Supplementary Information

I-motif sensor-based fluorometric detection of Mpox using multiplex-LAMP

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Contents

1. **Table S1:** Oligonucleotides used in this study.
2. **Supportive data as:**

- **Figure S1:** The Bar diagram represents the confirmation of pH change over different conditions of the LAMP.
- **Figure S2:** Confirming i-motif formation by colorimetric change.
- **Figure S3:** Temperature-dependent multiplex-LAMP gradient study to show LAMP primers suitability
- **Figure S4:** Fluorescence measurement of multiplex-LAMP mediated i-motif-TO fluorometric assay selectivity study by using nine different bacterial genomes.
- **Figure S5:** Analysis of amplification plot based on selectivity experiment of Mpox detection by Real-time PCR by using nine different bacterial genomes.
- **Figure S6:** Fluorescence measurement of multiplex-LAMP mediated i-motif-TO fluorometric assay sensitivity study.
- **Figure S7:** Analysis of sensitivity experiment of Mpox detection by Real-time PCR through 20% non-denaturing PAGE
- **Figure S8:** Analysis of sensitivity experiment of multiplex-LAMP mediated i-motif-TO fluorometric detection system with human serum samples to imitate real sample conditions.
- **Figure S9:** Fluorescence measurement of multiplex-LAMP mediated i-motif-TO fluorometric assay sensitivity study with human serum samples to imitate real sample conditions.

Target gene	LAMP Primers
B6R (204 mers): Envelope protein gene cgttgtgaagaaaaaaatggaaatacttcttggaatgatactgttacgtgtcctaatgcggaatgtcaatctcttcaattagatcacggatcgtgtcaaccagttaaaggaaaatactcatttgggggaacatataactatcaactgtgatgttggatatgaggttattggtgcttcgtacataacttgtacagctaattcttgg	**FIP:** CGATCCGTGATCTAATTGAAGAGATCTTCTTGGAATGATACTGTTACG **BIP:** AAGGAAAATACTCATTTGGGGAACACGAAGCACCAATAACCTCAT **F3:** CGTTGTGAAGAAAAAAATGGAA **B3:** CCAAGAATTAGCTGTACAAGTT
N3R (231 mers): Complement binding protein gene gtaagtagtcaatacgaggaattagaatattattattcatgtgattatactaacaaccgtcctacaattaaacaacattacttttataacggcgaagaatatactgaaattgatagatcgaaaaaagccactaataaaaacagttggttaattacttcaggctttagactacaaaaatggttcgatagcgaagattgtataatttatctcagatctttagttagaagaatg	**FIP:** TCAATTTCAGTATATTCTTCGCCGTATTCATGTGATTATACTAACAACCG **BIP:** AACAGTTGGTTAATTACTTCAGGCTAATTATACAATCTTCGCTATCGA **F3:** GTAAGTAGTCAATACGAGGAATT **B3:** CATTCTTCTAACTAAAGATCTGAGA
E9L (205 mers): DNA polymerase gene aaacagtaatcgtttaccacttaaatcgatataacagtaacttgtatgcgagattgggttaataaatacagaaggaaacttcttatcgaagtgacactctatatctagaaataagtacgatcttgggatatcgaatctaggtatttctttagcgaaacagttacgtggatcgtcacaatgataacatccattgttaatctttgtc	**FIP:** ACTTCGATAAGAAGTTTCCTTCTGTATCGATATAACAGTAACTTGTATGC **BIP:** AAGTACGATCTTGGGATATCGAATCTGTGACGATCCACGTAAC **F3:** AAACAGTAATCGTTTACCACTT **B3:** GACAAAGATTAACAATGGATGTT
Target gene	**RT-qPCR Primers**
B6R (92 mers): Envelope protein gene cggatcgtgtcaaccagttaaaggaaaatactcatttggggaacatataactatcaactgtgatgttggatatgaggttattggtgcttcgt	**Forward:** CGGATCGTGTCAACCAGTTA **Reverse:** ACGAAGCACCAATAACCTCATA
N3R (116 mers): Complement binding protein gene actaacaaccgtcctacaattaaacaacattacttttataacggcgaagaatatactgaaattgatagatcgaaaaaagccactaataaaaacagttggttaattacttcaggctt	**Forward:** ACTAACAACCGTCCTACAATTAAAC **Reverse:** AAGCCTGAAGTAATTAACCAACTG
E9L (141 mers): DNA polymerase gene acttgtatgcgagattgggttaataaatacagaaggaaacttcttatcgaagtgacactctatatctagaaataagtacgatcttgggatatcgaatctaggtatttctttagcgaaacagttacggtggatcgtcacaatg	**Forward:** ACTTGTATGCGAGATTGGGTTAATA **Reverse:** CATTGTGACGATCCACGTAACT
I-motif sequences (red colored one "Rb" mainly used in our detection system)	
❑ **H-telo:** TAACCCTAACCCTAACCCTAACCC ❑ **Rb: CCGCCCAAAACCCCCC** ❑ **HIF-1α :** CCCGCCCCCTCTCCCA ❑ **c-MYC:** CCCCACCTTCCCCACCCTCCCCACCCTCCCC	

