**SUPPORTING INFORMATION**

# Strand displacement probes combined with isothermal nucleic acid amplification for instrument-free detection from complex samples

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## MATERIALS AND METHODS

**Verification of *E. coli* primer tag effect on LFIA intensity.** We observed a difference in LFIA test line intensity based on whether standard *E. coli* LAMP reaction primers were tagged LF-FAM and LB-Biotin or LF-Biotin and LB-FAM. To confirm that our observation was a result of the tag configuration (and not some other experimental or pipetting error), we prepared a single master mix of LAMP reagents without the tagged loop primers. We then split the master mix into two aliquots and added the two sets of tagged loop primers for amplification. The LAMP reactions with LF-FAM and LB-Biotin yielded LFIA test bands 4 times the intensity of LAMP reactions with LF-Biotin and LB-FAM, indicating a significant difference in steric hindrance between the two configurations (Figure S-6).

**Verification of strand displacement probes' target specificity.** To demonstrate probes' specificity, a LAMP master mix (including F3, B3, FIP, BIP, and LB-biotin primers) optimized for *E. coli* was amplified with *E.coli* template and a probe designed for *V. cholerae*. Similarly, a LAMP master mix (inc. F3, B3, FIP, BIP, and LB-biotin primers) optimized for *V. cholerae* was amplified with *V. cholerae* template and a probe designed for *E. coli*. In both cases, despite successful amplification observed in agarose gel, no amplicons are captured on LFIA (Figure S-7A).

Next, to confirm specificity of primers and probes to their target, a LAMP master mix (including F3, B3, FIP, BIP, LB-Biotin primers and probe) optimized for *E.coli* was heated with *V. cholerae* template. Similarly, a LAMP master mix optimized for *V. cholerae* was heated with *E. coli* template. In neither case was non-specific amplification product observed in an agarose gel or in the respective LFIA strips (Figure S-7B).

**Table S1.** Gene target sequences.

| Gene Name | Sequence (5'-3') |
|---|---|
| *E. coli malB* | GCCATCTCCTGATGACGCATAGTCAGCCCATCATGAATGTTGCTGTCGATGACAGGTTGTTACAAAGG GAGAAGGGCATGGCGAGCGTACAGCTGCAAAATGTAACGAAAGCCTGGGGCGAGGTCGTGGTATCG AAAGATATCAATCTCGATATCCATGAAGGTGAATTCGTGGTGTTTGTCGGACCGTCTGGCTGCGGTAA AT |
| *V. cholerae ctxA* | GTTTTGATCAATTATTTTTCTGTTAAACAAAGGGAGCATTATATGGTAAAGATAATATTTGTGTTTTTTAT TTTCTTATCATCATTTTCATATGCAAATGATGATAAGTTATATCGGGCAGATTCTAGACCTCCTGATGAA ATAAAGCAGTCAGGTGGTCTTATGCCAAGAGGACAGAGTGAGTACTTTGACCGAGGTACTCAAATGAA TATCAACCTTTATGATCATGCAAGAGGAACTCAGACGGGATTTGTTAGGCACGATGATGGATATGTTTC CACCTCAATTAGTTTGAGAAGTGCCCACTTAGTGGGTCAAACTATATTGTCTGGTCATTCTACTTATTAT ATATATGTTATA |

