American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 6 (11): 951-962, 2011
ISSN 1557-4571 / DOI: 10.3923/ajft.2011.951.962

Validation of the Micro Biological Survey Method for Total Viable Count and *E. coli* in Food Samples

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ABSTRACT

The aim of this study was the validation of the Micro Biological Survey (MBS) method for microbiological analysis of food for Total Viable Count (TVC) and *Escherichia coli* (*E. coli*). The MBS method is a rapid quantitative alternative method for the detection and selective counting of bacteria in agro-food, in water and in environmental samples. It is based on colorimetric survey in mono-use disposable reaction vials that must be filled with the samples without any preliminary treatment (e.g., homogenization, dilution, etc.); the greater the number of bacteria presents into the sample, the faster the color change. However, an independent evaluation of the analytical results obtained with MBS method would be required before commercialization. Therefore, this alternative method was validated in comparison to the reference method. The general estimate of precision, reliability, uncertainty, linearity, accuracy and selectivity were determined. All the performance parameters have demonstrated total correlation between the alternative method and the reference method for the detection and counting of TVC and *E. coli* both in artificially contaminated and in naturally contaminated samples. MBS assay can be used as rapid and user friendly screening method for detection of TVC and *E. coli* in food industry.

Key words: Food microbiological analysis, food safety, alternative microbiological method, total viable count, *E. coli*

INTRODUCTION

Microbial analysis of food is an integrated part of the management of microbial safety in the food chain. The hygiene process criteria for many foodstuffs now include a test for Total Viable Count (TVC) and *Escherichia coli*. TVC gives a quantitative idea of the presence of mesophilic aerobic microorganisms of animal origin (ISO 4833, 2003). It serves as important criteria for evaluating the microbial quality of various foods and also degree of freshness of food (Nanu *et al*., 2007). *E. coli* is part of the normal microflora of the gastrointestinal tract of mammals and birds. As early as the 19th century, *E. coli* was recognized as a good indicator of faecal contamination. It is the only species in the coliform group found exclusively in the intestinal tract of humans and other warm-blooded animals, excreted in large numbers (ca., 10^9 CFU g^{-1}) in faeces (Cabral, 2010). Some strains have developed an ability to cause disease in the gastrointestinal,

urinary or central nervous system in even the most robust human hosts. The worldwide majority of the cases of the disease are caused by strains of serotype O157:H7, an enterohemorrhagic *E. coli* (EHEC). It can colonize the intestine of humans and cause diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome (Yin *et al*., 2011; Nataro and Ketalaper, 1998).

Both control authorities and individual food business operators use microbial analysis for the purpose of monitoring the microbiological quality of raw materials and finished products and the microbiological status of manufacturing procedure. Even for compliance testing or assessment of Hazard Analysis Critical Control Points (HACCP) management strategies, microbial analysis is a valuable tool (Nicolas *et al*., 2007; Jasson *et al*., 2010). Standardized methods (e.g. ISO methods) are acknowledged as the reference analytical methods for official control. These standardized methods are based on traditional microbiological culture standard methods that are widely used in food analysis laboratories. These techniques present several difficulties, such as the subjectivity in the interpretation of some biochemical or morphological tests and the possible interference of matrices, specially when they present high levels of contamination. In addition, they are characterized by the high cost of the method, both in terms of labor and supplies and above all, by the long time needed to obtain definitive results (from 3 to 7 days) (Thomas *et al*., 2009). These reasons have led to the development and refinement of alternative microbiological methods of analysis (Mandal *et al*., 2011). Such alternative methods are quicker and easier to perform than the corresponding reference method and some can also be automated (Feng, 1996). Therefore, the prerequisite for the sale and use of any alternative method is to provide evidence that this could yield results which are equivalent to those provided by the corresponding reference method. The suppliers of the alternative methods, the food and drink industry, the public health services and other authorities need a reliable common protocol for the validation of such alternative methods. As a result, ISO 16140 (2003) represents a key issue (Feinberg *et al*., 2009).

In this context, MBS srl (a spin-off of Roma Tre University, Rome, Italy) has developed an alternative method, called Micro Biological Survey (MBS) method. It is a colorimetric fast system for the detection and the selective counting of bacteria present in agro-food, in water and in environmental samples. The MBS method consist of an analytical kit utilizing disposable, ready-to-use reaction vials for fast microbiological analyses. The analysis is based on the color change of the vial content which is induced by the presence of bacteria. The analyses can be carried out by untrained personnel and anywhere where they are necessary, without the need for any other instrumentation than a thermostat provided on request. The MBS method measures the catalytic activity of the redox enzymes in the main metabolic pathways of bacteria (Shultz and Chan, 2001; Slater, 2003; Antonini *et al*., 2007) which allows an unequivocal correlation between the observed enzymatic activity and the number of viable cells present in the samples. The time required for a color change is inversely related to the log of bacterial concentration; like an enzymatic reaction, the greater the number of bacteria, the faster the color change (Berlutti *et al*., 2003). The goal of the present study was the primary validation of the MBS method in accord with ISO 4833 (2003) for both TVC and *E. coli* assays.

