Sep 15, 2024

Determination of Clostridium sp. contamination level

DOI
dx.doi.org/10.17504/protocols.io.bp2l64q2rvqe/v1

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Protocol Citation: Eva Petrova, Roey Angel 2024. Determination of Clostridium sp. contamination level. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l64q2rvqe/v1

Protocol status: Working
We use this protocol and it's working

Created: July 23, 2019

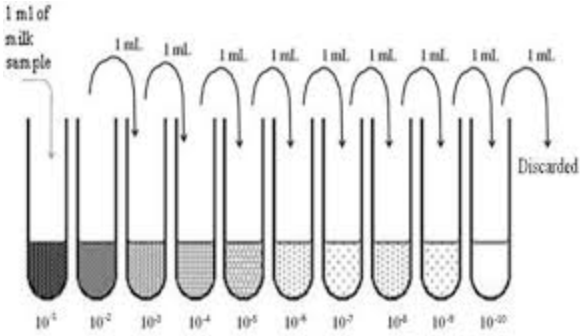
Last Modified: September 15, 2024

Protocol Integer ID: 26100

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 liguid 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.