A comparative study of automated TEMPO® rapid method with IS/ISO method for enumeration of microorganisms in different dairy products

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Abstract: Recently numerous automated microbiological methods have been developed which aims to provide accurate, precise, and rapid test results, but their performance needs to be established before being used for sample analysis. The objective of the study is to evaluate the comparison between an automated rapid technique “TEMPO” and a conventional method for enumeration of microorganisms in the Skimmed Milk Powder, Milk, Infant Milk Food and Paneer. The samples were artificially contaminated with reference microbial cultures for the study. The study was done for Total Aerobic Count as per IS 5402, enumeration of Coliform as per IS 5401 (Part-1), Yeast and Mould count as per IS 5403, and enumeration of *Escherichia coli* as per IS 5887 (Part-3). All samples were analyzed by both TEMPO and IS methods. The Acceptability Limit (AL) is set at $\pm 0.5 \log 10$ units as per ISO 16140-2: 2016. Acceptability Limit is expressed as a difference between the reference and the alternative method. Logarithmic differences between the results obtained from two methods are $\leq 0.5 \log$ for Aerobic count, Yeast and Mould count, Total Coliform count and *Escherichia coli* count. Statistical analysis of the results have shown good rate of agreement for all artificially contaminated samples for the two methods, when counted for Aerobic count at 30°C for 40-48 hours, Coliform count at 30°C for 24-27 hours, Yeast and Mould count at 25°C for 72-76 hours and *Escherichia coli* count at 37°C for 22-27 hours.

Keywords: Artificial contamination, Conventional methods, Rapid method and IS method, TEMPO®,

Introduction

Foodborne diseases have become a major public health problem worldwide due to the significantly increased incidence of foodborne illness over the last 20 years (Oliver et al. 2005). Although it is difficult to estimate the global incidence of foodborne illness as some of the cases are under-reported especially in developing countries, the increased incidence of foodborne diseases was reported in many parts of the world (Van de Venter, 2000).

Consumption of contaminated food by microorganisms has not only caused numerous infections but also resulted in foodborne outbreaks. Prevention of these foodborne diseases is a major concern in the food industry.

The presence of microorganisms in milk and dairy products is a major concern for safety, quality, regulations, and public health. Recent outbreaks of foodborne illness, implicating milk and dairy products contaminated with the microorganism like Coliform, *Escherichia coli*, Yeast and Mould, *Listeria spp.*, and *Salmonella* have underscored the need for rapid methods for microbiological analysis of dairy products. Microbiological analysis is a critical assessment for safety and quality conformation with standards or specifications, and regulatory compliances as well.

To control the microbial contamination of food and consequently to reduce foodborne illnesses, rapid and accurate microbiological detection methods are required for effective monitoring of microbial contamination in food supplies in a short time. Conventional methods for the enumeration of quality indicators such as Aerobic count, Yeast and Mould count, Coliform and *Escherichia coli* in foods are laborious and require more material, manpower and time. Besides, quality assurance in the food industry requires rapid test methods that allow a fast reaction to mitigate risks (Kawasaki et al. 2003).

For these reasons, several alternative rapid methods have been developed recently for the enumeration of quality indicators in

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foods. These methods are generally based on the utilization of chromogenic or fluorogenic substrates for the detection of specific enzyme activities (Manafi et al. 1991). Rapid methods are more time- efficient, labor-saving and are able to reduce human errors (Mandal et al. 2011).

Traditionally, these organisms have been enumerated using a pour plate or surface spread technique on selective agar and confirmed result is usually obtained in 8-10 days. To meet the need for rapid microbial analysis, different methods have been developed to reduce the time required for the detection of microorganisms. This study undertakes a comparison of the rapid technique versus conventional methods for various food matrices for four different parameters to ensure the effectiveness of rapid methods against conventional.