MATERIALS AND METHODS

This study was initiated in 2009, lasting about two years for conclusion.

Statistical analysis on artificially contaminated water samples: A comparison between the colorimetric MBS method and the plate count method for the detection and differential

count of TVC and *E. coli* in artificially contaminated samples was performed according to ISO/IEC 170 25 (2005) following the International Standards ISO/TR 13843 (2000), using as reference methods those described by ISO 9998 (1991).

Bacterial strains: All the strains used in this statistical analysis were available at ATCC (American Type Culture Collection). The strains used in this statistical analysis were *Salmonella typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 27853), *Listeria innocua* (ATCC 33090), *Escherichia coli* (ATCC 25992), *Escherichia coli* O157:H7 (ATCC 35150), *Staphylococcus aureus* (ATCC 12600) and *Enterococcus faecalis* (ATCC 29212).

Preparation of artificially contaminated samples: Sterile water samples were contaminated with a mixture of the above indicated microorganisms coming from overnight cultures with serial dilutions in sterile saline solution up to 10^{-8}. Ten different dilutions of ten different samples were analyzed for both TVC and *E. coli*. Each dilution was tested in duplicate with both the MBS method and the plate counting reference method.

Colorimetric MBS method procedure: The analytical procedure for the quantitative colorimetric MBS method is based on colorimetric survey, using a redox indicator of the change of the oxidoreductive state in the reaction medium. For the analysis by the MBS method, ready-to-use MBS vials, sterilised and containing the reagent for the analysis were used. Two different kinds of vials were used: the vials for TVC analysis and the vials for *E. coli* analysis. To carry out the analysis, 10 mL of sterile distilled water and 1 mL of the samples were added to a vial of one or another type, depending on the type of analysis to be carried out. The vial was shaken until all the reagent was dissolved. Later on, the vial was incubated at 30°C for TVC or at 44°C for *E. coli*. The TVC which were detected and counted by MBS are defined as aerobic microorganisms able to grow in a complete medium. The *E. coli* are defined, according to European Directive 91/492/CEE, as thermophilic coliforms that produce indole from tryptophan after incubation at 44±2°C for 24 h. The starting colors are blue for TVC vials and reddish for *E. coli* vials and in the presence of microorganisms, the colors of the vials changed to yellow color indicating a positive result. The time for the yellow color development is inversely related to the bacterial content of the analysed sample. The persistence of the starting color after 24 h indicates a negative result, i.e., absence of microorganisms. The *E. coli* confirmation test was carried out by adding few drops of the Kovac's reagent (isoamyl alcohol, p-Dimethylaminobenzaldehyde, concentrated hydrochloric acid) after color change from reddish to yellow occurred in *E. coli* MBS vials. The development of a red ring revealed the production of indole (MacFaddin, 1980).

Reference method ISO 9998 (1991): One hundred microliter of the different samples were plated on Tryptic Soy Agar (TSA; Sigma Chemical CO., St. Louis) for TVC and on Tryptone Bile X-Glucuronide Agar (TBX; Cultimed, Barcelona, Spagna) for *E. coli* (MacFaddin, 1980). Then the plates were incubated at 30°C for TVC and at 44°C for *E. coli*. During TVC plate count all colonies visible after 24 and 36 h on TSA were considered positive while during *E. coli* plate count only blue colonies on TBX were considered positive. For the statistical analysis, only the positive plates containing less than 300 colonies were utilized.

Statistical analysis: Statistical analysis was carried out according to ISO/TR 13843 (2000). One-way analysis of variance and two-way analysis of variance were performed to determine the

general estimate of precision in the colorimetric method when using it as compared to the reference method of plate counting. The reliability was calculated using statistical analysis of Coefficient of Variation (CV). Uncertainty was calculated using the statistical analysis of chi-square test (χ^2).

Primary validation: The primary validations of MBS method for TVC and for *E. coli* on food samples were made according to ISO 16140 (2003).

