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Development and validation of a LAMP assay for rapid detection of Escherichia coli associated UTIs with increased risk of urosepsis

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Abstract

Uropathogenic *Escherichia coli* (UPEC) are the leading cause of urinary tract infections (UTIs) and the virulence factor PapGII is linked to development of urosepsis. Current culture-based diagnostic assays are slow and do not include risk-assessment of the pathogen's potential invasiveness. Therefore, clinicians rely on empirical antibiotic prescription choices, which contributes to evolution of antimicrobial resistance. We aimed to develop and validate a Loop Mediated Isothermal Amplification (LAMP)-based assay for the rapid detection of *papGII*-positive *E. coli* directly from urine.

We designed specific primers targeting conserved regions of the *E. coli uidA* and *papGII* genes. To validate the assay, we collected 191 consecutive urine specimen samples over a three-day period from routine diagnostics. Culture results from routine diagnostics were used as reference for *E. coli* detection and whole genome sequencing for *papGII* detection.

Our assay showed a limit of detection of 10^3 CFU/ml, allowing detection at bacterial concentrations relevant for UTI. Among the clinical urine samples tested, the assay showed 87% sensitivity and 95% specificity for *E. coli*, and 100% sensitivity and specificity for *papGII*. Notably, all results were obtained within 40 minutes and without extensive sample preparation, highlighting the potential of early UPEC identification with an increased risk of invasiveness.

1. Introduction

Urinary tract infections (UTIs) affect more than 150 million individuals every year, making them one of the most prevalent infectious diseases globally [1]. While there are many causative pathogens, uropathogenic *Escherichia coli* (UPEC) account for up to 75% of UTIs [2,3]. The course of infection ranges from mostly mild cystitis to life-threating invasive infections with pyelonephritis and urosepsis [4,5]. Invasive infections caused by UPEC strains are correlated with various virulence factors such as adhesins, exopolysaccharides, iron uptake systems, and immunomodulating agents [6–8]. A particular important virulence factor is PapGII, the tip adhesin of pyelonephritis-associated pili (PAP). PapGII is linked to being a major risk factor for progression to an invasive infection [9]. UTIs with *papGII*-positive *E. coli* were associated in more than 40% of bacteremia, whereas only 7% of *papGII*-negative strains showed bacteremia. In particular, *papGII* positivity was the most significant factor in a multi-variable analysis also considering host factors such as age or immunosuppression [10]. Rapid detection of *papGII*-positive *E. coli* in urine could therefore help to identify UTIs with high risk of invasiveness.

However, current culture-based urine diagnostics remain slow and do not evaluate bacterial virulence. Standard diagnostics typically requires between 18 and 48 hours for pathogen identification and antimicrobial susceptibility testing (AST) [11]. Alongside culture, clinicians commonly use urine dipstick tests (nitrite and leukocyte esterase) for rapid urine assessment. While giving rapid results, these tests show a low positive predictive value for UTIs, and do not allow to identify the causative pathogen [12,13]. Therefore, clinicians need to rely on empirical antibiotic treatment before results are available [14].

However, empirical antibiotic therapy prescribed without pathogen identification can select for drug-resistant organisms and thereby contribute to the rise of antimicrobial resistance [15]. Uncomplicated infections may resolve without treatment, yet untreated UTIs have an increased risk of progression to an invasive infection [3,16]. Current guidelines recommend an initial antibiotic-sparing approach for uncomplicated lower UTI, reserving immediate antibiotic use for suspected pyelonephritis or systemic infection [17]. Therefore, rapid identification of the underlaying pathogen, and a risk-assessment of the potential invasiveness is critical for clinical management.

Loop Mediated Isothermal Amplification (LAMP) offers a rapid and affordable alternative to other diagnostic approaches. Unlike other nucleic-acid amplification methods such as polymerase chain reaction (PCR), LAMP does not involve a denaturation stage, due to the strand displacement activity of the Bst DNA polymerase [18]. This activity enables rapid DNA amplification at stable temperature (60–65°C) which removes the dependency on a thermocycler and therefore is significantly faster than classical PCR assays. LAMP assay results can be interpreted using qualitative detection methods, including colorimetric changes [19,20], turbidity [21], fluorescence [22], or gold nano-particles for colorimetric precipitation [23]. Additionally, LAMP products can be examined using quantitative methods, such as real-time fluorescence or turbidity intensity measurements [24], or with dPAD (measuring the migratory distance of fluorescent length) [25]. Furthermore, LAMP is more tolerant than PCR to inhibitors found in complex media such as urine [26,27]. This eliminates a potential DNA extraction step prior to the amplification [28], further reducing the required laboratory equipment and hands-on work.

We aimed to develop and validate a rapid LAMP-based assay to detect *E. coli* and *papGII* directly from urine. For the assay, we targeted conserved regions of the *E. coli uidA* (beta glucuronidase) and *papGII* genes. We selected the *uidA* housekeeping gene due to its high degree of conservation and specificity to *E. coli* [29].

2. Methods

2.1. Primers

We designed the primers used in this study with the NEB LAMP Primer Design Tool (New England Biolabs, Ipswich, MA, USA). All primers used in this study were synthesized by Microsynth (Microsynth AG, Balgach, Switzerland). We used two sets of primers targeting conserved regions of the *E. coli uidA* and *papGII* genes (Table 1). For each target, we prepared a 10x LAMP primer mix (2 µM F3, 2 µM B3, 16 µM FIP, 16 µM BIP, 4 µM LF, 4 µM LB).