Table S1: Oligonucleotides used in this study.

1. **Supportive data:**

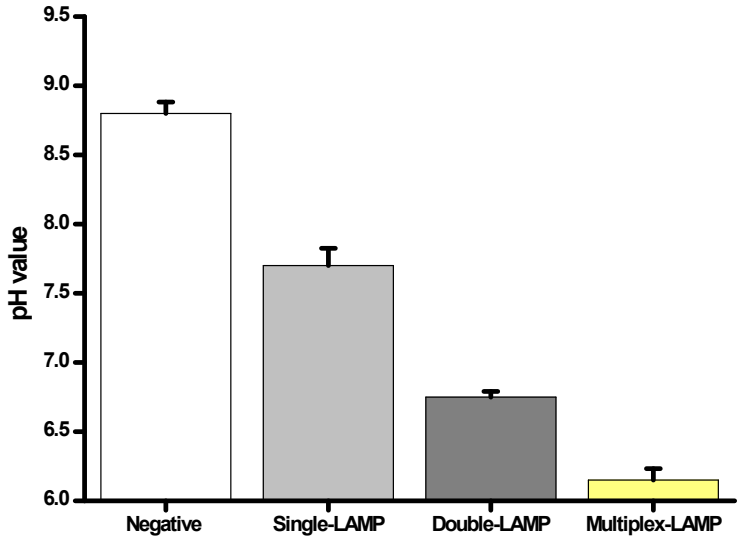

Figure S1: The Bar diagram represents the confirmation of pH change over different conditions of the LAMP (single, double, and multiplex) reaction. Data was measured from 5 replicates of each reaction; where error bars represent the standard deviations of five replicates.

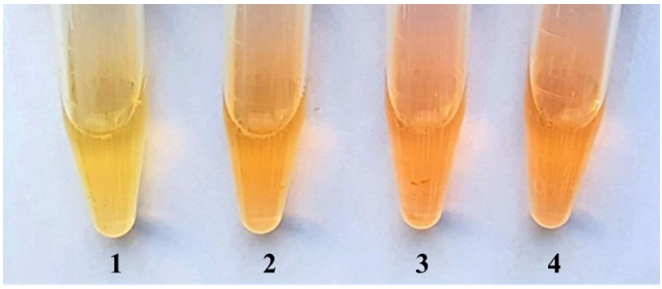

Figure S2: Confirming i-motif formation by the TO color change, whereas, after post-LAMP amplification, TO was added. Here, Lane 1: Multiplex LAMP without any target (negative control), Lane 2: Single-LAMP, Lane 3: Double-LAMP, Lane 4: Multiplex-LAMP (positive control). In Figure S2, we can clearly see the color difference between the negative and positive controls of multiplex-LAMP (lane 1 & lane 4). Additionally, from left to right (conversion from light yellow to dense yellow), while the color intensity difference is not significantly recognizable, still upon careful observation; even to the naked eye, the slight variations are more apparent.

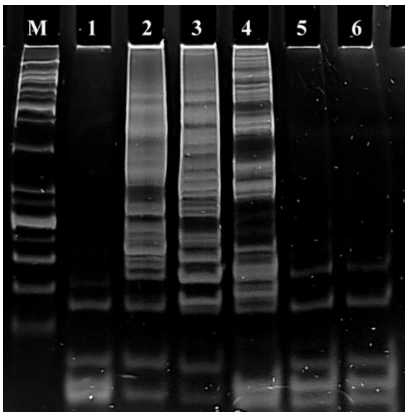

Figure S3: Temperature-dependent multiplex-LAMP gradient study to show LAMP primers suitability by 20% non-denaturing PAGE. Lane M: 25/100 bp DNA marker, Lane 1: at 65°C negative control, Lane 2: at 65°C positive control, Lane 3: at 60°C positive control, Lane 4: at 55°C positive control, Lane 5: at 60°C negative control, Lane 6: at 55°C negative control.