Bacterial strains: All the strains used in this validation were available at ATCC (American Type Culture Collection). The strains used in this validation were *E. coli* (ATCC 25992), *E. coli* O157: H7 (ATCC 35150), *C. freundii* (ATCC 43864), *K. pneumoniae* (ATCC 13883), *E. cloacae* (ATCC 13047), *E. sakazakii* (ATCC 51329), *S. enteritidis* (ATCC 13076) and *S. typhimurium* (ATCC 14028), *Y. enterocolitica* (ATCC 19543), *B. cereus* (ATCC 11778), *B. stearothermophilus* (ATCC 24567), *B. subtilis* (ATCC 6633), *L. innocua* (ATCC 33090), *L. ivanovii* (ATCC 19119), *L. monocytogenes* (ATCC 7644), *S. aureus* (ATCC 12600), *S. epidermidis* (ATCC 12228), *S. lentus* (ATCC 29070), *P. aeruginosa* (ATCC 27853), *R. equi* (ATCC 31543), *E. faecalis* (ATCC 29212), *L. delbrueckii* subsp. *lactis* (ATCC 12315), *C. perfringens* (ATCC 13124), *A. niger* (ATCC 9642) and *S. cerevisiae* (ATCC 9763).

Preparation of artificially contaminated samples: Sterile water samples were contaminated with a mixture of the above indicated microorganisms coming from overnight cultures with serial dilutions in sterile saline solution up to 10^{-8}. To verify the equivalence between the MBS method and the reference method the samples were simultaneously tested.

Preparation of different levels of contamination of naturally contaminated food samples: Five different food matrices were selected for validation. They were cheese, white meat, red meat, vegetable and fruit. Only the samples naturally contaminated were selected for the experiments. Baby food of the same foodstuffs typologies were used as a negative control, because they were definitely not contaminated. Five different levels of contamination of naturally contaminated samples were obtained as follows: 10±0.5 g of naturally contaminated samples were homogenized in 90 mL of peptone water by a stomacher according to ISO 16140 (2003). Then homogenates were incubated for different times and different temperatures obtaining different levels of contamination. To verify the equivalence between the MBS method and the reference method, the samples were simultaneously tested.

Colorimetric MBS methods procedure: Analysis with the MBS method was carried out as previously described.

Reference method: For TVC the reference method was plate count on Plate Count Agar (PCA; Liofilchem, Roseto degli Abbruzzi, Italy) after 72 h according to ISO 4833 (2003). For *E. coli* it was plate count on Tryptone Bile X-GLUC Agar (TBX; Cultimed, Barcelona, Spagna) after 24 h according to ISO 16649-2 (2001).

Data analysis: Data analysis was carried out according to ISO 16140 (2003). For the validation of MBS method 3 parameters were analyzed: linearity, accuracy and selectivity. The linearity of the method was assessed by analyzing the correlation using a plot of bacteria concentrations

(expressed as CFU mL^{-1}) against the time occurred for color change. The accuracy was assessed by analyzing the correlation using a plot of bacteria concentrations (expressed as log CFU mL^{-1}) obtained with the reference method with the alternative method MBS. Selectivity of the MBS method for *E. coli* was observed by comparing MBS method with the reference method on both artificially contaminated samples and naturally contaminated samples.

RESULTS

Statistical analysis on artificially contaminated water samples: Sterile water was initially used to avoid any chemical interference due to organic matrices. In MBS colorimetric method the change of the starting color of the vials from blue for TVC and reddish for *E. coli* to yellow color indicates a positive result, presence of microorganisms. The time occurred for color change is inversely related to bacteria content of analysed samples (Fig. 1a, b). The water samples were artificially contaminated (see Materials and Methods). The statistical analysis for the MBS method on TVC and on *E. coli* vials was carried out according to ISO/TR 13843 (2000) using as reference method the plate counting method ISO 9998 (1991) on ten different dilutions of ten different samples. MBS reliable operating limits were comparable to the reference methods for plate counts at concentrations between 1×10^7 and <10 CFU mL^{-1}. The results of the statistical analysis are shown on Table 1 and are expressed in terms of (1) Estimate of Precision; (2) Coefficient of Variance and (3) Uncertainty. General estimate of precision was made according to ISO/TR 13843 (2000) using Analysis of Variance (ANOVA) tests. Results obtained by both one-way analysis of variance and two-way analysis of variance have shown that there were no statistical differences on bacterial