Table 1.

Table 1
List of primers used in this study.

Primer	Sequence
F3_uidA	CCTGATCAACCACAAACCGT
B3_uidA	CGACAGCTGCAGTTTCATCA
FIP_uidA	TCGTGCACCATCAGCACGTTATACTTTACTGGCTTTGGTCGT
BIP_uidA	AATGGACTGGATTGGGGCCAATGTTCATCTGCCCAGTCGA
LF_uidA	CTTTGCCGCGCAAATCCG
LB_uidA	CGCATTACCCTTACGCTGAAGAG
F3_PapGII	ACACAGAAAGAGTCTGAGC
B3_PapGII	TGTTACAATTCCATACACTTCC
FIP_PapGII	TTAAGAATATCGGCGGATGCCGGCTATTAATAGACAGATCACCAT
BIP_PapGII	AACATTTCATTCTCTCTTGGGAGAGCATTTCGCGAGTTACTTGG
LF_PapGII	TCTGCACAGTCTCTGGAAATAAAGC
LB_PapGII	ATTGTATGGGATTTTAAAACGGGCC

2.2. Urine samples

We collected a total of 191 urine samples prospectively over three consecutive days (June 2025) from routine diagnostics (Bacteriology Division, Department of Diagnostics, Institute of Medical Microbiology, University of Zurich). All samples originated in three hospitals in the canton of Zurich, Switzerland: University Hospital Zurich (n = 161), Balgrist University Hospital (n = 8), and GZO Hospital Wetzikon (n = 22). All samples were anonymised.

We streaked out 10 µl of each urine sample on UriSelect 4 chromogenic agar plates (Bio-Rad Laboratories, Marnes-la-Coquette, France; #63726) and incubated for 18 hours at 37°C. We picked pink colonies, suggesting *E. coli*, and sub-cultured on Columbia Agar with 5% sheep blood (COS) plates (bioMérieux SA, Marcy-l'Étoile, France; #1011591500). After 18 hours incubation at 37°C, we identified the species using matrix assisted laser desorption ionization – time of flight (MALDI-TOF) mass spectrometry (MALDI Biotyper Sirius, Bruker Daltonics, Bremen, Germany) using the BDAL, version 2023 database (**Supplementary Table 1**).

For the proof-of-concept assays (Fig. 1) we used strains that were acquired before this study.

2.3. LAMP assay

For LAMP assay, we vortexed the sample and pipetted 100 µl of sample together with 100 µl of 800 mM GuHCl (Sigma-Aldrich, St. Louis, MO, USA; #SLCG0281) + 1% Triton-X (Sigma-Aldrich, CAS 9036-19-5) to

a clean tube. The addition of GuHCl + Triton-X was shown to increase the assay kinetics [30]. For every set of primers, we used nuclease free water (dH_2O) (Sigma-Aldrich, CAS 7732-18-5) instead of urine as a "no template control" (NTC) (Fig. 1, 1). We then put the tubes on a heat block for five minutes at 99°C to heat-lyse the cells and release the bacterial DNA (Fig. 1, 2). Subsequently, we transferred 2 µl of the lysate into the reaction tubes (Fig. 1, 3), prefilled with 2 µl of 10x LAMP primer mix, 10 µl of either WarmStart® Colorimetric LAMP 2X Master Mix with UDG (New England Biolabs, #M1804S) and 6 µl dH_2O for colorimetric LAMP (Fig. 1, 4**.1**); or 10 µl of WarmStart® Fluorescent LAMP/RT-LAMP 2X Master Mix with UDG (New England Biolabs, #M1708SVIAL), 0.4 µl fluorescent dye (New England Biolabs, B1700SVIAL) and 5.4 µl dH_2O for fluorescent LAMP (Fig. 1, 4**.2**). We then mixed the final reaction (20 µl) by gently flicking the tubes. For colorimetric LAMP, we incubated the reaction in a heat bath at 65°C and visually recorded the color change every five minutes for 35 minutes (Fig. 1, 5**.1**). For fluorescent LAMP, we incubated the reaction at 65°C in a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) and automatically measured the fluorescence every minute for 40 minutes using the SYBR channel (Fig. 1, 5**.2**).

2.4. Proof-of-concept study

For the proof-of-concept study, we performed colorimetric LAMP with seven strains from our own biobank and an NTC. For each strain, we spiked 100 µl of dH_2O with one bacterial colony. We then used colorimetric LAMP instead of fluorescent LAMP to demonstrate that the assay can be performed without a real time PCR system.

2.5. Limit of detection study

For the limit of detection assays, we prepared a dilution series (10^6 – 10^2 CFU/ml) of *papGII*-positive *E. coli* in 100 µl dH_2O. We measured three biological replicates on three different days (one replicate per day). To better quantify the results, we performed fluorescent LAMP.

