Rapid identification of bloodstream infection using MinION metagenomic sequencing

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INTRODUCTION:
Traditional culture based methods for diagnosing blood stream infection (BSI) have long turnaround times and poor sensitivity. Current molecular methods, most based on PCR, are rapid and accurate but not comprehensive, seeking only pre-set targets. We investigated the use of MinION metagenomics sequencing and WIMP™ automated bioinformatics analysis for the rapid diagnosis of BSI.

METHODS:
We developed a pathogen DNA enrichment method capable of removing the majority of human DNA in a 1 ml blood sample without significant loss of bacterial DNA. Pathogen DNA is extracted and whole-genome amplified (Qiagen REPLI-g) and sequenced. Sequences were identified using WIMP, real-time MinION sequence identification and classification software for pathogens. Antimicrobial resistance genes were identified using ARMA software (Oxford Nanopore Technologies, UK). Fifteen blood samples were analysed by culture, 16s rDNA PCR and NGS in parallel, 7 samples were collected and analysed prospectively and 8 retrospectively.

RESULTS:
Of the 7 prospective samples analysed, one was positive by the two molecular methods which identified *Streptococcus pyogenes*. This sample was negative by culture, however a sample taken from the same patient earlier that day was positive for *S. pyogenes*. The *S. pyogenes* isolate and the strain sequenced directly from the blood were the same. Five of the 8 retrospectively collected samples were concordant with culture and 3 were discordant.

Figure 1: Pathogen Detection workflow

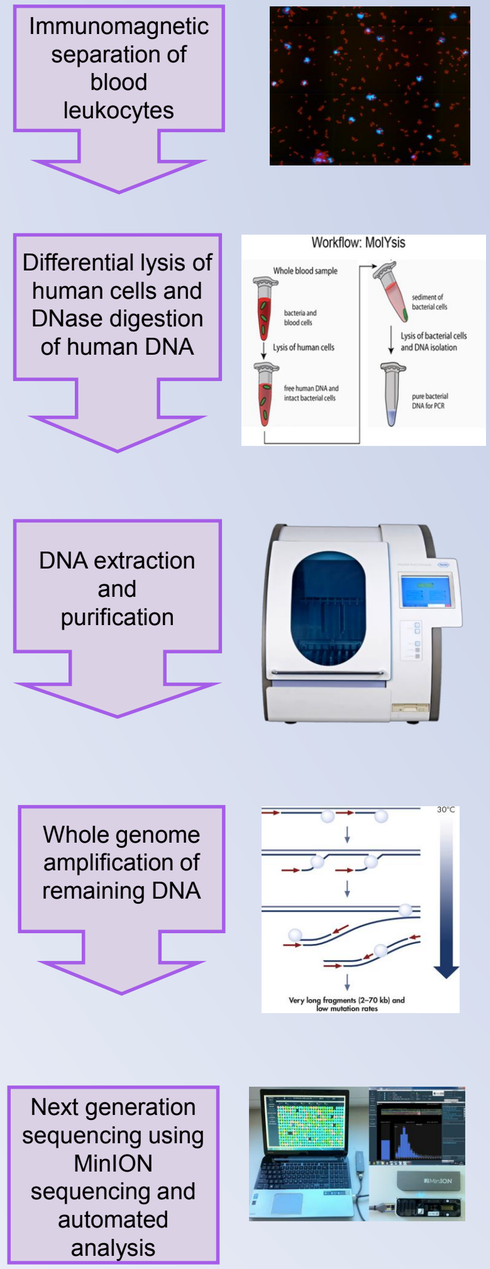

Table 1: Prospective study: Results of 16S rDNA PCR, MinION sequencing and blood cultures collected and analysed prospectively.

Patient number	Enriched sample (HumanC$_q$)	16S rDNA identification	Blood culture	MinION (WIMP)	Illumina
NGS 1	37.08	Negative	Negative	N/A	N/A
NGS 2	33.45	Negative	Negative	N/A	N/A
NGS 3	34.56	Negative	Negative	N/A	N/A
NGS 4	30.63	Positive	*S. pyogenes*	*S. pyogenes*	*S. pyogenes*
NGS 5	>45	Negative	Negative	N/A	N/A
NGS 6	36.19	Negative	Negative	N/A	N/A
NGS 7	34.02	-	Negative	N/A	N/A

Table 2: Retrospective study: Results of 16S rDNA PCR, MinION sequencing and blood cultures collected and analysed retrospectively.

Patient ID	Enriched Sample (HumanC$_q$)	16s rDNA (491 bp)	Septifast (PCR based sepsis test)	Blood culture	MinION (WIMP)	Illumina sequencing
Code 1	40	Negative	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	Negative	Negative
Code 2	>45	Negative	Negative	Negative	N/A	Negative
Code 3	32.28	Negative	Negative	Negative	N/A	Negative
Code 4	>45	Positive	*Enterococcus faecalis* Coagulase negative Staphylococcus	*Enterococcus faecalis*	*Enterococcus faecalis* *Staphylococcus haemolyticus*	*Enterococcus faecalis* *Staphylococcus haemolyticus*
Code 5	>40	Negative	Negative	*Negative*	*Escherichia coli*	*Escherichia coli*
Code 6	39.67	Negative	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	N/A	*Klebsiella pneumoniae*
Code 7	>40	Negative	*Staphylococcus aureus*	*Staphylococcus aureus*	N/A	*S. aureus* (detected but not most abundant bacterial reads)
Code 8	35.87	Negative	Negative	Negative	N/A	Negative

DISCUSSION:
The discordant samples containing Gram-negative species according to culture (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) were negative by 16S PCR analysis. Possible reasons for discordant results include loss of bacteria due to freeze - thaw or low bacterial load. WIMP identified the pathogen(s) within 15 minutes of beginning a MinION run and within 7 hours of receiving the sample.

CONCLUSION:
We have demonstrated the great potential of MinION metagenomics sequencing for the rapid, comprehensive diagnosis of BSI. The turnaround time for the method is 7 hours, which would enable clinicians to modify antibiotic treatment before second dose (usually 8 hours). Further work is required to determine whether the method can accurately detect Gram negative sepsis.

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