### TEMPO® EC (*E. coli*)

### For microbiological control only

TEMPO<sup>®</sup> EC (*E. coli*) is an automated test for use with the TEMPO system, for the enumeration of *Escherichia coli* in 22-27 hours in food products and environmental samples.

### **SUMMARY AND EXPLANATION**

The TEMPO EC test is intended for use exclusively with the TEMPO system for the enumeration of *Escherichia coli* in 22-27 hours in food products and environmental samples.

This test was developed in order to obtain performance levels similar to the standard EN ISO 16649-2 (1) and AOAC Official Methods 966.23 and 966.24.

Escherichia coli is a commensal host of humans and animals. This species may be responsible for food poisoning if development is profuse. Certain strains are considered pathogenic and can produce one or more toxins

#### **PRINCIPLE**

The TEMPO EC 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 (2, 3). The card is then hermetically sealed in order to avoid any risk of contamination during subsequent handling.

Based on ß-glucuronidase activity, *Escherichia coli* 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 instrument. Depending on the number and type of the positive wells, the TEMPO system calculates the number of *Escherichia coli* present in the original sample according to a calculation based on the MPN method.

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

TEMPO EC cards 2 x 24	CARDS	Ready-to-use, disposable cards with a transfer tube.		
TEMPO EC culture medium 2 x 24 vials		Each vial contains a single dose of dehydrated culture medium.  Dose for 4 mL.		
1 package insert downloadable from www.biomerieux.com/techlib				

### COMPOSITION OF THE TEMPO EC CULTURE MEDIUM

Theoretical formula in g/L of reconstituted solution.	
Bio-Soyase and nutrients	9 0.25 20.8 12.6 0.7 0.19 0.4
pH 7.4	

\* Signal Word: WARNING



(28.7% MOPS acid in the dehydrated medium)

### **Hazard statement**

H315: Causes skin irritation.

H319: Causes serious eye irritation.

H335: May cause respiratory irritation.

### Precautionary statement

P261: Avoid breathing dust/fume/gas/mist/vapours/spray. P280: Wear protective gloves/protective clothing/eye protection/face protection.

P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Material Safety Data Sheet.

### 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 for food samples:

- 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 (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<sup>®</sup> Neutralizing Buffer (Ref. 236210 Neutralizing Buffer for environmental samples)
- Letheen 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
- Mandatory diluent for milk powder protocol only: sterile MOPS buffer 0.4M, pH 7.5

MOPS buffer preparation (up to 500 mL):

- 13.60 g MOPS acid
- 31.21 g MOPS sodium salt

Make up to 500 mL with distilled water. Sterilize by autoclaving or filtration.

### 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 EN ISO 7218 (9)).
- 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 (28.7% MOPS). Refer to the hazard statements "H" and the precautionary statements "P" above.
- 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<sup>®</sup>
   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 EC kit at 2-25°C.
- It is recommended not to 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 human consumption and pet food.

### **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 (7) 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).

For the analysis of all milk powder samples, it is mandatory to use sterile MOPS buffer 0.4M, pH 7.5 as secondary diluent in the preparation of the TEMPO 1/40 dilution (the 1/400 dilution does not require the use of MOPS buffer).

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.

Protocol certified NF VALIDATION (No. BIO 12/13-02/05) according to the standard EN ISO 16140 (10)

### Test procedure for food samples

Preparation of a 1/40 dilution enabling enumeration between 10 and  $4.9 \times 10^4$  CFU/g. Only the ISO diluents listed on page 1 can be used with this protocol.

- Remove the required number of vials of culture medium (one vial per test sample) and allow to come to room temperature.
- 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.
- 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.
- 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.
- 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.
- 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<sup>®</sup> 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  $37 \pm 1^{\circ}$ C, in order to obtain performance levels similar to the standard EN ISO 16649-2 (1).

### Mandatory protocol for analysis of all milk powder samples

Follow steps 1 to 12 of the TEMPO procedure indicated above mandatorily using sterile MOPS buffer 0.4M, pH 7.5 as secondary diluent.

Incubate the cards for 24-27 hours at 37 ± 1°C.

**Note**: As the minimum incubation time authorized by the TEMPO software is 22 hours, the user must pay particular attention when performing the analysis in the context of NF VALIDATION certification to ensure that the 24-hour minimum incubation time is respected.

# Protocol for obtaining performance levels similar to those obtained according to AOAC Official Methods 966.23 and 966.24

The AOAC study included 7 different categories of food products:

- meat (fresh ground beef, frozen ground beef, raw ground veal, raw ground pork),
- poultry (raw fresh ground chicken, frozen turkey breast, raw ground turkey, frozen chicken nuggets),
- fish (raw white fish, frozen cooked fish, raw salmon),
- egg products (pasteurized egg),
- vegetables (fresh green beans, lettuce, strawberries),
- dairy (vanilla ice cream, raw milk, mozzarella, yogurt),
- miscellaneous (dry pet food).

Results were obtained using Butterfield's Phosphate as primary diluent.

Follow steps 1 to 12 of the TEMPO procedure indicated above then incubate the cards for 22-27 hours at  $35 \pm 1^{\circ}$ C.

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 22-27 hour time limit authorized by the software.

### Reading the cards at the end of incubation

- 1. Log on to the reading station.
- 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 EC cards may be deferred at the end of incubation by storing them at 2-8°C for a maximum of 48 hours (outside the scope of the NF VALIDATION certification). 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.
- At the end of the analysis, remove the cards from the rack and dispose of them into an appropriate receptacle.