2.6. Whole genome sequencing

We used whole genome sequencing (WGS) as reference method to determine the *papGII* presence. We extracted the DNA from *E. coli* using the Maxwell® RSC Cultured Cells DNA Kit (Promega, #AS1620). Subsequently, we performed WGS on an Oxford Nanopore Technologies GridION using R10.4.1 flow cells (FLO-MIN114) and the Rapid sequencing DNA V14 - barcoding kit (SQK-RBK114.24). We sequenced each run for 72 hours. Runs were controlled using MinKNOW v24.06.15. We enabled live base calling using the Dorado v7.4.14 super-accurate base calling model with a pass-threshold of Q9 and a minimum read length of 500 bp. These relatively low pass-thresholds were chosen to increase the total passed reads, thereby improving the ability to determine the presence or absence of the *papGII* gene.

2.7. Data analysis

For bioinformatic analysis, we used a de novo assembly of sequenced genomes with flye (version 2.9.1-b1780) and polished with medaka (version 1.7.2). We annotated genomes using Prokka (version 1.14.6).

We used BlastN (version 2.13.0) to identify *uidA* and to define the type of *papG* sequences found in each genome.

We used observational statistical analysis and show results with median and inter-quartile ranges. We analysed all LAMP assay in python 3.9.6. The following libraries were used: pandas, numpy, matplotlib, seaborn, and sklearn. We normalized fluorescence values for each sample to the first timepoint (fold-change) to reduce baseline differences. Diagnostic metrics were calculated according to supplementary table 4.

2.8. Ethics statement

All methods were carried out in accordance with relevant institutional guidelines and Swiss regulations, including the Swiss Federal Act on Research involving Human Beings (Human Research Act, HRA; SR 810.30). The study used fully anonymised residual urine samples, and no identifiable or coded health-related personal data were available to the investigators. Therefore, according to HRA Art. 2 para. 2, the study did not fall within the scope of the HRA and did not require ethics committee approval.[31] As only fully anonymised residual material was used, study-specific informed consent was not required.

3. Results

First, we performed a proof-of-concept colorimetric LAMP assay to assess if the selected primers show selectivity to the target genes for the detection of *E. coli* (*uidA*) and *papGII* (Fig. 2, A). For the selectivity assay, a selection of *papGII*-positive, *papGIII*-positive, and *papG*-negative *E. coli*, as well as *K. pneumoniae* and a NTC were chosen. Results depict, for both sets of primers, amplification between 10 to 15 minutes for the positive samples. We noted that false positive amplification tends to appear after 30 minutes of incubation. To better quantify the results and avoid manual photo documentation every five minutes, we performed all following assays using the fluorescence LAMP kit.

We showed that the limit of detection for this assay is between 10^3 and 10^4 CFU/ml. Primers for *uidA* detected one out of three samples with 10^3 CFU/ml, while we never detected *papGII* with a bacterial density below 10^3 CFU/ml (Fig. 2, B**/C**). Consistent with the colorimetric assays, false positives tend to arise after 30 to 35 minutes.

To validate the assay, we collected 191 clinical urine samples, identified the pathogens, and sequenced all *E. coli* isolates. While most samples depicted no microbial sign of bacterial growth, 63/191(33%) urines showed growth of at least one known UTI pathogen. Out of these urines, 38/63 (60%) were *E. coli* culture positive. Out of the *E. coli* isolated, WGS analyses identified 6/38 (16%) to be *papGII*-positive. (**Supplementary Table 1**)

Next, we conducted fluorescence LAMP assays on all 191 urine samples. Figure 3 depicts the median amplification curves for both target genes. Positive urine samples with *E. coli* tend to start amplifying after 20 minutes of incubation, while samples positive for *papGII* already show amplification after 15

minutes (Fig. 3). This difference in amplification time may be a result of the consistently high bacterial load of *papGII*-positive samples, compared to the wider spread of bacterial density of all *E. coli*-positive samples (**Supplementary Table 1**). Consistent with the preliminary data, false positive amplification tends to arise after 30 to 35 minutes of incubation.

To be able to state whether a sample is positive or negative based on the LAMP assay, thresholds for time and fluorescence fold-change must be set to fixed values. Since there are two non-identical sets of primers, different thresholds might be optimal for each target. To identify the most optimal thresholds for the detection of *uidA* (*E. coli*), we compared all possible combinations of time and fluorescence thresholds and ranked by descending Youden's index score, which summarizes sensitivity and specificity. Furthermore, we calculated positive- and negative predictive values (PPV, NPV), since the dataset was heavily skewed towards negative samples (80% *E. coli* negatives). Therefore, we chose the thresholds of 30 minutes and ≥ 3-fold change (second highest Youden's index score) to determine whether a sample is considered *E. coli* positive or negative (**Supplementary Table 2**). For the assessment of *papGII* LAMP assays, we determined the same fluorescence threshold of ≥ 3-fold change and a lower optimal time threshold of 23 minutes. (**Supplementary Table 3**). Figure 4 substantiates these decisions and highlights that thresholds need to be chosen carefully, depending on the research question asked, as well as the pre-test probability (Fig. 4). Overall, the areas under the ROC curves (0.95 for *E. coli*, 1.00 for *papGII*) show excellent discrimination between positives and negatives for both targets.

Out of all 191 clinical urine samples, the LAMP assay detected *E. coli* with a sensitivity of 0.95 and specificity of 0.87. Interestingly, after sequencing analysis it was found that one of the five false negative samples (LAMP107) carried a truncated *uidA*, which explains why there was no amplification. The remaining four false negatives have all been amplified, but only after the 30 minutes threshold and were therefore classified as negatives. For the *papGII* detection, this assay correctly identified all samples without any false positives or negatives (Fig. 5). Notably, all samples that showed a *papGII*-positive result were also correctly identified as *E. coli* by the *uidA* LAMP (**Supplementary Table 1**).

