Supplementary materials

ST131 quantification with ddPCR and limit of detection

A new ddPCR (BioRad, Hercules, USA) assay was designed to quantify the number of copies of ST131 using the same region targeted by ST131-LAMP. The new probe-based assay was designed with Primer3 Plus (1) and consisted of forward and reverse primers and FAM channel probe (Table).

Table. ST131-ddPCR primer and probe sequences.

ST131-ddPCR Primer	Sequence
Forward	AGTTTGGTACAACGTACCCG
Reverse	CGTAGCACCAGCAATGTTTC
Probe	ACCGCCGCACTGATGATGAA

ST131-ddPCR reaction mix was based on a previous study with modifications (2). Individual reactions consisted of 11 μL of ddPCR™ Supermix for Probes (Bio-Rad Laboratories, Hercules, CA, USA), 1.98 μL of 10 μM Forward and Reverse primers, 0.55 μL of 10 μM Probe, 4.29 μL of sterile nuclease-free water and 2.2 μL of DNA as template. A thermal gradient was tested during development of the ddPCR assay and the optimal annealing temperature was determined at 58.5 °C. Thermal cycling conditions consisted of initial denaturation for 10 min at 95°C, followed by 40 cycles of 94°C for 30 s and 58.5°C extension for 1 min, followed by enzyme deactivation at 98°C for 10 min and infinite hold at 4°C. Validation experiments were performed in triplicate and carried out using known concentrations of synthetic DNA fragments of the ST131-LAMP region (gBlocks™, IDT, Coralville, USA).

ddPCR reactions were separated into 12,000–20,000 droplets using a QX™ Droplet Generator (Bio-Rad Laboratories). Amplification was conducted using a C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories) using the cycling conditions above. ddPCR results were read using a QX200™ Droplet Reader (Bio-Rad Laboratories) and analyzed using QXManager Software version 2.1 (Bio-Rad Laboratories).

LOD on bacterial isolates was determined testing serial dilutions of pure ST131 cultures. To this end, ST131 cultures were standardized to absorbance value of 1.0 at optical density (OD) 600. For urine and stool specimens, LOD was determined testing serial dilutions of ST131-positive (non-contrived) specimens of each sample type. LOD studies on urine specimens were performed using crude urine samples. For stool specimens, a swab submerged in a stool sample was resuspended in 1 mL of TE buffer.

Ten-fold serial dilutions of ST131-positive isolates, urine and stool specimens were prepared using TE buffer. A total of 8 technical replicates per dilution was tested with ST131-LAMP. ddPCR was used to quantify gene copy concentration in each dilution. The LOD was defined as the highest dilution (lowest concentration) in which all replicates (8/8) showed a positive ST131-LAMP result.

References

1. **Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JA.** 2007. Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res **35:**W71-74.

2. **Srisutham S, Suwannasin K, Sugaram R, Dondorp AM, Imwong M.** 2021. Measurement of gene amplifications related to drug resistance in Plasmodium falciparum using droplet digital PCR. Malar J **20:**120.

Supplementary Table 1. LAMP-ST131 validation using whole genome sequencing as the gold standard for STc131 (n=84) *vs* known non-ST131 (n=176) isolates.

WGS	LAMP 36.79 cut-off		Total
	Positive	Negative	
STc131	84	0	84
Non-ST131	0	176	176
Total	84	176	260
PPV	100% (95% C.I. = [95.70% - 100%])		
NPV	100% (95% C.I. = [97.93% - 100%])		

This validation was performed using a Ct cut-off value of 36.79 Ct values.

Supplementary Table 2. LAMP-ST131 performance using PCR positive ST131 (n=111) as the gold standard *vs* unknown *E. coli* STs (PCR negative isolates [n=343]).

PCR	LAMP 35.77 cut-off		Total
	Positive	Negative	
Positive	111	0	111
Negative	6	343	349
Total	117	343	460
PPV	94.84% (95% C.I. = [89.26% - 97.60%])		
NPV	100% (95% C.I. = [98.93% - 100%])		

This validation was performed using a Ct cut-off value of 35.77 cycles.

Supplementary Table 3. LAMP-ST131 validation on urine specimens using ST131 culture positives (n=42) as gold standard vs ST131 culture negatives (n=508).

Culture	LAMP 60.05 cut-off		Total
	Positive	Negative	
Positive	41	1	42
Negative	39	469	508
Total	80	470	550
PPV	51.3% (95% C.I. = [44.90% - 60.01%])		
NPV	99.8% (95% C.I. = [98.47% - 99.97%])		

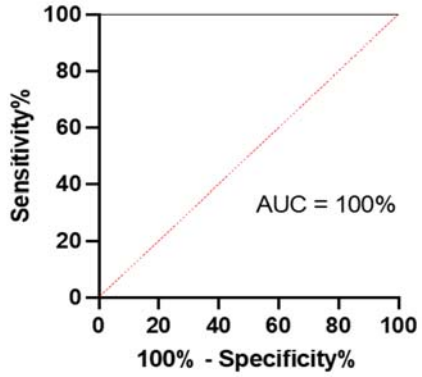
This validation was performed using a Ct cut-off value of 60.05 cycles.

