

PCA WITH SKIMMED MILK

ENUMERATION OF TOTAL MICROORGANISMS IN DAIRY PRODUCTS

1 INTENDED USE

Plate Count Agar with skimmed milk is used in food and dairy bacteriology to enumerate aerobic bacteria in milk powders and dairy products.

It is also used in the enumeration of psychrotrophic microorganisms.

The typical composition conforms to that defined in the standards NF ISO 17140, NF EN ISO 4833-1 & 2 and XP V08-034 ; ISO 8552, ISO 8553, ISO 14461-1 & 2 and ISO 6730 for the control of dairy products.

2 HISTORY

Plate Count Agar is prepared with the same ingredients as those originally used by Buchbinder *et al.* These authors compared several batches of yeast extract and showed that the results obtained (without milk added to the medium) were satisfactory for the enumeration of bacteria that were contaminating samples of raw and pasteurized milk. The transparency of the medium and the relative size of colonies formed led to uncomplicated enumeration.

3 PRINCIPLES

The nutrients supplied by skimmed milk and Tryptone, vitamins from yeast extract and glucose used as energy source favor the growth of most bacteria.

The skimmed milk allows responds to the recommendations of the standards in terms of dairy bacteriology.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone	5,0 g
- Yeast extract	2,5 g
- Glucose	1,0 g
- Powdered skimmed milk (exempt from inhibitory substances).....	1,0 g
- Bacteriological agar.....	12,0 g

pH of the ready-to-use media at 25 °C : 7,0 ± 0,2.

5 PREPARATION

Preparation from dehydrated media :

- Dissolve 21,5 g of dehydrated media (BK161) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, with constant agitation until complete dissolution.
- Dispense in tubes or vials.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47°C.
- For surface inoculation, let the plates solidify on a cold, flat surface before use.

✓ **Reconstitution :**
21,5 g/L

✓ **Sterilization :**
15 min at 121 °C

Use of ready-to-melt media :

- Melt the media (if it was prepared in advance as above) or use the ready-to-melt media (BM086) and melt the agar for the minimum amount of time necessary to achieve total liquefaction.

6 INSTRUCTIONS FOR USE

Surface inoculation (NF EN ISO 4833-2) :

- Dry the plates in an incubator with the covers partially removed.
- On the surface of the plates, transfer 0,1 mL of the sample to test and its serial dilutions.
- Inoculate the surface using a sterile triangle or « hockey stick ».
- Incubate at 30 ± 1 °C for 72 ± 3 hours.

✓ **Inoculation :**
0,1 mL on surface

✓ **Incubation :**
72 h at 30 °C

NOTES

- A Spiral inoculation system can be used.
- For the detection of psychrotropic microorganisms in food microbiology, acc. to standard (NF ISO 17410), incubate the plates for roughly 10 days at 6,5 °C.

Inoculation in pour plates (NF EN ISO 4833-1):

- Transfer the inoculum and its serial dilutions to successive empty Petri plates.
- Pour roughly 15 mL of media held in molten state at 44-47 °C, per plate.
- Mix well and let solidify on a cold, flat surface.
- Incubate at 30 ± 1 °C for 72 ± 3 hours.

✓ **Inoculation :**
1 mL in pour plates

✓ **Incubation :**
72 h at 30 °C

NOTES

In the case where invasive colonies are suspected on the surface of the agar, it is possible to pour a second layer of agar after the first layer solidifies (around 4 mL additional agar).

For all other uses, refer to the reference or standard in vigor.

7 RESULTS

Count plates containing at least 10 and a maximum of 300 colonies, according to the standards being applied. Caseinolytic bacteria form a clear halo around each colony (proteolysis of milk casein).

8 QUALITY CONTROL

Dehydrated media : beige powder, free-flowing and homogeneous.

Prepared media : amber agar.

Typical culture response after 72 hours of incubation at 30 °C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity ratio : P_R)
<i>Escherichia coli</i>	WDCM 00013	$P_R \geq 70$ %
<i>Staphylococcus aureus</i>	WDCM 00034	$P_R \geq 70$ %
<i>Bacillus subtilis</i>	WDCM 00003	$P_R \geq 70$ %

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.

Ready-to-melt media in vials : 2-25 °C.

The expiration dates are indicated on the labels.

Prepared media in tubes or vials (*) : 180 days at 2-25 °C.

Prepared media in plates (*) : 30 days at 2-8 °C.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :

500 g bottle BK161HA

Ready-to-melt media :

10 x 200 mL vials BM08608

11 BIBLIOGRAPHY

NF ISO 17410. Novembre 2001. Microbiologie des aliments. Méthode horizontale pour le dénombrement des micro-organismes psychrotrophes.

NF EN ISO 4833-1. Octobre 2013. Microbiologie des aliments. Méthode horizontale pour le dénombrement des micro-organismes. Partie 1 : comptage des colonies à 30 °C par la technique d'ensemencement en profondeur.

NF EN ISO 4833-2. Octobre 2013. Microbiologie des aliments. Méthode horizontale pour le dénombrement des micro-organismes. Partie 2 : comptage des colonies à 30 °C par la technique d'ensemencement en surface.

XP V 08-034. Septembre 2010. Microbiologie des Aliments. Dénombrement des microorganismes par comptage des colonies obtenues à 30°C après ensemencement par la méthode spirale.

ISO 8552 / IDF 132. Mai 2004. Lait. Estimation des micro-organismes psychrotrophes. Technique par comptage des colonies à 21°C (méthode rapide).

ISO 8553 / IDF 131. Mai 2004. Lait. Dénombrement des micro-organismes méthode de l'anse sur boîtes de Petri à 30°C.

ISO 14461-2 / IDF 169-2. Avril 2005. Lait et produits laitiers. Contrôle de qualité en laboratoires microbiologiques. Partie 2 : Détermination de la fiabilité des comptages de colonies en boîtes parallèles et des dilutions décimales suivantes.

ISO 14461-1 / IDF 169-1. Mai 2005. Lait et produits laitiers. Contrôle de qualité en laboratoires microbiologiques. Partie 1 : Evaluation de la performance des analystes effectuant les comptages de colonies.

ISO 6730 / IDF 101. Septembre 2005. Lait. Dénombrement des unités formant colonies de micro-organismes psychrotrophes - Technique par comptage des colonies à 6,5°C.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : PCA WITH SKIMMED MILK_ENV8
Creation date : 04-2003
Updated : 03-2016
Origin of revision : General update