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LAMP-based hydrogen ion selective electrochemical sensor for highly sensitive detection of *Mycoplasma pneumoniae*

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Supplementary Information

Primers (Table S1) specific to gene of *M. pneumoniae* were designed and evaluated using the NUPACK web tool (http://www.nupack.org/) and NCBI primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast). The primers were synthesized by Sangon Biotech (Shanghai, China) .

Table S1. Sequences of nucleic acids used in this work

Name		Sequence (5'-3')
q-PCR primer set ([a] CP017343.1)	F	CGTAAACGATAGATACTAGCTGTC
	R	CCATAACTTTGCCAAGGATGTCA
LAMP primer set ([a] CP017341.1)	F3	TGTTAGCGCGGTTAATGC
	B3	TTCCAAATCACTAGGTGGATT
	FIP	GAGGAGACCCGAAACTGAGTTAGTGAACCAAAAGTGAAAAATGAC
	BIP	ACTGTTAGGTCAAACTGAAAATGGCAAAGGACATACATACAAATCGTG
	LF	TTGCACTGTCTTGCGGT
	LB	GTTCTGAAATACCAAGAGT

[a] GenBank accession number.

Procedure of q-PCR and LAMP

The reaction system of q-PCR with a total volume of 20 μL consisted of 2 μL sample, 0.4 μL dNTPs (10 mM each), 0.8 μL forward and backward primer (10 μM each), 0.2 μL *Taq* DNA polymerase (5 U/μL), 2.0 μL 10× *Taq* buffer, 0.5 μL EvaGreen Dye and 13.3 μL DEPC-treated water. The reaction procedure included denaturation at 95℃ for 3 min, and 40 cycles consisting of 95℃ for 15 s, and 60℃ for 10 s for amplification. The real-time fluorescence LAMP system exhibited a similar composition to the electrochemical LAMP system described in Section 2.4 of the main text. The primary distinction between the two lay in the additional incorporation of 0.625 μL of EvaGreen Dye for facilitating real-time fluorescence detection in the former. Notably, this volume of dye was deducted from the water content of the system. Real-time fluorescent LAMP reaction at 63℃ for 40 minutes. Both q-PCR and real-time fluorescence LAMP assays were carried out by a CFX Connect™ Real-Time PCR System (Bio-Rad, CA, USA). The fluorescence signal of the q-PCR was read after each thermal cycling, while that of the real-time fluorescence LAMP was monitored at 1-min intervals.

The interface connecting the portable device to a smartphone, as depicted in Figure S1, allowed users to operate and control the instrument via the smartphone. The built-in program maintained a temperature range of 20-80°C, with temperature fluctuations accurately controlled within ±0.3°C once the instrument reached the set temperature and stabilized, ensuring the stability and accuracy of the experiments. As shown in Figure S1(a), it was the interface for connecting the instrument to a smartphone via Bluetooth, while Figure S1(b) depicts the real-time detection interface of real samples, with red indicating a positive result and green indicating a negative result, providing users with intuitive access to the test results.

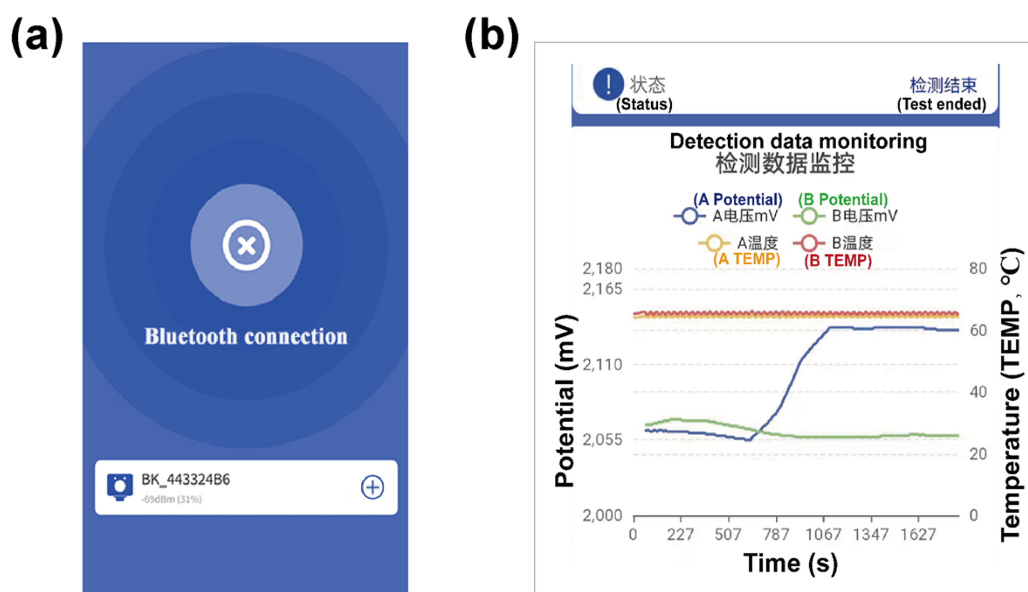

Figure S1 Smartphone real reaction interface. (a) Bluetooth connection interface. (b) Detection completion interface.