By investigating the time-to-fluorescence threshold, depicted in Fig. 6, it becomes evident that false amplification already tends to start at the 25 minutes mark (Fig. 6). While this did not influence the results for the *papGII* detection, where the threshold was set to 23 minutes, it is more problematic for the *E. coli* detection. The bacterial load of *papGII*-negative *E. coli* samples had a higher distribution than the consistently high load of *papGII*-positive *E. coli*. Therefore, consistent with the proof-of-concept experiment, samples with lower bacterial density showed delayed amplification. This led to a higher optimal time threshold, and therefore more false negatives and false positives. In our setting, the PPV was 0.80 and the NPV was 0.97 (**Supplementary Table 2**)

4. Discussion

In this study, we developed a LAMP based assay to detect *papGII*-positive *E. coli* directly from urine. Among the clinical urine samples tested, the assay showed 87% sensitivity and 95% specificity for *E. coli*,

and 100% sensitivity and specificity for *papGII*, compared to the reference methods. The diagnostic performance of our assay is comparable to that of commercial LAMP-based diagnostic platforms, which are specifically designed for rapid detection of uropathogens and resistance markers directly from urine [32,33].

The key advantages of LAMP lie in its operational simplicity. Unlike PCR, LAMP runs at a constant temperature, eliminating the need for thermocyclers or complex laboratory infrastructure [34]. Even though a real-time PCR system was used in this study for quantification, the assay can also be performed with a simple heat bath and colorimetric detection. Furthermore, the tolerance of the Bst polymerase to inhibitors allowed for direct testing from urine without DNA extraction, reducing the turnaround time and simplifying the workflow [27]. This makes LAMP particularly suitable for primary care settings, or low-resource environments. While colorimetric LAMP highlights the assay's ease of use and practical applicability, fluorescent real-time LAMP enables a more quantitative assessment of amplification but requires more expensive laboratory equipment.

As for other molecular methods, pathogen identification with LAMP also relies on detection of a target gene. Therefore, the target gene must be species specific and highly conserved within the target species, which is challenging itself. This being a potential limitation of LAMP was shown in this study, where one of the false negative *E. coli* carried a truncated *uidA* (LAMP107), which led to no amplification. However, an analysis for the presence of *uidA* in 751 UPEC genomes [10] showed that only 0.4% did not carry a complete *uidA*. Furthermore, primers must be designed carefully to cover different gene variants without losing specificity. In this study, all samples carrying *papGII* were identified successfully, while no other *papG* alleles were detected incorrectly.

The tolerance to primer mismatches of the Bst polymerase helps for more robust detection of different variants of the target gene within the target species. However, this tolerance may also lead to false amplification of similar genes, or amplification of primer dimers [35]. Notably, the false amplification, as shown in this study, usually occurs at a later stage compared to amplification of the target gene. Therefore, by choosing a lower time threshold, true positives and false positives can be segregated, as demonstrated for the *papGII* detection. However, while a lower time threshold for the *uidA* amplification reduced false positives, it also induced false negatives, especially for samples with a bacterial load of less than 10^4 CFU/ml. Although relatively high, this value is within the range observed in most clinically relevant urinary tract infections [36]. To improve the detection time of low-bacterial density samples, the urine could first be centrifuged, adding minimal hands-on work to the sample preparation. To further separate positives and negatives, a target gene specific fluorescent probe could be included instead of an intercalating dye [37].

However, while there is room for improvement, this intentionally simplified assay still depicted good to excellent differentiation between positives and negatives, highlighting the potential of LAMP as a rapid, low-resource tool for UTI diagnostics. While multiple LAMP-based assays for UTI detection directly from urine have been developed, most of them focus on species identification and detection of AMR genes

[32,38,39]. In contrast, by specifically targeting *papGII*, a key virulence gene in *E. coli* for progression to an invasive infection, our assay shifts the emphasis to virulence profiling. Early detection of *papGII*-positive *E. coli* could help identify patients at higher risk of invasive infection and guide more personalized antibiotic management. Although only six clinical *papGII*-positive *E. coli* samples were evaluated in this study, the consistent detection across these samples highlights the potential of the assay and supports its further assessment in larger, clinically diverse collections.

Declarations

Data availability

Raw reads of all sequenced isolates are publicly available via the European Nucleotide Archive (Project Accession PRJEB106156).

All codes to reproduce the analytical results are available open source (https://github.com/JanisRog/LAMP_ESCOL_PapGII_analysis/).

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Additional Information

Author contributions

J.R. conducted all experiments, wrote the main manuscript text, and prepared the figures. A.GL. supervised J.R. in data curation and statistical analysis. O.N., C.Q., V.H., and N.KG. collected clinical urine samples, identified species and determined bacterial densities. M.B. performed preliminary experiments. M.B. and A.E. acquired funding. J.R., M.B. and A.E. conceptualized this work. A.E. supervised this work.

Declaration of competing interest

The authors declare no competing interests.

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40. Additional & Information.

