TECHNICAL DATA SHEET

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, **with neutralizers:**

- Tryptone ..... 5,0 g
- Autolytic yeast extract ..... 2,5 g
- Glucose ..... 1,0 g
- triphenyl tetrazolium chloride (TTC)……...0,1 g
- Neutralizers mixture ……7,2 g
- Sodium thiosulfate…… 0,5 g
- Bacteriological agar..... 12,0 g

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

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. 500g for 10s 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.

See ANNEX 1: PHOTO SUPPORT.

7 QUALITY CONTROL

Typical culture response after 72 of incubation at 30°C :

Microorganisms		Growth (Productivity Ratio : P_R)
Escherichia coli	WDCM 00012	$P_R \geq 70$ %
Staphylococcus aureus	WDCM 00193	$P_R \geq 70$ %
Bacillus subtilis ssp. spizizenii	WDCM 00003	Orange colonies

8 STORAGE / SHELF LIFE

Ready-to-use media: 2-8 °C
Expiry dates are indicated on the labels.
Sealed off bags can be stored for 30 days at 25°C

9 PACKAGING

Pre-poured media in plates (Ø 65 mm):
20 plates BM20608

10 BIBLIOGRAPHY

Rozier, J.; Pantaléon, J. 1969. Méthode simple et rapide d'appréciation des flores microbiennes de surface (note préliminaire). Académie vétérinaire de France, Paris (FRA).

NF EN 1040. April 2006. Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics - Test method and requirements (phase 1)

NF EN ISO 18593. July 2018. Microbiology of the food chain - Horizontal methods for sampling techniques from surfaces using contact plates and swabs

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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ANNEX 1 : PHOTO SUPPORT

CONTACT PCA + TTC + NEUTRALIZERS

Enumeration of total microorganisms.

Reading:

Growth obtained after 48 hours of incubation at 30 °C.