
CONTACT PCA + TTC + NEUTRALIZERS

ENUMERATION OF TOTAL MICROORGANISMS

1 INTENDED USE

Plate Count Agar (PCA) supplemented with TTC and neutralizers is a ready-to-use media. The media is used for the detection and enumeration of microorganisms present on surfaces allowing its use to control critical points in industry (examples: protected areas, microbiological monitoring programs of surfaces and industrial environments).

2 HISTORY

The agar for enumeration is prepared with the same ingredients as those originally used by Buchbinder et al. In their work, they compared several batches of yeast extract and showed that the results obtained (without milk added to the media) were satisfactory for counts of contaminating germs in raw and pasteurized milk samples. The transparency of the media and the satisfactory size of the colonies obtained facilitated counting.

According to J. Rozier and J. Pantaléon, the assessment of surface microbial flora is of primary interest for food hygiene controls. They cite the example of meat to show that it is the germs spread on the aponeurotic coating of the carcasses or on the surfaces of the equipment and instruments which very quickly produce undesirable "microbism" in this foodstuff. They study and compare the techniques of enumeration of surface germs. They consist of swabbing, sampling of superficial tissue flaps, taking decals with cork, rubber, cloth, or paper swabs, or applying agar plates. The main disadvantage is the complexity of use. The operator, who must be competent, usually needs aseptic equipment to perform the sampling properly. Furthermore, a laboratory, even if poorly equipped, is necessary to take advantage of this preliminary operation. They therefore propose a simple method that consists of using plastic plates containing various types of nutrient agar. These are poured in such a way as to form a meniscus of 1 to 2 mm thickness that covers the bottom of the plate. They can thus be easily applied to the surfaces to be controlled.

3 PRINCIPLES

The media forms a convex meniscus that allows direct application of the agar to the control areas, whether on walls, floors, utensils, or staff. The media contains several neutralizers that inhibit any disinfectant residues on the surfaces to be tested, in order to assess the levels of contamination before and after disinfection of the food chain environment.

The neutralizers are selected to inactivate disinfectant residues that may be present on the surfaces, such as aldehydes and phenols, quaternary ammoniums, and oxidising compounds.

TTC (triphenyl tetrazolium chloride) is reduced to insoluble formazan by bacteria (including *Proteus* and *Pseudomonas*), giving a red coloration to colonies.

4 TYPICAL COMPOSITION

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

For 1 liter of media :

- Tryptone	5.0 g
- Autolytic yeast extract	2.5 g
- Glucose	1.0 g
- Triphenyl tetrazolium chloride (TTC)	0.1 g
- Sodium thiosulfate	0.5 g
- Bacteriological agar	12.0 g

pH of ready-to-use media at 25 °C: 7.0 ± 0.2 .

A mixture of neutralizing agents is added according to the specific characteristics of the medium.

NOTE : Triphenyl tetrazolium chloride (TTC) is used to facilitate colony reading.

5 INSTRUCTIONS FOR USE

- Use the culture media at room temperature and on a dry surface.
- Open the plate and apply the agar directly to the surface to be tested and maintain uniform pressure over time (e.g. 500 g for 10 s according to NF EN ISO 18593). Then close the plate. Keep the agar at 1 to 8°C in a suitable transport container and incubate within 48 hours.
- Clean the sample surface to remove any traces of nutrients, moisture and chemical or physical elements resulting from the application of the agar.
- Incubate at 30°C for 48 to 72 hours.

✓ **Incubation :**

48-72h h at 30 °C

NOTE: It is recommended that a control of the efficiency of the mixture of neutralizers present in the media be carried out in relation to the disinfectant product used, given the diversity of antiseptics existing on the market.

6 RESULTS

Proceed with the counting of all the colonies. The grid at the bottom of the plates facilitates counting.

Divide the number of characteristic colonies by the area of the sampled surface and deduce the number of colonies forming units (CFU) per square centimetre of surface.

7 QUALITY CONTROL

Typical culture response at 72h of incubation at $30 \pm 1^\circ\text{C}$ (NF EN ISO 11133):

Microorganisms		Growth (Productivity Ratio : P_R)
<i>Bacillus subtilis</i>	WDCM 00003	$P_R \geq 50 \%$, dark red colonies
<i>Escherichia coli</i>	WDCM 00012	$P_R \geq 50 \%$, dark red colonies
<i>Staphylococcus aureus</i>	WDCM 00032	$P_R \geq 50 \%$, dark red colonies

8 STORAGE / SHELF LIFE

Ready-to-use media: 2-25 °C

Expiration dates are indicated on the labels.

9 PACKAGING

Pre-poured media in plates (Ø 65 mm):

30 plates BM20609

10 BIBLIOGRAPHY

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NF EN ISO 11133. July 2014. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

NF EN ISO 11133/A1. March 2018. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

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11 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.

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