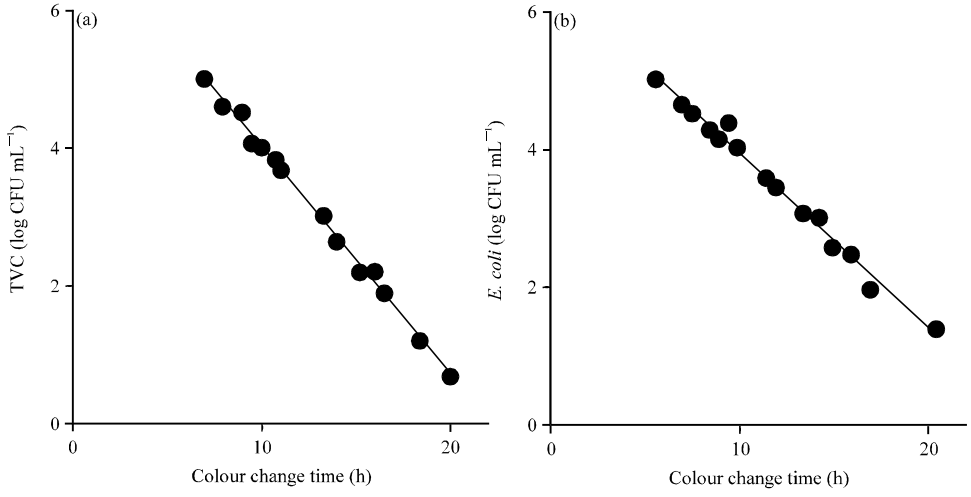

Fig. 1(a-b): Linearity: correlation line between analytes (TVC and *E. coli*) concentration with the time occurred for color change in the MBS vials. Using naturally contaminated food samples, bacteria numbers (expressed as the log of CFU mL^{-1}) obtained with the reference method are plotted against the time occurred for color change of the identical samples analyzed with MBS method. A linear inverse relationship between the time occurred for color change in the MBS vials and the bacteria concentration could be observed on five different food matrices with either TVC vials or *E. coli* vials. The correlation factor (R^2) is 0.95 for TVC and 0.98 for *E. coli*

Table 1: Statistical analysis carried out on the MBS method on TVC and *E. coli* according to the ISO/TR 13843 (2000)

Analysis	TVC	*E. coli*
Estimate of precision		
One-way analysis of variance		
DF 1.30 (limit 1% = 4.17)	F = 1.13	F = 0.2615
Two-way analysis of variance		
DF 7.28 (limit 1% = 4.17)	F = 1.8576	F = 1.16
Coefficient of Variance (CV)		
Plate count	0.1815	0.4533
MBS	0.0295	0.0628
Uncertainty (χ^2)		
Plate count		
DF 9 (limit 0.5% = 4.17)	χ^2<0.25	χ^2<1.2
MBS		
DF 9 (limit 0.5% = 4.17)	χ^2<0.22	χ^2<0.30

F: ANOVA F-test, CV: Coefficient of variation, DF: Degrees of freedom, χ^2: Chi-square test

count between the results obtained with MBS method and the results obtained with the reference method. The reliability of the bacterial count using MBS method was also assessed by statistical analysis using Coefficient of Variation (CV) analysis according to ISO/TR 13843 (2000). It appeared that the MBS method was more reliable than the reference method. Likewise, the uncertainty of the bacterial count using MBS method was less than that of the reference method as determinated by χ^2 statistical test according to ISO/TR 13843 (2000).

Primary validation: The primary validation of the MBS method for TVC and for *E. coli* was made according to ISO 16140 (2003). The main performance parameters which the alternative method must demonstrate are: linearity, accuracy and selectivity.

Linearity is the ability of the method when used with a given matrix to give results that are in proportion to the amount of analyte present in the sample. An increase in analyte should correspond to a linear or proportional increase in results (ISO 16140, 2003). This was achieved graphically as illustrated in Fig. 1 by plotting bacteria numbers (expressed as the log of CFU mL^{-1}) obtained with the reference methods for TVC and *E. coli* with the time occurred for color change of the identical samples analyzed with MBS methods for TVC and *E. coli*. A linear inverse relationship between the MBS methods and the bacteria concentration, with a correlation factor (R^2) close to 1.00, confirming the linearity of the data, can be observed.

