addition of raw milk to enhance the coliform counts in the samples of homogenised pasteurised milk has been considered irrelevant in this occurrence; in fact, false negative results were found almost totally in samples showing low coliform counts, in which 1 mL of raw milk per litre at the most was added.

Finally, the change of incubation temperature from 39 °C to 30 °C does not significantly amend the accomplishment of Colifast® Milk, though a small increase in correspondence between the two methods took place. The recovery of coliforms by Colifast® Milk improved at 30 °C (288 samples), as the correspondence and the coefficient of determination, respectively, reached 72% and r^2 = 0.760 (P = 0.89) if compared with 56% and r^2 = 0.735 (P = 0.87) when the incubation temperature was 39 °C (224 samples).

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