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Loop-Mediated Isothermal Amplification Assay for Rapid Detection of Common Strains of *Escherichia coli*‡

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We developed a highly sensitive and specific LAMP assay for *Escherichia coli*. It does not require DNA extraction and can detect as few as 10 copies. It detected all 36 of 36 *E. coli* isolates and all 22 urine samples (out of 89 samples tested) that had *E. coli*. This assay is rapid, low in cost, and simple to perform.

*Escherichia coli* can cause infections of a variety of extraintestinal sites such as the urinary tract, meninges, and bloodstream (3, 5, 8, 10–12, 21). Diagnosis of most extraintestinal *E. coli* (ExPEC) infections requires bacterial culture, requiring 1 to 2 days of incubation, and subsequent confirmatory testing (6, 17). Guidance as to the presence or absence of an ExPEC infection at initial presentation could be useful for a patient, especially when attempting to reduce excessive use of antibiotics. Although conventional DNA amplification using PCR can provide fast results, it is not widely used partly because it requires considerable skill and expensive equipment. Recently, loop-mediated isothermal amplification (LAMP), a rapid technique for amplifying DNA has been reported (14, 15). It requires incubation at a constant temperature (60 to 66°C for 30 to 60 min) and eliminates the need for specialized equipment or expertise. We report here the development and characterization of a LAMP assay to detect urinary *E. coli*.

Three sets of primers (outer, loop, and inner) were required for the *E. coli* LAMP assay that were designed by analyzing a conserved region of the *E. coli malB* gene with Primer Explorer version 3 software (http://primerexplorer.jp/elamp3.0.0/index.html) (Fig. 1). This gene is conserved across diverse lineages of *E. coli* and is not shared by other gram-negative bacteria except *Shigella* spp., based on BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) (18). We considered this region to be appropriate because of its conservation, as well as the rarity of *Shigella* as a cause of extraintestinal infections.

The LAMP reaction was conducted using methods described previously, with minor modifications (14, 15). The final LAMP reaction (total, 25 μl) contained the three primer pairs in the following concentrations: 0.2 μM outer primers, 0.8 μM loop primers, and 1.6 μM inner primers. The reaction mix also contained 2.5 μl of 10× *Bst* DNA polymerase reaction buffer (1× containing 20 mM Tris-HCl, 10 mM KCl, 10 mM (NH2)2SO4, 2 mM MgSO4, 0.1% Triton X-100), 1 μl of an 8-U/μl concentration of *Bst* DNA polymerase (New England Biolabs, Inc., MA), 2 mM MgSO4 (2 μl), 5 μl of betaine (Sigma-Aldrich, St. Louis, MO), and 5 μl of sample. We generated and quantified a plasmid containing the target *E. coli* sequence to determine the sensitivity of the LAMP assay. The target sequence in *E. coli* was amplified by PCR using the primers F3 and B3 of the LAMP assay and cloned by using a StratataClone PCR cloning kit (Stratagene, La Jolla, CA). The copy number of the solution containing the *E. coli* clone was determined by UV spectrophotometry (2). The analytical sensitivity of LAMP was determined by using 10-fold serial dilutions of the *E. coli* clone.

The LAMP reaction was performed in a heating block (Lab-Line, Iowa). For comparison, it was also performed using a conventional thermal cycler (Bio-Rad, California), and both machines performed equally well. The cost per reaction was ca. 50¢ (U.S. currency).

A positive *E. coli* LAMP reaction typically required incubation for 60 min at 66°C to produce a ladder pattern on agarose gel (Fig. 2i). The *E. coli* LAMP products from several of the bands in the gel were excised and sequenced. The amplicons matched the *E. coli malB* gene segment spanned by F3/B3 primers. The *E. coli* LAMP products were also detected without electrophoresis using ambient light after the addition of a number of DNA dyes to the reaction tube. These dyes included propidium iodide, ethidium bromide, methylene blue, acridine orange, and Sybr green. Of the various dyes, Sybr green (1:10...
dilution of a 10,000× stock solution) and propidium iodide (1:10 dilution of 10-mg/ml stock solution) produced the best visual discrimination. For both the dyes, 1 μl of the diluted dye was added to 25 μl of the reaction mix to develop the color reaction (Fig. 2ii). UV transillumination of the reaction mix containing the DNA dye improved the differentiation further.

The specificity of the assay was evaluated by testing eight gram-negative bacteria (Acinetobacter spp., Citrobacter freundii, Enterobacter cloacae, Haemophilus influenzae, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, and Serratia marcescens) and six gram-positive bacteria (Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, and Streptococcus viridans). No ladder pattern was seen with any of the other non-E. coli bacteria or with the negative “no-target” control (Fig. 2i). The sensitivity of E. coli LAMP was found to be approximately 10 copies per reaction when an incubation period of 60 min was used. Larger copy numbers required shorter incubation times (as low as 15 to 20 min for >1,000,000 copies).