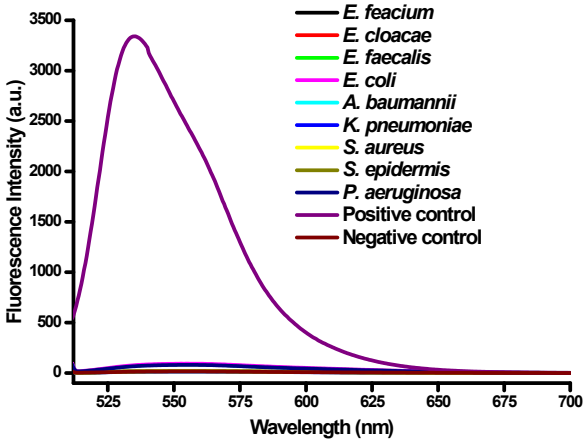

Figure S4: Fluorescence measurement of multiplex-LAMP mediated i-motif-TO fluorometric assay selectivity study by using nine different bacterial genomes**;** including negative and positive control of our system. All fluorescence emission peaks were measured at 535 nm (Thiazole Orange; TO) wavelength.

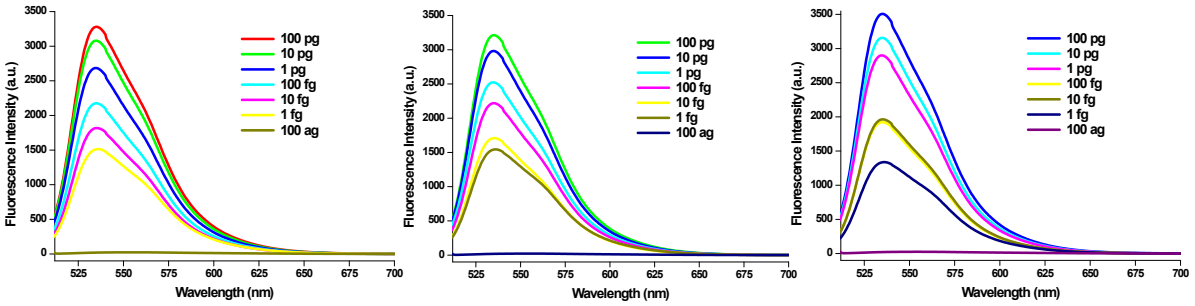

Figure S5: Fluorescence measurement of multiplex-LAMP mediated i-motif-TO fluorometric assay sensitivity study**.** A, B, and C represent the three-replication study of sensitivity experiments. All fluorescence emission peaks were measured at 535 nm (Thiazole Orange; TO) wavelength.

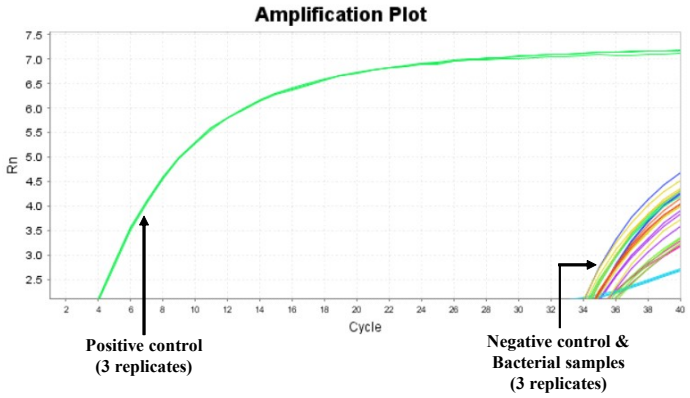

Figure S6: Analysis of amplification plot based on selectivity experiment of Mpox detection by Real-time PCR by using nine different bacterial genomes.

