Alternative methods for agribusiness
Analytical performances certified

VALIDATION CERTIFICATE FOR ALTERNATIVE ANALYTICAL METHOD ACCORDING TO STANDARD EN ISO 16140: 2003

Certificate No.: BIO 12/28 – 04/10

Validation date:	**01.04.2010**
Renewal date:	**30.01.2014**
End of validity:	**01.04.2018**

The company (Head office)	**bioMérieux SA** Chemin de l'Orme 69280 Marcy l'Etoile France	**Production site**	**bioMérieux** 3, route de Port Michaud 38390 La Balme Les Grottes France

is hereby authorized to refer to this **NF VALIDATION certificate** for the following alternative **qualitative** analysis method:

TEMPO® STA

Protocol reference: Ref. 80 002 - 12595 version **G**

SCOPE

All human food products and pet food.

RESTRICTIONS

None

REFERENCE METHOD

EN ISO 6888-2 (1999) and its **amendment A1** (2003) – Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 2: technique using rabbit plasma fibrinogen agar.

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PRINCIPLE OF THE METHOD

TEMPO® STA methods allows the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus*) in 24-27 hours. It is an automated test on TEMPO® system based on Most Probable Number (MPN) method, associating an enumerating card with a specific medium.

The culture medium contains a fluorescent substrate which, when its pH is neutral, emits a signal detected by the TEMPO® Reader. During incubation, the strains of coagulase-positive staphylococci present in the card assimilate the nutrients in the culture medium, resulting in a decrease in pH and the extinction of the fluorescent signal. Depending on the number and size of positive wells in the card, the TEMPO® system deduces the number of strains present in the original sample according to a calculation based on the MPN method (Most Probable Number).

The versions of TEMPO® software usable in the context of NF VALIDATION are: R5 and all prior versions.

Note (History of validation): In January 2014, the certification of TEMPO® STA was renewed without performing additional tests, because neither the alternative method, nor the reference method and nor the validation protocol has changed since the previous validation study.

LINEARITY AND relative ACCURACY
Comparison of performances of the alternative method and the reference method

Linearity study:

Tests were performed in 2009 on the 5 "food product/strain" combinations and for the food categories given in the table below.

The samples were analyzed **in duplicate** with each of the **two methods**, at the five following artificial contamination levels: 200 – 1,000 – 5,000 – 25,000 – 125,000 CFU/g.

The following results were obtained with combined dilutions (1/40 - 1/400 - 1/4,000) and usual dilutions (1/40 - 1/400):

Combined dilutions:

Food category	Strain	Regression equation
Minced meat	*Staphylococcus aureus* Ad 160	Y = 1.086 X - 0.371
« Pâté » for dog	*Staphylococcus aureus* Ad 155	Y = 1.106 X - 0.513
Liquid egg	*Staphylococcus aureus* Ad 159	Y = 1.096 X - 0.324
Pasteurized milk	*Staphylococcus aureus* Ad 468	Y = 1.096 X - 0.324
Fish filet	*Staphylococcus aureus* A00M072	Y = 1.077 X - 0.322

Y = log (N alternative method)
X = log (N reference method)

Usual dilutions:

Food category	Strain	Regression equation
Minced meat	*Staphylococcus aureus* Ad 160	Y = 1.095 X - 0.337
« Pâté » for dog	*Staphylococcus aureus* Ad 155	Y = 1.075 X - 0.256
Liquid egg	*Staphylococcus aureus* Ad 159	Y = 1.105 X - 0.344
Pasteurized milk	*Staphylococcus aureus* Ad 468	Y = 1.100 X - 0.318
Fish filet	*Staphylococcus aureus* A00M072	Y = 1.057 X - 0.264

Y = log (N alternative method)
X = log (N reference method)

Accuracy study:

Tests were performed in 2009. The statistical interpretation was conducted on 65 results, including 36 naturally contaminated samples and 29 artificially contaminated samples, belonging to the following major food categories:

Meat products, pet food, dairy products, seafood products, miscellaneous (ready-cooked dishes, pastry, etc.), and dehydrated products.

The samples were analyzed **in duplicate** with each of the **two methods**.