We further tested a range of E. coli isolates by this assay. A total of 36 isolates of E. coli from unique patients (n = 23) or the Escherichia coli Reference (ECOR) Collection (n = 13) were tested. ECOR (established in 1984) contains 72 strains and five phylotypes (16). A LAMP assay was performed on an aliquot of bacterial solution without extraction of DNA. It was able to detect all of the 36 E. coli isolates (Table 1). Finally, 89 fresh urine samples were tested by LAMP assay, and the results were compared to cultured urine samples obtained from microbiology laboratories of the Children’s Hospital of Pittsburgh and the All India Institute of Medical Sciences from patients suspected with urinary tract infections (UTI). The culture was considered positive if there was growth of individual bacteria in a concentration of >100,000 colonies/ml. Growth of multiple bacteria was considered contamination. The samples were stored at 4°C after plating of urine and were analyzed by LAMP assay within 1 to 3 days of collection. Initial LAMP testing, performed on three known E. coli-positive urine samples, showed positive results both with and without DNA extraction using a QIAmp DNA Mini-Kit (Qiagen, California). Subsequent tests were performed on unextracted urine, i.e., 5 μl of unprocessed urine was directly added to the LAMP reaction mix, followed by incubation for 60 min at 66°C. Of the 89 urine samples, 22 were positive for E. coli, all of which tested positive by the LAMP assay. In addition, 36 samples had no bacterial growth, and the LAMP assay results were negative in all of these samples. Finally, in 31 samples, organisms other than E. coli grew, including 13 samples that pro-
duced ≥1 bacterial species. In 29 of these 31 samples, the LAMP assay was negative. Two non-*E. coli* samples that had tested positive by the LAMP assay had grown multiple organisms and probably had *E. coli* as one of the contaminants (Tables 2 and 3).

We describe here a new, rapid, and economical assay to detect *E. coli* in human urine in 1 h (or less), which makes this assay amenable for point-of-care or outpatient settings. There are several advantages of the LAMP over PCR, the conventional method for DNA amplification. This assay only needs a heating block and obviates the need for a thermal cycler. Also, DNA extraction is not required for LAMP. In contrast, unprocessed urine normally requires DNA extraction for PCR, since urinary urea can inhibit PCR at concentrations of >50 mM and the normal concentration of urea in adult urine is >330 mM (1, 4, 9, 20). Hence, the LAMP assay, by eliminating the step of DNA extraction, is more “user friendly” than PCR.

We focused on urine as the analyte for the present study and obtained consistent results for all of the culture-positive samples. Although we did not test other biological fluids (e.g., blood, cerebrospinal fluid, etc.) it is likely, based on our recent studies on BK virus LAMP, that *E. coli* LAMP would perform well with these types of specimens (2).

Another advantage of this assay is that identification of a positive reaction does not require any special processing or electrophoresis. It could be detected by looking for a color change of the reaction mix in ambient light, when a DNA-binding dye is used. This visualization can be further enhanced by UV transillumination. We also showed that a simple chemical such as propidium iodide can be used for detecting a positive reaction. To our knowledge, this is the first report of using propidium iodide to visualize LAMP products. Pro-

![FIG. 2. LAMP assay reactions. (i) A gel image showing LAMP products from *E. coli* and other bacteria. The lane numbers correspond to the following specimens. Lanes: 1, *E. coli*; 2, 100-bp ladder; 3, *Proteus mirabilis*; 4, *Pseudomonas aeruginosa*; 5, *Enterobacter faecalis*; 6, *Staphylococcus aureus*; 7, *Streptococcus pyogenes*; 8, *Streptococcus pneumoniae*; 9, *Streptococcus viridans*; 10, no target control. (ii) Visualization of LAMP products by inspection. (A) Propidium iodide without UV illumination. The color changes from a deep red-orange in the negative reaction to a light (almost clear) pink in the positive reaction. (B) Propidium iodide with UV illumination showing bright transillumination in the positive reaction. (C) Sybr green without UV illumination. The color changes from orange in the negative to green in the positive reaction. (D) Sybr green with UV illumination showing bright transillumination in the positive reaction.](http://jcm.asm.org/)

![Figure 2](http://jcm.asm.org/)
Although LAMP assays for various specific \textit{E. coli} strains have been reported, these studies were designed to detect virulence loci or markers of specific strains, such as verotoxin (7, 19, 22). However, for detection of UTI-associated \textit{E. coli} strains, a less discriminatory approach is intended. Our studies extend the role of LAMP to the rapid diagnosis of such infections, using this less-biased approach. In addition to the advantages described here, there are some limitations of the assay in its current format. This assay is suited for qualitative but not quantitative information. Possibly, semiquantitative assays can be developed where the time required to develop a positive reaction could be used as a surrogate for the starting copy number. Also, LAMP assays do not provide antimicrobial susceptibility information and since bacteria demonstrate increasing resistance to antimicrobial agents, this assay is more likely to be an adjunct rather than a replacement for culture. Finally, in contrast to culture, this assay detects only a single organism. However, LAMP assays for other common bacterial pathogens, including \textit{Staphylococcus} spp., have recently been reported (13); these assays may potentially be combined with this assay to screen for common infections. Further studies involving a larger number of patients and different specimens are needed in order to evaluate the clinical utility of this assay.