Figures

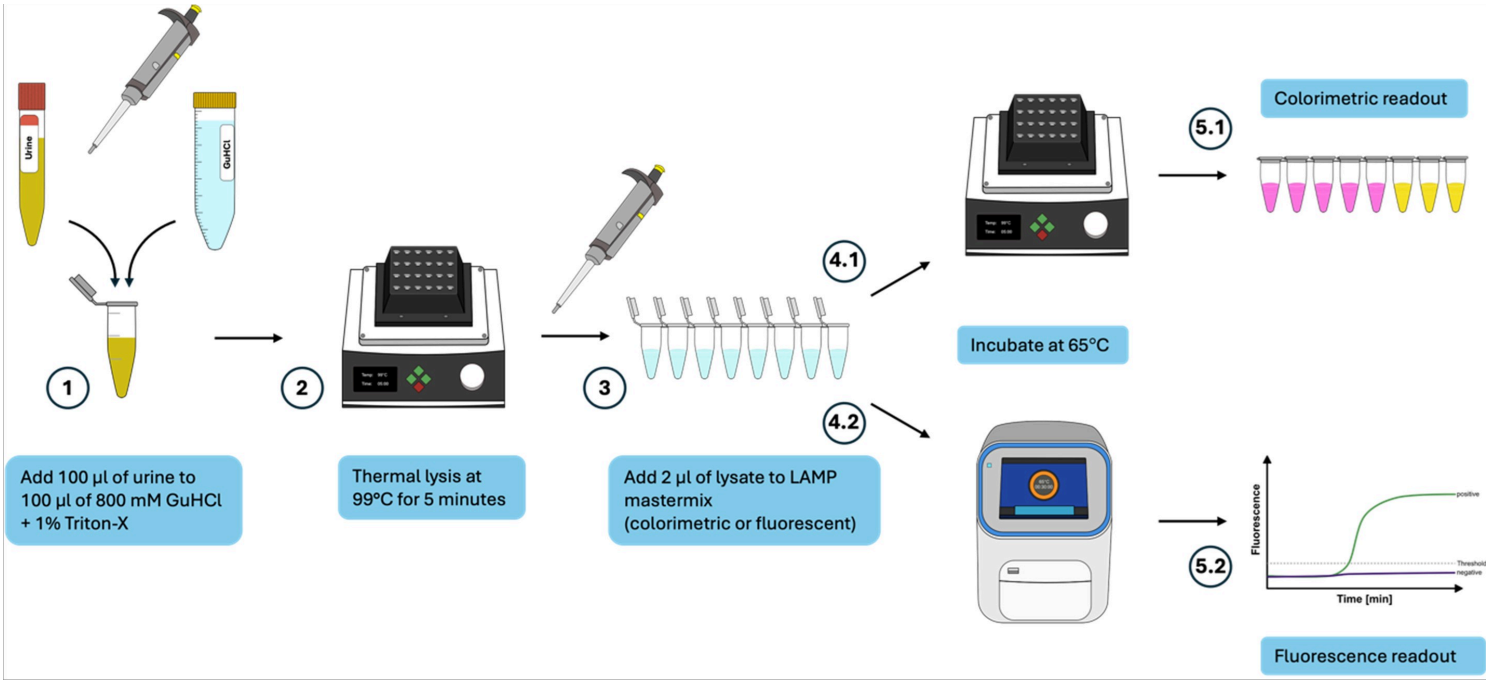


Figure 1

LAMP workflow.

