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Determination of Clostridium sp. contamination level

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Eva Petrova¹, Roey Angel¹

¹Soil and Water Research Infrastructure



Eva Petrova

Soil and Water Research Infrastructure

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Protocol status: Working

We use this protocol and it's working

Created: July 23, 2019

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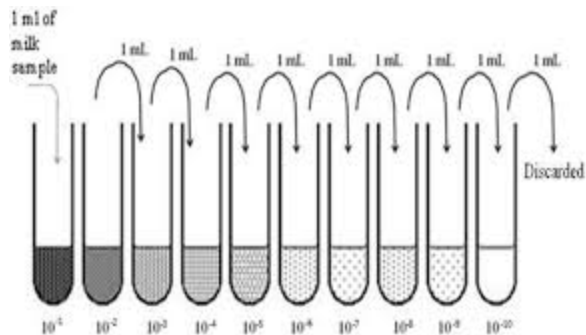
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Abstract

adopted from Dr Tomasz Grenda (2018)

Guidelines



Example of serial dilutions preparation



- 1 Prepare 10g of sample and resuspend it in 90ml of saline solution (0.85% NaCl).

 10 g soil sample

 90 mL saline solution

- 2 Subject suspension to decimal dilutions in saline solution (see Guidelines), prepare at least 5 levels.

- 3 After sample preparation, dispense 10ml of TPGY broth into tubes (number of tubes depends on the number of samples multiplied by prepared dilutions, each tube corresponds to examined level). Transfer 1ml of each dilution to tubes with dispensed and preregenerated TPGY broth.

 10 mL TPGY media

 1 mL diluted sample

- 4 Subject the samples to pasteurization process at 70°C for 15 min.

 70 °C pasteurization

 00:15:00

- 5 Incubate inoculated tubes at 30°C or 37°C for 48h, under anaerobic conditions.

 37 °C anaerobic incubation

 48:00:00

- 6 After incubation check the turbidity of TPGY and the ability to gas production.

- 7 Spread 10µl of liquid culture onto surfaces of FAA and Willis–Hobbs agar media. Incubate inoculated plates at 37°C for 48h, under anaerobic conditions.

 10 µL liquid culture

 37 °C anaerobic incubation

 48:00:00

- 8 After incubation evaluate characteristic phenotypic features of obtained cultures and determine the contamination level with clostridia adequately to dilution at which their

growth was noticed.