**Table S2.** Oligonucleotide primers and probes' sequences.

| | Oligonucleotide | Sequence (5'-3') |
|---|---|---|
| *E. coli* | F3 primer | GCCATCTCCTGATGACGC |
| | B3 primer | ATTTACCGCAGCCAGACG |
| | Forward inner primer (FIP) | CATTTTGCAGCTGTACGCTCGCAGCCCATCATGAATGTTGCT |
| | Backward inner primer (BIP) | CTGGGGCGAGGTCGTGGTATTCCGACAAACACCACGAATT |
| | Forward loop primer (LF-FAM) | /56-FAM/CTTTGTAACAACCTGTCATCGACA |
| | Forward loop primer (LF-Biotin) | /5Biosg/CTTTGTAACAACCTGTCATCGACA |
| | Backward loop primer (LB-FAM) | /56-FAM/ATCAATCTCGATATCCATGAAGGTG |
| | Backward loop primer (LB-Biotin) | /5Biosg/ATCAATCTCGATATCCATGAAGGTG |
| | Strand displacement probe | GTCGATGACAGGTTGTTACAAAGGGAGAAGGGCATGGCGA/36-FAM/ |
| | Quencher (displaced) strand | /5IABkFQ/TCGCCATGCCCTTCTCCCTTTGTAACAAC/3InvdT/ |
| *V. cholerae* | F3 primer | TCGGGCAGATTCTAGACC |
| | B3 primer | GTGGGCACTTCTCAAACT |
| | Forward inner primer (FIP) | TTGAGTACCTCGGTCAAAGTACTTCCTGATGAAATAAAGCAGTCA |
| | Backward inner primer (BIP) | TCAACCTTTATGATCATGCAAGAGGGGAAACATATCCATCATCGTG |
| | Forward loop primer (LF-FAM) | /56-FAM/CCTCTTGGCATAAGACCACC |
| | Backward loop primer (LB-Biotin) | /5Biosg/AACTCAGACGGGATTTGTTAGG |
| | Strand displacement probe | /56-FAM/CTGCAGGTGGTCTTATGCCAAGAGGACAGAGTG/3InvdT/ |
| | Quencher (displaced) strand | TCTTGGCATAAGACCACCTGCAG/3IABkFQ/ |

Note that quencher probes were modified with Iowa Black Dark Quencher for real-time PCR detection (data not shown) of probes' strand displacement during amplification. The fluorescence observed when the probe is displaced from the quencher strand is proximity-dependent. In order to meet the required toehold strength for optimal strand displacement and to maintain a close proximity between the fluorophore and quencher when duplexed, the *E. coli* probe's fluorophore was placed on the 3' end and the *V. cholerae*'s probe was placed on the 5' end.

