67651 Y. enterocolitica Millichrome™ plus Agar Base

For qualitative detection, differentiation and presumptive identiification of pathogenic biotypes of *Yersinia enterocolitica*.

Composition:

Ingredients	Grams/Litre
Peptones	20.0
Salts	5.0
Chromogenic mix*	1.3
Agar	15.0

Final pH 7.0 +/- 0.2 at 25°C

* confidential mix with chromogenic substrates

Store prepared media at 2-8°C, protected from direct light and dehydration (max. 1 month) or 1 day at room temperature. Store dehydrated powder, in a dry place, in tightly sealed containers at 2-25°C.

Preparation:

Step 1 (Preparation of 1L Y. enterocolitca Millichrome™ plus Agar base)

- Disperse slowly 41.3 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. DO NOT HEAT TO MORE THAN 100 °C. DO NOT AUTOCLAVE AT 121 °C.

Warning 1: If using an autoclave, do so without pressure.

Advice 1: 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).

- Cool in a water bath to 45-50 °C +/- 2 °C.

Step 2 (Preparation of the Y. enterocolitica Millichrome™ plus Supplement)

- Prepare a stock solution of the supplement: Add 100 mg to 1 mL of purified water.
- Swirl well until complete dissolution. Filter sterilize at 0.45 µm.

Warning 2: This supplement stock solution should be used immediately after preparation or can be stored at -20 °C and used within 15 days.

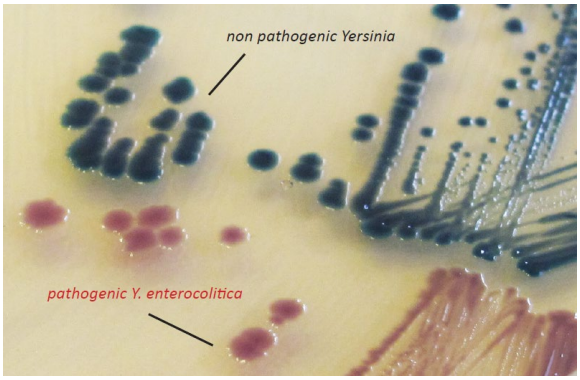
Step 3 (Mixing of the prepared base and the prepared supplement)

- Add 1 mL of the prepared supplement solution to the prepared base cooled at 45-50 °C +/- 2°C.
- Swirl gently to homogenize.
- Pour into sterile Petri dishes.
- Let it solidify and dry.

Principle and Interpretation:

Among the Yersinia genus, Yersinia enterocolitica is one of the most common food borne pathogen. In several countries, Y. enterocolitica has eclipsed Shigella and approaches Salmonella and Campylobacter as the predominant cause of acute bacterial gastroenteritis. Its ability to grow at

refrigeration temperature makes it an increasing concern in terms of food safety. Only a few strains of *Y. enterocolitica* cause illness in humans. Those pathogenic *Y. enterocolitica* strains belong to biotypes 1B, 2, 3, 4, and 5, whereas biotype 1A strains are non-pathogenic and widespread in the environment. The major animal *Y. enterocolitica* reservoir causing illnesses are pigs.
Compared to CIN agar the Y. enterocolitica Millichrome™ plus Agar Base inhibits remarkable more background flora and gives less false positive results. It clearly differentiate pathogenic from non-pathogenic *Yersinia enterocolitica* species and also from other bacteria. Pathogenic *Yersinia enterocolitica* species appear as mauve colonies, other *Yesinia* species and other Enterobacteriaceae grow as metallic blue colonies or are inhibited. Gram positive bacteria are inhibited.



Peptones provide nitrogenous nutrients for growth and other essential growth factors.
Salts are needed for the osmotic balance and provide essential ions. The chromogenic mix contains chromogenic substrates for the color differentiation based on the ability to cleave the substrate by characteristic enzymes. Agar is added as the solidifying agent.

Limitation and further testing

- Some *Y. enterocolitica* could have a poor or no growth on the media. Some rare strains of non-pathogenic *Yersinia* could appear as mauve colonies (*Y. bercovieri*, *Y. mollareti*, *Y. kristensenii*, *Y. rohdei* etc.).
- Final confirmation as pathogenic *Y. enterocolitica* must be done by appropriate methods.
- Final identification may require additional testing such as biochemical tests or mass spectrometry (e.g. MALDI-TOF) which can be done directly from the suspicious colonies observed on the medium.

Quality control:
Cultural characteristics after 36-48h at 30±2°C under aerobic conditions.

Organisms (ATCC/WDCM)	Growth	Colony color
Y. enterocolitica pYV+ (23715/)	+++	mauve
Y. enterocolitica pYV- biotype 1A	+++	metallic blue
Escherichia coli (25922/00013)	-	-
Enterococcus faecalis (29212/)	-	-
Pseudomonas aeruginosa (9027/)	-	-
Citrobacter freundii (8090/)	-/+	metallic blue

References:

1. T. Råsbäck et al.. Prevalence of human pathogenic Yersinia enterocolitica in Swedish pig farms, Acta Vet. Scand., 60:39 (2018)
2. J. Karhukorpi. M. Päivänurmi, Differentiation of Yersinia enterocolitica biotype 1A from pathogenic Yersinia enterocolitica biotypes by detection of b-glucosidase activity: comparison of two chromogenic culture media and Vitek2, Journal of Medical Microbiology, 63, 34–37 (2014)

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.