Comparing alternative methods to enumerate total aerobic flora

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Shanghai Entry-Exit Inspection and Quarantine Bureau (Shanghai CIQ) is one of the important public food testing centres in China. It is responsible for ensuring the safety of food products imported to and exported via the city of Shanghai where the biggest commercial centre in China is located.

The Bureau is also responsible for building and maintaining a platform for the application of food safety technology like the establishment of Chinese S/N standard methods and the transfer of technical support to the export food industry. The Bureau faces a number of challenges. There is an ever increasing volume of food samples to be tested. At the same time there is a demand for even earlier test results to identify potential food microbiological contamination.

Furthermore, it is important that the team achieves and maintains international recognition of the laboratory results and that it uses methods that are comparable and compatible with those accepted in the foreign import countries.

TEMPO vials and cards on a filler tray.

Fig. 1. The general protocol.

Sample 1/10 dilution
in a stomacher bag
Diluent: peptone-salt
↓ 1/400 dilution
3.9ml distilled sterile water
+
0.1ml suspension 1/10 in a TEMPO TVC vial
↓
Incubation 40-48 hrs at 36°C ± 1°C
↓
Reading
The dilution 1/400 allows 100 to 490 000 cfu/g enumeration.
The dilution can be increased according to the contamination level expected.

Chinese Reference Method
SN 0168-92
(plate count for bacterial colonies in food for export, China)
Sample 1/10 dilution
Decimal dilutions in peptone salt
↓
1ml per plate (2 plates per dilution)
↓
Addition PCA agar
↓
Incubate 48 hrs ± 2 hrs
at 36°C ± 1°C
↓
Count colonies

Utilising rapid methods

The Bureau's laboratory has long been utilising rapid methods to handle the daily food samples for routine microbiological testing, but these methods have only been applied to the pathogen testing which is a minor part of the total workload.

In early 2006 the laboratory was pleased to have a chance to evaluate the TEMPO system.

The system had the potential to automate the enumeration of food quality indicator organisms and it was hoped that the laboratory might benefit from the system's ability to reduce test handling time and achieve a faster time to result.

It would be applied to the quality indicator testing that accounts for more than 80% of the laboratory's routine testing volume.

Used in combination with a rapid method for the detection of microbial pathogens (bioMérieux VIDAS system), it was hoped that the laboratory could achieve total automation and a standardised approach, which would ensure its capability to guarantee the good food quarantine level.

In China, the hygiene target test of total bacterial count is commonly used to determine the level of food contamination and reflects the compliance with hygiene requirements during production, storing, transportation and sales of food products.

In the food industry, enumeration of total aerobic flora is performed by a labour intensive traditional poured plate count method (so called S/N method in China).

The TEMPO system was developed to improve laboratory efficiency and replace the traditional method. In this study, the TEMPO TVC (Total Viable Count) method was compared to SN 0168-92 (plate count for bacterial colonies in food for export, China) by testing a variety of food products including raw and processed poultry, fish, seafood, and vegetables.

The results of the two methods were compared for 190 samples. Very good correlation was shown between the two methods for TVC (the rate of agreement is 97.4%).

The TEMPO method was found to be simpler, less time consuming and generated less waste volume than the SN method and proved to be a valuable alternative.

The new system developed by bioMérieux is an automated

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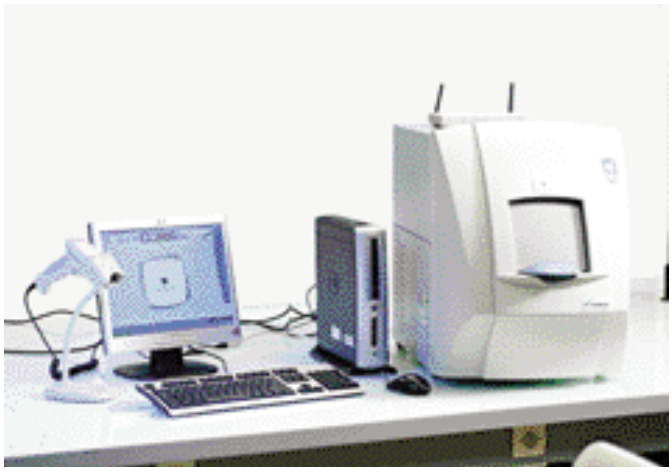

The Tempo reader.

