

**TEMPO® STA***For microbiological control only*

TEMPO® STA is an automated test for use with the TEMPO system, for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus*) in 24-27 hours in food products.

**SUMMARY AND EXPLANATION**

TEMPO STA is intended for use exclusively with the TEMPO system for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus*) in 24-27 hours in food products, with no need for confirmation.

This test was developed in order to obtain performance levels similar to the standard EN ISO 6888-2 (1) and chapter 12 of the Bacteriological Analytical Manual (BAM) (2).

Staphylococci are naturally present on the skin and mucous membranes of humans and animals. The presence of this microorganism indicates food contamination from humans or animals. Growth of staphylococci in food products is a public health risk since certain strains produce enterotoxins, which if ingested can cause staphylococcal food poisoning. By regularly performing bacteriological analyses, it is possible to monitor the contamination level and prevent accidents.

**PRINCIPLE**

The TEMPO STA test consists of a vial of culture medium and a card, which are specific to this 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 (3, 4). The card is then hermetically sealed in order to avoid any risk of contamination during subsequent handling.

The culture medium contains a fluorescent pH indicator which, when its pH is neutral, emits a signal detected by the TEMPO Reader instrument. The staphylococci present in the card assimilate the nutrients in the culture medium during incubation, resulting in a decrease in pH and the extinction of the fluorescent signal. Depending on the number and type of the positive wells, the TEMPO system calculates the number of staphylococci present in the original sample according to a calculation based on the MPN method.

**CONTENT OF THE KIT (48 TESTS):**

TEMPO STA cards 2 x 24	Ready-to-use, disposable cards with a transfer tube.
TEMPO STA culture medium 2 x 24 vials	Each vial contains a single dose of dehydrated culture medium. Dose for 4 mL.
1 package insert provided in the kit or downloadable from <a href="http://www.biomerieux.com/techlib">www.biomerieux.com/techlib</a>	

**COMPOSITION OF THE TEMPO STA CULTURE MEDIUM****Theoretical formula in g/L of reconstituted solution.**

Animal (bovine and porcine) and vegetable peptones.....	12.5
Sugars and growth supplements.....	11
Buffer system.....	10
Selective agents*.....	10.25
Fluorescent pH indicator.....	0.06
Anti-foaming agent.....	0.4

pH 7.2

\* *Medium Xi*: IRRITANT (22.7 % LiCl in the dehydrated medium)

- **R36/37/38**: Irritating to eyes, respiratory system and skin.
- **S36/37/39**: Wear suitable protective clothing, gloves and eye/face protection.

For more detailed information consult the material safety data sheet available on request.

**MATERIAL AND REAGENTS REQUIRED BUT NOT PROVIDED****Material:**

- TEMPO Bags - Bags with lateral filter (bioMérieux Ref. 80 015)
- Paddle blender
- Pipettes to dispense exactly 0.10 mL or 1.0 mL of sample
- Vortex-type mixer
- Laboratory incubator (under metrology)

*The references below are given as a guide only:*

**Primary diluents recommended:**

- Peptone water / Peptone Saline Diluent (90 mL - Ref. AEB611494)
- Buffered peptone water (90 mL - bioMérieux Ref. 42 042)
- Sodium citrate solution or dipotassium hydrogen phosphate solution following EN ISO 6887-5: 2010 point 5.3 (5)
- Butterfield's phosphate-buffered dilution water (2)
- Or any other diluent which has first been validated by the user as compatible for use with the TEMPO system

**Secondary diluents recommended:**

- Sterile distilled water or equivalent purified water validated by the user
- *Diluent for dehydrated product protocol only*: sterile 0.07 M D-Alanine solution (D-Alanine Sigma Ref. A7377). Refer to protocol in paragraph "Instructions for Use"

**Material recommended for quality control:**

- Densimat (bioMérieux Ref. 99 234)
- Trypcase Soy Agar [TSA] (bioMérieux Ref. 43 011)

## WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
- Comply with Good Laboratory Practice (e.g., standard ISO 7218 (8)).
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- The dehydrated culture medium contains an irritant agent (22.7% LiCl). Refer to the risk phrases "R" and the safety advice "S" given under the composition of the culture medium.
- The culture medium should not be used as a manufacturing material or component.
- All samples and inoculated media should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure; refer to the Laboratory Biosafety Manual – WHO – Geneva – Latest edition, or the current regulations in the country of use.
- Do not use reagents or disposables after the expiry date indicated on their label.
- Before use, check that the packaging and components are intact.
- Only use culture media which appear to be homogeneous (no agglomerates or moisture).
- Do not use visibly deteriorated cards.
- **Do not allow the sample to come into direct contact with the culture medium (in powder form) before the medium has been reconstituted.**
- Any cards which have not been sealed by the TEMPO® Filler instrument must not be used.
- The TEMPO card is not intended for performing subcultures from positive wells.
- Do not write on the card wells or the barcodes.
- Do not stick any labels on the card.
- The TEMPO Reader instrument, the TEMPO Filler instrument and the racks should be regularly cleaned and decontaminated (see the User's Manuals).
- Any change or modification in the procedure may affect the results and must be validated by the laboratory. bioMérieux will not be held liable for results obtained following any changes or modifications in procedures not validated by bioMérieux. In addition, such changes or modifications may void all warranties.

## STORAGE CONDITIONS

- Store the TEMPO STA kit at 2-25°C.
- Do not leave the cards exposed to light (on the workbench or the media stand) for more than 15 days.
- Avoid directly exposing the cards to ultraviolet light.
- If stored according to the recommended conditions, all components are stable until the expiry date indicated on their label.

## SAMPLES

### Sample type

The TEMPO system can be used for the analysis of a large variety of food products for human consumption and pet foods.

### Preparation

Allow the primary and secondary diluents to come to room temperature (18-25°C) (refer to list of diluents recommended in the paragraph "Material and reagents required but not provided").

Follow the recommendations in the current ISO Standards [or BAM (2) if applicable] for performing sample collection and preparing the stock solution. In particular

- for butter and margarine: separate the aqueous and lipid phases according to standards EN ISO 6887-5: 2010 (5) and EN ISO 6887-4 point 9.4.2.2 (7).
- for acidic products, ensure that the pH is restored to neutral when the solution is prepared (EN ISO 6887-4 point 8.2) (6).
- for all aromatic herbs, spices, teas and herbal teas, which may have an inhibitory effect, a minimal dilution of 1/400 should be used (EN ISO 6887-4 point 9.5.4.4) (6).

To prepare the samples, dilute the sample 1/10 (**primary dilution**), using one of the primary diluents recommended. For example, aseptically add 10 g or 10 mL of sample to 90 mL of Peptone water. Homogenize in the TEMPO bag (see instructions for using the TEMPO bag in the User's Manual for the TEMPO Preparation Station).

**The interval between the homogenization of the primary dilution and its transfer into the TEMPO card must not exceed 45 minutes**, unless otherwise indicated in the specific international Standard (7).

## INSTRUCTIONS FOR USE

*For complete instructions, see the TEMPO system User's Manuals.*

### Protocol certified NF VALIDATION (N° BIO 12/28-04/10) according to the standard EN ISO 16140 (9)

### Test procedure

Example for the preparation of a 1/40 dilution enabling enumeration between 10 and  $4.9 \times 10^4$  CFU/g. The dilution can be modified according to the expected level of contamination.

1. Remove the required number of vials of culture medium (one vial per test sample) and allow to come to room temperature.
2. Set the dispenser containing the secondary diluent to 3 mL and prime the pump by eliminating the first two volumes dispensed.
3. Log on to the TEMPO preparation station.
4. Following the instructions of the preparation station user interface, identify the sample to be tested, either by manually entering the identifier via the keyboard or using the preparation station barcode reader.
5. Reconstitute the culture medium by dispensing 3 mL of secondary diluent per vial using the dispenser.

