International Journal of Infectious Diseases 101(S1) (2021) 180–203

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Diagnostics & Molecular Technologies

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Cerebrospinal fluid MinION sequencing of 16S rRNA gene for rapid and accurate diagnosis of bacterial meningitis

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Background: Bacterial meningitides cause substantial morbidity and mortality worldwide. Rapid and accurate identification of the microorganisms is essential for early initiation of appropriate antimicrobial therapy, thereby improving clinical outcome. Current routine diagnostic assays may require several days to complete, but often fail to identify the bacteria in the majority of patients. Meanwhile in recent years, advanced sequencing technologies (including Oxford Nanopore Technologies) have greatly improved our capacity to detect the causative agents of infectious diseases in clinical samples.

Methods and materials: Cerebrospinal fluid (CSF) samples used were derived from an ongoing prospective diagnostic study conducted in the brain infection ward of the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam. Complete 16S rRNA gene was amplified and sequenced using 16S Barcoding Kit (SQK-RAB204, ONT) and MinION Nanopore sequencer. MinION reads were first basecalled using Albacore v2.1.7 (ONT), followed by demultiplexing using Porechop (https://github.com/rrwick/Porechop). Determination of bacterial genus/species composition in the obtained reads was carried out using Epi2Me interface (Metrichor, Oxford, UK), a platform for cloud-based analysis of MinION data.

Results: MinION sequencing of 16S rRNA gene was successfully used to confirm *Streptococcus agalactiae* in CSF of a 59 y old patient falling meningitis treatment.

To further assess the utility of the assay, six additional CSF samples positive for *S. pneumoniae*, *S. suis* or *Neisseria menigitidis* by Gram stain, culture and PCR combined were used. Subsequently, the MinION sequencing of 16s rRNA gene base assay was able to accurately detect the respective bacterial pathogens in all 6 included CSF samples. In contrast, conventional methods like Gram stain and bacterial culture could not detect the pathogens in 3/6 CSF samples.

Conclusion: MinION sequencing of 16S rRNA gene could accurately detect diverse bacterial pathogens in CSF samples. Additionally, the bacterial species information generated by the analysis of 16S rRNA sequences can be useful for disease surveillance and vaccine evaluation. Thus, the application of the method would be relevant for both patient management and epidemiological research. Because the whole procedure only takes ≤6 h to operate, same day diagnosis is theoretically achievable. Prospective studies are urgently needed to assess its translational potential.

https://doi.org/10.1016/j.ijid.2020.09.481

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Development and validation of Loop-Mediated Isothermal Amplification (LAMP) detection limit using blood matrix spike for *Candida glabrata* detection

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Background: Rapid and accurate identification of yeast responsible for invasive candidiasis (IC) allows for focused and early initiation of therapy for good clinical outcome. *Candida glabrata* is increasingly important, and the diagnostic sensitivity of microbial culture method "gold standard" is low and could miss up to 50% of the IC patients. To develop and validate LAMP technique detection limit in comparison to microbial culture and PCR techniques using blood matrix spike for *C. glabrata* detection.

Methods and materials: Blood spiking experiment was conducted using healthy blood sample. *Candida glabrata* ATCC 2001 reference strain colonies were re-suspended in phosphate-buffered saline (PBS) buffer to 0.5 McFarland standard. Ten-fold serial dilutions of yeast suspensions in 1x PBS was made from 10^6 to 10^1 cfu/mL concentrations. A 100 μL from each dilution was plated