Towards ultra-rapid microbial cfDNA nanopore sequencing for the identification of sepsis-causing pathogens

Ana Martinez-Lopez[1], Kazuhiro Horiba[2,3], Linda Marriott[1], Amanda Warr[4], Jacob N. Phulusa[5], Jamie Rylance[5,6], Yoshinori Ito[7], Maïwenn Kersaudy-Kerhoas[1, 8]

❶ Introduction

Bloodstream infections lead to long hospitalizations and create severe socio-economic burden globally, but most critically, in developing nations. The current standard for pathogen identification for diagnosis is through blood culture, which lacks specificity, sensitivity, and has time to results often exceeding three to five days, or longer.

Microbial cell-free DNA (cfDNA), released from lysed pathogens in the infected human circulation, has been shown to have high sensitivity for detection.

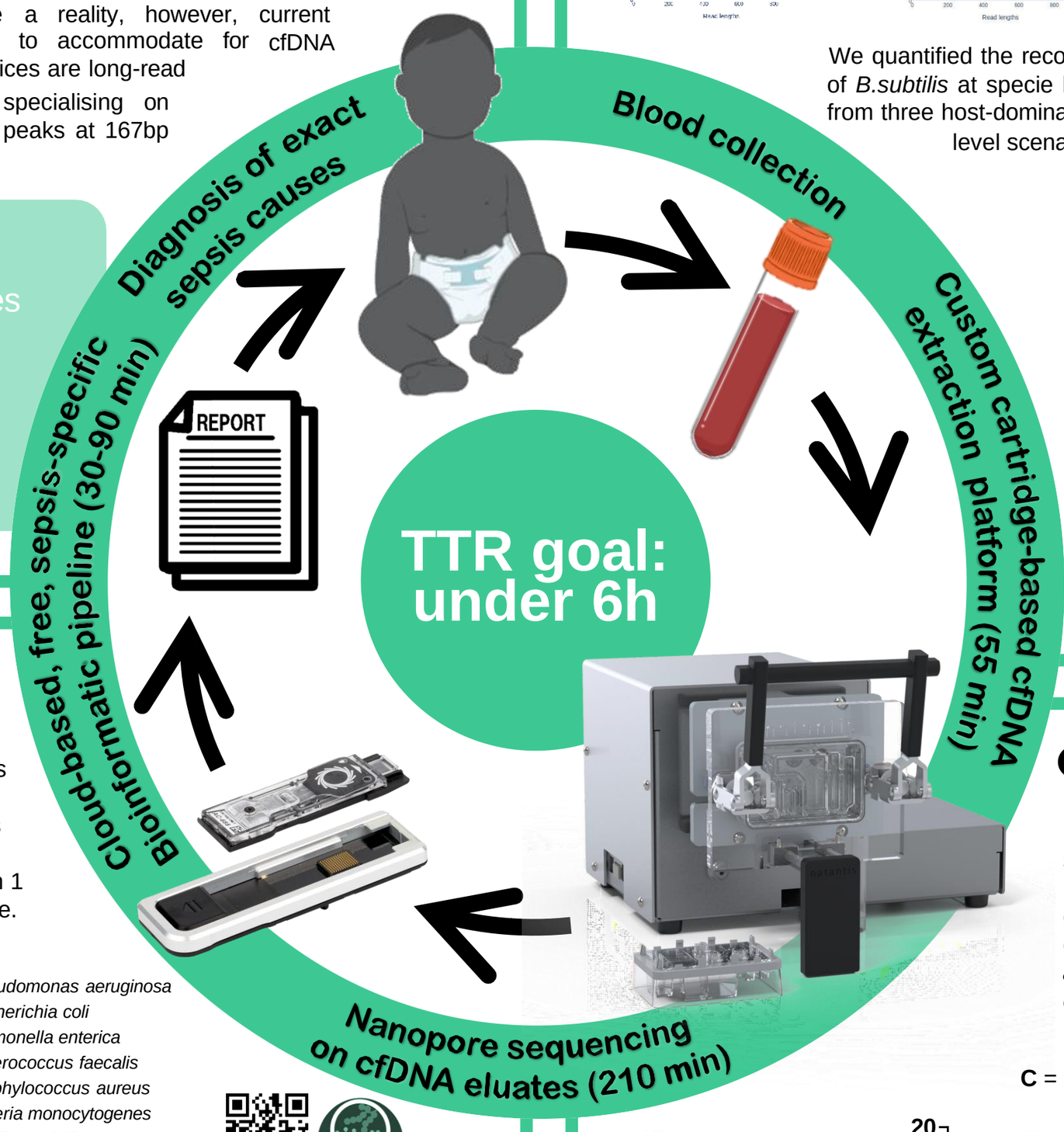
Nanopore sequencing could offer a culture-free, sensitive and specific diagnosis while being quicker and more cost-effective than current proposed solutions.