\begin{table}[h]
\centering
\caption{Statistical comparison of LAMP and culture results from urine samples obtained from patients with suspected UTI}
\begin{tabular}{lcccc}
\hline
Comparison standard & \% Sensitivity & \% Specificity & PPV & NPV \\
\hline
LAMP vs culture & 91.7 & 100 & 1.00 & 0.97 \\
Culture vs LAMP & 100 & 97 & 0.917 & 1.00 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Description of isolates of \textit{E. coli} evaluated by LAMP}
\begin{tabular}{lcccc}
\hline
Source & Sample no. & Phylogenetic group & Antigen(s) & Sample Syndrme & LAMP positive \\
\hline
Clinical & C1 & A & O147 & Urine & Cystitis & Yes \\
C2 & A & O6 & Urine & Pyelonephritis & Yes \\
C3 & A & 025:K2:H2 & Blood & Urosepsis & Yes \\
C4 & A & O74 & Feces & NA & Yes \\
C5 & B1 & O75 & Urine & Cystitis & Yes \\
C6 & B1 & O149 & Urine & Pyelonephritis & Yes \\
C7 & B1 & O64:H21 & Blood & Urosepsis & Yes \\
C8 & B1 & O86 & Feces & NA & Yes \\
C9 & B2 & O6 & Urine & Cystitis & Yes \\
C10 & B2 & ON & Urine & Cystitis & Yes \\
C11 & B2 & O2 & Urine & Pyelonephritis & Yes \\
C12 & B2 & O2 & Urine & Pyelonephritis & Yes \\
C13 & B2 & O1:K1:H7 & Blood & Urosepsis & Yes \\
C14 & B2 & O6:K2:H1 & Blood & Urosepsis & Yes \\
C15 & B2 & ON & Blood & Abdominal & Yes \\
C16 & B2 & O1 & Blood & Abdominal & Yes \\
C17 & B2 & O1 & Blood & Pulmonary & Yes \\
C18 & B2 & O1 & Blood & sepsis & Yes \\
C19 & B2 & O6 & Feces & NA & Yes \\
C20 & B2 & ON & Feces & NA & Yes \\
C21 & D & O17:77 & Urine & Pyelonephritis & Yes \\
C22 & D & O7:K1:H1 & Blood & Urosepsis & Yes \\
C23 & D & O21 & Feces & NA & Yes \\
\hline
ECOR & ECOR-11 & A & O6 & Urine & Cystitis & Yes \\
ECOR-5 & A & O79 & Feces & NA & Yes \\
ECOR-3 & A & O1 & Feces & NA & Yes \\
ECOR-72 & B1 & O144 & Urine & Pyelonephritis & Yes \\
ECOR-28 & B1 & O104 & Feces & NA & Yes \\
ECOR-33 & B1 & O79 & Feces & NA & Yes \\
ECOR-45 & B1 & ON & Feces & NA & Yes \\
ECOR-55 & B2 & O25 & Urine & Pyelonephritis & Yes \\
ECOR-61 & B2 & O25 & Feces & NA & Yes \\
ECOR-57 & B2 & ON & Feces & NA & Yes \\
ECOR-48 & D & ON & Urine & Cystitis & Yes \\
ECOR-39 & D & O79 & Feces & NA & Yes \\
ECOR-37 & E & ON & Feces & NA & Yes \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Clinical comparison of LAMP and culture results from urine samples obtained from patients with suspected UTI}
\begin{tabular}{lcccc}
\hline
Culture result & No. of samples: & Tested & Positive by LAMP & Negative by LAMP \\
\hline
Positive for \textit{E. coli} (pure culture and \( \geq 100,000 \) CFU/ml) & 22 & 22 & 0 \\
No growth & 36 & 36 & 0 & 36 \\
Growth of bacteria other than \textit{E. coli}, including gram-positive, gram-negative, fungus, and multiple organisms & 31 & 0 & 29 \\
Gram positive (\( n = 15 \)) & 1 & 0 & 1 \\
\textit{Enterococcus faecalis} & 1 & 0 & 1 \\
\textit{Lactobacillus} spp. & 1 & 0 & 1 \\
\textit{Staphylococcus saprophyticus} & 2 & 0 & 2 \\
\textit{Streptococcus} spp. (alpha-hemolytic) & 2 & 0 & 2 \\
\textit{Streptococcus} spp. (group B, beta-hemolytic) & 8 & 0 & 8 \\
Gram-positive cocci (not otherwise specified) & 1 & 0 & 1 \\
Gram negative (\( n = 2 \)) & 1 & 0 & 1 \\
\textit{Klebsiella oxytoca} & 1 & 0 & 1 \\
Fungus (\( n = 1 \)) & 1 & 0 & 1 \\
\textit{Candida albicans} & 1 & 0 & 1 \\
Multiple organisms (\( n = 13 \)) & 13 & 2 & 11 \\
Total & 89 & 24 & 65 \\
\hline
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\end{table}
References