As an indication, the contamination (concentration) ranges were as follows:

Food category	Contamination range (in log CFU/g)
Meat products	1.32 to 4.81
Pet food	1.32 to 5.54
Dairy products	1.00 to 4.69
Miscellaneous	1.32 to 5.23
Seafood products	2.04 to 5.53
Dehydrated products	1.32 to 5.54

The equation of the regression straight line between the alternative method and the reference method, for all combined categories, is as follows:

$$Y = 1.003X - 0.120$$

Y = log (N alternative method)
X = log (N reference method)

The repeatability standard deviation for both methods and the bias between the two methods were determined according to the method of calculation used for the interlaboratory study (see sections 6.3.5 and 6.3.6 of the standard EN ISO 16140/A1). These results provide additional information for the accuracy criterion.

The repeatability standard deviations (in log) obtained for the alternative method and the reference method are as follows:

Alternative method	Reference method
Sr alt. = 0.352	**Sr ref. = 0.176**

NB: Limit of repeatability r = 2.8 Sr, with Sr: repeatability standard deviation

The bias (in log) between the two methods (alternative method - reference method) is as follows:

D = - 0,080 log CFU/g

This bias is low and not significant.

Conclusion for linearity and relative accuracy:

Linearity and accuracy studies showed that the results obtained with the TEMPO® STA method are comparable to the results obtained with the reference method however the repeatability limit was higher with the alternative method than for the reference method.

Storage of TEMPO cards during 72 hours at 4°C

TEMPO® cards were kept at 4°C for 24 hours and 48 hours and results obtained after storage of the cards were compared to those obtained just before incubation of TEMPO® cards.

Results after storage of TEMPO® cards were identical to those obtained directly after incubation.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)

- 30 strains of coagulase-positive staphylococci were detected out of 31 tested. One strain of *S. intermedius* CIP 81.60 did not grow using the alternative method. A second strain of *S. intermedius* was tested (CIP 81.67) and gave a negative result with both methods. Note that *S. aureus* 6 and *S. aureus* 605 strains were enumerated with TEMPO® STA method only using peptone water suspension adding milk.

- The study of 24 strains not belonging to the genus of coagulase-positive staphylococci did not detect the presence of cross-reactions.

PRACTICABILITY

Use of alternative method only

- **Time to results :**
  - **Positive** and **negative** results are obtained in 1 day with the alternative method against 1 to 2 days with the reference method.

- **The major interest of the TEMPO® STA method consist in :**
  - important labour saving for both analysis and reading, the use of TEMPO® STA methods reducing by 2 the time of analysis in case of high series (20 samples)
  - space saving during incubation of the TEMPO® STA cards and facilitated management of waste
  - complete traceability of analysis ensured by TEMPO® Filler and TEMPO® Reader

INTERLABORATORY STUDY

The interlaboratory study was conducted in 2010 with 16 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Staphylococcus aureus* strain at the 4 following levels:

- level 0 : 0 CFU/ml
- level 1: 10 – 100 CFU/ml
- level 2: 100 – 1 000 CFU/ml
- level 3: 1 000 – 10 000 CFU/ml

The laboratories, using each of the **two methods**, tested **two replicates per contamination level**.

The results calculated in accordance with the EN ISO 16140/A1 standard were the following:

Contamination level	Number of samples taken into account *	Reference method		Alternative method		Bias
		Repeatability standard deviation S_r	Reproducibility standard deviation S_R	Repeatability standard deviation S_r	Reproducibility standard deviation S_R	
Level 1	13*	0.165	0.165	0.150	0.207	-0.181
Level 2	15*	0.057	0.133	0.139	0.159	-0.262
Level 3	16	0.127	0.194	0.118	0.147	0.005

** Three laboratories did not perform the tests with dilution 1/10 for the reference method. The results were not interpretable for the three laboratories at level 1, and for two laboratories at level 2.*

Remark: The bias is significant for levels 1 and 2 but, the results stay acceptable.

NB: Limit of repeatability $r = 2.8\ S_r$, with S_r: repeatability standard deviation
Limit of reproducibility $R = 2.8\ S_R$, with S_R: reproducibility standard deviation

Conclusion

The interlaboratory study shows that the results obtained with the alternative method are comparable to those obtained with the reference method.

Please send any queries concerning the performance of the validated method to AFNOR Certification.
You may download a summary document on the preliminary and inter-laboratory studies on http://nf-validation.afnor.org/en/