TEMPO® YM (Yeasts/Molds)

For microbiological control only

TEMPO YM (Yeasts/Molds) is an automated test for use with TEMPO, for the enumeration of yeasts and molds in 72-76 hours in food products and environmental samples.

SUMMARY AND EXPLANATION

TEMPO YM is intended for use exclusively with the TEMPO system for the enumeration of yeasts and molds in 72-76 hours in food products and environmental samples.
This test was developed in order to obtain performance levels similar to the standard EN ISO 21527 (1) and chapter 18 of the Bacteriological Analytical Manual (BAM) (2).
Molds are agents which can be responsible for the contamination and deterioration of certain food products. Some molds develop mycotoxins which diffuse into food and may, if present in sufficient number, cause acute or chronic food poisoning.
Yeasts may produce changes affecting food quality and saleability, such as cloudiness, strange smell, unusual taste (ethanol, modified pH, etc.), or bloated products and/or packaging (CO_2), etc.

PRINCIPLE

The TEMPO YM 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.
The yeasts and molds 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 yeasts and molds present in the original sample according to a calculation based on the MPN method.

CONTENT OF THE KIT (48 TESTS):

TEMPO YM cards 2 x 24	Ready-to-use, disposable cards with a transfer tube.
TEMPO YM 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 YM CULTURE MEDIUM

Theoretical formula in g/l of reconstituted solution.

Glucose	30
Nutrients (bovine and porcine)	10
Buffer system and inhibitors *	4.9
Substrate	0.075
Anti-foaming agent	0.4

pH 5.0

* *Medium T: TOXIC* (Chloramphenicol ≥ 0.1 % in the dehydrated medium)
- **R45:** May cause cancer.
- **R42/43:** May cause sensitisation by inhalation and skin contact.
- **S22:** Do not breathe dust.
- **S36/37/39:** Wear suitable protective clothing, gloves and eye/face protection.
- **S46:** If swallowed, seek medical advice immediately and show this container or label.

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

MATERIAL AND REAGENTS REQUIRED BUT NOT PROVIDED

Material:
- TEMPO Bags - Bags with lateral filter (bioMérieux Ref. 80 015)
- 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:
- 0.1 % (mass concentration) peptone water broth (1)
- 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 (2)
- 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 (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)
- Sabouraud Dextrose Agar [SDA] (bioMérieux Ref. 43 555)
- Trypcase Soy Agar [TSA] (bioMérieux Ref. 43 011)
- BioBall® MultiShot 550 containing 550 UFC of *Aspergillus niger* (bioMérieux Ref. 56 011)

WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
- Comply with Good Laboratory Practice (e.g., standard ISO 7218 (8)).
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- The dehydrated culture medium contains a toxic agent (Chloramphenicol ≥ 0.1 %). Refer to the risk phrases “R” and the safety advice “S” given under the composition of the culture medium.
- The culture medium should not be used as a manufacturing material or component.
- All samples and inoculated media should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the microbial 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 YM 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 [or BAM (2) 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) (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.

Protocol validated by AOAC Research Institute (Certification No. 041001)

Test procedure for food samples

Example for the preparation of a 1/40 dilution enabling enumeration between 10 and $4.9x10^4$ CFU/g. The dilution can be modified according to the expected level of contamination.
Please refer to the paragraph "Limitations of the method" for information concerning the primary diluents and recommended dilutions.

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 72-76 hours at **25 ± 1°C**, in order to obtain performance levels similar to the standard EN ISO 21527 (1) and chapter 18 of the BAM (2).

The TEMPO method was compared to Chapter 18 of the BAM (2). The AOAC study included the following categories of food products:
- fruit (orange juice, apple juice, frozen strawberries),
- dairy products (cheddar cheese, frozen yogurt, milk-based infant formula),
- miscellaneous (flour tortillas, cornmeal, almonds, pecans).

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 72-76 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.
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 1 : The TEMPO cards should be handled observing the same safety precautions as for incubated plates due to the risk of mold spreading.

Note 2 : Remove the incubation rack from the reader immediately after reading and dispose of the cards as soon as the 76 hour time limit has expired.

Note 3 : After each use, the incubation racks must be decontaminated using a fungicide or following the decontamination procedure described in the chapter "Cleaning and decontamination" (paragraph "Filling rack and incubation/reading rack") of the Preparation Station User's Manual.