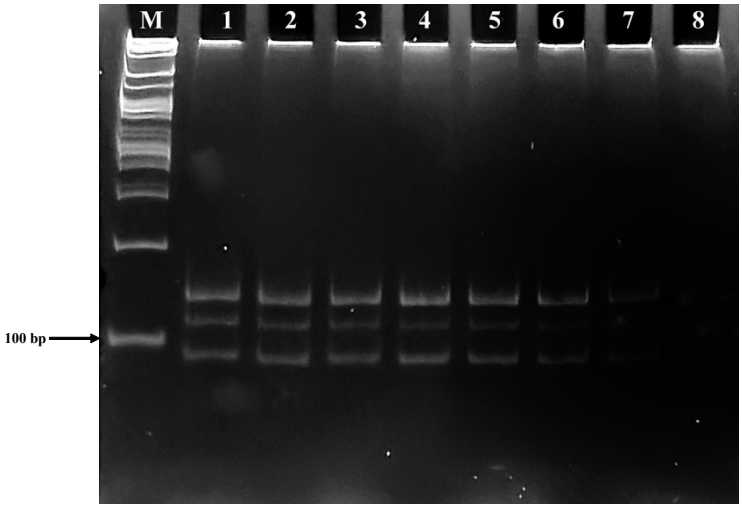

Figure S7: Analysis of sensitivity experiment of Mpox detection by Real-time PCR through 20% non-denaturing PAGE; where B6R gene (92 mers), N3R (116 mers), and E9L (141 mers). Here, Lane M: 100 bp DNA marker, Lane 1: 100 pg, Lane 2: 10 pg, Lane 3: 1 pg, Lane 4: 100 fg, Lane 5: 10 fg, Lane 6: 1 fg, Lane 7: 100 ag, Lane 8: negative control (without any target).

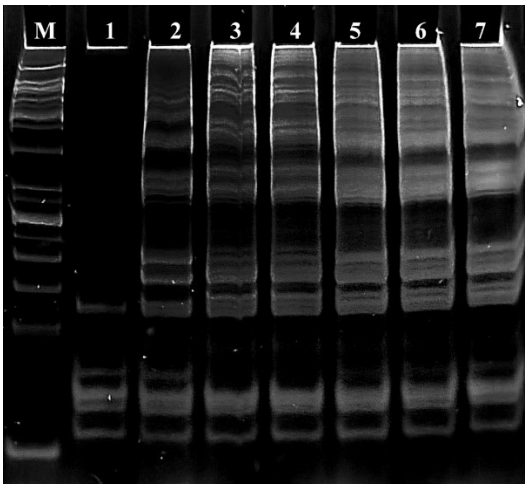

Figure S8: Analysis of sensitivity experiment of multiplex-LAMP mediated i-motif-TO fluorometric detection system with human serum samples to imitate real sample conditions by 20% non-denaturing PAGE. Here, Lane M: 25/100 bp DNA marker, Lane 1: 100 ag, Lane 2: 1 fg, Lane 3: 10 fg, Lane 4: 100 fg, Lane 5: 1 pg, Lane 6: 10 pg, Lane 7: 100 pg.

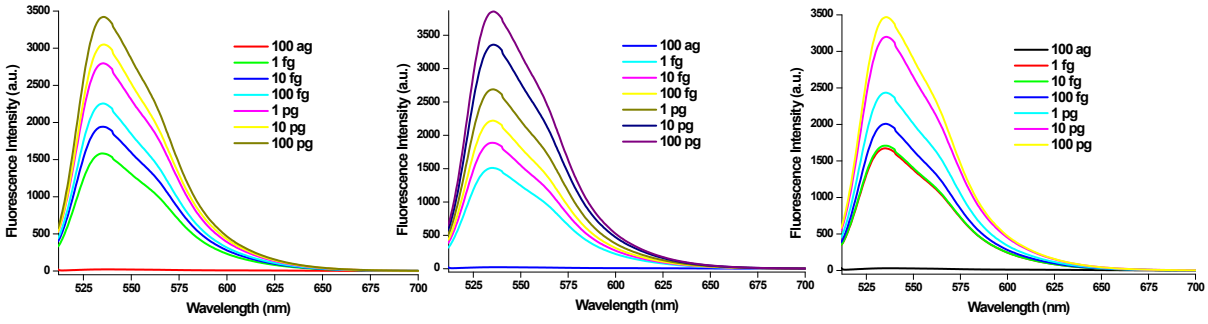

Figure S9: Fluorescence measurement of multiplex-LAMP mediated i-motif-TO fluorometric assay sensitivity study with human serum samples to imitate real sample conditions**.** A, B, and C represent the three-replication study of sensitivity experiments. All fluorescence emission peaks were measured at 535 nm (Thiazole Orange; TO) wavelength.