Supplementary Materials

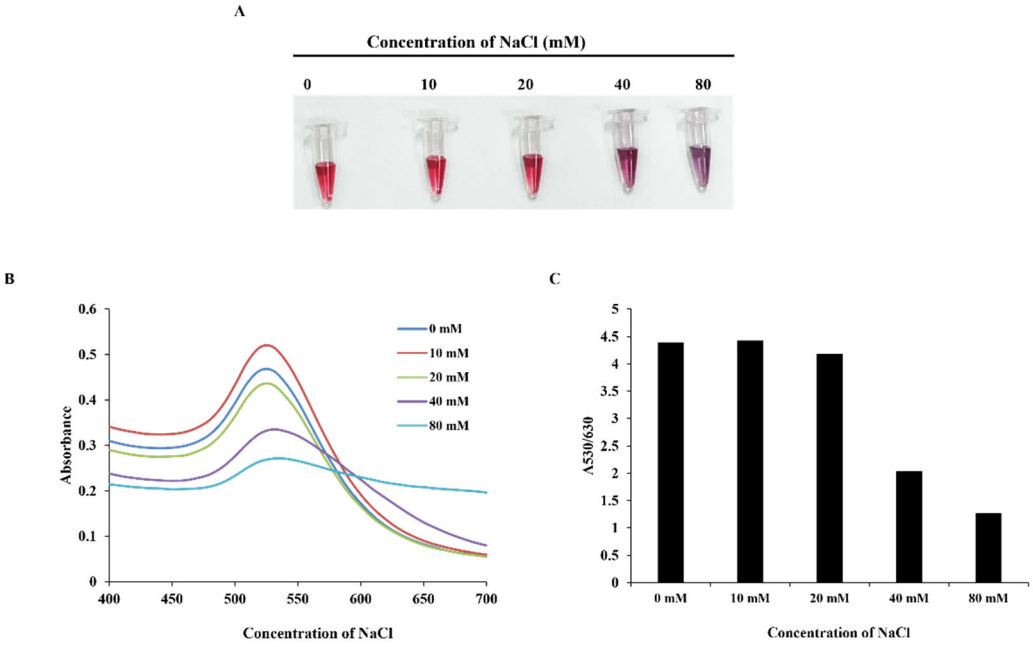


Figure S1. Determination of NaCl concentration. (A) Colorimetric detection of RPA products using unmodified AuNPs; (B) The UV-Vis absorbance spectra of the AuNP aggregation; and (C) A quantitative A_{530}/A_{630} absorbance ratio illustrating the degree of AuNP aggregation