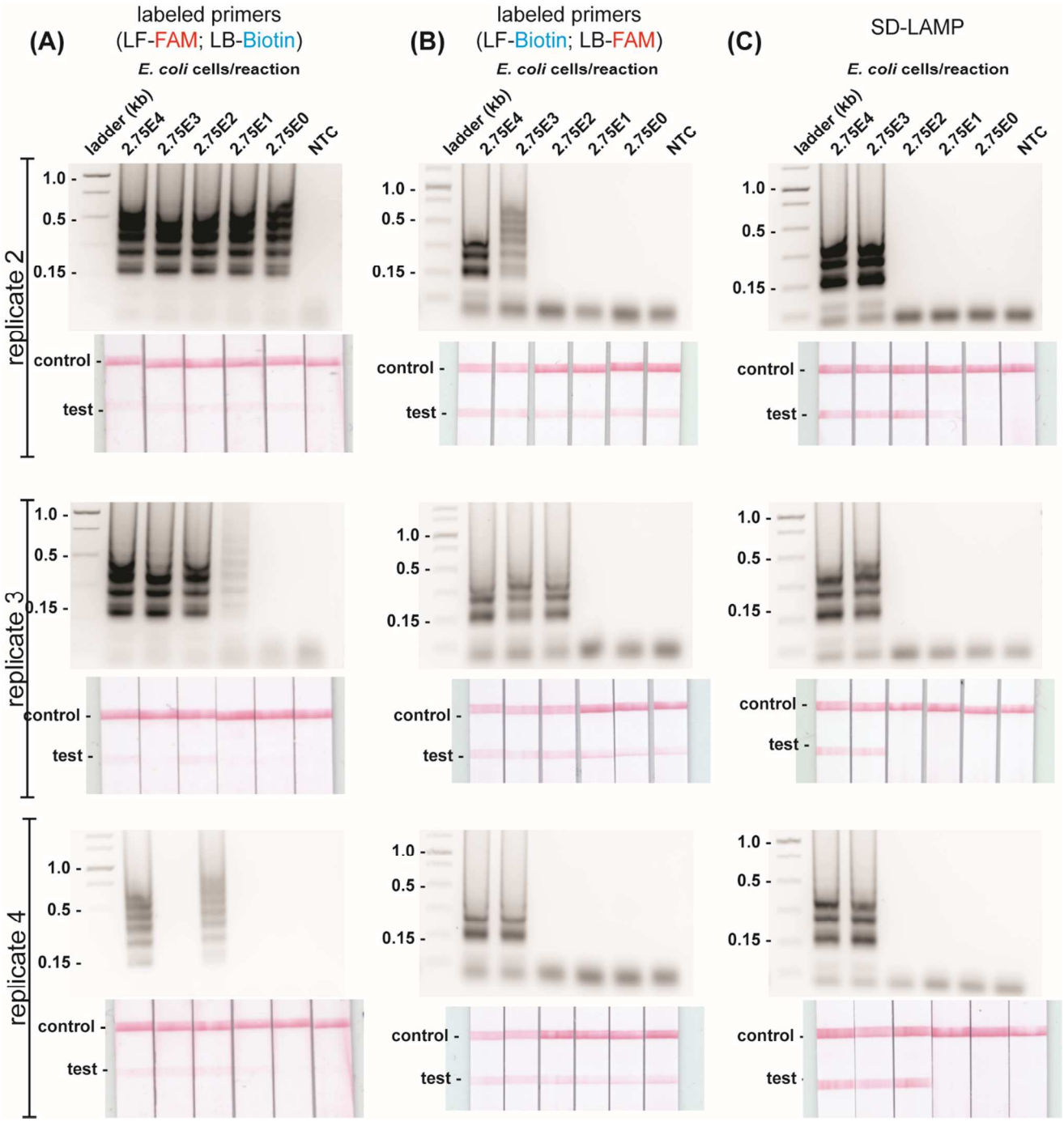


**Figure S1**. *E. coli* LAMP amplicons detectable on lateral flow immunoassays (replicates of Figure 2)

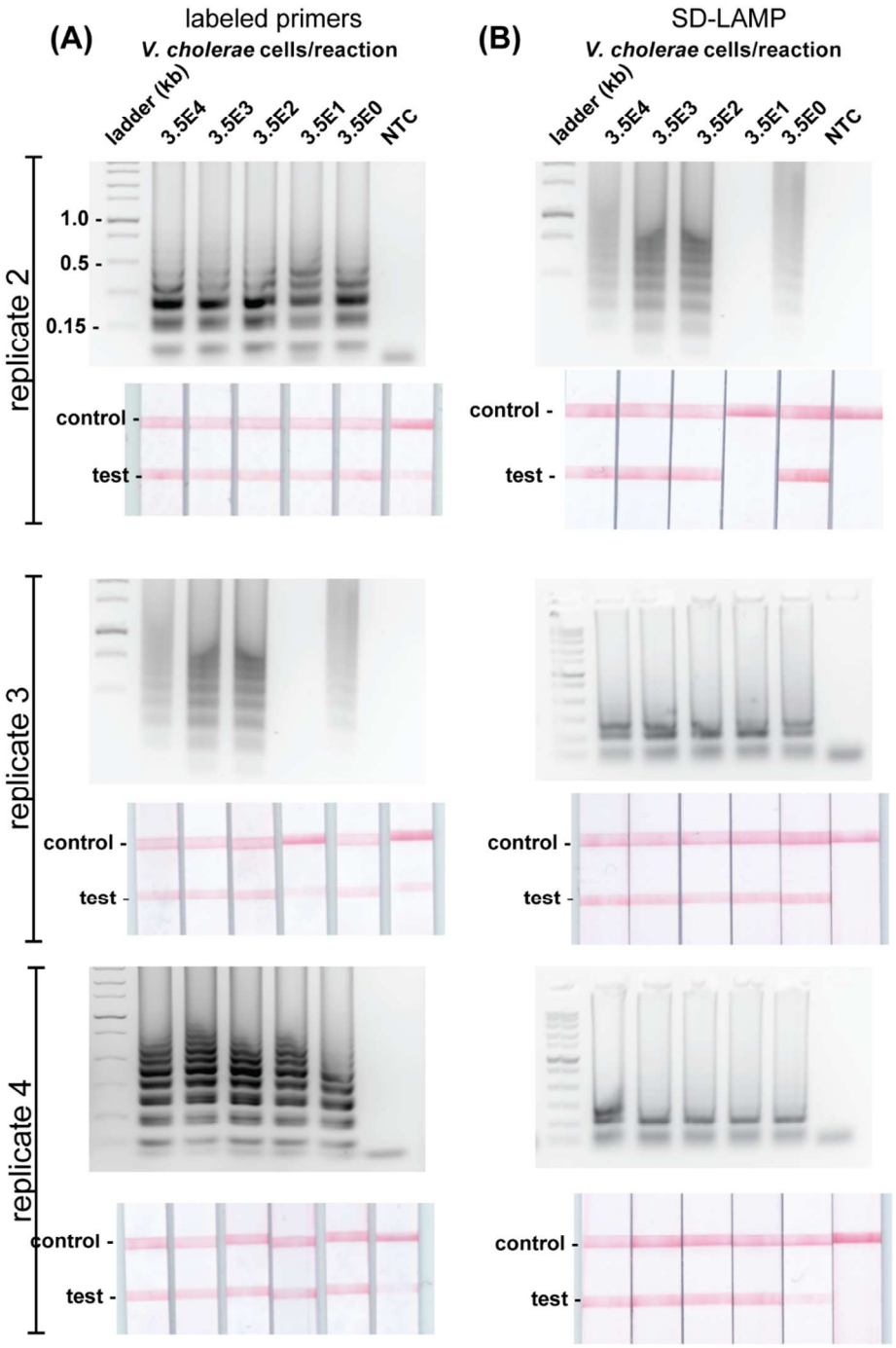


**Figure S2.** *V. cholerae* LAMP amplicons detectable on lateral flow immunoassays (replicates of Figure 4)

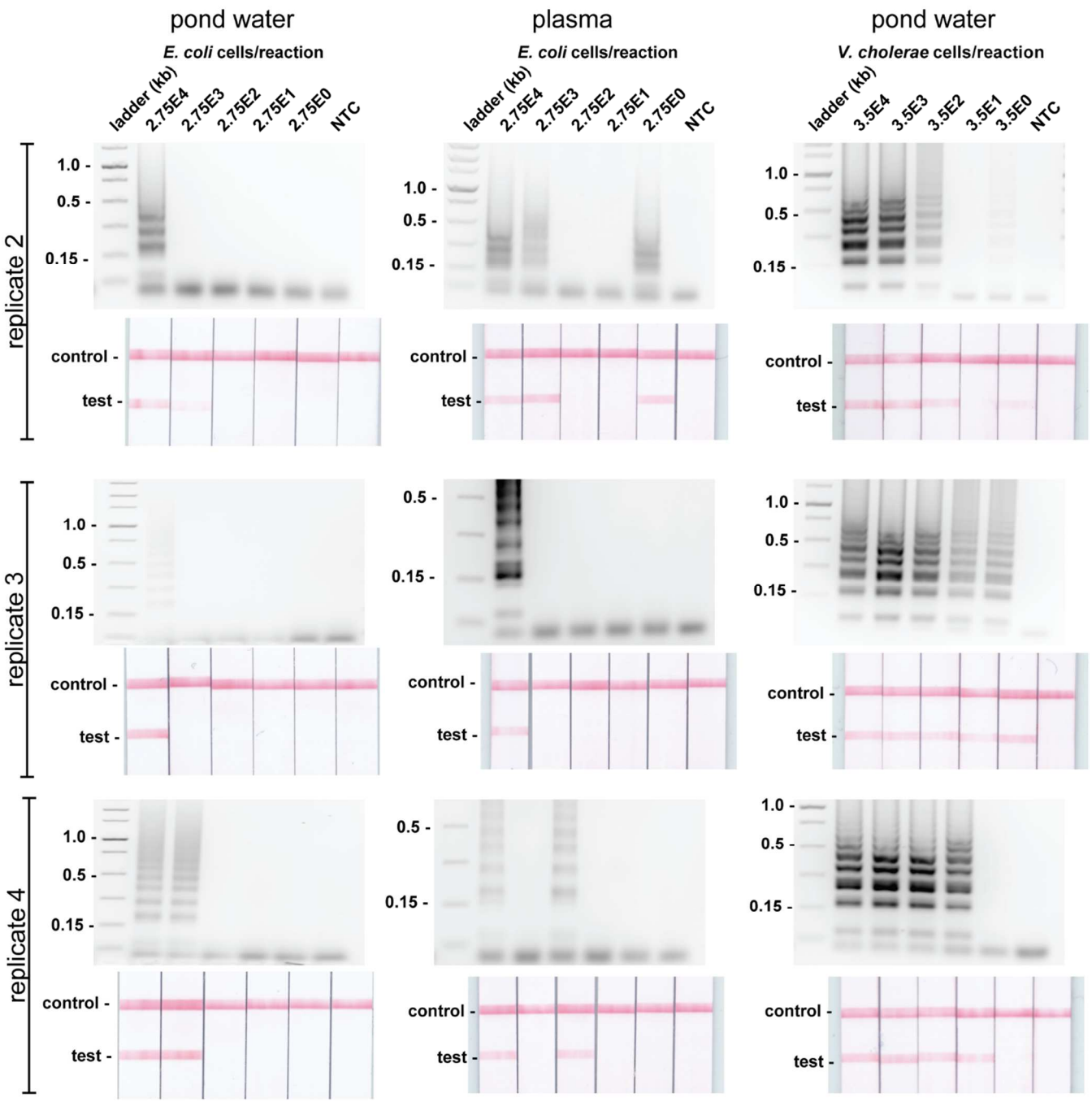


**Figure S3.** Probed strand displaced LAMP amplicons detectable from complex matrices (replicates of Figure 5)

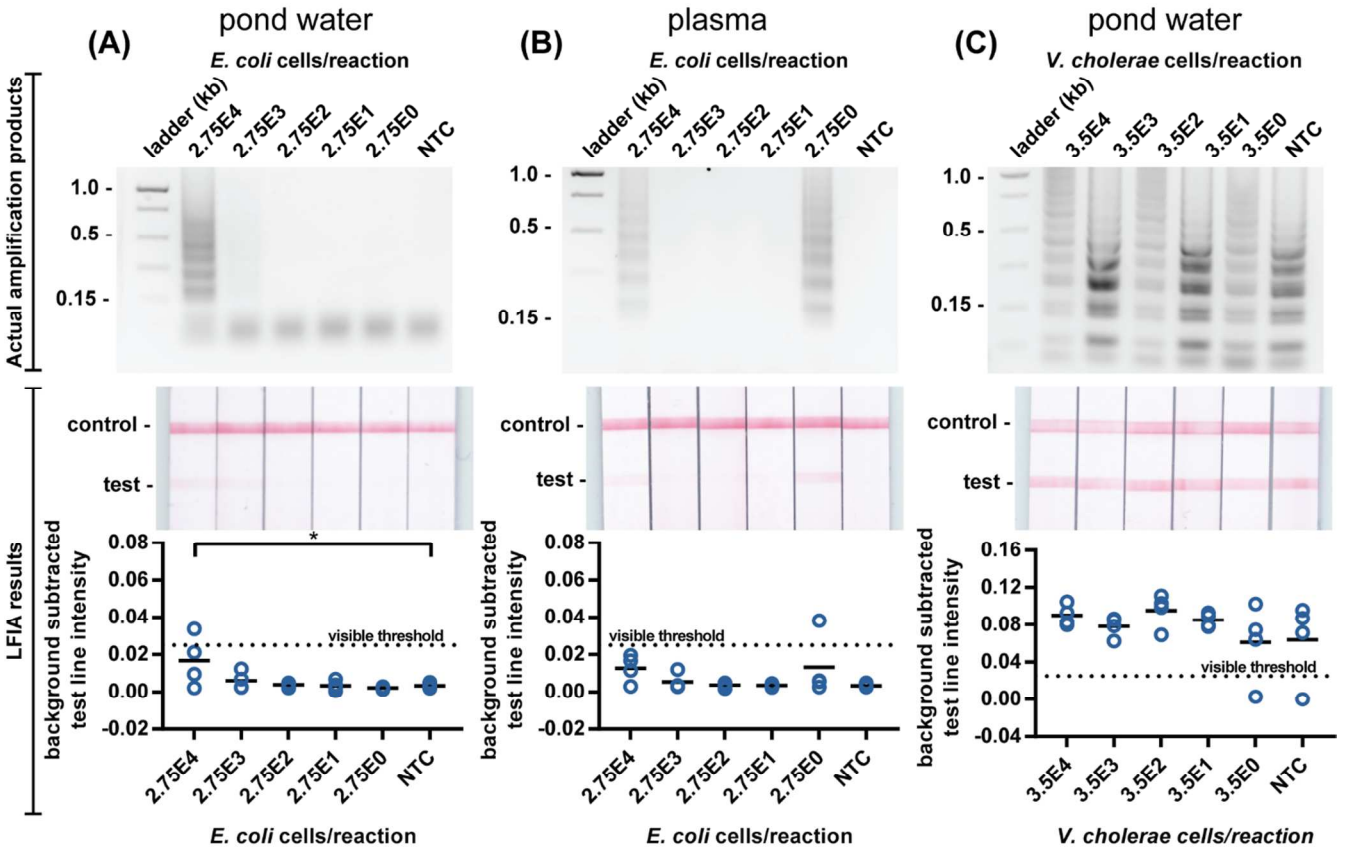


**Figure S4.** Detection of standard LAMP reactions in complex matrices. (top) Electrophoresis gels verifying amplification and indicating inhibited amplification in the presence of pond water and plasma. (middle) LFIA test results and (bottom) LFIA test line quantification. Standard LAMP reactions yield faint LFIA results for *E. coli* diluted in (A) pond water and (B) human plasma. Standard LAMP reactions yield false positive LFIA results for (C) *V. cholerae* diluted in pond water in no template control (NTC) reactions). n=4, replicates indicated by each circle. * indicates p-value ≤ 0.05.