Accuracy is the degree of correspondence between the response obtained by the reference method and the response obtained by the alternative method on identical samples. The term relative accuracy used here is complementary to the “accuracy” and “trueness” as defined in ISO 5725-1:1994/COR 1 (1998). This states that accuracy is “the closeness of agreement between a test result and the accepted reference value” and that trueness is "the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value". For the purpose of this standard, the accepted reference values are chosen as the values obtained by the reference method. Thus, the term "relative" implies that the reference method does not automatically provide the accepted reference value as indicated by ISO 16140 (2003). In Fig. 2a and b the bacteria numbers (expressed as log CFU mL^{-1}) obtained with the reference counting methods for TVC and *E. coli* are plotted against the bacteria numbers (expressed as log

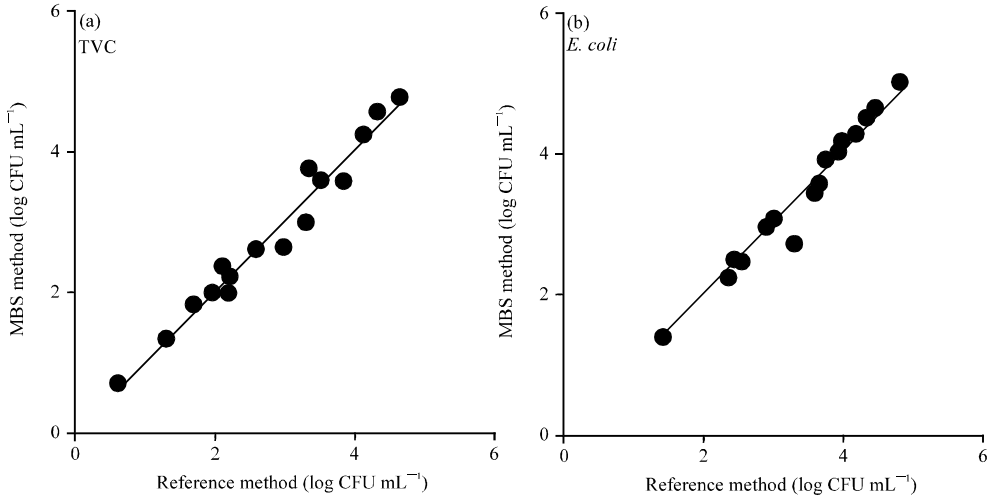

Fig. 2(a-b): Accuracy: correlation line between alternative MBS methods and reference methods (TVC and *E. coli*). Using naturally contaminated food samples, bacteria numbers (expressed as the log of CFU mL^{-1}) obtained with the reference method are plotted against bacteria numbers (expressed as the log of CFU mL^{-1}) obtained with MBS method on identical samples. A good correlation between the bacteria numbers (expressed as log CFU mL^{-1}) obtained with the traditional counting method and the alternative MBS method could be observed. In fact the slope is close to the theoretical value 1.00 (i.e. 1.00 for TVC and 0.99 for *E. coli*). The correlation factor (R^2) is 0.94 for TVC and 0.99 for *E. coli*

CFU mL^{-1}) obtained with the alternative MBS method for TVC and *E. coli*. The straight lines in both graphs show a perfect correlation between the reference methods and the MBS methods. In fact the slopes are close to theoretical value of 1.00.

Selectivity is the ability of an alternative method to detect the target analyte from a wide range of strains and is the lack of interference from a relevant range of non-target strains of the alternative method as indicated by ISO 16140 (2003). Among different inoculations only the vial inoculated with *E. coli* shows a change of the color from red to yellow. Table 2 shows the lowest detection limit (expressed as CFU mL^{-1}) of the MBS vials for TVC and for *E. coli* towards different bacterial strains in artificially contaminated samples. The lowest detection limit represents the minimal bacterial quantity required for inducing the color change in either MBS vials for TVC or for *E. coli*. The lowest detection limit is utilized to assess of the MBS vial selectivity. It could be noted that, using the *E. coli* vials, very high concentrations of all the bacteria other than *E. coli* were required for inducing the color change (i.e., a positive result of the test) while just one *E. coli* cell (on average) was sufficient to induce the color change of the same vials. These results indicate that the *E. coli* vials are selective for *E. coli*, although the *E. coli* vials showed a lower selectivity towards other coliforms strains, medium level selectivity towards *Enterobacteriaceae* and a higher selectivity towards Gram-positive bacteria. On the contrary, using TVC vials, just one cell of all the aerobic bacteria strains (on average) was sufficient to induce the color change, indicating the very low selectivity of the TVC vials.

Table 3 shows the results obtained with MBS method and reference method to detect *E. coli* in naturally contaminated samples of 5 different food matrices. Both methods have identified 100 target strains on 100 as positives, with total absence of false negatives; moreover both have identified 25 non target strains on 25 with a total absence of false positives.

