TECHNICAL DATA SHEET

ORANGE SERUM AGAR

DETECTION AND ENUMERATION OF YEASTS AND ACID-TOLERANT BACTERIA

1 INTENDED USE

Orange Serum Agar is used for the growth, isolation and enumeration of yeasts, molds and acid-tolerant bacteria (*Bacillus*, lactobacilli, *Leuconostoc*, *Streptococcus*, *Clostridium*), which are responsible for deteriorations in fruit juices and citrus concentrates. It has also been used for hygiene controls of industrial equipment used to prepare fruit-based beverages.

2 HISTORY

In 1951, the medium was described by Hayes for the enumeration and isolation of microorganisms causing alteration in frozen orange juice concentrates and subsequently by Murdock *et al*. for lemon juice concentrates.

3 PRINCIPLES

The addition of clarified orange juice to the peptones and extracts in the formula leads to a satisfactory recovery of microorganisms which can resist the acidity of the fruit juices they contaminate.

4 TYPICAL COMPOSITION

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

For 1 liter of media :

- Tryptone ..... 10,0 g
- Yeast extract ..... 3,0 g
- Orange extract ..... 5,0 g
- Glucose ..... 4,0 g
- Dipotassium phosphate..... 3,0 g
- Bacteriological agar..... 17,0 g

pH of the ready-to-use media at 25 °C : 5,5 ± 0,2.

5 PREPARATION

- Dissolve 42,0 g of dehydrated media (BK103) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes or vials.
- Sterilize in an autoclave at 115 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.

✓ **Reconstitution :**
42,0 g/L

✓ **Sterilization :**
15 min at 115 °C

6 INSTRUCTIONS FOR USE

- Transfer 1 mL of the product to analyze and its serial dilutions to sterile Petri plates.
- Pour in roughly 15 mL of molten media per plate.
- Homogenize by swirling and let solidify on a cool, flat surface.
- Incubate for 3 to 5 days at 25 °C or at 30 °C, depending on the type of microorganism being sought.

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

✓ **Incubation :**
3 to 5 jours at 25 or 30 °C

Note : Do not overheat the medium in order to avoid browning and the loss of the gelling properties of the agar. The medium should be used preferentially the day of its preparation.

7 RESULTS

Separately enumerate yeasts, molds and bacteria. Carry out a microscopic examination and identification tests on each type of colony found.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : cream-white powder, free-flowing and homogeneous.
Prepared media : amber agar.

Typical culture response after 72 hours of incubation at 25 °C :

Microorganisms		Growth (Productivity Ratio : P_R)
Lactobacillus plantarum	ATCC® 8014	$P_R \geq 70$ %
Leuconostoc mesenteroides subsp. *mesenteroides*	WDCM 00016	$P_R \geq 70$ %
Saccharomyces cerevisiae	WDCM 00058	$P_R \geq 70$ %
Candida albicans	WDCM 00054	$P_R \geq 70$ %
Aspergillus brasiliensis	WDCM 00053	$P_R \geq 70$ %

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in vials (*) : 180 days at 2-25 °C.
Prepared media in plates (*) : 15 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK103HA

11 BIBLIOGRAPHY

Hays, G.L.. 1951. The isolation, cultivation and identification of organisms which have caused spoilage in frozen concentrated orange juice. Proceedings of the Florida State Horticultural Society, **54** : 135.

Murdock, D.I., Folinazzo, J.F. and Troy, V.. 1951. Evaluation of plating media for citrus concentrates. Food Technology, **6** : 181-185.

Hays, G.L. and Reister, D.W.. 1952. The control of "off-odor" spoilage in frozen concentrated orange juice. Food Technology, **6** : 386-389.

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 : ORANGE SERUM AGAR_ENv8.
Creation date : 09-2000
Updated : 05-2016
Origin of revision : General update.

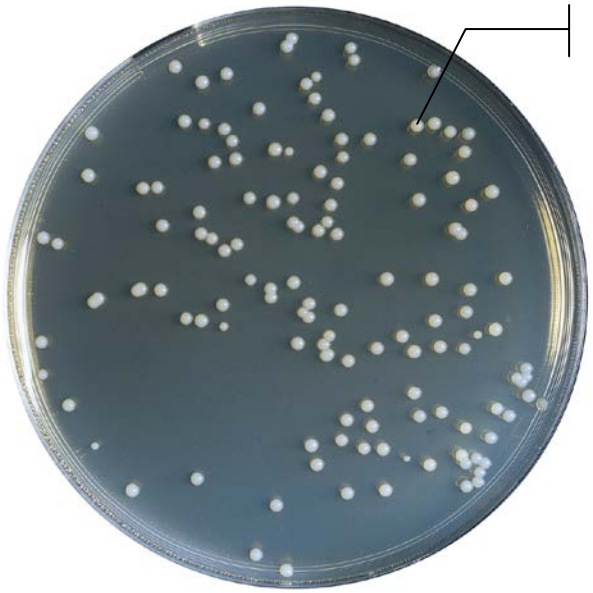
ANNEX 1 : PHOTO SUPPORT

Orange Serum agar

Detection and enumeration of yeasts and acid-tolerant bacteria.

Results :

Growth obtained after 72 hours of incubation at 25 °C.



Characteristics : good growth of yeasts and acid-tolerant bacteria.