

TEMPO® AC (Aerobic Count)*For microbiological control only*

TEMPO® AC (Aerobic Count) is an automated test for use with the TEMPO system, for the enumeration of viable aerobic mesophilic flora in food products and environmental samples.

SUMMARY AND EXPLANATION

TEMPO AC is intended for use exclusively with the TEMPO system for the enumeration of viable aerobic mesophilic flora in food products and environmental samples.

This test was developed in order to obtain performance levels similar to the standard EN ISO 4833 (1), the AOAC Official Method 966.23 and the American Public Health Association's Standard Methods for the Examination of Dairy Products (SMEDP) (2).

The enumeration of this flora is used to determine the sanitary quality of the product and can express its state of freshness or deterioration. For products which are handled or which undergo various technological processes, the total flora count can be used to judge the conditions in which the food was produced, transported and stored.

PRINCIPLE

The TEMPO AC 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 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 instrument. 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 AC cards 2 x 24	Ready-to-use, disposable cards with a transfer tube.
TEMPO AC 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 AC CULTURE MEDIUM**Theoretical formula in g/L of reconstituted solution.**

Nutrient and growth supplements (bovine/porcine origin).....	27
Substrate.....	0.08
Anti-foaming agent.....	0.4
pH 7.2	

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 (5)
- Butterfield's phosphate-buffered dilution water (7)
- 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:

- Difco Neutralizing Buffer (Ref. 236210 Neutralizing Buffer for environmental samples)
- Lethen Broth, Modified (8)
- 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 EN ISO 7218 (10)).
- 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 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 AC 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.

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 (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) (9).
- for all aromatic herbs, spices, teas and herbal teas, which may have an inhibitory effect, a minimal dilution of 1/400 should be used, even after applying the standard EN ISO 6887-4 point 9.5.4.4 (9).

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 (10).

PROCEDURE FOR OBTAINING RESULTS EQUIVALENT TO THE ISO 4833 REFERENCE METHOD AT 30°C (1)

For complete instructions, see the TEMPO system user's documentation (available at www.biomerieux.com/techlib).

Sample	Test mode
All food matrices (standard protocol)	40-48h
Raw meat including poultry (specific protocol)	24-28h
Ready-to-eat meal (specific protocol)	24-28h
Production environment including carcasses (specific protocol)	24-28h
Fruit and vegetables (specific protocol)	24-28h

Test procedure for food samples

Example for the preparation of a 1/400 dilution enabling enumeration between 100 and 4.9×10^5 CFU/g. The dilution can be increased or reduced, 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 3.9 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 5 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 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 at $30 \pm 1^\circ\text{C}$ for the time indicated for the chosen test mode, in order to obtain performance levels similar to the standard EN ISO 4833 (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. Reading must always be performed within the 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 AC 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 (approximately 5-15 minutes) before introducing them into the reader. The activation of refrigeration management and type must have been previously configured in **TEMPO Admin**.
This functionality enables the warning « card read too late » obtained for a test result, to be replaced by a message indicating that the card has 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 various types of environmental samples, including swabs, cleaning wipes, process water, dust and carcasses. Given the diversity of environmental samples, users should first validate this protocol or any other protocol.

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").

If using a collection device which already contains a neutralizing diluent, ensure that the diluent is listed in the paragraph "Material and reagents required but not provided". If it is not listed, users should first validate this collection device.

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 (CFU/S). The dilution can be adjusted 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 5 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:

- *Escherichia coli* ATCC® 25922™,
- *Bacillus subtilis* ATCC® 6633™.

Recommended protocol:

- The different incubation steps should be performed at $30 \pm 1^\circ\text{C}$.
- Using a 24-hour old culture on Trypcase Soy Agar, prepare a suspension in Peptone water and adjust to approximately 10^8 CFU/mL for *E. coli* and 10^7 CFU/mL for *B. subtilis* (which corresponds to a turbidity between 0.4 McFarland and 0.8 McFarland on 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 each strain, transfer 1 mL of this suspension into a vial of culture medium which has been reconstituted beforehand with 3 mL of sterile distilled water.
- Modify the default dilution in the TEMPO software by entering "4" in order to obtain a 1/4 dilution.
- Modify the default unit in the TEMPO software by selecting "No unit".
- 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.
- At the end of incubation of the plates, count the number of colonies on TSA.

Range of expected results:

For the 2 strains, calculate the ratio R:

$$R = \frac{\text{TEMPO result}}{\text{no. of colonies on TSA}}$$

R should be between 0.1 and 10.

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.

