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

The culture-independent Bcc NAD method for the rapid detection and quantification of Bcc in water

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ABSTRACT

Here we present a rapid (<4 hours from sample in to result out) culture-independent Bcc NAD method, incorporating a quantitative real-time Polymerase Chain Reaction (qPCR) assay. This method can be used to detect and simultaneously identify the Bcc species directly from water samples. This culture-independent Bcc NAD method is validated to the testing method equivalent of the ISO/TS 12869:2019 standard (a widely used rapid culture-independent NAD method for detecting Gram-negative *Legionella* species in water) in terms of specificity, sensitivity, accuracy and turnaround time to result compared to Bcc microbial culture diagnostic methodologies.

We propose that the rapid culture-independent Bcc NAD method presented could gain widespread acceptance and be applied as a valuable diagnostic tool supporting a water risk management strategy in the pharmaceutical sector to assure the safety and quality of these water systems and water used for manufacturing.

The development and validation of this culture-independent Bcc NAD method has been published in:

CITATION

Duong H, Fullbrook S, Reddington K, Minogue E, Barry T (2023). Design, Development, and Validation of a Culture-Independent Nucleic Acid Diagnostics Method for the Rapid Detection and Quantification of the Burkholderia cepacia Complex in Water with an Equivalence to ISO/TS 12869:2019..

LINK

https://doi.org/10.5731/pdajpst.2021.012728

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GUIDELINES

- The laboratory workspace and equipment were sterilized by UV-light, DNase solution, and 70% ethanol.
- Due to the sensitivity of the assays, extra care must be paid to avoid sample contamination during analysis, and as such Good Laboratory Practice must be followed. Wear laboratory coats and gloves at all times.
- Filter pipet tips were used in all steps of the laboratory work.
- The material to be used (tubes, tips, water, etc.) must be free of nucleases.

MATERIALS

- Filtration apparatus (mounted either on an air pump or on a vacuum flask), suitable for sterilization by steam (autoclave)
- Filter funnel has visible graduations that are appropriate to the volumes of the sample typically analysed (should be sterilized by autoclaving before use)
- A vacuum pump provides a pressure which permits the suction of filtration through the membrane filter
- A vessel to collect the filtered water (e.g. 1 litre bottle)
- Sterile forceps for handling membrane filters
- 0.4 µm hydrophilic polycarbonate membrane filters of 47 mm diameter (Isopore membrane filters, Merck Millipore) with a low absorption capacity for extracellular nucleic acids using a vacuum pump. The membrane filters shall be sterilized.

CITATION

Majaneva M, Diserud OH, Eagle SHC, Boström E, Hajibabaei M, Ekrem T (2018). Environmental DNA filtration techniques affect recovered biodiversity..

LINK

https://doi.org/10.1038/s41598-018-23052-8

- Quick-gDNA™ MiniPrep Kit (Zymo Research)

WATER FILTRATION

1 Water preparation:
Water is collected in a sterile container containing sterile 0.1% v/v sodium thiosulfate pentahydrate solution to inactivate any residual disinfectant chlorine (if needed) and immediately used for experimental analysis upon arrival in the laboratory (within approximately 2 hours of collection).

2 Ethanol-flame sterilise the forceps and, with them, remove the sterile membrane filter from its packaging.

Note

To sterilise forceps, dip forceps in alcohol and flame in an alcohol burner. Cool before use.

3 Place the sterile hydrophilic polycarbonate membrane filter on the sterile membrane filter base of the filtration apparatus, taking care that only the outer edge of the membrane filter is grasped with sterile forceps.

4 Fit the sterile funnel securely on the filter base of the apparatus.

Note

Do not touch the inside of the funnel.

5 Flame the pouring lip of the sample container and pour the desired volume of test water sample to be studied (e.g. 100 ml) into the funnel.

6 Turn on the vacuum pump (pressure differential of 30-50 kPa (kiloPascals)) and allow the complete separation of filtrate through the membrane filter.

Note

It may be advisable to rinse the funnel's interior with one to three 10 ml to 30 ml portions of sterile water while the filter is still in place to remove organisms adhering to the funnel.

7 Power off the vacuum pump and remove the funnel.

8 With ethanol-flame sterile forceps, carefully remove the membrane filter and aseptically place the membrane filter into a prepared sterile 20-ml tube.

9 The funnel may be re-used without disinfection for the same sample so that the smallest volumes and/or the most diluted samples are filtered first.
To filter another sample, a separate sterile apparatus shall be used, or where appropriate, the funnel can be disinfected by rinsing the funnel with at least 100 ml of sterile water and then with alcohol to prevent cross-contamination.

> **Note**
>
> In every batch of experiments, always include a processing control sterile water confirmed to be free of Bcc, to be used as a negative control to indicate any contamination during the process.

BACTERIAL gDNA EXTRACTION AND PURIFICATION

10 Add 2 ml of Genomic Lysis Buffer of the Quick-gDNA MiniPrep Kit to each filter in the 20-ml tube and incubate at room temperature on a rolling platform for 30 mins.

11 Each tube is vortexed for 1 minute.

12 The remainder of the DNA extraction and purification protocol follows steps 2-4 of the section "Cell Suspensions and Proteinase K Digested Samples" from the Quick-gDNA MiniPrep Kit (Zymo Research) manufacturer's instructions to extract total gDNA.
https://files.zymoresearch.com/protocols/_d3024_d3025_quick-dna_miniprep_kit.pdf

13 The column is transferred to a new tube and centrifuged again at maximum speed for 1 minute to ensure the complete removal of remnants of the washing solution from the column before DNA elution.

14 Transfer the spin column to a clean microcentrifuge tube. Add 25 µl nuclease-free water to the spin column. Incubate 5 minutes at room temperature and then centrifuge at top speed for 30 seconds to elute the DNA.

Note

- It is advisable to incubate the column with resuspension solution for 5 minutes to increase DNA yield. Centrifuge again; the eluted DNA in solution is collected in the tube, and the column is discarded.
- The eluted DNA can be used immediately for molecular-based applications or stored at ≤-20°C for future use.

THE Bcc qPCR NAD ASSAY

15 The eluted DNA samples are used as templates in the previously described Bcc qPCR NAD assay.
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