6. Using a sterile pipette, take up 1 mL from the filtered compartment of the TEMPO bag and transfer it into the vial containing the reconstituted culture medium. Homogenize for approximately 3 seconds using a vortex-type mixer. The 4 mL of inoculated medium obtained corresponds to a 1/40 dilution of the sample.
7. Remove one card for each vial of inoculated medium, **without touching** the tip of the transfer tube. Check that the codes (colors and abbreviations) on the card and the vial of inoculated medium match.
8. Associate the identifier of the test sample with the barcodes of the corresponding inoculated medium and card using the preparation station barcode reader, following the instructions of the preparation station user interface.
9. Put the vial containing the inoculated medium in the filling rack. Insert the card in the slot opposite the vial, placing the transfer tube of the card inside the vial. The rack can hold up to 6 vials + cards and enables 1-6 TEMPO cards to be filled simultaneously.
10. Place the rack in the TEMPO® Filler instrument and start the filling cycle. The inoculated medium is completely aspirated into the card. After the cards have been filled, the TEMPO Filler instrument cuts and seals the transfer tubes. All these operations are performed automatically and take 3 minutes. The filling cycle is the same for all the parameters and enables cards for different parameters to be filled at the same time.
11. Remove the filling rack from the TEMPO Filler instrument and visually check that the vials are empty. Take the cards out of the rack and transfer them into the incubation racks: insert the cards into the slots, with the label on the card facing the user (towards the rack handle). Cards which are to be incubated at the same temperature should be grouped together on the same rack. Each rack can hold up to 20 cards. Do not insert cards in between the slots.
12. Dispose of the used vials and transfer tubes into an appropriate receptacle.
13. Incubate the cards for 24-27 hours at  $37 \pm 1^\circ\text{C}$ , in order to obtain performance levels similar to the standard EN ISO 6888-2 (1).

**Protocol for analysis of dehydrated products (e.g., milk powder, dehydrated soups, cake mixes, cereals, etc.) tested at a 1/40 TEMPO dilution**

Dehydrated products diluted to 1/40 have a high level of residual solids and therefore require a specially adapted protocol. This protocol is not required for the 1/400 dilution.

Specific diluent preparation:

- Use D-Alanine (Sigma Ref. A7377) or an equivalent product from another supplier and prepare a 0.07 M solution (6 g/L) using purified or distilled water.
- Sterilize by autoclaving (15 mins at  $121^\circ\text{C}$ ) or filtration ( $0.22 \mu\text{m}$ ).
- Store the diluent at  $2-8^\circ\text{C}$ . Expiry date = 1 day after the diluent is used the first time.

Follow steps 1 to 12 of the TEMPO procedure indicated above **using the sterile specific diluent as secondary diluent** then incubate the cards for **24-27 hours** at  $35 \pm 1^\circ\text{C}$  or  $37 \pm 1^\circ\text{C}$ .

**Protocol for obtaining performance levels similar to those obtained according to AOAC Official Method 975.55**

The AOAC study included 5 different categories of food products:

- dairy products (raw milk, vanilla ice cream, cheddar cheese, nonfat dry milk),
- meat (fresh ground beef patties, filet of beef, prosciutto, cooked diced chicken, chicken salad),
- seafood-based products (tuna salad, smoked salmon),
- fruit and vegetables (frozen mixed vegetables),
- miscellaneous (chocolate eclairs, frozen egg omelette, frozen hash browns).

Results were obtained using Butterfield's Phosphate as primary diluent and a range of dilutions from 1/40 to 1/4000 (enabling enumeration between 10 and 49 million CFU/g).

Follow steps 1 to 12 of the TEMPO procedure indicated above then incubate the cards for 24-27 hours at  $35 \pm 1^\circ\text{C}$  in order to obtain performance levels similar to the BAM (2).