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. 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:
Saccharomyces cerevisiae ATCC® 9763
Aspergillus niger ATCC 16404
Escherichia coli ATCC 8739

Recommended protocol:

- Using a
  - 48 to 72-hour old culture on Sabouraud Dextrose Agar incubated at 25°C for *Aspergillus niger* and *Saccharomyces cerevisiae*,
  - 24 to 72-hour old culture on Trypcase Soy Agar incubated at 30°C for *Escherichia coli*,

  prepare a suspension in Peptone water and, using the Densimat (see "Material and reagents required but not provided"), adjust to:
  - 0.3 McFarland, i.e. approximately 10^6 CFU/ml for *S. cerevisiae*,
  - 1 McFarland, i.e. approximately 10^6 CFU/ml for *A. niger*,
    (for this strain, the correspondence between optical density and suspension concentration may vary greatly depending on the status of strain development (see Note below)),
  - 0.4 McFarland, i.e. approximately 10^8 CFU/ml for *E. coli.*
- Perform serial decimal dilutions in Peptone water until a suspension with the following theoretical concentration is obtained:
  - approximately 10^2 CFU/ml for *A. niger* and *S. cerevisiae*
  - approximately 10^3 CFU/ml for *E. coli*.

  Alternatively, a suspension of *Aspergillus niger* can be obtained using the strain in the form of a BioBall® (bioMérieux Ref. 56 011) which contains 550 CFU of *Aspergillus niger*. Place the BioBall in 5.5 ml of Peptone water and vortex until the ball has completely dissolved (10-20 seconds minimum). A suspension with a theoretical concentration of approximately 10^2 CFU/ml is obtained directly.
  For the yeasts and molds, transfer 1 ml of the suspension with a theoretical concentration of approximately 10^2 CFU/ml into a vial of culture medium which has been reconstituted beforehand with 3 ml of secondary diluent. Follow the same procedure for *E. coli*, 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 SDA for *S. cerevisiae*
  - 0.5 ml of the 10^2 CFU/ml suspension on two plates of SDA for *A. niger*
  - 0.1 ml of the 10^3 CFU/ml suspension on TSA for *E. coli*.
- Incubate as indicated above.
- After incubation, perform card reading.
  Count the number of colonies on SDA.
  For *A. niger*, count the number of colonies on both plates and add together.
  Check for the presence of *E. coli* on TSA.

Note: For *Aspergillus niger* obtained on SDA, in order to ensure an effective concentration of approximately 10^2 CFU/ml, it is recommended to inoculate 3 TEMPO cards using theoretical 10^4, 10^3 and 10^2 CFU/ml successive dilutions. At the same time, check the concentration of each of the suspensions by streaking 0.5 ml of each suspension used to inoculate the cards on two plates of SDA. Interpret the results of the card inoculated with the suspension which is closest to the expected 10^2 CFU/ml inoculum.

Range of expected results:

Yeast and mold strains

Calculate the ratio R:

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

R should be between 0.01 and 1.

Escherichia coli should be totally inhibited by TEMPO YM (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 YM 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 YM test, a dilution of these matrices at least equivalent to 1/400 is recommended.
- It is not recommended to use TEMPO YM for the enumeration of yeasts and molds in yogurts.
- For products which are highly contaminated with total flora (fermented products, products at the end of shelf-life), a minimal dilution of 1/400 is recommended.
- As the sodium citrate solution and dipotassium hydrogen phosphate solution primary diluents affect the pH of the TEMPO YM medium, a dilution lower than 1/400 is not authorized.
- For the buffered peptone water primary diluent, a minimal dilution of 1/400 is recommended.

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

The TEMPO YM test, for the enumeration of yeasts and molds in a variety of food products, was validated by the AOAC Research Institute in April 2010 (Certification No. 041001).

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE
LICENSE NUMBER 041001

041001 – 04/07/10
PERFORMANCE TESTED METHOD
Certified by AOAC Research Institute
www.aoac.org

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 EN ISO 21527 (2008) – Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds.
   • ISO 21527-1 Part 1: Colony count technique in products with water activity greater than 0,95.
   • ISO 21527-2 Part 2: Colony count technique in products with water activity less than or equal to 0,95.
2. Bacteriological Analytical Manual Online
   BAM Chapter 18 “Yeasts, molds and mycotoxins”
   (January 2001).
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 Online
   BAM Media M79 (January 2001).
7. International Standard EN ISO 6887-4 – Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products.
8. International Standard EN ISO 7218 – Microbiology of food and animal feeding stuffs – General rules for microbiological examinations.

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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