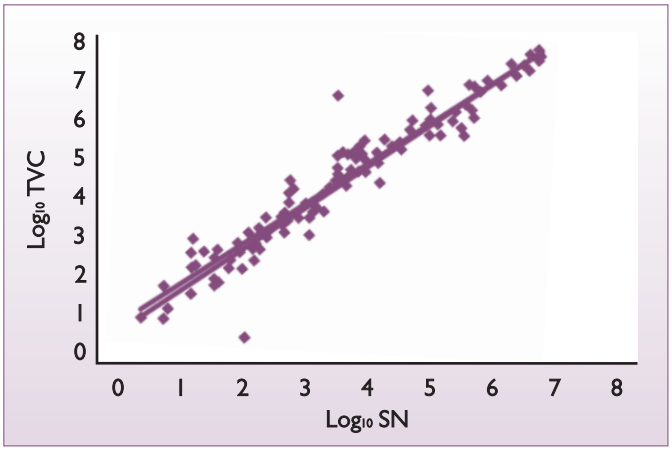

Fig. 2. Linear regression of Log_{10} total viable counts/g by TEMPO TVC to S/N with 190 food samples.

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enumeration system based on the MPN method derived from the company's card technology. The system interprets the microbiological growth and calculates the enumeration results in cfu/g (colony forming units per gram).

There are two distinct activity stations:

- The preparation station where a medium vial is inoculated with the diluted food sample and the TEMPO filler instrument is used to fill and seal the TEMPO cards.
- The reading station which uses the TEMPO reader to read and interpret the cards.

The general protocol is presented in Fig. 1.

In the comparative study a number of naturally contaminated samples were taken from a variety of different foods and these were used to evaluate the TEMPO TVC test.

Detailed results are presented in Table 1.

The following protocol was used:

- Homogenise 25g of food samples in 225ml of MRD (primary 1/10 dilution) in the stomacher bag with filter. Then prepare decimal dilutions of 10-N transferring 1ml of previous dilution to 9ml of peptone salt, and shake well.

In parallel, using:

- **SN 0168-92 (plate count for bacterial colonies in food for export-China) method:** Choose two appropriate serial dilutions depending on the degree of contamination, pipette 1.0ml of each dilution into duplicate Petri dishes, add PCA. After 48+2 hours incubation at 36+1°C, colonies were counted by visual inspection.
- **TEMPO method:** 0.1ml of the primary dilution was transferred in a TEMPO TVC medium vial previously reconstituted with 3.9ml of sterile distilled water to achieve a final volume of 4ml. The system ensured automatic filling of the enumeration card, and after a 40-48 hours incubation at 35°C, reading and MPN calculation by TEMPO reader.

To measure and ensure that the results given were comparable to the laboratories S/N standard method, even though TEMPO is AFNOR/ISO 16140 validated, it was necessary to use log difference of Log_{10} TVC (TEMPO) - Log_{10} SN <1 as a within range result for satisfactory compliance between two methods.

Use one kind of food (frozen dumplings), by repeat testing 10 samples by two above mentioned protocols, counting bacterial colonies with both methods. Collect the data and calculate (see Table 2).

In conclusion, the trial indicated that the performance of the TEMPO TVC test in different food samples was equivalent to the corresponding S/N method with very good rate of agreement at 97.4%. Moreover, the uncertainty of measurement is similar between two methods.

The system offers improved standardisation of testing with automation of different analysis steps such as sample inoculation and objective result interpretation by the automated system reader.

The system additionally complied with various operating requirements such as providing quicker results.

The TEMPO method takes 45 minutes, whereas traditional S/N method consumes 2.5 hours for 50 food samples on TVC testing. It also has true random access and flexible result reading schedule that allows better use of time.

Last but not least, the one card plus one vial for TEMPO analysis versus three test tubes plus four Petri dishes per sample for the Pour Plate method offers better environmental protection by producing less waste.

Without a doubt, the Bureau concluded this would be a good alternative method for enumerating quality indicators in food samples. ■

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Methods	S/N	TEMPOTVC
Average count (cfu/g)	2.5×10^4	3.7×10^4
Measure uncertainty (cfu/g)	1.5×10^4~3.3×10^4	2.7×10^4~4.5×10^4

Table 2. Uncertainty of measurement of the methods.

Table 1. Enumeration of total aerobic flora of different food categories with the methods.

Food category	Total number of samples	Within agreement criteria results	Out of agreement criteria results	Agreement between methods (%)
Frozen vegetables	25	24	1	96.0
Quick frozen food made of wheat flour and rice	30	29	1	96.7
Poultry, beef and chicken products	58	56	2	96.5
Seafood	12	12	0	100
Dairy products	14	14	0	100
Flavouring	44	33	1	97.7
Feather and down	7	7	0	100
Total	190	161	5	97.4

Within agreement criteria: Log_{10} TVC- Log_{10} SN <1
Out of agreement criteria: Log_{10} TVC- Log_{10} SN >1