

# Direct dilution of cell aliquot for high temporal resolution bacterial surface charge measurement

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## Conflicts of interest

The authors declare no conflict of interest.

## Authors' contributions

Wenfa Ng conceived the idea, designed and performed the experiments, analyzed the data, and wrote the abstract. Yen-Peng Ting mentored Wenfa Ng, as well as discussed and analyzed the data.

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# Abstract

Bacterial surface charge mediates important cell-environment and microbe-host interactions, and its accurate and precise measurement by microelectrophoresis requires removing metabolites adhered to the cell surface - where repeated centrifugation and washing by buffers is the gold standard sample preparation method. Unfortunately, the need for time-consuming centrifugation limits the temporal resolution of sampling and profiling of experimental dynamics; especially for samples requiring immediate treatment after sampling. Herein, the feasibility of diluting cell aliquots with buffer as a one step sample preparation technique for surface charge measurement was investigated by characterizing the effects of dilution ratio, cation type, and buffer conductivity on measuring surface charge of *Escherichia coli* DH5 $\alpha$  (ATCC 53868) grown in LB Lennox medium. Results indicated that dilution ratio was critical to accurate surface charge measurement since low signal-to-noise ratio in high or low cell concentration samples generated substantial error. Type of buffer cation was also important since putative binding of high affinity cations to the cell surface underestimated surface charge of negatively charged bacteria. Finally, high conductivity buffers enabled greater removal of adsorbed metabolites through increased charge screening. However, a broader statistical distribution of measured surface charge was also observed – which, at extreme conductivity values, led to inaccurate data; probably due to removal of both intrinsic cell surface ions and exogenous adsorbed metabolites. Altogether, one step dilution of cell aliquot with deionized water reliably reproduced *E. coli* surface charge values obtained via the gold standard approach. But, since the ensemble of secreted metabolites is bacteria and/or medium specific, distinct diluent and experiment parameters exist for each system. The described methodology may find use in preparing samples for cell surface characterization studies, where it would help reduce sample preparation time – and thus, improve temporal resolution at which scientific questions can be probed and answered.

**Keywords:** adsorption; desorption; zeta potential; microelectrophoresis; ionic strength; non-specific adsorption;

**Subject areas:** Microbiology, Environmental Sciences; Biotechnology;