Table 2: Results of selectivity tests

Bacteria strains		Lowest detection limits for *E. coli* vials (CFU mL^{-1})	Lowest detection limits for TVC vials (CFU mL^{-1})
Enterobacter cloacae	ATCC 13047	$>10^5$	1
Enterobacter sakazakii	ATCC 31329	$>10^5$	1
Pseudomonas aeruginosa	ATCC 27853	$>10^6$	1
Salmonella enteritidis	ATCC 13076	$>10^6$	1
Salmonella. enterica ser. *typhimurium*	ATCC 14028	$>10^4$	1
Yersinia enterocolitica	ATCC 19543	$>10^6$	1
Citrobacter freundii	ATCC 43864	$>10^3$	1
Klebsiella pneumoniae	ATCC13883	$>10^3$	1
Eschierica coli	ATCC 25922	1	1
Eschierica coli O157:H7	ATCC 35150	1	1
Enterococcus faecalis	ATCC 29212	$>10^6$	1
Bacillus cereus	ATCC 11778	$>10^6$	1
Bacillus stearothermophylus	ATCC 24567	$>10^6$	1
Bacillus subtilis	ATCC 6633	$>10^6$	1
Listeria innocua	ATCC 33090	$>10^6$	1
Listeria ivanovii	ATCC 19119	$>10^6$	1
Listeria monocytogenes	ATCC 7644	$>10^6$	1
Rhodococcus equi	ATCC 31543	$>10^6$	1
Sthaphylococcus aureus	ATCC 12600	$>10^6$	1
Sthaphylococcus epidermidis	ATCC 12228	$>10^6$	1
Staphylococcus lentus	ATCC 29070	$>10^6$	1
Lactobaccilus delbrueckii subsp. *lactis*	ATCC 12315	$>10^6$	$>10^3$
Clostridium perfringens	ATCC 13124	$>10^6$	$>10^6$

The lowest detection limit represents the minimal bacterial quantity of each bacterial strain required for inducing the color change in the corresponding MBS vials for TVC or *E. coli*

Table 3: Results for the selectivity test on *E. coli* vials using naturally contaminated samples of five different food matrices

	Reference method		
MBS method	Present	Absent	Total
Positive	100	0	100
Negative	0	25	25
Total	100	25	125

The food matrices utilized were cheese, white meat, red meat, vegetable, fruit. Both methods have been used to identify 100 target strains on 100 as positives, with total absence of false negatives; moreover both have identified 25 non target strains on 25 with total absence of false positives

DISCUSSION

In recent years, the need for the food industry to rapidly assess the microbiological quality of raw materials and finished products, has led to the development and refinement of alternative microbiological methods of analysis. Such alternative methods are quicker and easier to perform than the corresponding reference method (Feinberg *et al*., 2009). In this context, the goal of the present study was the primary validation of the Micro Biological Survey (MBS) method for both TVC and *E. coli*, defined, according to European Directive 91/492/CEE, as thermophilic coliforms that produce indole from tryptophan after incubation at 44±2°C for 24 h.

The MBS method is a colorimetric fast system for the detection and selective counting of bacteria in agro-food, in water and in environmental samples. The MBS method measures the catalytic activity of redox enzymes of the main metabolic pathways of bacteria (Shultz and Chan, 2001; Slater, 2003; Antonini *et al*., 2007), allowing an unequivocal correlation between the observed enzymatic activity and the number of viable cells present in the samples. The time required for color change is inversely related with the log of bacterial concentration; like an enzymatic reaction, the greater the number of bacteria, the faster the color change (Berlutti *et al*., 2003). The results reported in this study further support the previous findings concerning the existence of a stringent correlation between metabolic activity of bacteria and the number of viable cells.

Validation aims to compare the results obtained with an alternative method, in this case the MBS method, with the results obtained with the reference method verifying the equivalence between the two methods by looking at linearity, accuracy and selectivity. The results were statistically analyzed and compared according to the norm ISO/IEC 17025 (2005) and ISO 16140 (2003) verifying the equivalence between the two methods. All the performance parameters indicated a total equivalence between the reference method and the MBS method for detection and counting of TVC and *E. coli* in artificially contaminated water samples and in naturally contaminated food samples. When a method is validated for environmental sample analysis, it is important to include naturally contaminated samples. In this study, we have selected five different food matrices: cheese, vegetable, white meat, red meat and fruit.

The validation of the MBS method strongly supports its use as an alternative method for food analysis. The linearity over a range of bacterial concentrations was excellent. The selectivity was more than satisfactory with the absence of false negatives and false positives. The accuracy, evaluated on 125 naturally contaminated samples, showed a high correlation between the MBS method and the reference methods.