TEMPO was used as a rapid technique for microbial analysis of Total Aerobic Count, *Escherichia coli*, Yeast & Mould and Coliform parameters and its performance was checked by comparing its results with the results obtained from conventional methods based on IS/ISO methods. This study was conducted using 4 different food products, which was artificially contaminated for better and controlled evaluation of TEMPO. In this paper, we propose a fully automatic rapid technique TEMPO and compared its performance with conventional methods.

Materials and Methods

Samples

In this study, 4 samples i.e., Milk, Infant Milk Food, Paneer & Skimmed Milk Powder (SMP) were used. All samples were collected from the local market of Anand, Gujarat, India. These samples were analyzed by both

TEMPO and IS methods respectively.

Bacterial strains and growth conditions

Escherichia coli (MTCC-1687) was used to contaminate the samples artificially for the verification test of Total Aerobic Count, *Escherichia coli* and Total Coliform count and *Candida albicans* (NCPF 3179) was used to artificially contaminate the samples for verification test of Yeast and Mould count.

Escherichia coli working culture was prepared in Nutrient broth, incubated at 37°C for 24 hours and *Candida albicans* working culture was prepared in Sabouraud Dextrose Broth, incubated at 25°C for 72 hours. Serial tenfold dilutions of each test organism were prepared; 1ml from the 10⁻⁴ dilution from each culture tube was transferred to 90 ml diluent containing 10 gm sample.

Sample preparation

10 gm or ml of sample was homogenized with 90 ml of sterile

peptone salt solution in a sterile bag with a lateral filter using stomacher and further analyzed by TEMPO and IS methods respectively.

Conventional Method (IS/ISO method)

Total aerobic count

Total aerobic count was performed as per IS 5402: 2012. Plate Count Agar (PCA) was previously cooled at 44°C - 46°C. 1.0 ml aliquots of the dilutions were transferred to petri plate in duplicates and PCA was poured. After solidification, plates were incubated at 30 ± 1°C for 72 hours. After completion of the incubation period, colonies were counted.

Coliform count

Coliform count was performed as per IS 5401 (Part-1):2012. Violet Red Bile Agar (VRBA) was previously cooled at 44°C - 46°C. 1.0 ml aliquots of the dilutions were transferred to petri plate in duplicates and VRBA was poured. After solidification of media, overlay was done with VRBA. Plates were incubated at 37 ± 1°C for 24 hours. After completion of the incubation period, colonies were counted.

Yeast and mould count

Yeast and Mould count was performed as per IS 5403: 1999. CYGA (Chloramphenicol Yeast Glucose Agar) was previously cooled at 44°C - 46°C. 1.0 ml aliquots of the dilutions were transferred to petri plates in duplicate and CYGA was poured. Plates incubated at 25 ± 1°C for 5 days. After completion of the incubation period, colonies were counted.

Escherichia coli count

Escherichia coli was performed as per IS 5887 (Part-3): 1976. 1.0 ml of aliquot was spread over the three Tergitol-7 agar plates. The plates were incubated at 37°C for 24 hours. After completion of the incubation period, yellow colonies were counted.

TEMPO method

The TEMPO test kit consists of a vial of a specific culture medium and a card, which are specific to a particular test. The culture medium is inoculated with the sample to be tested. The inoculated medium is transferred by the TEMPO Filler instrument into the card containing 48 wells of three different volumes. The card contains 3 sets of 16 wells (small, medium and large wells) with a one log difference in volume for each set of wells. The card is designed to simulate the Most Probable Number (MPN) method (Cochran et al. 1950). The card is then hermetically sealed to avoid any risk of contamination during subsequent handling. The specific organism present in the card reduces the substrate in the culture medium during incubation and causes a fluorescent

signal to appear, which is detected by the TEMPO Reader instrument. Depending on the number and type of the positive wells, the TEMPO system calculates the number of microorganisms present in the original sample according to a calculation based on the MPN method.

Primary diluents use for sample preparation

Peptone Saline Diluent (90 ml) and di-potassium hydrogen phosphate (for dried milk products).