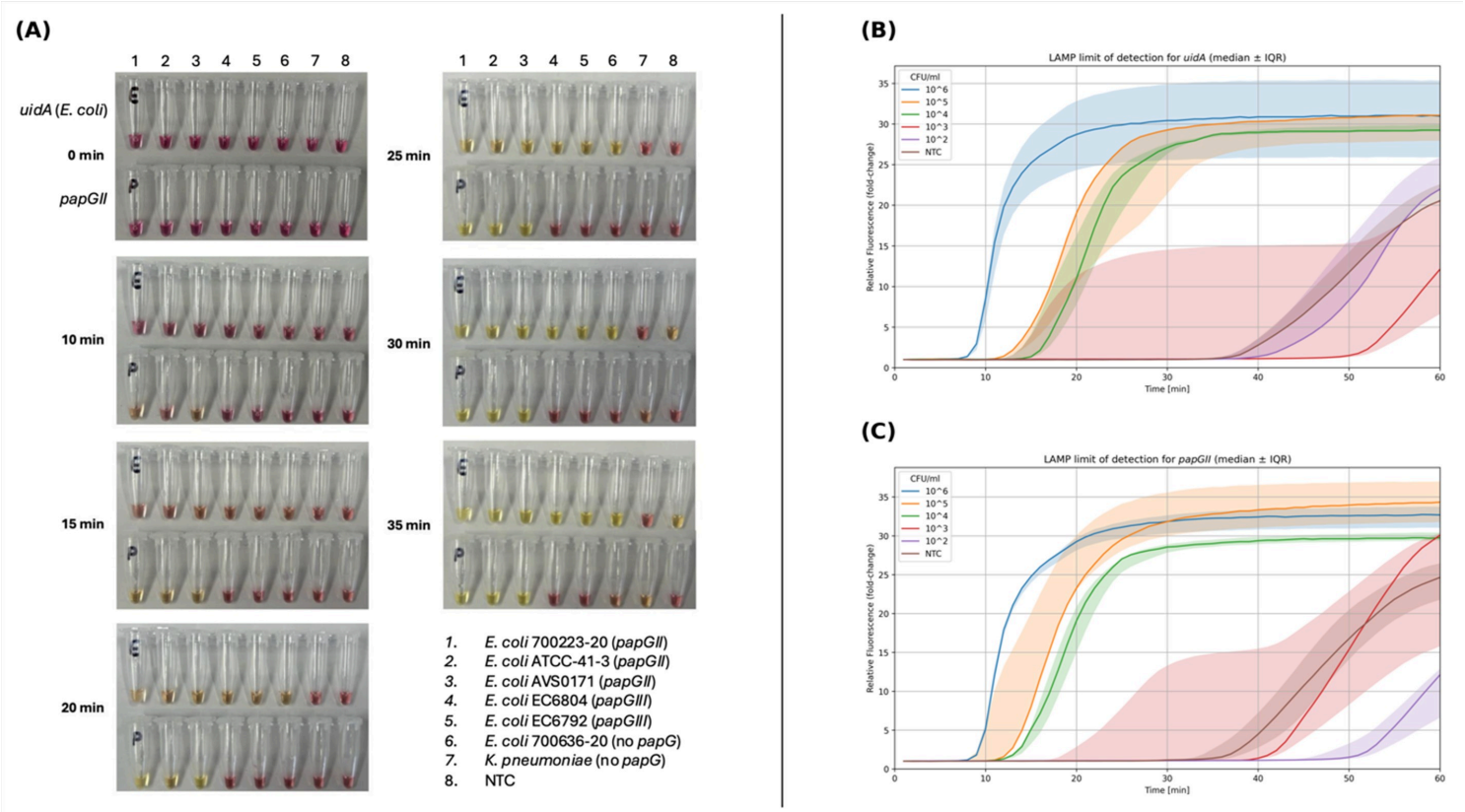


Figure 2

Proof-of-concept for the detection of *uidA* (*E. coli*) and *papGII*. (**A**) Colorimetric LAMP assays for eight samples with images recorded every five minutes over 35 minutes. Pink tubes indicate no amplification, yellow tubes indicate amplification. (B, C) Fluorescence LAMP amplification plots for a dilution series of *papGII*-positive *E. coli* with primers for (**B**) *uidA* and (**C**) *papGII*. We measured three biological replicates. The x-axis indicates relative change in fluorescence (F/F_0), where F_0 is the first measurement, and the y-axis indicates time [min]. Curves are stratified by bacterial load. Solid lines indicate median, shaded bands indicate median values ± interquartile range.

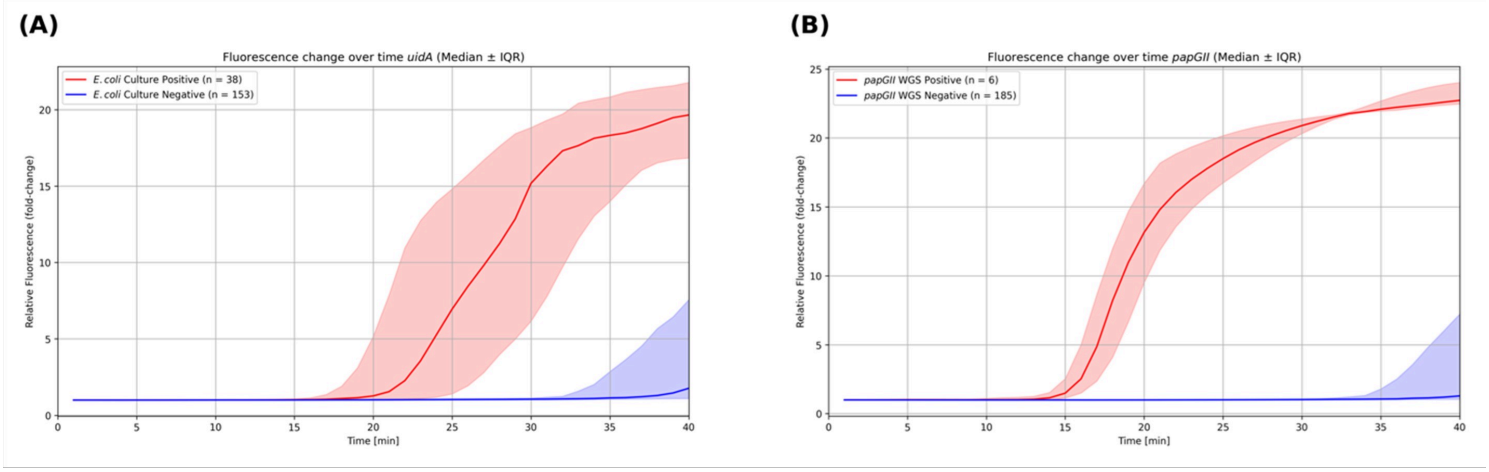


Figure 3

Fluorescence kinetics of LAMP assays for the detection of (A) *uidA* (*E. coli*) and (B) *papGII* in clinical urine samples (n = 191). Fluorescence values were normalized to the first time point (fold-change). Curves are stratified based on the reference method (red = positive, blue = negative; culture for *E. coli*, WGS for *papGII*). Within each plot, solid lines indicate median and shaded bands indicate variability (± interquartile range).

