

67616 Enterobacteria Millichrome™ plus Agar

For detection and enumeration of Enterobacteriaceae

Composition:

Ingredients	Grams/Litre
Peptone and yeast extract	22.0
Mineral salts	4.9
Chromogenic and selective mix*	0.7
Growth factors	3.7
Agar	11.0
Final pH 7.4 +/- 0.2 at 25°C	

* confidential mix with chromogenic substrates and selective agents

Store prepared media below 8°C, protected from direct light (max. 1 month) or 1 day at room temperature. Bottles can be stored for up to 6 months at 2-8°C. Store dehydrated powder, in a dry place, in tightly sealed containers at 2-25°C.

Preparation:

Step 1 (Preparation of the mix):

- Disperse slowly 42.3 g of powder base in 1 L of purified water.
- Stir until agar is well thickened.
- Heat and bring to boil (100 °C) while swirling or stirring regularly.

Advice: For the 100°C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, stir gently, then return to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam).

DO NOT HEAT TO MORE THAN 100 °C.
DO NOT AUTOCLAVE AT 121 °C.

Warning: If using an autoclave, do so without pressure.

Step 2 (Cooling):

- Cool in a water bath to 45-50 °C.
- Swirl or stir gently to homogenize.

Step 3 (Pouring in plates):

- Pour medium into Petri dishes.
- Let solidify.
- Let plates dry.

Inoculation with pour plate or surface technique.

Principle and Interpretation:

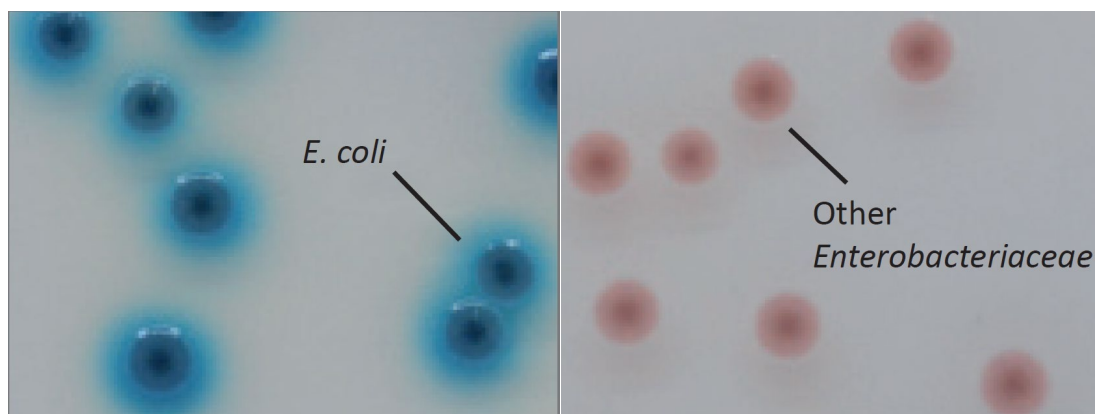
Enterobacteriaceae is a large family from the Gram-negative bacteria. Several genera are seen as important indicator organisms used by the food industry. ISO 21528 describes the detection and enumeration of Enterobacteriaceae for food using VRBG as culture medium.[1,2] Enterobacteria Millichrome™ plus Agar is a medium that was developed to drastically reduce the workload of the laboratory compared to the very labour intense traditional VRBG. Differentiation between *E. coli* and other Enterobacteriaceae is easy because of different colors and the clear background of the media, resulting in a better contrast. The high sensitivity and specificity of the medium lead to a higher detection rate of Enterobacteriaceae:

Sensitivity : 100 % *

Specificity : 100 % *

* Specificity and sensitivity from scientific study: "Validation of an alternative method of Analysis for the ISO 21528-2 (2017)" Laboratoire de Touraine, 2018.





Typical colony appearance

Peptone and yeast extract provide nitrogenous nutrients for growth and other essential growth factors. Mineral salts are needed for the osmotic balance and to provide essential ions. Chromogenic and selective mix contains chromogenic substrates for the color differentiation based on the ability to cleave the substrate by characteristic enzymes and contains selective agents to inhibit other organisms. The medium contains growth factors to support the growth of Enterobacteriaceae. Agar is added as the solidifying agent.

Limitation and further testing

- Final identification may require additional testing.
- Some strains of *Pseudomonas* may appear as pink colonies. However, they can be differentiated by an Oxidase test.

Quality control:

Cultural characteristics after 24h at 37°C (psychotropic Enterobacteriaceae, at 30°C).

Organisms (ATCC/WDCM)	Growth	Colony color
<i>Escherichia coli</i> (25922/00013)	+++	blue
<i>Klebsiella pneumoniae</i> (13883/00097)	+++	pink
<i>Pseudomonas aeruginosa</i> (9027/00026)	-	inhibited
<i>Enterococcus faecalis</i> (29212/00087)	-	inhibited

References:

1. ISO 21528-1:2017, Microbiology of the food chain — Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 1: Detection of Enterobacteriaceae
2. ISO 21528-2:2017, Microbiology of the food chain — Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 2: Colony-count technique.
3. F.M. Pilar, S.R. Scott, O. Howard, Phylogenetic Relationships of Bacteria with Special Reference to Endosymbionts and Enteric Species, The Prokaryotes, Springer New York, pp. 41–59 (2021)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

