

Applicability of the TEMPO® Automated System to the Microbiological Analysis of Dairy Products

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ABSTRACT

Rapid and reliable methods play an essential role in routine microbiological analysis of foods. Due to this development there is growing interest in having available analytical tools that are faster and less time-consuming than traditional techniques and that are also of enhanced precision and reliability in daily laboratory routine. Most Probable Number (MPN) procedures usually involve extensive preparatory work, much time and glassware, need sufficient space for incubation and the results (e.g., gas bubbles) are not always clearly indicated and therefore are not easy to interpret. The TEMPO[®] System (Biomerieux, Marcy L'Etoile, France) is an automatically working apparatus, which has been designed to overcome these disadvantages through performing fluorogenic-based readings with the so called TEMPO[®] cards filled with serially diluted samples at a smaller format. The instrumental equipment consists of a preparatory and a reading station. This system also includes a PC based automatic calculation of the most probable number counts based on statistical evaluation of the readings. The purpose of this study was to apply and to evaluate the TEMPO[®] system for the microbiological examination of a variety of dairy products. The main focus of this work was laid on the detection and enumeration of the coliform group and of E.coli. In addition, cheese samples artificially contaminated with selected bacterial strains were measured at different cell densities. Corresponding IDF standard methods (IDF 170-1:2005, IDF 100B:1991) were used for reference purposes. It could be shown that the mean findings obtained with both methodologies were in good agreement. Since the TEMPO[®] system allows measurements based on a 16-tubes MPN-method, the precision of the results is higher compared to the conventional MPN technique usually involving three replicate tubes per dilution, e.g., equipped with Durham vials. Thus the confidence intervals of the TEMPO[®] results are smaller and indicate improved precision. The method also exhibits a better capability than conventional MPN tests. Selected examples for different applications in dairy product examination are presented. From the results it can be concluded that the TEMPO[®] system can be advantageously applied in routine analysis.

METHODS

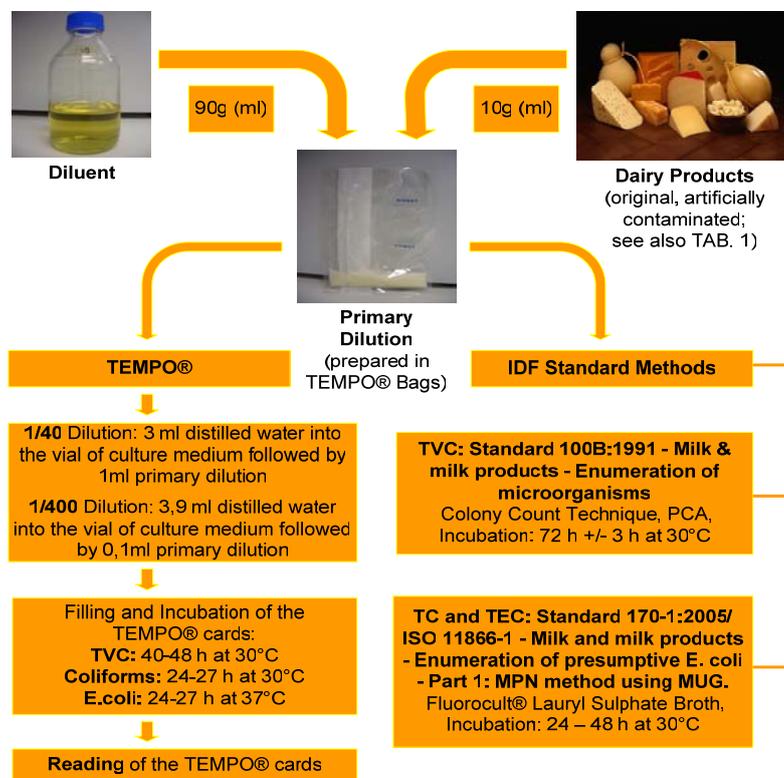


Figure 1: Protocol of the methods

RESULTS and CONCLUSIONS:

Total viable counts of the samples examined were low, indicating a high quality of these products. For coliforms and E.coli, the correlation between the results obtained by the TEMPO[®] and those of the standard method is depicted as scattergrams of the corresponding log CFU/g values (FIG 2).

The results of the matrix calibration for coliforms and E.coli show a better repeatability of the TEMPO[®] method, obviously due to the 16-tubes MPN approach. Differences to the standard method were marked for E.coli, but not so pronounced in terms of the enumeration of coliforms. Both TEMPO[®] applications possess a proper linearity and homogeneity of variance. Regression analyses using the orthogonal mode were applied to calculate the correlation coefficients. Both TEMPO applications exhibited a high correlation with the corresponding standard methods within the tested concentration range.

Hence, the results indicate that the Tempo[®] method can be advantageously applied for the examination of coliforms and E.coli in dairy products. Data are in good agreement with those of routine reference techniques. This experience is also in accordance with former studies [1]. As far as the practicability is considered, the Tempo[®] method allows higher throughput and is less time-consuming than traditional methods, as it helps to reduce manipulation (time), glassware, space and culture material. In addition, the software allows easy handling and good traceability [2].

Samples	Number of Samples	TVC	Coliforms	E. coli
Soft Cheese	60		•	•
Fresh Cheese	40		•	•
Semi hard Cheese	10		•	•
Milk and Milk Drinks	29	•		
Butter	3	•		
Cream	5	•		
Ice Cream	3	•		
Skimmed Milk Powder	3	•		
Artificially contaminated soft cheese	3		•	•
TOTAL	156			

Table 1: Samples and analysed parameters

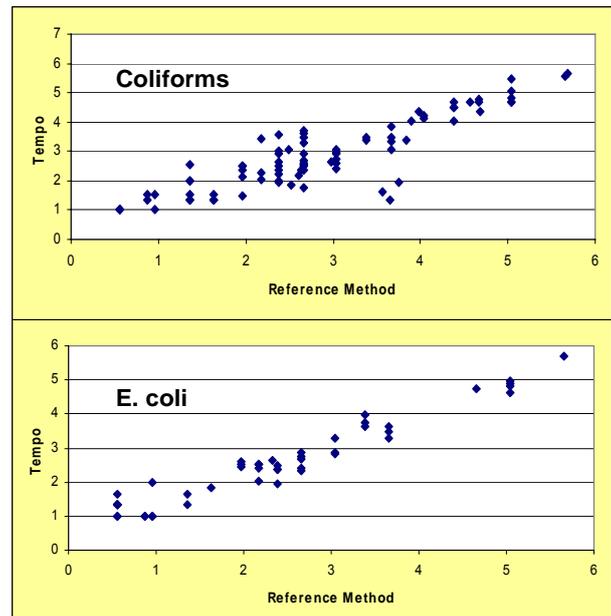


Figure 2: Results [logCFU/g] obtained by TEMPO[®] vs. Ref. Method

Additionally, a matrix calibration was performed. For this purpose, a fresh cheese sample was artificially contaminated with increasing concentration levels of E. coli within a pre-defined concentration range. For results see TAB 2.

Matrix Calibration Conc.: 500 - 10 ⁵ CFU/g	n	TEMPO [®]	Reference Method	Correlation Coefficients
Coliforms	12	r _{rep.} = 0,43	r _{rep.} = 0,49	r _{corr.} = 0,99
E. coli	12	r _{rep.} = 0,36	r _{rep.} = 0,49	r _{corr.} = 0,99

Table 2: Repeatability and Correlation Coefficients

References: [1]: Sohler et al.: TEMPO[®] System: AFNOR Validation according to the ISO 16140 Standard (2005); [2]: Betts: New Food 4: 70-74 (2005)

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