

TEMPO® EB (*Enterobacteriaceae*)*For microbiological control only*

TEMPO EB (*Enterobacteriaceae*) is an automated test for use with TEMPO, for the enumeration of enterobacteria in 22-27 hours in food products.

SUMMARY AND EXPLANATION

TEMPO EB is intended for use exclusively with the TEMPO system for the enumeration of enterobacteria in 22-27 hours at $35 \pm 1^\circ\text{C}$ in food products with no need for confirmation.

This test was developed in order to obtain performance levels similar to the standard ISO 21528-2 (1).

The *Enterobacteriaceae* family is a major indicator in the food industry as it includes both intestinal pathogens and important food spoilage agents and remains one of the most widely used indicators of hygiene and food safety.

Although many members are not referred to as pathogens, the *Enterobacteriaceae* family includes numerous species such as *Salmonella* spp., *Shigella* spp. and *Escherichia coli*, which can cause a wide range of human diseases.

PRINCIPLE

The TEMPO EB 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 (2, 3). 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. The enterobacteria 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 enterobacteria present in the original sample according to a calculation based on the MPN method.

CONTENT OF THE KIT (48 TESTS):

TEMPO EB cards 2 x 24	Ready-to-use, disposable cards with a transfer tube.
TEMPO EB 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 www.biomerieux.com/techlib	

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

Vegetable peptones.....	10
Sugars.....	26
Sodium chloride.....	5
Growth supplements.....	0.5
Buffer system.....	16
Sodium deoxycholate* (bovine and ovine).....	0.75
Fluorescent pH indicator.....	0.05
Anti-foaming agent.....	0.4

pH 7.4

* The vial of culture medium contains > 1% Sodium deoxycholate.
A Safety Data Sheet is available on request.

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
- $35 \pm 1^\circ\text{C}$ 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:* dipotassium hydrogen phosphate solution following EN ISO 8261:2001 point 5.3 (4)
- Butterfield's phosphate-buffered dilution water (5)
- 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)
- Lethen Broth, Modified (6)
- 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)
- 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 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 unopened TEMPO EB kit at 2-8°C.
- After opening the kit:
 - Carefully reseal the packaging (pouch or blister pack) containing the remaining cards each time cards are removed from the kit.
 - **The reagents can be stored at a temperature greater than 8°C up to 25°C for up to 1 month.** Record the new expiry date (date on which the reagents were placed at room temperature + 1 month) in the box printed for this purpose on the inner vial container label (☞). This date must not under any circumstances exceed the initial expiry date indicated on the label.
- 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.
- All components are stable if stored according to the recommended conditions.

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 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 8261:2001 point 8.2.6 (4) 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) (7).
- 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) (7).

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 (8).

INSTRUCTIONS FOR USE

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

Protocole certified AFNOR Validation according to the standard ISO 16140 (9) (N° BIO 12/21-12/06) and certified by AOAC Research Institute (attestation N° 050801)

Test procedure for food samples

Example for the preparation of a 1/40 dilution enabling enumeration between 10 and 4.9x10⁴ CFU/g. The dilution can be modified according to the expected level of contamination. Only the ISO diluents listed on page 1 can be used with this protocol.

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 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 **22-27 hours** at $35 \pm 1^\circ\text{C}$, in order to obtain performance levels similar to the standard ISO 21528-2 (1).

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, in order to adhere strictly to the real incubation time (in the $35 \pm 1^\circ\text{C}$ incubator) which must be at least 22 hours. Reading must always be performed within the 22-27 hour time limit authorized by the software.

The TEMPO method was compared to the Compendium of Methods for the Microbiological Examination of Foods:

- Meat (raw ground pork, fresh ground beef, heat processed cooked roast beef),
- Poultry (fresh ground chicken, frozen cooked chicken, heat processed grilled chicken),
- Fish (frozen catfish, heat processed frozen fish, raw cod),
- Vegetables (bagged salad, frozen green beans, hashbrown potatoes),
- Dairy products (vanilla ice cream, pasteurized milk, milk powder),
- Miscellaneous (pasteurized egg, rice, dry pet food).

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.
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.

Note : The TEMPO EB cards may be stored at $2-8^\circ\text{C}$ after inoculation, for a maximum of 48 hours, before incubating for 22-27 hours at $35^\circ\text{C} \pm 1^\circ\text{C}$ (outside the scope of the AFNOR Validation certification). 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.

ENVIRONMENTAL SAMPLES (outside the scope of the AFNOR Validation certification).

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. 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:

Enterococcus faecalis ATCC® 29212
Escherichia coli ATCC 25922
Salmonella enterica serovar *typhimurium* ATCC 14028

Recommended protocol

- The different incubation steps should be performed at $35 \pm 1^\circ\text{C}$.
- 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 10^8 CFU/ml using the Densimat (see "Material and reagents required but not provided"). Perform serial decimal dilutions in Peptone water until a suspension with a theoretical concentration of approximately 10^2 CFU/ml is obtained. For *E. coli* and *S. enterica* serovar *typhimurium*, 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 *E. faecalis*, but transfer 1 ml of the 10^6 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 suspension which was used to inoculate the TEMPO card by streaking 0.1 ml of the 10^3 CFU/ml suspension on TSA. Incubate.
- After incubation, perform card reading. Count the number of colonies of *E. coli* and *S. enterica* serovar *typhimurium* and check for the presence of *E. faecalis* on TSA.

Range of expected results

Non-enteric strain

E. faecalis ATCC® 29212 should be totally inhibited by TEMPO EB (in this case, the TEMPO software indicates: enumeration < 1 CFU/g).

Enteric strains

This concerns *E. coli* ATCC 25922 and *S. enterica* serovar *typhimurium* ATCC 14028. Calculate the ratio R :

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

For both strains, R should be between 0.01 and 1.

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 EB 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 EB 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 EB parameter was approved according to the standard EN ISO 16140 (9) as an alternative method for the analysis of all food products for consumption by humans and domestic animals, with the exception of drinks and cattle feed. The TEMPO EB method was certified AFNOR Validation by comparison with the reference method described in the international standard ISO 21528-2 (1).

The BIO 12/21-12/06 validation certificate can be obtained from our Technical Assistance Dept. or from AFNOR Certification. The date of end of validity for the AFNOR Validation certification is indicated on the certificate.



BIO 12/21-12/06
 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
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The TEMPO EB test, for the enumeration of enterobacteria in a variety of food products, was validated by the AOAC Research Institute in May 2008 (Certification No. 050801).



050801 – 05/07/08
PERFORMANCE TESTED METHOD
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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. International Standard ISO 21528-2 (2004) – Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of *Enterobacteriaceae*. Part 2: Colony-count method.
2. Cochran W.G.
Estimation of bacterial densities by means of the "Most Probable Number".
(1950) *Biometrics* 6, 105-116.
3. Woodward R.L.
How probable is the most probable number ?
(1957) *J. Am. Water Works Assoc.*, 49, 1060,1068.
4. 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.
5. *Bacteriological Analytical Manual*,
(1998) 8th Edition, Revision A, Chapter 1, B.6, R11.
6. *Bacteriological Analytical Manual*
(1998) 8th Edition, Revision A, Appendix 3, M.79.
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 limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests

WARRANTY

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bioMérieux SA
 RCS LYON 673 620 399
 69280 Marcy-l'Étoile / France
 Tel. 33 (0)4 78 87 20 00
 Fax 33 (0)4 78 87 20 90
www.biomerieux.com

bioMérieux, Inc
 Box 15969,
 Durham, NC 27704-0969 / USA
 Tel. (1) 919 620 20 00
 Fax (1) 919 620 22 11