A microfluidic platform for sensitive bacterial detection in blood through whole genome sequencing within 4 hours

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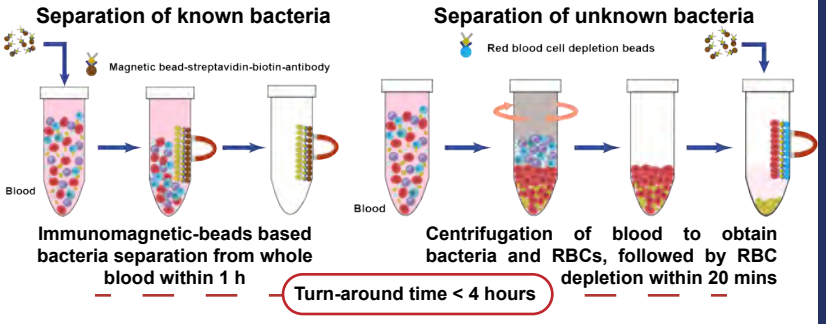
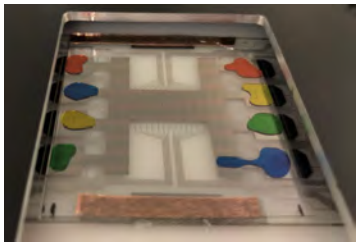
Background

Bacterial diagnosis often relies on detecting the DNA of various microorganisms such as clinical bacterial pathogens and harmful foodborne microbial species. Standard bacterial detection requires laborious microbial culture, identification and antimicrobial susceptibility testing of isolated microorganisms, and it usually takes days or weeks. Whole genome sequencing (WGS) has emerged as a promising tool for microbial diagnosis and analysis, since it provides bacterial classification as well as additional genomic information such as antibiotic resistance. However, broader implementation of this methodology for clinical specimens such as blood, is hindered by the complex sample preparation and extended turn-around time. Moreover, human cells typically contain abundant DNA which can compromise bacterial detection.

We have developed an integrated platform for bacteria separation from blood, whole genome amplification and library preparation in a microfluidic device, followed by nanopore-based WGS. This platform is able to detect bacteria DNA as low as 10fg within 4 hours.

Overall Workflow





Automated digital microfluidic device (DMF) for WGA of bacterial DNA within 2 h

Hand-held nanopore-based whole genome sequencing within 30 mins

Results

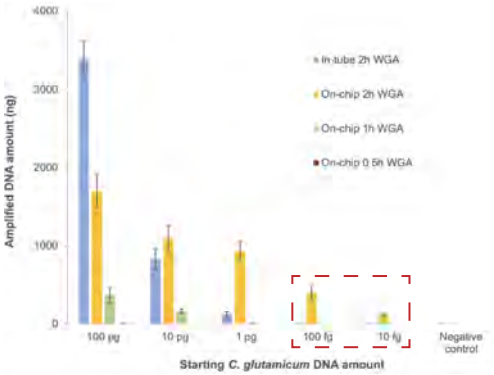
• Immunomagnetic-beads based *staphylococcus aureus* separation from whole blood:

Bacteria in blood (CFU/mL)	10^2	10^3	10^4	10^5
Capture efficiency	>95%	>95%	>85%	>85%

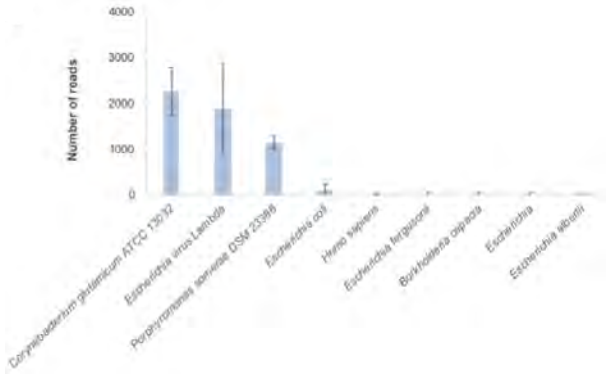
• On-chip vs. in-tube genome coverage (%) of *C. glutamicum* DNA after 30 mins sequencing in MinION:

	100pg	10pg	1pg	100fg	10fg
In-tube	74.5%	58.6%	50.0%	N/A	N/A
On-chip	76.8%	43.6%	16.9%	18.6±1.5%	10.5±5.0%

• Comparison between the amount of *Corynebacterium glutamicum* DNA amplified on-chip and in-tube :



• Detection of *C. glutamicum*, *P. somerae* and *E. coli* bacteriophage lambda DNA in a mock sample:



• Immunomagnetic beads-based bacteria separation mechanism demonstrates > 95% capture rate of *s. aureus* from blood at a limit of detection of 10^2 CFU/mL, and this method can be expanded and used for separation of other species.

• Digital microfluidic device provides an outstanding DNA amplification resolution: 10fg, which is equivalent to the amount of DNA within a single bacterial cell.

• The platform allows simultaneous detection of multiple bacteria species, as well as differentiation of target bacteria DNA from low-abundance contaminants.

Conclusion and Prospectives

• We have developed an integrated platform for the capture of target bacteria from blood using immunomagnetic beads, and whole genome amplification of bacterial DNA in a palm-sized DMF device, following nanopore sequencing and obtained the results within 30mins.

• The whole process holds outstanding operational efficiency requiring minimal human intervention within 4 hours turnaround time, and can potentially be used to identify low-abundance bacteria in urgent settings.

• Future investigation should focus on the non-specific capture of bacteria from biofluids by integrating of human cell depletion strategies, expanding the usability of our platform on bacteria species identification to translational applications.

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