### **ENVIRONMENTAL SAMPLES** (outside the scope of the NF 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 4.9x10<sup>4</sup> CFU/surface swabbed. The dilution can be increased according to the expected level of contamination.

- Remove the required number of vials of culture medium (one vial per test sample) and allow to come to room temperature.
- 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.
- 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:

Escherichia coli ATCC<sup>®</sup> 25922<sup>TM</sup> Escherichia coli ATCC<sup>®</sup> 8739<sup>TM</sup>

Pseudomonas aeruginosa ATCC® 27853<sup>TM</sup>

### **Recommended protocol:**

- The different incubation steps should be performed at  $37 \pm 1^{\circ}$ C or  $35 \pm 1^{\circ}$ 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"). Perform serial decimal dilutions in Peptone water until a suspension with a theoretical concentration of approximately 10<sup>2</sup> CFU/mL is obtained. For *E. coli*, 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 the strains of *Pseudomonas*, but transfer 1 mL of the 10<sup>7</sup> 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<sup>3</sup> CFU/mL suspension on TSA. Incubate.
- After incubation, perform card reading. Count the number of colonies of *E. coli* and check for the presence of *Pseudomonas* on TSA.

### Range of expected results:

Strains of E. coli

Calculate the ratio R:

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

R should be between 0.01 and 1.

<u>Pseudomonas aeruginosa</u> should be totally inhibited by the TEMPO EC test (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® EC 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 EC test, a dilution of these matrices at least equivalent to 1/400 is recommended.
- It is not recommended to use the TEMPO EC test for the microbiological analysis of products with strong enzymatic activity. This restriction applies in particular to raw mollusks and raw red offals.

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

#### **PERFORMANCE**

During approval of the TEMPO EC parameter, according to the standard EN ISO 16140 (10), the preliminary study gave the following results:

- Selectivity: out of the 30 E. coli strains tested, the 28 strains which produced characteristic colonies with the reference method were detected by the TEMPO EC test. For the 2 E. coli strains (β-glucuronidase negative) which produced non-characteristic colonies on TBX Medium, enumeration was not obtained using the TEMPO EC test.

The study of 20 non-*E. coli* strains did not reveal any cross-reactivity.

 Relative accuracy: the analysis was based on 50 samples (including 25 naturally contaminated samples) tested in duplicate and simultaneously using the TEMPO EC test and the EN ISO 16649-2 (1). The linear regression equation when comparing the 2 methods is:

log TEMPO EC = 0.99 log ISO + 0.35

The bias observed between the 2 methods of 0.26 log, in favor of the TEMPO EC method, is due to a better coverage of *E. coli*.

The TEMPO EC parameter was approved according to the standard EN ISO 16140 (10) 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 EC method was certified NF VALIDATION by comparison with the reference method described in the international standard EN ISO 16649-2 (1).

The BIO 12/13-02/05 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 12/13 – 02/05
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
www.afnor-validation.org
www.afnor-validation.com

The TEMPO EC parameter has been validated and certified by the AOAC Official Methods (2009.02) for the enumeration of Escherichia coli. The alternative method was compared in a multi-laboratory collaborative study to AOAC Official Method 966.24. Six food types were artificially contaminated with E. coli: raw ground beef, bagged lettuce, cooked chicken, pasteurized crabmeat, frozen green beans and pasteurized whole milk.

The TEMPO EC test, for the enumeration of Escherichia coli in a variety of food products, was validated by the AOAC Research Institute in August 2006 (Certification No. 080603).



080603 - 08/08/06
PERFORMANCE TESTED METHOD
Certified by AOAC Research Institute
www.aoac.org

### **WASTE DISPOSAL**

Dispose of unused reagents following procedures for hazardous chemical waste.

Dispose of used 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

- International Standard EN ISO 16649-2 (2001) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive Escherichia coli. Part 2: Colony-count technique at 44°C using 5-bromo-4chloro-3-indolyl β-D-glucuronide.
- Cochran W.G.
   Estimation of bacterial densities by means of the "Most Probable Number".
   (1950) Biometrics 6, 105-116.
- Woodward R.L. How probable is the most probable number ? (1957) J. Am. Water Works Assoc., 49, 1060,1068.
- International Standard EN ISO 6887-5 (2010) 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.
- Bacteriological Analytical Manual Online BAM Reagent R11 (January 2001).
- Bacteriological Analytical Manual Online BAM Media M79 (January 2001).
- Bacteriological Analytical Manual Online Chapter 4 "Enumeration of Escherichia coli and the Coliform Bacteria" (September 2002).
- 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.
- International Standard EN ISO 7218 Microbiology of food and animal feeding stuffs – General rules for microbiological examinations.
- 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		
REF	Catalog number		
•••	Manufacturer		
	Temperature limit		
	Use by date		
LOT	Batch code		
[]i	Consult Instructions for Use		
Σ	Contains sufficient for <n> tests</n>		
M	Date of manufacture		

### **WARRANTY**

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### **REVISION HISTORY**

### Change type categories:

N/A Not applicable (First publication)

Correction Correction of documentation anomalies

Technical change Addition, revision and/or removal of information related to the product Administrative Implementation of non-technical changes noticeable to the user

Note: Minor typographical, grammar, and formatting changes are not included in the revision history.

Release date	Part Number	Change Type	Change Summary
2015/01	12597L	Administrative	Content of the kit
			Index of symbols
			Creation of the revision history table
		Technical change	Composition of the TEMPO EC culture medium
			Warnings and precautions
			Storage conditions

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