Supplementary Table 4. LAMP-ST131 validation on stool swab specimens using ST131 culture positives (n=30) as gold standard vs ST131 culture negatives (n=248).

Culture	LAMP 57.94 cut-off		Total
	Positive	Negative	
Positive	30	0	30
Negative	40	208	248
Total	70	208	278
PPV	42.9% (95% C.I. = [36.59% - 50.44%])		
NPV	100% (95% C.I. = [98.24% - 100%])		

This validation was performed using a Ct cut-off value of 57.94 cycles.

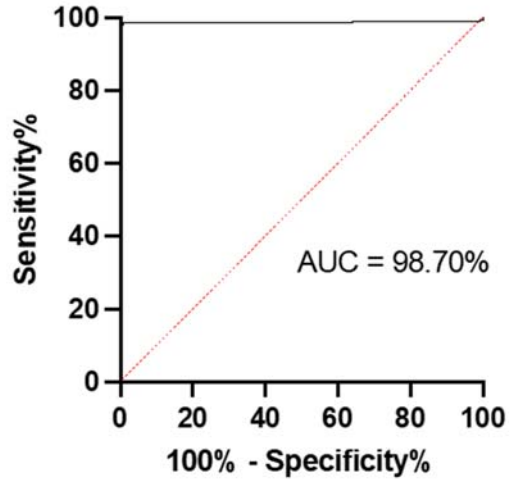
Supplemental Figure 1. Receiver Operational Characteristic (ROC) curve of the ST131 (n=84) *vs* well-characterized non-ST131 (n=176) isolates.



The red dotted line depicts an AUC = 50, equivalent to discrimination by chance.

The optimal cut-off value was 36.79 cut-off cycle threshold.

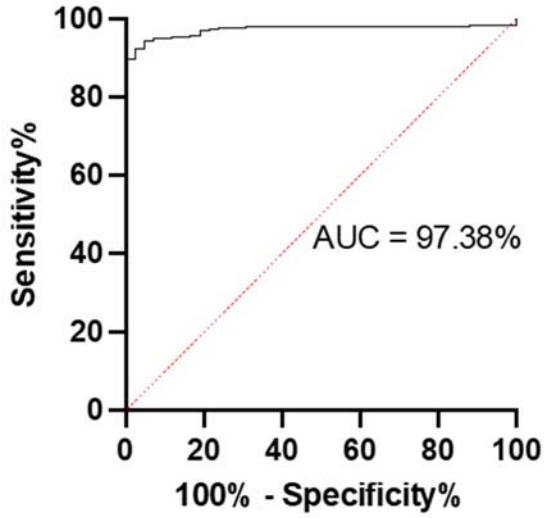
Supplemental Figure 2. Receiver Operational Characteristic (ROC) curve of ST131 (PCR positive) [n=111] *vs* unknown *E. coli* STs (PCR negative) [n=343].



The red dotted line depicts an AUC = 0.50, equivalent to discrimination by chance.

The optimal cut-off value was 35.77 cut-off cycle threshold.

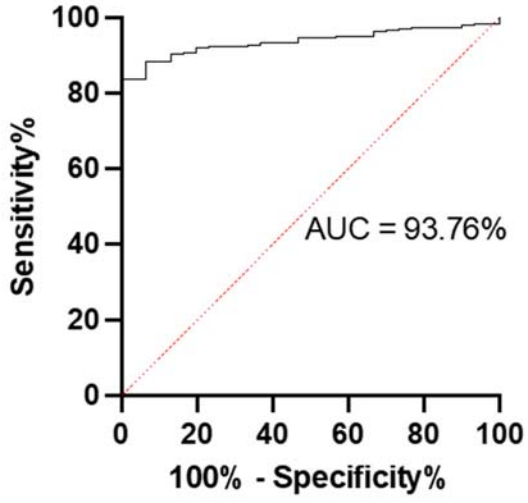
Supplemental Figure 3. Receiver Operational Characteristic (ROC) curve of urine specimens (ST131 culture positives [n=43] vs ST131 culture negatives [n=508]).



The red dotted line depicts an AUC = 50, equivalent to discrimination by chance.

The optimal cut-off value was 60.05 cut-off cycle threshold.

Supplemental Figure 4. Receiver Operational Characteristic (ROC) curve of stool swab specimens (ST131 culture positives [n=30] vs ST131 culture negatives [n=248]).



The red dotted line depicts an AUC = 50, equivalent to discrimination by chance.

The optimal cut-off value was 57.94 cut-off cycle threshold.