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An integrated DNA barcode assay microdevice for rapid, highly sensitive and multiplex pathogen detection at the single-cell level

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Here we report an integrated microdevice consisting of an efficient passive mixer, a magnetic separation chamber, and a capillary electrophoretic microchannel in which DNA barcode assay, target pathogen separation, and barcode DNA capillary electrophoretic analysis were performed sequentially within 30 min for multiplex pathogen detection at the single-cell level. The intestine-shaped serpentine 3D micromixer provides a high mixing rate to generate magnetic particle-pathogenic bacteria-DNA barcode labelled AuNP complexes quantitatively. After magnetic separation and purification of those complexes, the barcode DNA strands were released and analyzed by the microfluidic capillary electrophoresis within 5 min. The size of the barcode DNA strand was controlled depending on the target bacteria (Staphylococcus aureus, Escherichia coli O157:H7, and Salmonella typhimurium), and the different elution time of the barcode DNA peak in the electropherogram allows us to recognize the target pathogen with ease in the monoplex as well as in the multiplex analysis. In addition, the quantity of the DNA barcode strand (~104) per AuNP is enough to be observed in the laser-induced confocal fluorescence detector, thereby making single-cell analysis possible. This novel integrated microdevice enables us to perform rapid, sensitive, and multiplex pathogen detection with sample-in-answer-out capability to be applied for biosafety testing, environmental screening, and clinical trials.

Keywords: DNA barcode assay, microdevice, multiplex detection, pathogen detection, single-cell analysis