The secondary diluent used for Aerobic Count (AC), Total Coliform (TC) and Yeast and Mould (YM) Sterile distilled water.

The secondary diluent used for *Escherichia coli* is sterile distilled water. The Mandatory diluent used for milk powder is MOPS buffer (0.4M). MOPS buffer was prepared by dissolving 13.60 gm MOPS acid and 31.21 gm MOPS sodium salt in 500 ml distilled water. Sterilized by autoclaving.

Total aerobic count

TEMPO AC medium vial reconstituted by dispensing 3.9 ml of secondary diluent per vial using the sterile pipette. 0.1 ml of prepared sample in primary diluent added into the reconstituted 3.9 ml vial. Then card was filled by the TEMPO filler system automatically. Filled card incubated at $30 \pm 1^\circ\text{C}$ for 40–48 hours. It is recommended to incubate card at $30 \pm 1^\circ\text{C}$ upto 48 hours for pasteurized milk. (TEMPO Aerobic Count package insert 9301732B-en-2013/01).

Total coliform count

TEMPO TC medium vial reconstituted by dispensing 3.0 ml of secondary diluent per vial using a sterile pipette. 0.1 ml of prepared sample in primary diluent added into the reconstituted 3.0 ml vial. Then card was filled by the TEMPO filler system automatically. Filled card incubated at $30 \pm 1^\circ\text{C}$ for 24–27 hours. (TEMPO Total Coliform package insert 12599H-en-2014/03)

Yeast and mould count

TEMPO YM culture medium vial reconstituted by dispensing 3.0 ml of secondary diluent per vial using a sterile pipette. 1.0 ml of prepared sample in primary diluent added into the reconstituted

3.0 ml vial. Then Card was filled by the TEMPO filler system automatically. Filled card incubated at $25 \pm 1^\circ\text{C}$ for 72–76 hours. (TEMPO Yeast and Mould package insert 12594E-en-2010/12)

***Escherichia coli* count**

TEMPO EC culture medium vial reconstituted by dispensing 3.0 ml of secondary diluent per vial using the sterile pipette. 1.0 ml of prepared sample in primary diluent added into the reconstituted 3.0 ml vial. Then card was filled by the TEMPO filler system automatically. Filled card incubated at $35 \pm 1^\circ\text{C}$ for 22–27 hours. (TEMPO *E. coli* package insert 12587F-en-2007/06). After completion of incubation time, the instrument read cards, calculates and gives the result in CFU/gm.

Statistical analysis

All samples were analyzed in three replicates using both the methods for all parameters. For the statistical analysis, decimal logarithms of the results were used. The statistical equivalence of the two method groups converted in log₁₀ values and evaluated by their difference in log values.

Results and Discussion

The variation in data obtained by using the TEMPO technique and IS methods for all 4 parameters (Total Aerobic Count, Total Coliform Count, Yeast and Mould Count and *Escherichia coli* Count) in triplicates are shown in tables.

The log difference calculated in table 1 for Total Aerobic Count is -0.20 in SMP, 0.11 in Milk, 0.03 in Infant Milk Food and -0.26 in Paneer. The log difference calculated in table 2 for the enumeration of *Escherichia coli* is -0.05 in SMP, -0.02 in Milk, and 0.00 in Infant Milk Food and -0.07 in Paneer. The log difference calculated in table 3 for Total Coliform Count is -0.14 in SMP, -0.05 in Milk, -0.10 in Infant Milk Food and -

0.17 in Paneer. The log difference calculated in table 4 for Yeast and Mould Count is 0.13 in SMP, -0.11 in Milk, 0.09 in Infant Milk Food and 0.02 in Paneer. The log difference of results between the IS method and the TEMPO method are not exceeded 0.5 logarithmic units in any case. Hence, the above data indicate that the results correlated with the two levels of artificial

Table 1 Represents the enumeration results of Total Plate Count obtained by IS 5402:2012 and TEMPO