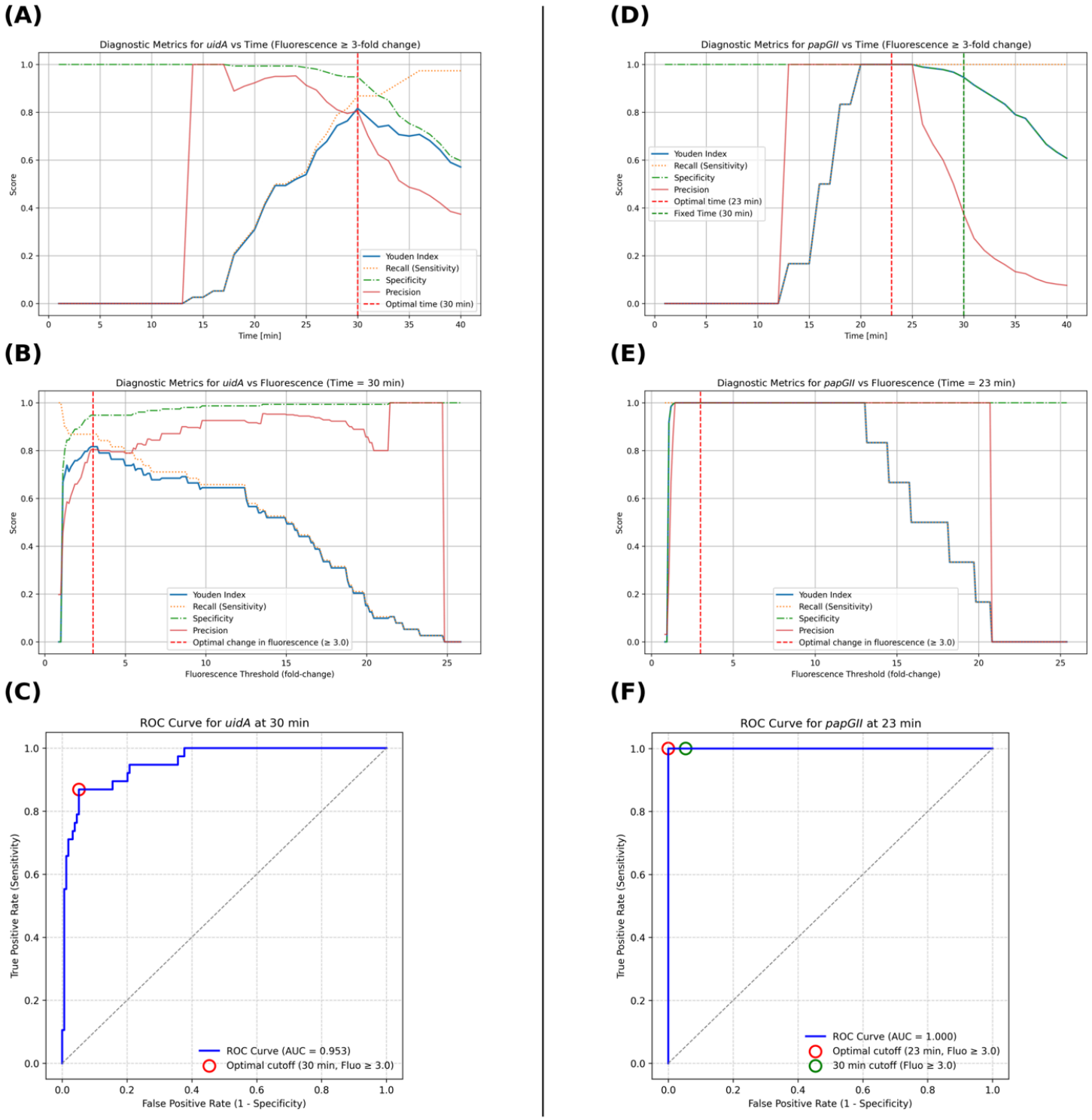


Figure 4

Diagnostic performance of LAMP assays (n = 191) **for *uidA* (*E. coli*) und *papGII***. (A-C) *uidA*: (**A**) metrics vs. time at fixed fluorescence threshold (≥ 3-fold change; optimal by Youden's index; red dotted line indicates optimal time cutoff), (**B**) metrics vs fluorescence at fixed time (30 min; red dotted line indicates optimal fluorescence cutoff), (**C**) ROC curve with AUC and optimal cutoff marked in red. (D-F) *papGII*: corresponding analyses; green dotted line in (**D**) and green circle in (**F**) indicate the optima for *uidA* for

reference. In (**E**) the time is fixed at 23 min. Metrics shown are Youden's Index, sensitivity, specificity, and precision.

