TEMPO® LAB (Lactic Acid Bacteria)

For microbiological control only

TEMPO LAB (Lactic Acid Bacteria) is an automated test for use with TEMPO, for the enumeration of Lactic Acid Bacteria in 40-48 hours in food products.

SUMMARY AND EXPLANATION

TEMPO LAB is intended for use exclusively with the TEMPO system for the enumeration of Lactic Acid Bacteria in 40-48 hours in food products.
This test was developed in order to obtain performance levels similar to the standard NF ISO 15214 (1) and the recommendations of the American Public Health Association's Compendium of methods for the Microbiological Examination of Foods (2).

PRINCIPLE

The TEMPO LAB 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 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 microorganisms present in the card reduce the substrate in the culture medium during incubation and cause a fluorescent signal to appear, which is detected by the TEMPO Reader. 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.

CONTENT OF THE KIT (48 TESTS):

TEMPO LAB cards 2 x 24	Ready-to-use, disposable cards with a transfer tube.
TEMPO LAB culture medium 2 x 24 vials	Each vial contains a single dose of dehydrated culture medium. Dose for 4 ml.

COMPOSITION OF THE TEMPO LAB CULTURE MEDIUM

Theoretical formula in g/l of reconstituted solution.

Glucose	14
Nutrients (bovine and porcine)	4
Other nutrients	12.5
Buffer system and inhibitors	17.4
Substrate	0.05
Anti-foaming agent	0.4

pH 5.7

MATERIAL AND REAGENTS REQUIRED BUT NOT PROVIDED

Material:
- TEMPO Bags - Bags with lateral filter (bioMérieux Ref. 80 015)
- Stomacher (Model 400 or equivalent)
- 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 for food samples:
- Peptone water / Peptone Saline Diluent (90 ml - bioMérieux Ref. 42 021)
- Buffered peptone water (90 ml - bioMérieux Ref. 42 042)
- *Dairy products only*: sodium citrate solution or dipotassium hydrogen phosphate solution following EN ISO 8261 : 2001 point 5.3 (5)
- Butterfield's phosphate-buffered dilution water (6)
- Or any other diluent which has first been validated by the user as compatible for use with the TEMPO system

Primary diluents recommended for environmental testing (swabs – cleaning wipes):
- Difco Neutralizing Buffer (Ref. 236210 Neutralizing Buffer for environmental samples)
- Letheen Broth, Modified (7)
- 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

Material recommended for quality control:
- Densimat (bioMérieux Ref. 99 234)
- Columbia agar with sheep blood [COS] (bioMérieux Ref. 43 041)
- Trypcase Soy Agar [TSA] (bioMérieux Ref. 43 011)

WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
- **Comply with Good Laboratory Practice.**
- 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 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 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, TEMPO Filler and 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 LAB kit at 2-25°C.
- After opening the kit, and each time cards are removed from the kit, carefully reseal the packaging (pouch or blister pack) containing the remaining cards.
- 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.

FOOD SAMPLES

Sample type

The TEMPO system can be used for the analysis of a large variety of food products for consumption by humans and domestic animals.

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 (6) if applicable] for performing sample collection and preparing the stock solution. In particular

- for acidic products, ensure that the pH is restored to neutral when the solution is prepared (EN ISO 6887-4 point 8.2) (8).
- 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) (8).

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 (9).

INSTRUCTIONS FOR USE

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

Protocol for obtaining performance levels similar to those obtained according to the standard NF ISO 15214 (1)

Test procedure for food samples

The 1/400 dilution is the minimal default dilution recommended enabling enumeration between 100 and $4.9x10^5$ CFU/g. A 1/40 dilution enabling enumeration between 10 and $4.9x10^4$ CFU/g should only be used for products which are slightly contaminated with total flora (for example, salad dressings). Only the following primary diluents: Peptone water / Peptone Saline Diluent and Butterfield's phosphate-buffered dilution water, are authorized for the 1/40 dilution (see "Limitations of the method").

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 3.9 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 3.9 ml of secondary diluent per vial using the dispenser.
6. Using a sterile pipette, take up 0.1 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 1/400 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 and start the filling cycle. The inoculated medium is completely aspirated into the card. After the cards have been filled, the TEMPO Filler 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 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 40-48 hours at 30 ± 1°C, in order to obtain performance levels similar to the standard NF ISO 15214 (1).

Protocol for obtaining performance levels similar to the Compendium chapter 19 (2)

Follow steps 1 to 12 of the TEMPO procedure indicated above then incubate the cards for 40-48 hours at 35 ± 1°C, in order to obtain performance levels similar to the enumeration protocol using MRS pH 5.5, incubated in anaerobic conditions for 72 ± 3 hours at 35 ± 1°C.

Note 1: 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 40-48 hour time limit authorized by the software.