Comparing the MBS method to other analytical methods currently in use the following considerations come to light. With traditional count plate methods bacteria replication can be observed with the naked eye but greater expertise in the operators and operational complexity are required. On the other hand, alternative methods often turn out to be very expensive also requiring highly equipped laboratories. The use of immunological or genetic probes (with the assistance of PCR to increase sensitiveness) had a great impact in microbial analysis (Thacker *et al*., 1996; Sherfi *et al*., 2006; Settanni and Corsetti, 2007; AL-Haj *et al*., 2008; Parekh and Subhash, 2008; Cook *et al*., 2011; Loongyai *et al*., 2011). Indeed they are very quick and sensitiveness can be improved by using automated o semi-automated systems. The disadvantages are not only related to the need for specialized personnel and equipment but also for an high limit of sensitiveness (immunological methods) and/or complexity and high costs of analysis (genetic methods). In addition the exact quantification of the number of bacteria over a large range of concentrations is not always possible. Colorimetric methods currently available are mainly based upon microorganisms secondary metabolism measuring. One of these methods detects the presence of *E. coli* on the basis of the activity of the enzyme β-glucuronidase (Al-Turki and El-Ziney, 2009). However, it should be mentioned that using this method it is not possible to detect the pathogenic, although relatively uncommon forms *E. coli* O157: H7 verocytotoxin producers (Ling *et al*., 2000; Donkor *et al*., 2008) which do not exhibit β-glucuronidase activity (Thompson *et al*., 1990; Karmali *et al*., 2010). Instead, *E. coli* O157: H7 is detected by the MBS method on the basis of its indole production from tryptophan.

For the above reported reasons, the MBS method can represent a worthy aid in food screening without replacing the analysis carried out with traditional methods which are very precise though often long and complex.

CONCLUSIONS

The validation here reported provided evidence that the MBS method for TVC and *E. coli* gives similar results and is in agreement with the reference methods, also confirming the better reproducibility, specificity and selectivity of the MBS method. MBS method could therefore become a valid support for the control procedures for all the food farming companies willing to do a microbiological screening over their products to ensure utterly complete hygienic production.

ACKNOWLEDGMENTS

This study was supported by a grant from Italian Ministry of University and Research (Decreto No. 2933/2006 ex art.11 DM 593/2000).

REFERENCES

AL-Haj, N.A., N.S. Mariana, A.R. Raha and Z. Ishak, 2008. Detection of diarrheagenic *Escherichia coli* isolated using molecular approaches. Res. J. Microbiol., 3: 359-367.

Al-Turki, A. and M.G. El-Ziney, 2009. Evaluation of commercial colilert 18-quantitray® method by iso techniques for enumeration and quantification of total coliforms and *Escherichia coli* in drinking-water of Buraidah, Saudi Arabia. J. Applied Sci., 9: 3357-3363.

Antonini, G., F. Malatesta and P. Sarti, 2007. Twenty-five years of cytochrome oxidase research in Rome with Maurizio Brunori. IUBMB Life, 59: 570-577.

Berlutti, F., F. Rosso, P. Bosso, F. Giansanti and M. Ajello *et al*., 2003. Quantitative evaluation of bacteria adherent to polyelectrolyte HEMA-based hydrogels. J. Biomed. Mater. Res., 67A: 18-25.

Cabral, J.P.S., 2010. Water microbiology. Bacterial pathogens and water. Int. J. Environ. Res. Public Health, 7: 3657-3703.

Cook, P.E., K.L. Beers and K.W. Beers, 2011. Efficacy of the freshlight® 210 ultraviolet light system[1] for control and elimination of *Escherichia coli* O157:h7 in a commercial beef brine solution (Flow rate = 10 gpm and turnover time = 2.5 min). Int. J. Meat Sci., 1: 79-79.

Donkor, E.S., R. Lanyo, M.L. Akyeh, B.B. Kayang and J. Quaye, 2008. Monitoring enterohaemorrhagic *Escherichia coli* O157: H7 in the vegetable food chain in Ghana. Res. J. Microbiol., 3: 423-428.

Feinberg, M., D. Sohier and J.F. David, 2009. Validation of an alternative method for counting *Enterobacteriaceae* in foods based on accuracy profile. J. AOAC Int., 2: 527-537.

Feng, P., 1996. Emergence of rapid methods for identifying microbial pathogens in foods. J. AOAC Int., 79: 809-812.

ISO 16140, 2003. Microbiology of food and animal feeding stuffs-Protocol for the validation of alternative methods. International Organization for Standardisation, Geneva, Switzerland.

ISO 16649-2, 2001. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*- Part 2: Colony-count technique at 44 degrees Celsius using 5-bromo-4-chloro-3-indolyl β-D-glucuronide. International Organization for Standardisation, Geneva, Switzerland.