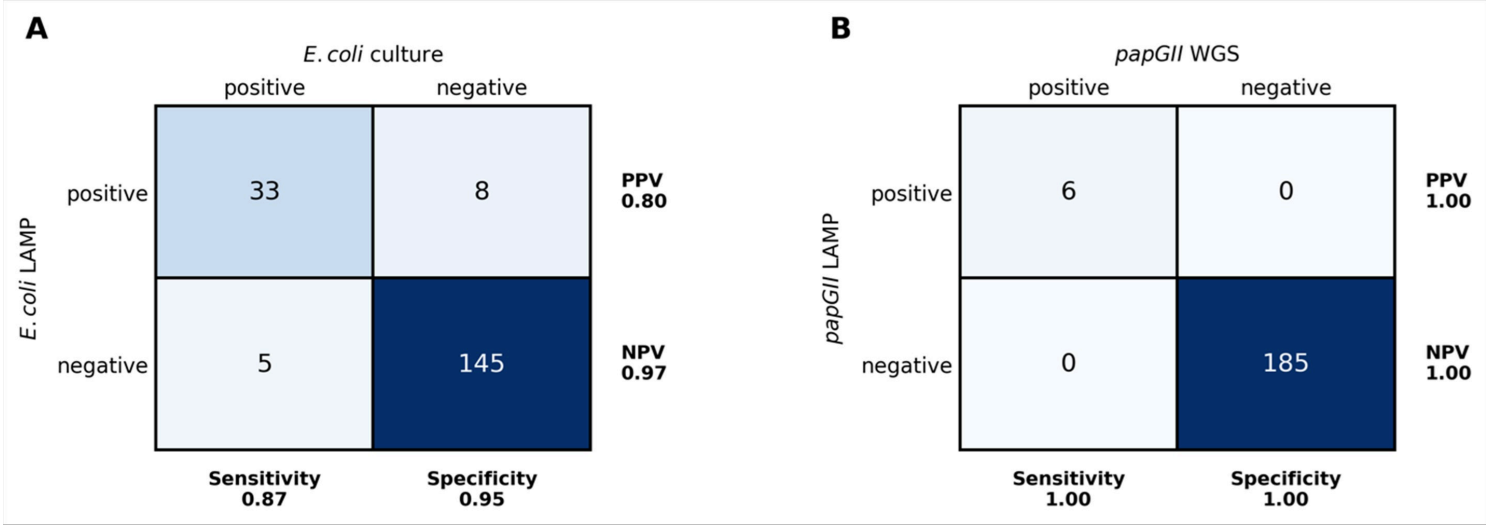


Figure 5

Confusion matrix for (A) time = 30 min and fluorescence fold-change ³ 3 for *E. coli*, and (B) time = 23 min and fluorescence fold-change ³ 3 for *papGII*.

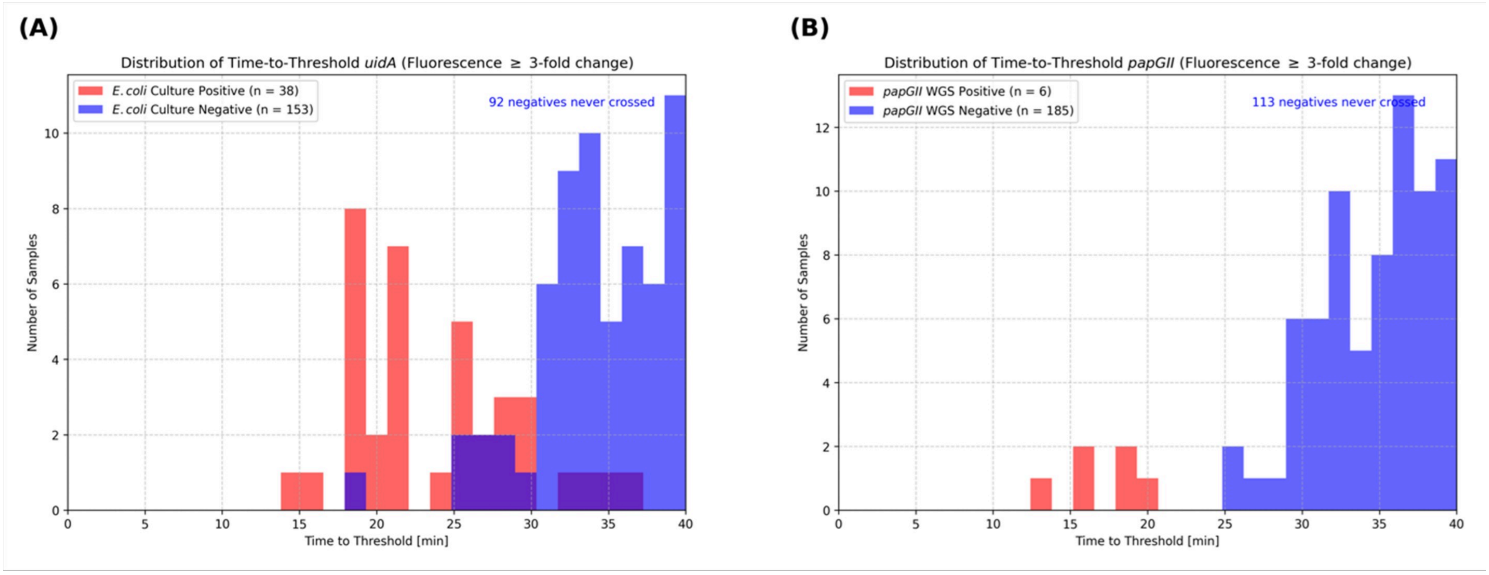


Figure 6

Time-to-threshold distribution of LAMP assays (n = 191) for (A) *uidA* and (B) *papGII*. Bar heights indicate the number of samples that crossed the fluorescence threshold (≥ 3-fold change) at the same time. Red indicates positives based on the reference method; blue indicates negatives based on the reference method.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformation1.xlsx
- LAMPsupplementaryinformation2.docx