**Note: The incubation time for the test is managed by the TEMPO Read software which integrates a theoretical interval of 15 minutes between the reading of the card barcode and the start of incubation.**

**If the real interval is greater than 15 minutes (without exceeding 2 hours), this extra time must be added to the remaining incubation time displayed by the TEMPO Read software. Reading must always be performed within the 24-27 hour time limit authorized by the software.**

Reading the cards at the end of incubation

1. Log on to the reading station.
2. Introduce the incubation rack containing the cards to be read into the reader. The reader scans the barcode of each card and interprets the results of fluorescence in the wells. It automatically associates the sample identifier with the type of test, the dilution and the enumeration results.

Reading of the TEMPO STA cards may be deferred at the end of incubation by storing them at  $2-8^\circ\text{C}$  for a maximum of 48 hours. In this case, allow the cards to come to room temperature (approximately 5-15 minutes) before introducing them into the reader. It should be emphasized that the result obtained will include the annotation "The card was read too late". The user can specify in the comment text box that the cards were read after having been refrigerated.

3. Editing the results: on the reading station screen, the number of colony forming units (CFU) per gram or milliliter of initial product is associated with the sample identifier, the parameter tested and the analysis date.
  4. The reading station user interface enables the results to be printed out or transmitted to the laboratory information management system (LIMS). It also enables the records of the results obtained the previous days to be consulted.
  5. At the end of the analysis, remove the cards from the rack and dispose of them into an appropriate receptacle.
- Modify the default dilution in the TEMPO software by entering "4" in order to obtain a 1/4 dilution.
  - Fill one card per vial of medium and incubate.
  - At the same time, check the concentration of the suspensions which were used to inoculate the TEMPO cards by streaking 0.1 mL of the 10<sup>3</sup> CFU/mL suspension on TSA. Incubate.
  - After incubation, perform card reading.
  - At the end of incubation of the plates, count the number of colonies of *S. aureus* and check for the presence of *S. epidermidis* and *E. coli* on TSA.

#### **Range of expected results:**

##### *S. aureus*

Calculate the ratio R:

$$R = \frac{\text{TEMPO result (CFU/g)}}{10 \times \text{no. of colonies on TSA}}$$

R should be between 0.01 and 1.

*E. coli* and *S. epidermidis* should be totally inhibited by TEMPO STA (in this case, the TEMPO software indicates: enumeration < 1 CFU/g).

If the enumeration results obtained deviate from the expected values, please contact bioMérieux SA or its local representative.

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

## **RESULTS AND INTERPRETATION**

Once the reading is completed, the results are automatically analyzed by the computer which determines which wells are positive.

The number of positive wells obtained, in relation to the volume of the wells and the dilution of the sample, gives the enumeration result in CFU per gram or milliliter for the original sample, using the MPN tables.

## **QUALITY CONTROL**

The TEMPO® reagents are systematically quality controlled at various stages of their manufacture. For users who wish to perform their own quality control tests to ensure that the TEMPO method has been carried out correctly, the following strains can be used:

*Staphylococcus aureus* ATCC® 25923™

*Escherichia coli* ATCC® 8739™

*Staphylococcus epidermidis* ATCC® 12228™

### **Recommended protocol:**

- The different incubation steps should be performed at 37 ± 1°C or 35 ± 1°C.
- Using a 24-hour old culture on Trypcase Soy Agar, prepare a suspension in Peptone water and adjust to 0.5 McFarland, i.e. approximately 10<sup>8</sup> CFU/mL using the Densimat (see "Material and reagents required but not provided").

The TEMPO STA medium was developed for use with food matrices. When pure strains are used, a specific protocol (milk) is required:

#### Milk protocol:

Perform serial decimal dilutions in Peptone water until a suspension with a theoretical concentration of approximately 10<sup>3</sup> CFU/mL is obtained.

