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Proceedings Article

Detection of *Pseudomonas aeruginosa* in Whole Blood using a portable Magnetic Particle Spectrometer - a feasibility study

Lukas Heinen[a,*] · Alexander Simonis[b,c,d] · Jan Rybniker[b,d] · Johanna Günther[e] · Patrick Vogel[e] · Christina Janko[a] · Christoph Alexiou[a] · Stefan Lyer[a] · Rainer Tietze[a,*]

[a]Department of Otorhinolaryngology – Head and Neck Surgery, Section of Experimental Oncology and Nanomedicine (SEON), Else Kröner Fresenius Foundation Professorship (EKFS), Universitätsklinikum Erlangen, Erlangen, Germany
[b]Department I of Internal Medicine, Faculty of Medicine and University Hospital Cologne, Cologne, Germany
[c]Center for Molecular Medicine Cologne, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany
[d]German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, Cologne, Germany
[e]Department of Experimental Physics 5 (Biophysics), Julius-Maximilians-University Würzburg, Würzburg, Germany
*Corresponding authors, email: {lukas.heinen, rainer.tietze}@uk-erlangen.de

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Abstract

Rapid identification of WHO critical priority pathogens, such as *Pseudomonas aeruginosa*, is essential for managing sepsis, where standard blood cultures cause diagnostic delays of 24-72 hours. To address this, we developed a rapid, culture-independent magnetic immunoassay using a portable Magnetic Particle Spectroscopy (MPS) device for the direct detection of *P. aeruginosa* in blood. We evaluated two SPION formulations and found that our developed formulation, SPION-152, demonstrated excellent specificity, generating a strong signal change with virulent *P. aeruginosa* strains while showing negligible cross-reactivity with *E. coli*. Specificity for a virulence-associated factor was confirmed using a knockout mutant. These findings validate the MPS platform as a promising tool for accelerating pathogen identification in patients with suspected sepsis.

I. Introduction

Pseudomonas aeruginosa, a WHO critical priority pathogen, is a leading cause of life-threatening sepsis, where diagnosis is delayed by 24-72 hours due to reliance on blood cultures [1]. To address this diagnostic bottleneck, we applied a portable Magnetic Particle Spectrometer (MPS) [2, 3] for the rapid, culture-independent detection of whole bacterial cells in blood. This method utilises superparamagnetic iron oxide nanoparticles (SPIONs) functionalised with specific antibodies to generate a detectable signal change upon binding to *P. aeruginosa*, aiming to significantly accelerate pathogen identification in patients with suspected sepsis.

II. Materials and Methods

SPIONs were synthesised using a co-precipitation approach, coated with polyacrylic acid, purified via tangential flow filtration, and functionalised with anti-*P.*

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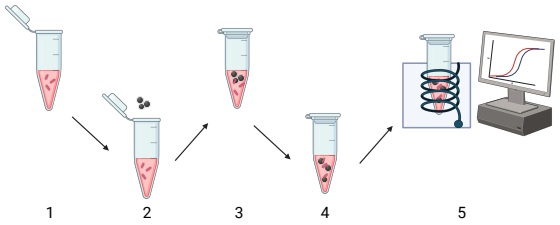

Figure 1: MPS assay workflow. 1 A blood sample (50 µl) doped with *P. aeruginosa* is mixed with **2** antibody-modified SPIONs (100 µl total volume) , **3-4** incubated for 10 min, and **5** measured.

aerugniosa antibodies [4] (100 µg Ab per mg Fe) and stored in phosphate buffered saline. Table 1 summarises the properties of the utilised SPION clusters.

The wash-free assay workflow is depicted in Figure 1. A blood sample (50 µl) doped with *P. aeruginosa* or *E. Coli* (10^5 bacteria/ml) was mixed (vortex) with 50 µl of functionalised SPIONs (0.5 mg/ml iron). The mixture was incubated for 10 minutes at room temperature before measurement in a self-made portable MPS device (sinusoidal excitation 20 kHz, amplitude ~34 mT).

Table 1: SPIONs used.

	SPION-152	SPION-106
z-avg. (DLS) / nm	152.3 ± 0.8	105.7 ± 0.9
PDI / -	0.185 ± 0.005	0.153 ± 0.012
ζ-potential / mV	-36.9 ± 0.7	-40.6 ± 0.8
Core size / nm	12.2 ± 2	12.2 ± 2
M_s (TEM) / $Am^2 \cdot kg^{-1}$	77.4 ± 0.4	76.6 ± 0.4

III. Results and Discussion

To develop a specific assay, we evaluated two antibody-functionalised SPION formulations. The initial formulation, SPION-106, showed significant non-specific binding to the negative control, *E. coli*, rendering it unsuitable for diagnostic use (Figure 2, top panel). In contrast, the optimised SPION-152 formulation demonstrated excellent specificity and a robust signal response (Figure 2, bottom panel). Its baseline signal remained low in the presence of *E. coli* but increased strongly upon incubation with virulent *P. aeruginosa* strains (PAO1 and PA14). Specificity was confirmed using a knockout mutant, which lacks the target antigen and consequently produced a significantly reduced signal. To further validate the physical binding of nanoparticles to the bacterial surface, we performed flow cytometry (data not shown), which revealed a significant increase in side scatter signal upon SPION binding.

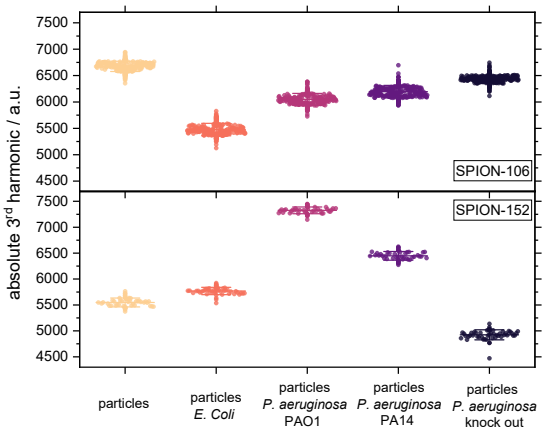

Figure 2: Specificity screening of antibody-functionalised SPIONs for *P. aeruginosa* detection.

IV. Conclusion

This work demonstrates the feasibility of a rapid (< 15 min), culture-independent MPS-based assay for detecting *P. aeruginosa* directly in blood. The promising results with the SPION-152 formulation highlight the potential to significantly reduce diagnostic time compared to conventional methods. Future work will focus on optimising particle surface chemistry and implementing full harmonic analysis to enhance sensitivity especially for samples with lower bacteria concentration. The platform's inherent modularity suggests it can be readily adapted for other critical pathogens, warranting further investigation as a future diagnostic tool.

Acknowledgments

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Author's statement

Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: ethic vote 21-383_2-B. The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee. Use of AI: Readability of this manuscript was improved with the assistance of AI tools (Google AI Studio, DeepL).

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