Product	Mean Counts of IS/ISO Method, log cfu/g	Mean Counts of TEMPO Method, log cfu/g	log difference
SMP	4.37	4.57	-0.20
Milk	4.22	4.11	0.11
Infant Food	4.34	4.30	0.03
Paneer	4.34	4.59	-0.26

Table 2 Represents the enumeration results of *Escherichia coli* obtained by IS 5887 (Part-3): 1976 and TEMPO

Product	Mean Counts of IS Method, log cfu/g	Mean Counts of TEMPO Method, log cfu/g	log difference
SMP	4.37	4.42	-0.05
Milk	3.89	3.91	-0.02
Infant Food	2.43	2.43	0.00
Paneer	4.57	4.64	-0.07

Table 3 Represents the enumeration results of Total Coliform obtained by IS 5401 (Part-1):2012 and TEMPO

Product	Mean Counts of IS/ISO method, log cfu/g	Mean Counts of TEMPO Method, log cfu/g	log difference
SMP	4.25	4.39	-0.14
Milk	3.93	3.97	-0.05
Infant Food	4.01	4.10	-0.10
Paneer	4.14	4.30	-0.17

Table 4 Represents the enumeration results of Yeast & Mould count obtained by IS 5402:1999 and TEMPO

Product	Mean Counts of IS method, log cfu/g	Mean Counts of TEMPO Method, log cfu/g	log difference
SMP	3.01	2.88	0.13
Milk	2.87	2.97	-0.11
Infant Food	3.16	3.07	0.09
Paneer	4.43	4.41	0.02

contamination, with no significant difference between the counts estimated by the two methods.

To ensure the quality check of food, we require rapid techniques that can help to achieve quick and reliable results. Recently, different rapid methods for microbiological analysis with high sensitivity and specificity have been developed to overcome the limitations of conventional methods. We have studied 4 different microbiological parameters of various food products using the TEMPO method. To evaluate the efficiency of TEMPO results, we analyzed all the 4 parameters of 4 different food products in IS methods (conventional methods) as well; 4 readings in triplicates are taken for each food product for individual parameters. The results obtained from both the methods of TEMPO and IS are compared statically and are highly correlated with less significant variation. Log difference for the results of TEMPO and IS methods were not exceeding 0.5 logarithmic units for all the tested parameters, which confirms the excellent performance of TEMPO for artificially contaminated samples. The results are comparable and within acceptable criteria, as can be seen from the above-mentioned data. Thus, from the study, we can conclude that the TEMPO method can help to achieve the results more rapidly and precisely, but it is not advisable to use rapid techniques without its verification. Sometimes it is found that the results may vary in the TEMPO method due to food matrixes effect, sample homogeneity, sample weighing error, pipetting error, etc. It is better to verify the rapid technique first for various parameters in different food products and assure confidence for the rapid techniques, than it may be used for daily analysis and can be replaced by conventional methods. Thus, the TEMPO system

eliminates the disadvantages of the conventional methodology while at the same time allowing precise results. The number of miniaturized tubes in the TEMPO card increases the enumeration range and the precision of results compared to a traditional methodology. The TEMPO system can give results over quite a wide enumeration range. The advantages of the MPN methodology and the chromogenic substrate are combined in the TEMPO method.

Conclusions

The comparison of the TEMPO technique for Total Aerobic Count, Total Coliform Count, Yeast & Mould Count, and *Escherichia coli* Count was equivalent to the corresponding IS/ISO methods with a very good rate of agreement, manufacturer instructions and GLP are strictly followed. This system offers improved standardization as well as economic savings in terms of manpower and time by eliminating serial dilutions, media preparation, tedious plate reading, calculations, confirmation tests, etc. Many samples can be analyzed quickly and precisely, involving minimum manpower and man-day's; thereby reducing the turnaround time (TAT). Hence, from the study, it can be concluded that the TEMPO method is a suitable and reliable alternative method for microbiological testing of various food products provided its performance is verified for respective food matrixes.

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