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WILLIS -HOBBS AGAR

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Roey Angel¹, Ana Lara-Rodriguez¹

¹Soil and Water Research Infrastructure

Anaerobic and Molecular Microbiology Lab, Biology Centre CAS

Tech. support email: eva.petrova@bc.cas.cz



Ana Lara

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External link: <http://himedialabs.com/TD/M1375.pdf>

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Protocol status: Working

We use this protocol and it's working

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Abstract

For the diferential isolation of Clostridium sp.

Guidelines

taken from: <http://himedialabs.com/TD/M1375.pdf>

Materials

MATERIALS

⊗ Agar

⊗ Peptone A FROM MEAT **Bio Basic Inc. Catalog #G213.SIZE.500g**

⊗ D-Lactose, monohydrate **Bio Basic Inc. Catalog #LC4280.SIZE.500g**

⊗ Neutral red **Bio Basic Inc. Catalog #NB0652.SIZE.25g**

⊗ Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S3014**

⊗ Meat Extract **Merck Millipore (EMD Millipore) Catalog #70164-100G**




⊗ Egg Yolk Emulsion **Catalog #414722,1608**

Safety warnings

⚠ Use temperature resistant gloves for taking the media out of the autoclave and during pouring of the petri dishes.



Before start

- Make a 10% skim milk solution and sterilize it by autoclaving at 15 lbs pressure  121 °C for  00:15:00 .
- Turn ON the water bath to  50 °C
- Before taking the media from the autoclave, open the petri dish bags and organize them on the flow box. Mark the petri dishes as W-H agar.



- 1 For the preparation of 1L of media (50 to 66 petri dishes depending of depth) weigh:

🧪 10 g Peptic Digest from Animal tissue

🧪 10 g Meat Extract

🧪 5 g NaCl

🧪 12 g Lactose

🧪 0.032 g Neutral Red

🧪 10 g Agar

🧪 30 mL Egg yolk Emulsion

Final pH (at 25°C) 7.0±0.2

- 2 Heat to boiling to dissolve the medium completely.
- 3 Sterilize by autoclaving at 15 lbs pressure 🌡️ 121 °C for ⌚ 00:15:00 .
- 4 Cool to 🌡️ 50 °C
- 5 Add 🧪 120 mL sterile skimmed milk 10% solution.
- 6 Aseptically add 🧪 30 mL Egg Yolk Emulsion.
- 7 Pour 🧪 15 mL or 🧪 20 mL of media in petri dishes depending on desired depth .
- 8 Let cool the open petri dishes under UV light for ⌚ 00:10:00 to ⌚ 00:15:00 .
- 9 Close petri dishes and keep at 🌡️ 4 °C until use. (2 weeks max)