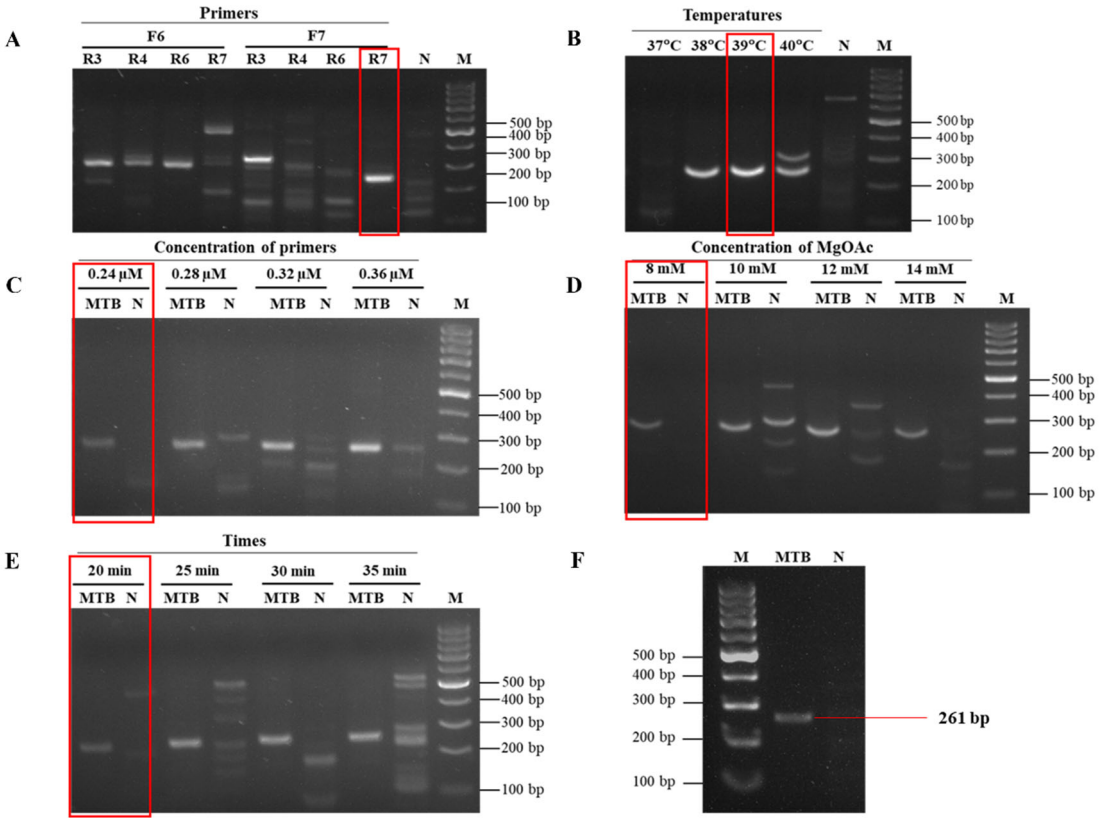


Figure S2. Optimization of the RPA assay demonstrated by agarose gel electrophoresis of RPA products. M: 100-bp DNA marker; MTB: *M. tuberculosis* H37Rv genomic DNA; and N: negative control. (A) Representative primers selection experiment performed using forward primers F6 and F7 and reverse primers R3, R4, R6, and R7; (B) Optimization of reaction temperatures; (C) Optimization of primer concentration; (D) Optimization of MgAOc concentration; (E) Optimization of incubation time; and (F) RPA products under optimal conditions. Red boxes indicate achieved optimal condition.

Table S1. A comparative summary of RPA-based MTB detection assays with TB-GoldDx assay.

Assay/ Method	Principle	Detection system/ Time	Target	LOD	Samples	Sensitivity	Specificity	Reference
TB-GoldDx	RPA + unmodified AuNPs	Colorimetry ~ 40 min	IS*6110*	0.001 ng (~210 bacilli) per 25-µL reaction	Culture (*n* =100) (72 Mtb and 13 NTM 15 non-mycobacteria) Sputum (*n* =140)	95.83% vs. Culture+ Mol ID 81.43% vs AFB smear 82.98% vs. LPA	100% vs. Culture+ Mol ID 58.57% vs. AFB smear 55.56% vs. LPA	This study
RPA-S	RPA + SYBR Green I	Fluorescence 15 min RPA	IS*6110* or IS*1081*	0.05 ng 0.5 ng	Culture (*n* = 146) (146 Mtb and 24 NTM isolates)	97.95% or 99.32%	100% vs.	Singpanomchai et al. (2019) [11]
RT RPA and RPA-LF	0 Real time RPA 0 RPA-Lateral flow	Fluorescence and Lateral flow ~ 40 min	IS*1081* or IS*6110*	25 fg/µL 5 fg/µL	Sputum (*n* =62)	85.1% 53.2%	93.3% 93.3%	Sciaudone et al. (2023) [11]
MCMD	0 RPA-CRISPR–Cas12a-based + Real time fluorescence 0 Lateral flow	Fluorescence and Lateral flow within 1h	IS*6110* and IS*1081*	4 copies/µL	Sputum (*n* =147)	74.8%	100%	Xiao et al. (2023) [29]
Dual-mode MTB sensing	Asymmetric RPA-triggered PAM-free CRISPR system + DNA-functionalized AuNPs	Fluorescence and Colorimetry 30 min RPA + 40 min detection	N/A*	1 copy/µL	Sputum (*n* =32)	100%	100%	Zhang et al. (2025) [22]
MyTRACK	RPA-CRISPR system	Fluorescence 30 min RPA + ~ 20 min detection	*rpoB*	1–100 copies/ reaction	Culture (*n* = 82) Sputum (*n* = 20)	92.59-100% -	100% -	Compiro et al. (2025) [28]

*N/A: The assay target is described in the supplementary materials; however, access to the document is currently restricted.