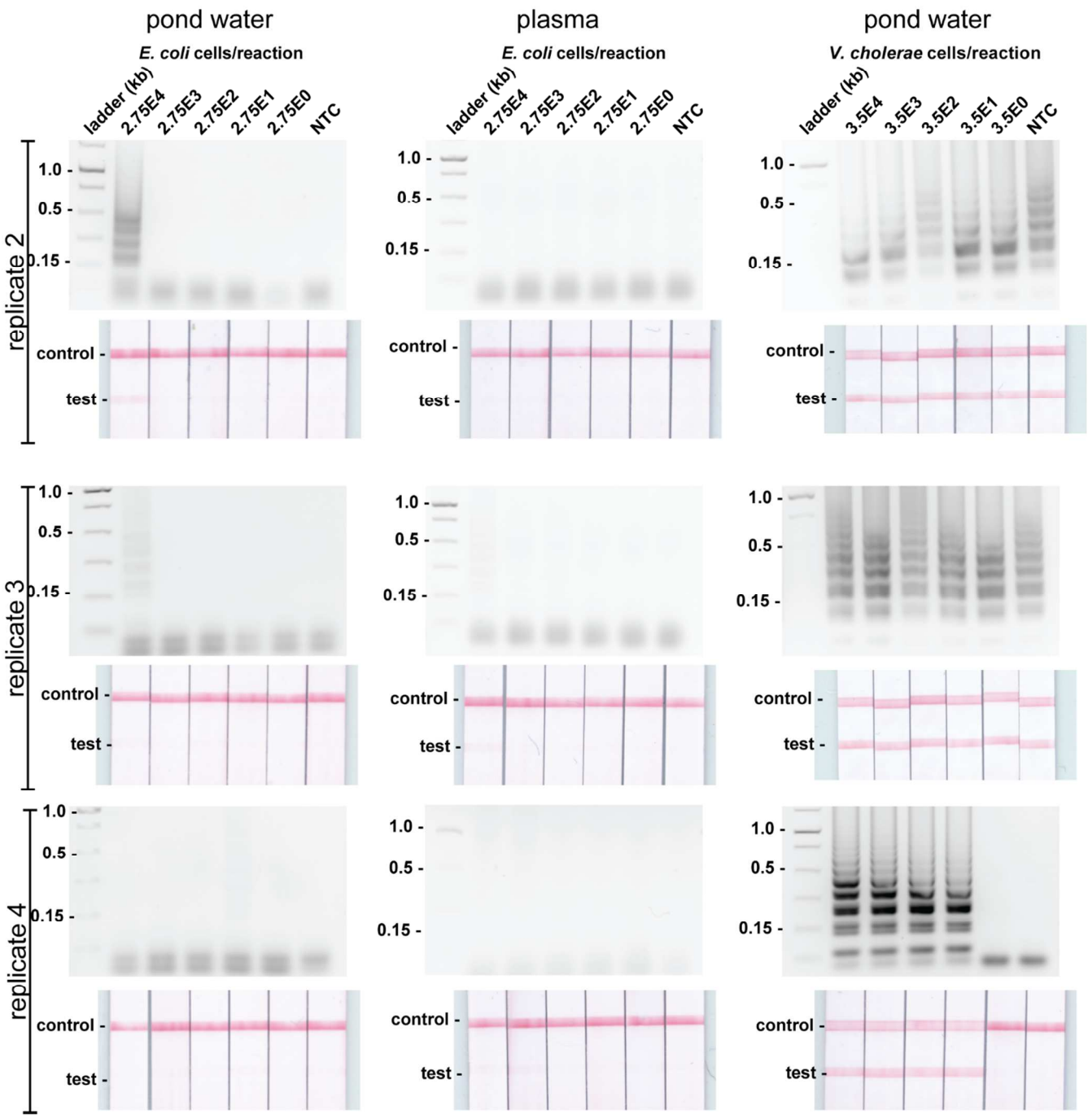


**Figure S5**. Standard LAMP amplicons detectable from complex matrices (replicates of Figure S4)

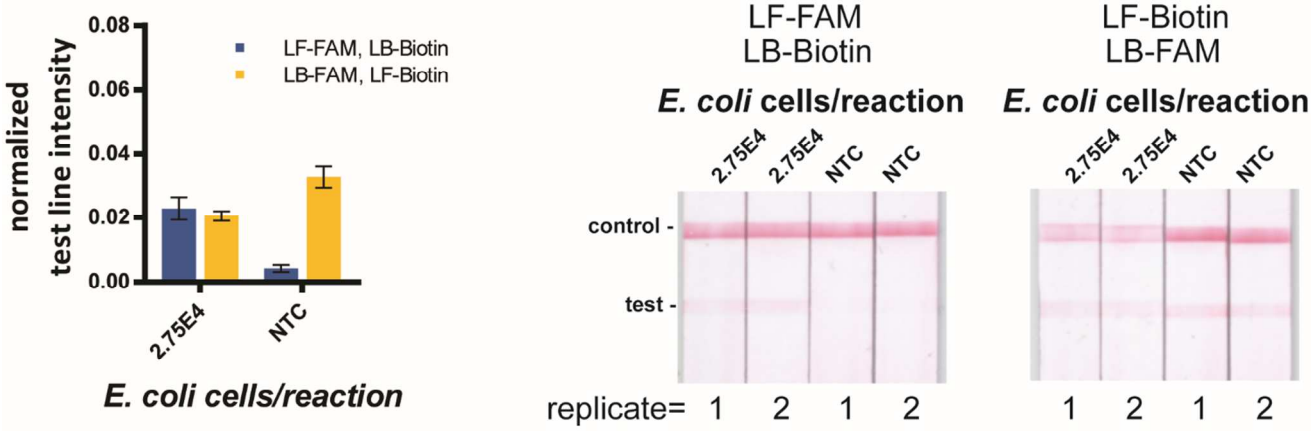


**Figure S6**. *E. coli* LAMP with opposite primer tag configurations.

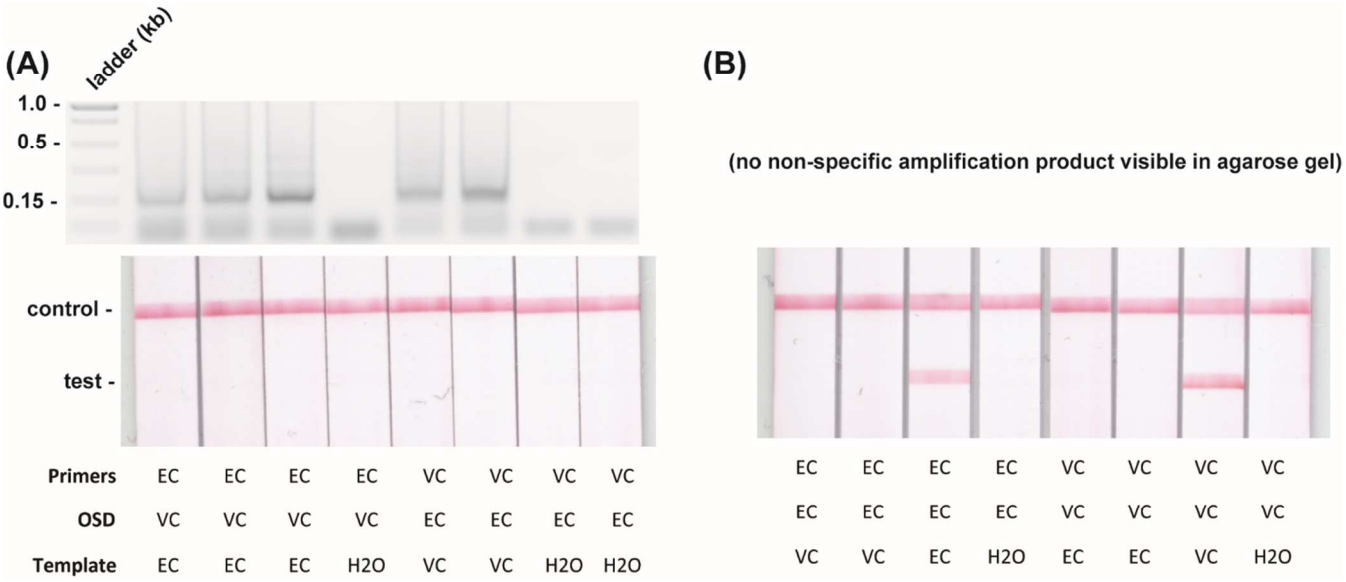


**Figure S7.** Strand displacement probes' target specificity.

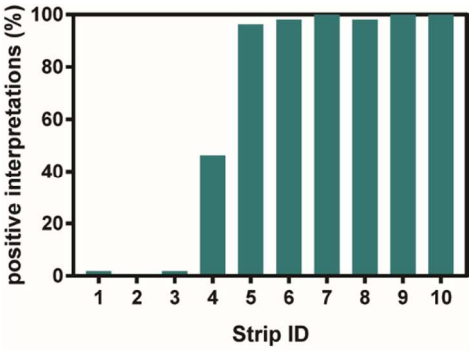


**Figure S8.** The percentage of times a test strip was interpreted as positive. Each strip was evaluated 54 times total (each strip was evaluated indoors, outdoors in the sun, and outdoors in the shade by all 18 participants). Only strips 7, 9, and 10 were interpreted positive all 54 times. Note that only participants with no previous instruction interpreted test strips 5, 6, and 8 as negative.

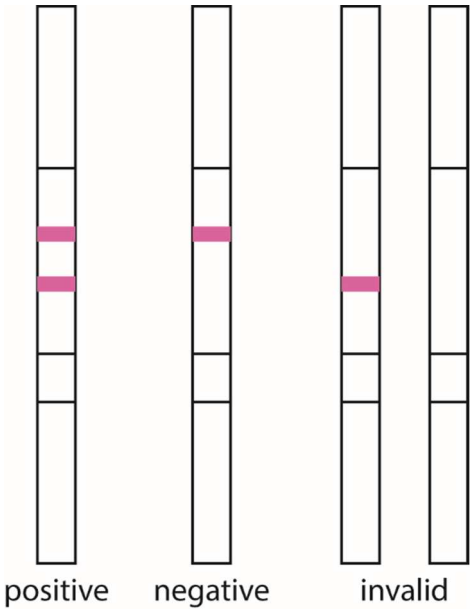


**Figure S9.** Schematic provided to test subjects to guide their interpretation of lateral flow tests.

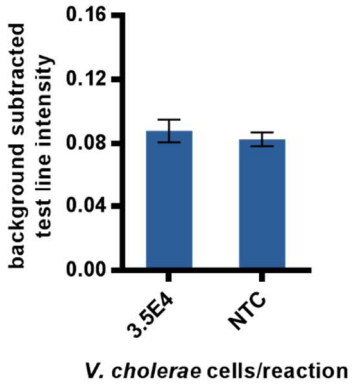


**Figure S10.** Standard LAMP reaction of *V. cholerae* with labeled primers containing 0.2 μM each F3 and B3 primers, 1.6 μM each FIP, BIP, LB-biotin primers and 0.05μM LF-FAM primers (the same concentration as FAM tagged strand displacement probe in SD-LAMP reactions of Figure 4 in the main text). When amplification products are loaded to the lateral flow strips, the test line intensity of negative control reactions remains indistinguishable from positive reactions containing template DNA (n=2). This confirms that the decrease in NTC signal observed in SD-LAMP reactions is due to the strand displacement mechanism and not because there is a lower concentration of FAM tagged oligonucleotide.