For such a system to become a reality, however, current methodologies must be updated to accommodate for cfDNA fragment size: nanopore-based devices are long-read sequencers with methodologies specialising on long DNA fragments, while cfDNA peaks at 167bp and rarely exceed 500bp.

SEPSIS BURDEN

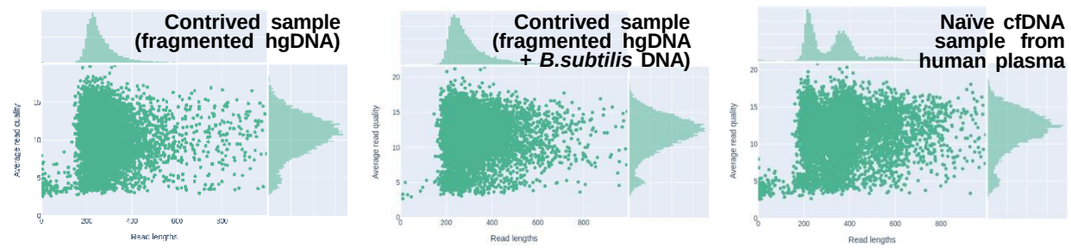
47 000 000 cases per year.

Survivors may face lifelong consequences

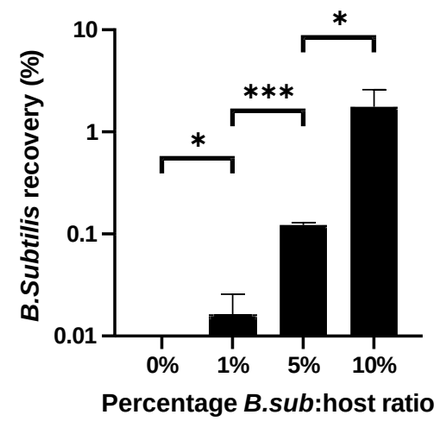


❷ Microbial cfDNA sequencing: Use of contrived samples for assay development

We set out to determine key variables in replicability and recovery of a particular bacteria species, *B.subtilis*, in an ideal human cfDNA sample.



We quantified the recovery of *B.subtilis* at specie level from three host-domination level scenarios



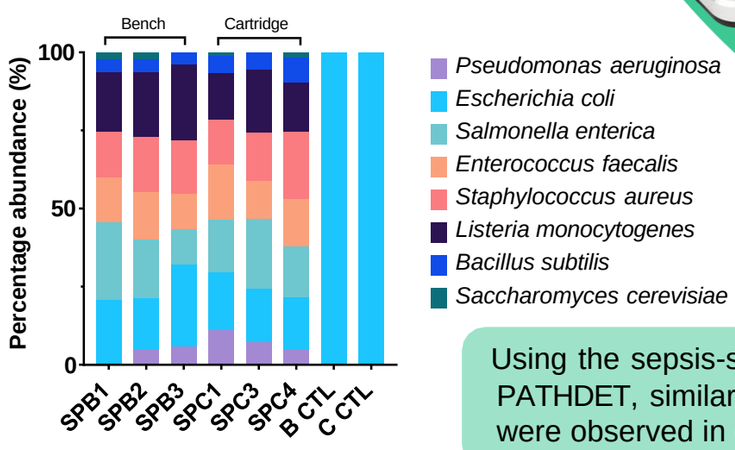
- Contrived samples resemble plasma samples in average quality and quality-to-fragment size distribution.
- We recovered *B:subtilis* down to 1% pathogen:host ratio

❸ cfDNA extraction solution

Poor or delayed preparation leads to nucleic acid from host cells swamping the microbial cfDNA in biological samples, reducing the diagnostic test accuracy. We have developed a ***portable platform for automated and fast extraction of cfDNA*** directly from blood samples

