

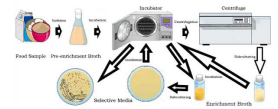


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Concentration technique for Viable but Non-Culturable organisms

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Muhammad Muhsin Fathuddin¹, Solomon John Obidah²

¹Ahmadu Bello University; ²Modibbo Adama University



Muhammad Muhsin Fathuddin

Ahmadu Bello University

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Oxoid (2024) *CM0862 Listeria Enrichment Broth*. (n.d.). Oxoid. Retrieved May 17, 2024, from http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0862&c=UK&lang=EN

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Protocol status: Working

We use this protocol and it's working

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Keywords: Centifugation, Selective Medium, Concentration, VBNC, Resuscitation, resuscitating microbe, microbes via resuscitation, isolating microbe, concentration of the microbe, concentration technique, medium to centrifugation, selective isolation, centrifugation, concentration, microbe, selective broth, selective agar, subsequent growth on selective agar, isolation, selective growth, resuscitation

Abstract

This protocol is based on existing protocols, such as those for resuscitating microbes, freeze-drying, and selective isolation. It merges the parts of the three protocols to create a new protocol for isolating microbes via resuscitation, centrifugation, and selective isolation. The first part involves resuscitating the microbes; the second part subjects the medium to centrifugation (concentration of the microbes); the third and final part involves the selective growth in a selective broth and subsequent growth on selective agar.

Image Attribution

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The flowchart represents the process of bacterial isolation and cultivation from a food sample for laboratory analysis. The steps involved include resuscitation, centrifugation, and selective isolation. The first part involves resuscitating the microbes; the second part subjects the medium to centrifugation (concentration of the microbes); the third and final part involves the selective growth in a selective broth and subsequent growth on selective agar.

Guidelines

Laboratory protocols must be followed, and the environment must be kept as sterile as possible.

Materials

Petri-dish, Centrifuge Machine, Falcon tubes, Wire-loop, bunsen burner, spatula, vortex shaker, incubator

Safety warnings

 Aseptic techniques should strictly be followed

Ethics statement

No animals are harmed/used in this experimental setup

Before start

Wear necessary safety gear before commencing the work



Resuscitation of Microorganism

- 1 To the resuscitation broth media (e.g., peptone water, tryptic soy broth, Luria-Bertani broth, etc.), add the sample, making a 10% solution, i.e.,  10 mg in  90 mL .
- 2 Incubate the broth culture at 37°C for 24-48 hours

1d



Centrifugation of the resuscitation media

30m

- 3 The grown broth culture is then centrifuged  5000 rpm, 4°C, 00:30:00
- 4 Then, concentrate the cell suspension and decant the culture supernatant.

30m



5m

Selective medium growth of desired organism

2d

- 5 Mix cell pellets with the desired selective medium broth. Suspend cells thoroughly by using a vortex mix  100 rpm, 00:05:00 until complete cell resuspension is achieved aseptically.
- 6 Then, incubate at the desired temperature  24:00:00 , which can be extended up to  168:00:00 .
- 7 From the above selective broth, subculture onto a selective agar for the desired organism.
- 8 incubate the selective agar plate at the required temperature for 24-48 hours. then observe for the desired growth and colony morphologies
- 9 Store the isolate for further tests (Biochemical, Molecular, etc.)

1w 1d

1d



Protocol references

- Chauhan, A., & Jindal, T. (2020). *Microbiological methods for environment, food and pharmaceutical analysis*. Springer Nature.
- Fonseca, F., Cenard, S., & Passot, S. (2015). Freeze-drying of lactic acid bacteria. *Cryopreservation and freeze-drying protocols*, 477–488.
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