- For *S. aureus*, transfer 0.1 ml of the 10<sup>4</sup> CFU/mL suspension into a tube containing 9 mL of Peptone water and 1 mL of sterilized milk (e.g., UHT). Transfer 1 mL of this 10<sup>2</sup> CFU/mL suspension into a vial of culture medium which has been reconstituted beforehand with 3 mL of water.
- For *E. coli* and *S. epidermidis*, transfer 0.1 mL of the 10<sup>7</sup> CFU/mL suspension into a tube containing 9 ml of Peptone water and 1 mL of sterilized milk (e.g., UHT). Transfer 1 mL of this 10<sup>5</sup> CFU/mL suspension into a vial of culture medium which has been reconstituted beforehand with 3 mL of water.

## **LIMITATIONS OF THE METHOD**

- Invalid results may appear if the card has not been filled correctly (presence of empty wells and/or liquid remaining in the vial after the filling cycle): for example, **use of a filtering bag other than the one recommended** (see paragraph "Material and reagents required but not provided").
- Improper preparation or storage of the samples may lead to incorrect results.
- It is recommended to use Buffered Peptone Water as primary diluent for products containing severely stressed microbial flora (stressed pure strains or combination of different types of stress).
- **Warning:** The TEMPO STA parameter was evaluated using numerous food matrices, excluding soft drinks. However, given the diversity of food matrices and manufacturing processes, users should check that the composition of the matrices tested does not affect result accuracy. In particular, the fluorescent signal may be affected if the primary dilution is strongly colored (e.g., fruit purées and cocoa); or if it becomes colored during incubation in the card due to an oxidation reaction (e.g., raw mushrooms): for the TEMPO STA test, a dilution of these matrices at least equivalent to 1/400 is recommended.

See the TEMPO User's Manuals for more complete information.

**The TEMPO® STA parameter was approved according to the standard EN ISO 16140 (8) as an alternative method for the analysis of all food products for human consumption and pet food, with the exception of drinks and cattle feed. The TEMPO STA method was certified NF VALIDATION by comparison with the reference method described in the international standard EN ISO 6888-2 (1).**

**The BIO 12/28-04/10 validation certificate can be obtained from our Technical Assistance Dept. or from AFNOR Certification. The date of end of validity for the NF VALIDATION certification is indicated on the certificate.**



BIO 12/28 – 04/10  
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS  
Certified by AFNOR Certification  
www.afnor-validation.org  
www.afnor-validation.com

**The TEMPO STA test, for the enumeration of Staphylococcus aureus in a variety of food products, was validated by the AOAC Research Institute in December 2009 (Certification No. 120901).**



120901 – 12/24/09  
PERFORMANCE TESTED METHOD  
Certified by AOAC Research Institute  
www.aoc.org

## WASTE DISPOSAL

Unused reagents may be considered as non hazardous waste and disposed of accordingly.

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

## LITERATURE REFERENCES

1. International Standard EN ISO 6888-2 (1999) - 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 medium.
2. Bacteriological Analytical Manual Online, BAM Chapter 12 "*Staphylococcus aureus*" (January 2001).
3. Cochran W.G. Estimation of bacterial densities by means of the "Most Probable Number". (1950) Biometrics 6, 105-116.

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4. Woodward R.L. How probable is the most probable number ? (1957) J. Am. Water Works Assoc., 49, 1060,1068.
5. International Standard EN ISO Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 5 : Specific rules for the preparation of milk and milk products.
6. Bacteriological Analytical Manual Online BAM Media M79 (January 2001).
7. International Standard EN ISO 6887-4 – Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products.
8. International Standard EN ISO 7218 – Microbiology of food and animal feeding stuffs – General rules for microbiological examinations.
9. International Standard EN ISO 16140 (2003) - Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods.

## INDEX OF SYMBOLS

Symbol	Meaning
	GB: Catalogue number US: Catalog number
	Manufacturer
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests

## WARRANTY

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