PROCEDURE FOR OBTAINING RESULTS EQUIVALENT TO THE BAM REFERENCE METHOD AT 35°C (SMEDP REFERENCE METHOD AT 32°C) (2)

For complete instructions, see the TEMPO system user's documentation (available at www.biomerieux.com/techlib).

Sample	Test mode
All food matrices and production environment	22-28h

Test procedure for food samples

Example for the preparation of a 1/40 dilution enabling enumeration between 10 and 4.9×10^4 CFU/g. The dilution can be adjusted according to the expected level of contamination. Only the 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 5 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 22-28 hours
 - at $35 \pm 1^\circ\text{C}$, in order to obtain performance levels similar to those obtained according to AOAC Official Method 966.23.
 - at $32 \pm 1^\circ\text{C}$, in order to obtain performance levels similar to the Standard Methods for the Examination of Dairy Products (SMEDP) (2).

The TEMPO method was compared to AOAC Official Method 966.23 and SMEDP (2). The AOAC study included the following categories of food products:

- meat (fresh raw ground beef, cooked deli roast beef),
- poultry (fresh ground chicken breast, chicken carcass rinse),
- seafood-based products (raw fresh cod, heat processed frozen white fish),
- fruit and vegetables (fresh fruit salad, fresh tomatoes),
- dairy (pasteurized milk, vanilla ice cream),
- miscellaneous (almonds, pasteurized liquid eggs, dry onions),
- animal feed (dry pet food),
- environmental (stainless steel surfaces).

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 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 AC 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 (approximately 5-15 minutes) before introducing them into the reader. The activation of refrigeration management and type must have been previously configured in **TEMPO Admin**.

This functionality enables the warning « card read too late » obtained for a test result, to be replaced by a message indicating that the card has 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 (CFU/S). 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 5 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® 25922™,
- *Bacillus subtilis* ATCC® 6633™.

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 approximately 10^8 CFU/mL for *E. coli* and 10^7 CFU/mL for *B. subtilis* (which corresponds to a turbidity between 0.4 McFarland and 0.8 McFarland on 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 each strain, transfer 1 mL of this suspension into a vial of culture medium which has been reconstituted beforehand with 3 mL of sterile distilled water.
- Modify the default dilution in the TEMPO software by entering "4" in order to obtain a 1/4 dilution.
- Modify the default unit in the TEMPO software by selecting "No unit".
- 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.
- At the end of incubation of the plates, count the number of colonies on TSA.

Range of expected results:

For the 2 strains, calculate the ratio R:

$$R = \frac{\text{TEMPO result}}{\text{no. of colonies on TSA}}$$

R should be between 0.1 and 10.

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® AC parameter was evaluated using numerous food matrices. 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 AC test, a dilution of these matrices at least equivalent to 1/400 is recommended.
- For food products with strong reducing activity, e.g., foods rich in vitamin C and raw mussels, a dilution greater than 1/40 is recommended.
- The TEMPO AC reagent enables the enumeration of total aerobic mesophilic flora as described in the standardized protocols. Incubation conditions may not be optimum for slow-growing bacteria, such as certain lactic bacteria. Laboratories testing products which may contain this type of microorganism, e.g., fermented products, are therefore advised to perform an internal verification.

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

The TEMPO AC parameter was approved according to the standard EN ISO 16140 (11) as an alternative method for the analysis of all food products for human consumption, pet food and environmental samples. The TEMPO AC method was certified NF VALIDATION by comparison with the reference method described in the international standard EN ISO 4833 (1).

The BIO 12/35 - 05/13 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 12/35 – 05/13
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
www.afnor-validation.org
www.afnor-validation.com

The TEMPO AC test, for the enumeration of total aerobic mesophilic flora in a variety of food products and environmental samples, was validated by the AOAC Research Institute in December 2012 (Certification No. 121204).



121204 – 12/19/12
PERFORMANCE TESTED METHOD
Certified by AOAC Research Institute
www.aoac.org

WASTE DISPOSAL

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








Dispose of used reagents or unused vials of TEMPO AC culture medium 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 hazardness 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 4833 (2003) - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony-count technique at 30°C.
2. American Public Health Association (2004) 17th Edition. Standard Methods for the Examination of Dairy Products, APHA Washington DC.
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 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.
6. Bacteriological Analytical Manual Online, BAM Chapter 3 "Aerobic Plate Count" (January 2001).
7. Bacteriological Analytical Manual Online BAM Reagent R11 (January 2001).
8. Bacteriological Analytical Manual Online BAM Media M79 (January 2001).
9. 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.
10. International Standard EN ISO 7218 – Microbiology of food and animal feeding stuffs – General rules for microbiological examinations.
11. 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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