❹ Microbial cfDNA recovery from contrived sepsis samples

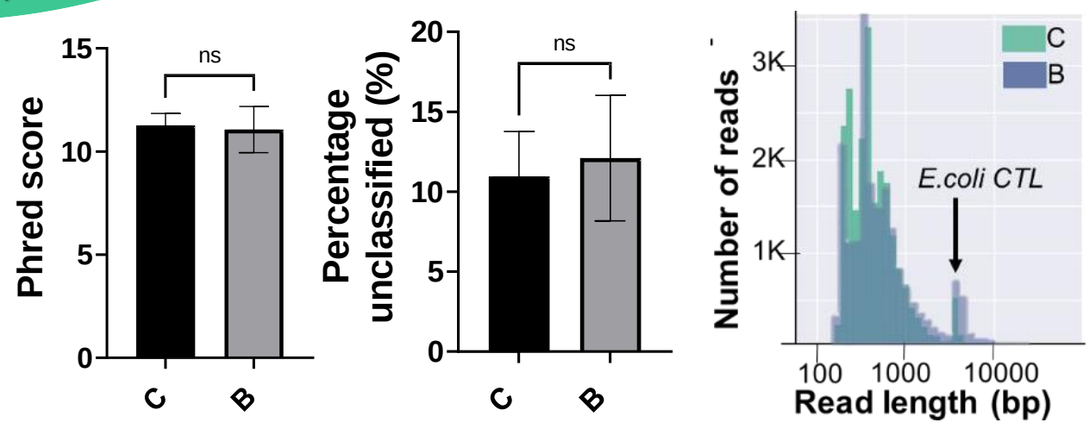
We created contrived mock samples by mixing fragmented microbial community mix containing genomes from 8 microbes (7 bacteria and 1 fungus) (D6322, Zymo Research) in 1 mL of healthy human plasma sample.



PATHDET

Using the sepsis-specific bioinformatic pipeline PATHDET, similar pathogen recovery patterns were observed in cartridge and bench eluates

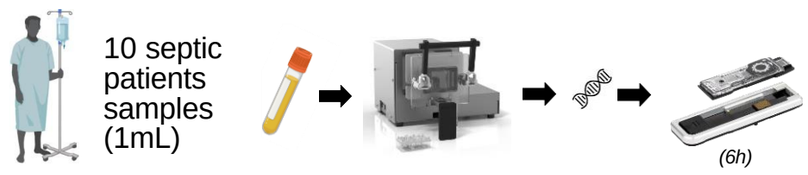
C = Cartridge **B** = Bench (manual)



We observed no significance difference between our cartridge and bench method eluates across three quality indicators

❺ Proof-of-concept study on adult patient samples

We acquired ten patient samples and applied the complete workflow



Sam-ple #	Total non-human reads	% of non-human reads
2	15907	6.9
4	8154	9.7
5	2061	4.4
6	23903	11.4
7	19067	6.5
8	27838	8.7

- Culture results "*no growth but GPC seen*"
- 29y male,
- Fever, cough (inc. coughing sputum), chest pain, and difficulty breathing.
- Lactate at 2.63 mmol/L
- **Organism identified: *Streptococcus pneumoniae* (8% total reads) – consistent with clinical presentation**

❻ Conclusion

We have demonstrated a workflow combining a custom cfDNA extraction platform and nanopore sequencing (Flongle) for a microbial cfDNA sepsis diagnostic approach.

This workflow could help diagnose the exact causes of sepsis under 6h in clinical settings. It could provide sepsis patients with appropriate treatment, decrease the prescription of broad-spectrum antibiotics and reduce the burden of antibiotic re-sistance in the hospital environment, and avoid the use of antibiotics altogether, if cause is viral, fungal or parasitic

(1) The School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, United Kingdom (2) Department of Genetics, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan (3) Department of Human Genetics and Molecular Biology, Nagoya University Graduate School of Medicine, Nagoya, Japan (4) The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, UK (5) Malawi-Liverpool Wellcome Clinical Research Programme (6) Liverpool School of Tropical Medicine (7) Department of Pediatrics, Nagoya University (8) Infection Medicine, University of Edinburgh

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