



Comparison of a New TEMPO® Method for the Enumeration of Lactic Acid Bacteria in Food Products with the ISO 15214 Method

Grégory Devulder, Audrey Maingue, Cidem Ilter, Marie--Thérèse Lescure
bioMérieux, Chemin de l'Orme, 69280 Marcy l'Etoile, France

PURPOSE

Lactic Acid Bacteria (LAB) are widely distributed through-out nature. They are very important in both production and spoilage of food products. The enumeration of these microorganisms is used in food industry. The traditional method is based on the De Man Rogosa Sharpe agar incubated 72h at 30°C (ISO 15214). The purpose of this study is to evaluate a new LAB enumeration method adapted to the TEMPO® system, developed for the enumeration of Quality Indicators in Food and Environmental samples. The comparison between both methods was performed on three different sites with more than one thousand results from products naturally contaminated. From the primary dilution, both methods were performed in parallel.

MATERIALS AND METHODS

Sample Preparation:

Different food categories (316 food products) were tested: raw and cooked meat, poultry, fish and seafood, vegetables, dairy products, bakery, delicatessen, pet food, ready-to-eat meals. A 1:10 primary dilution was prepared for each sample using TEMPO blender bags. Depending on the level of contamination, decimal dilutions were performed in Tryptone salt (Maximum Recovery Diluent).

Reference Method:

Rehydrated MRS was prepared according to manufacturer's instructions. The pH of the medium was adjusted to 5.7 ± 0.1 after sterilization according to ISO 15214. Plates were inoculated using pour plating procedure with 1mL of the successive dilutions and incubated aerobically at 30°C for 72 ± 3 hours.

TEMPO LAB Method:

TEMPO LAB is a method for Lactic Acid Bacteria enumeration, based on the familiar format of the TEMPO MPN procedure. The method uses a new selective dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the MPN. Filled cards were incubated aerobically at 35°C for 40-48 hours.

Analysis:

The comparison in terms of enumeration (T-test, bias and regression) was performed on within range data: this means that for both methods, the log decimal transformation is possible. The analysis of below and above range was performed using the rate of agreement results. Results for the bias, with the 95% confidence interval on the within range data set are detailed in Table 2.

RESULTS AND DISCUSSION

Table 1: Percentage of agreement between both tested methods

	Total	Agreement results (%)	Discrepant results (%)
Within range	348	309 (89%)	39
Below/Above range	684	633 (93%)	51
Total	1032	942 (91%)	90

The overall percent agreement between the TEMPO LAB and reference method was calculated by determining the number of data points which exhibited log differences of less than one. The percent agreement within one log for this study was 91%.

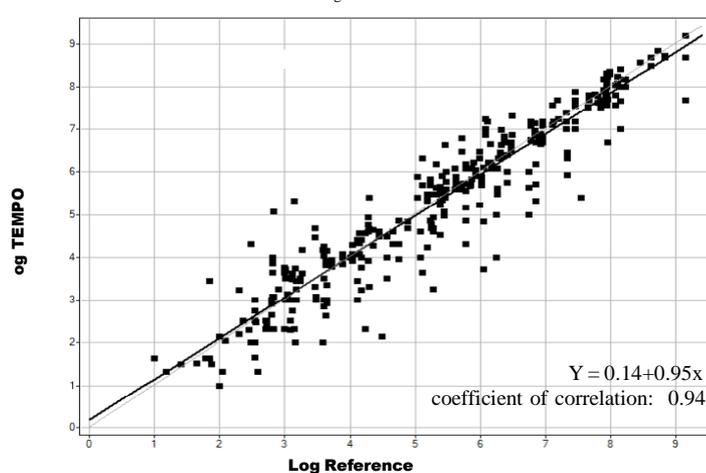
The majority of the discrepant results were due to TEMPO under-enumeration. To understand this under-enumeration, we performed an additional study including the confirmation of five colonies on MRS. For 11 samples tested out of 105, yeasts were detected in the MRS plates whereas they were normally counted as bacteria. As suggested in the ISO 15214, it is recommended to add sorbic acid to the MRS medium, incubated at 30°C, to limit any risk of over enumeration for samples suspected to be contaminated with yeasts. In this study, TEMPO LAB shows a better selectivity than MRS media.

Table 2: Bias and confidence interval between both tested methods

	Bias	Confidence interval	P. Value
TEMPO LAB vs. Reference Method	-0.07	[-0.014; 0.00]	0.056

No significant bias between both methods with a P-Value higher than 0.05 and 0 is included in the 95% confidence interval

Figure 1: Correlation between both tested methods



In term of enumeration, the comparison does not show any significant bias on the data compared using a paired t-test. With a correlation coefficient of 0.94, the intercept on the y axis and the slope are respectively close to 0 and 1.

CONCLUSION

With a high degree of automation and standardisation for food laboratories, this new method for the enumeration of Lactic Acid Bacteria performs as well as the ISO 15214 reference method and reduces the time to result from 72h to 40-48h.

Results were not statistically different than those obtained by plate count on MRS agar, showing a satisfactory agreement between the methods.

In this study, the automated MPN method demonstrated a better specificity than MRS on food products containing yeasts.

TEMPO LAB is less fastidious than the reference method, limiting the number of dilutions and allowing significant savings due to an optimized laboratory workflow.