Nanopore-based metagenomic sequencing: a diagnostic tool in respiratory tract infection

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To the Editor:

We read the recent *ERJ Open Research* article "Advances in diagnostic tools for respiratory tract infections: from tuberculosis to COVID-19 – changing paradigms?" by Stojanovic *et al.* [1] with great interest. The article represents a comprehensive review of current best practice in diagnosing respiratory tract infection (RTI), as well as highlighting the future of diagnostic tools in this area. We pay particular interest to the section on pathogen identification and the exciting developments described.

Although significant progress has been made, RTI remains a leading cause of mortality globally [2], and pulmonary tuberculosis still represents a burden on healthcare systems across the world [3]. The coronavirus disease 2019 (COVID-19) pandemic highlighted the importance of time-efficient, accurate and cost-effective diagnostic tools in managing RTIs. Quicker confirmation of the right diagnosis leads to patients receiving the correct treatment earlier in their illness and thus increases the probability of a positive outcome. Within their section on pathogen identification, Stojanovic *et al.* [1] describe microbiological diagnostics as "critical" in the process of ensuring the correct antimicrobial agent is started as soon as possible to maximise treatment benefit and reduce unnecessary drivers of antimicrobial resistance. We believe that nanopore metagenomic sequencing can have a significant impact as a diagnostic tool in patients with suspected bacterial RTI.

Nanopore sequencing utilises a membrane protein pore as a biosensor to sequence RNA or DNA molecules as they pass through the pore [4]. Nanopore sequencing has been used, for example, throughout the COVID-19 pandemic to sequence severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomes [5]. But nanopore sequencing is not yet a routine clinical practice as part of RTI diagnostic panels, despite the fact that nanopore sequencing has several clinical advantages over culture, which remains the gold standard diagnostic tool for RTIs [6]. Turnaround time, for example, is much shorter: nanopore-based methods have been shown to report pathogen and acquired resistance gene identification within 8 h of receiving a sample, compared to 48–72 h or more for culture-based methods [7]. Such rapid turnaround would allow patients to receive targeted antimicrobial therapy on the same day as clinical diagnosis, as well as reduce the need for prolonged broad-spectrum antibiotic coverage. This advantage is compounded in mycobacterial infections, in which traditional culture methods can take up to 6 weeks.

It was thought initially that the much shorter turnaround time would mean that nanopore sequencing is a less accurate method of pathogen identification than traditional culture methods. However, sensitivity and specificity comparable to those of culture have been reported. In one cohort, for example, nanopore sequencing identified pathogens in almost 40% of culture-negative samples [8]. Findings such as these verify the accuracy of nanopore sequencing-based methods and indicate that there are clear advantages over culture-based methods in identifying RTIs. This level of accuracy and time-efficiency has been replicated in studies focused on the identification of *Mycobacterium tuberculosis* (including drug-resistant variants) [9].

Nanopore sequencing is highly accessible. Limited sample preparation is required, often with no need for substrate amplification. Capital outlay for the MinION (Oxford Nanopore Technologies, Oxford, UK), the smallest nanopore sequencing device (smaller than the average mobile phone), is very low relative to other sequencing platforms, with starting prices advertised from USD 1000 per device. Consumable costs are already reportedly below EUR 100 per sample [10], with improvements in this area expected.

Shareable abstract (@ERSpublications)
This correspondence highlights the burden of respiratory tract infection and focuses on nanopore sequencing as a promising approach in diagnostics https://bit.ly/3fgs8zg

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In conclusion, we agree with Stojanovic *et al.* [1] that diagnostic tools, and particularly pathogen identification, remain at the centre of RTI management and that their improvement is integral to advancing patient care and outcomes. We have highlighted the potential impact of nanopore sequencing as a feasible method of pathogen identification in RTI and we believe it has multiple advantages over traditional culture, particularly time to result (including antibiotic resistance markers). We hope that this correspondence makes clear the strengths of nanopore sequencing-based technology within this field and encourages further clinical study in this area.

Robert Chapman [1], Alberto D'Angelo[2] and Stefan Bagby[2]

[1]Princess Alexandra Hospital NHS Trust, Harlow, UK. [2]Department of Life Sciences, University of Bath, Bath, UK.

Corresponding author: Robert Chapman (robert.chapman2@nhs.net)

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