Note 2: The ± 1°C tolerance for the incubation temperature must be strictly adhered to.

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 LAB cards may be deferred at the end of incubation by storing them at 2-8°C for a maximum of 48 hours. In this case, allow the cards to come to room temperature 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.

ENVIRONMENTAL SAMPLES

Sample type

The proposed protocol can be used for swabbing equipment, countertops or hands with pre-moistened swabs or for wiping countertops with cleaning wipes or sponges. Given the diversity of environmental samples, users should first validate this protocol or any other protocol.

Preparation

Immediately after swabbing or wiping the countertop, transfer the used swab or wipe/sponge directly into a tube containing a given volume of one of the recommended primary diluents. The dilution obtained is the primary dilution of the sample.

Example of test procedure for environmental swabs

Transfer the swab into a tube containing 10 ml, to obtain a dilution which corresponds to a 1/10 dilution of the sample **(primary dilution)**. Homogenize the suspension carefully by shaking the swab in the diluent. Press out the solution by rotating the swab against the inside edge of the tube. It is recommended to test the samples at a dilution of at least 1 in 40 which will enable enumeration between 10 and 4.9×10^4 CFU/surface swabbed. The dilution can be increased 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 tube containing the suspension obtained after swabbing 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 environmental sample collected from the swabbed surface.
7. Modify the default dilution in the TEMPO software by entering "40" in order to obtain a 1/40 dilution.
8. Follow the TEMPO procedure in the paragraph "Test procedure for food samples" from step 7 onwards.

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:
Lactobacillus plantarum ATCC® 14917
Bacillus subtilis ATCC 6633

Recommended protocol:

- The different incubation steps should be performed at 30 ± 1°C or at 35 ± 1°C, in the conditions recommended in the standard used by the laboratory. For *Lactobacillus plantarum*, the COS agar plates should be incubated under microaerophilic or anaerobic conditions.
- Using a 48-hour old culture on Columbia agar with sheep blood [COS] for *Lactobacillus plantarum*, prepare a suspension in Peptone water and adjust to 0.6 McFarland, i.e. approximately 10^8 CFU/ml using the Densimat (see "Material and reagents required but not provided"). Using a 24-hour old culture on Trypcase Soy Agar [TSA] for *Bacillus subtilis*, prepare a suspension in Peptone water and adjust to 1 McFarland, i.e. approximately 10^8 CFU/ml using the Densimat. Perform serial decimal dilutions in Peptone water until a suspension with a theoretical concentration of approximately 10^3 CFU/ml is obtained. For *Lactobacillus plantarum*, transfer 1 ml of this suspension into a vial of culture medium which has been reconstituted beforehand with 3 ml of sterile distilled water. Follow the same procedure for *Bacillus subtilis*, but transfer 1 ml of the 10^5 CFU/ml suspension.
- 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 10^3 CFU/ml suspension on COS (*Lactobacillus plantarum*) or TSA (*Bacillus subtilis*). Incubate as indicated above.
- After incubation, perform card reading. Count the number of colonies of *Lactobacillus plantarum* on COS and check for the presence of *Bacillus subtilis* on TSA.

Range of expected results:

Strain of *Lactobacillus plantarum*

Calculate the ratio R:

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

R should be between 0.1 and 10.

The *Bacillus subtilis* strain should be totally inhibited by TEMPO LAB (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.

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.
- **Warning:** The TEMPO LAB 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): for the TEMPO LAB test, a dilution of these matrices at least equivalent to 1/400 is recommended.
- This test should not be used for the enumeration of thermophilic starter cultures.
- For certain products which contain technological, thermophilic lactic acid flora (e.g., products containing pressed cooked cheese, etc.), an underestimation of counts may be observed at 30°C.
- For the 1/40 dilution, buffered peptone water and dipotassium hydrogen phosphate solution (primary diluents) should not be used as they affect the pH of the TEMPO LAB medium.

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

WASTE DISPOSAL

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. Standard NF ISO 15214 (1998) - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30°C.
2. American Public Health Association
(2004) 4th Edition. Compendium of methods for the Microbiological Examination of Foods, chapter 19, Acid-Producing Microorganisms, §19.522 Acidified MRS Agar.
3. Cochran W.G.
Estimation of bacterial densities by means of the "Most Probable Number".
(1950) Biometrics 6, 105-116.
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 8261 (2001) – Milk and milk products – General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination.
6. Bacteriological Analytical Manual
(1998) 8th Edition, Revision A, Chapter 1 “Food Sampling and Preparation of Sample Homogenate”.
7. Bacteriological Analytical Manual
(1998) 8th Edition, Revision A, Appendix 3, M.79.
8. 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.
9. International Standard EN ISO 7218 – Microbiology of food and animal feeding stuffs – General rules for microbiological examinations.

INDEX OF SYMBOLS

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

WARRANTY

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