ISO 4833, 2003. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of microorganisms-Colony-count technique at 30 degrees Celsius. International Organization for Standardisation, Geneva, Switzerland.

ISO 5725-1:1994/COR 1, 1998. Accuracy (trueness and precision) of measurement methods and results- Part 1: General principles and definitions. International Organization for Standardisation, Geneva, Switzerland.

ISO 9998, 1991. Water quality-practices for evaluating and controlling microbiological colony count media used in water quality tests. International Organization for Standardisation, Geneva, Switzerland.

ISO/IEC 17025, 2005. General requirements for the competence of testing and calibration laboratories. International Organization for Standardisation, Geneva, Switzerland.

ISO/TR 13843, 2000. Water quality-Guidance on validation of microbiological methods. International Organization for Standardisation, Geneva, Switzerland.

Jasson, V., L. Jacxsens, P. Luning, A. Rajkovic and M. Uyttendaele, 2010. Alternative microbial methods: An overview and selection criteria. Food Microbiol., 27: 710-730.

Karmali, M.A., V. Gannon and J.M. Sargeant, 2010. Verocytotoxin-producing *Escherichia coli* (VTEC). Vet. Microbiol., 140: 360-370.

Ling, O.W., S. Radu, G. Rusul, M.I.A. Karim, E. Purwati and S. Lihan, 2000. Enterobacterial repetitive intragenic consensus (ERIC) genotyping of *Escherichia coli* O157:H7. Pak. J. Biol. Sci., 3: 35-37.

Loongyai, W., B. Wiriya and N. Sangsawang, 2011. Detection of *Salmonella* and *Escherichia coli* in egg shell and egg content from different housing systems for laying hens. Int. J. Poult. Sci., 10: 93-97.

MacFaddin, J. F., 1980. Biochemical tests for Identification of Medical Bacteria. 2nd Edn., Williams and Wilkins, U.S.A.

Mandal, P.K., A.K. Biswas, K. Choi and U.K. Pal, 2011. Methods for rapid detection of foodborne pathogens: An overview. Am. J. Food Technol., 6: 87-102.

Nanu, E., C. Latha, B. Sunil, Prejit, M. Thomas and K.V. Menon, 2007. Quality assurance and public health safety of raw milk at the production point. Am. J. Food Technol., 2: 145-152.

Nataro, J.P. and J.B. Kaper, 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev., 11: 142-201.

Nicolas, B., B.A. Razack, I. Yollande, S. Aly and O.C.A. Tidiane *et al.* 2007. Street-vended foods improvement: Contamination mechanisms and application of food safety objective strategy: Critical review. Pak. J. Nutr., 6: 1-10.

Parekh, T.S. and R. Subhash, 2008. Molecular and bacteriological examination of milk from different milch animals with special reference to coliforms. Current Res. Bacteriol., 1: 56-63.

Settanni, L. and A. Corsetti, 2007. The use of multiplex PCR to detect and differentiate food and beverage-associated microorganisms: A review. J. Microbiol. Meth., 69: 1-22.

Sherfi, S.A., I.E. Aradaib and H.A. Dirar, 2006. Evaluation of polymerase chain reaction for rapid detection of *E. coli* strains: A preliminary study. Asian J. Cell Biol., 1: 9-13.

Shultz, B.E. and S.I. Chan, 2001. Structures and proton-pumping strategies of mitochondrial respiratory enzymes. Annu. Rev. Biophys. Biomol. Struct., 30: 23-65.

Slater, E.C., 2003. Keilin, cytochrome and the respiratory chain. J. Biol. Chem., 278: 16455-16461.

Thacker, J.D., E.S. Casale and C.M. Thucker, 1996. Immunoassays (ELISA) for rapid, quantitative analysis in the food-processing industry. J. Agric. Food Chem., 44: 2680-2685.

Thomas, D., A. Rodrigo, M. Hernandez and M.A. Ferrus, 2009. Validation of real-time PCR and enzyme-linked fluorescent assay-based methods for detection of *Salmonella* spp. in chicken faces samples. Food Anal. Methods, 2: 180-189.

Thompson, J.S., D.S. Hodge and A.A. Borczyk, 1990. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* O157. J. Clin. Microbiol., 28: 2165-2168.

Yin, X., J. Zhu, Y. Feng, J.R. Chambers, J. Gong and. C.L. Gyles, 2011. Differential gene expression and adherence of *Escherichia coli* O157: H7 *in vitro* and in ligated pig intestines. PLoS One, Vol. 6.