# Supplementary Material for

# Single-step Duplex CRISPR Coupled with Lateral Flow Assay for Point-of-care Detection of Human Immunodeficiency Virus and Treponema Pallidum

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**Supplementary Table 1. Information of the materials used in this study.**

| Reaction | Reagent | Manufacturer | Cat.no. | Stock Concentration |
|---|---|---|---|---|
| Single-step duplex CRISPR | LbCas12a | Tolo Biotech Co.,Ltd | 32108 | 10 μM |
| | LwaCas13a | Tolo Biotech Co.,Ltd | 32117 | 10 μM |
| | RPA lyophilized pellets | TwistDx™ | TABAS03KIT | / |
| | Rehydration buffer | TwistDx™ | TABAS03KIT | / |
| | Magnesium acetate | TwistDx™ | TABAS03KIT | 280 mM |
| | T7 RNA polymerase | New England Biolabs® Inc. | M0251 | 50 U/μL |
| | RNase Inhibitor | Solarbio® Life Science | R8061 | 40 U/μL |
| | rNTP mix | New England Biolabs® Inc. | N0466 | 25 mM |
| | SuperScript™ IV Reverse Transcriptase | Thermo Fisher Scientfic™ | 18090010 | 200 U/μL |
| | FAM-CCCCCC-BHQ1 | Sangon, China | Synthesis | 100 μM |
| | ROX-UUUUU-BHQ2 | Sangon, China | Synthesis | 100 μM |
| In vitro transcription | HiScribe ™ T7 High Yield RNA Synthesis Kit | New England Biolabs® Inc. | E2040 | / |
| | Standard Taq buffer | New England Biolabs® Inc. | B9014 | 10× |
| | RNA Clean & Concentrator-5 Kit | ZYMO RESEARCH | R1013 | / |
| | DNase I | New England Biolabs® Inc. | M0303 | 2 U/μL |
| | Qubit™ RNA Broad Range Assay Kits | Thermo Fisher Scientfic™ | Q10211 | / |
| LFA | HybriDetect 2T | Milenia Biotec | MGHD2 1 | / |
| | FAM-TTTTTTT-Biotin | Sangon, China | Synthesis | 100 μM |
| | FAM-UUUUUU | Sangon, China | Synthesis | 100 μM |

| | | | | |
|---|---|---|---|---|
| | U-Digoxin | | | |
| Nucleic acids extraction | TIANamp Virus RNA Kit | Tiangen Biotech | DP315-R | / |
| Sample Lysis | Lysis buffer | GenDx | NR215 | / |
| Lyophilization | Trehalose | Solarbio® Life Science | IT0870 | / |
| | Mannitol | Solarbio® Life Science | SM8120 | / |
| Sample | HIV-1 gag gene RNA standard | National Institute of Metrology, China | NIM-RM5212 | / |
| | TP tpp47 gene DNA standard | National Institute of Metrology, China | NIM-RM4602 | / |
| | HBV | Fubio, China | FNDV3704 | / |
| | HCV | Fubio, China | FNRV5393 | / |
| | HPV16 pseudovirus | Fubio, China | FNDV4994 | / |
| | HSV | GeneWell | GW-IBF1203 | / |

**Supplementary Table 2. Sequences of oligonucleotides used in this study.**

| Name | Oligo type | Sequences (5'-3') |
|---|---|---|
| TP-RPA-F | RPA primer | *cctctaatacgactcactatagggG*GTTCCTCATGAATTAAAAGGGATTGCAAA |
| TP-RPA-R | RPA primer | TGCTGCTTACCTTACGTGCAGAAAAACTAT |
| TP-crRNA | crRNA | CGGAGAATACCAACGGCCTTAAGACAAT*gtttta gtccccttcgtttttggggtagtctaaatcccctatagtgagtcgtattaatt tc* |
| HIV-RPA-F | RPA primer | TGCAHTCTATCCCATTCTGCAGCTTCCTCA |
| HIV-RPA-R | RPA primer | CCRCAAGAYTTAAACACCATGCTRAACACA |
| HIV-crRNA | crRNA | CAAATGTTAAAAGAGACCAT*atctacacttagtagaa attaccctatagtgagtcgtattaatttc* |
| T7 promoter oligo | Oligo for IVT | GAAATTAATACGACTCACTATAGGG |

**Supplementary Table 3. Information of clinical samples**.

| Sample No. | Pathogen | Specimen | HIV Ct | TP Ct |
|---|---|---|---|---|
| S1 | Negative | Whole blood | / | / |
| S2 | Negative | Whole blood | / | / |
| S3 | TP | Serum | / | 31.22 |
| S4 | TP | Serum | / | 31.57 |
| S5 | TP | Serum | / | 31.84 |
| S6 | Negative | Whole blood | / | / |
| S7 | Negative | Whole blood | / | / |
| S8 | Negative | Whole blood | / | / |
| S9 | Negative | Whole blood | / | / |
| S10 | HIV | Serum | 27.65 | / |
| S11 | Negative | Whole blood | / | / |
| S12 | Negative | Whole blood | / | / |
| S13 | HIV | Serum | 33.05 | / |
| S14 | Negative | Whole blood | / | / |
| S15 | Negative | Whole blood | / | / |
| S16 | HIV | Serum | 27.1 | / |
| S17 | TP | Whole blood | / | 30.38 |
| S18 | HIV | Serum | 27.3 | / |
| S19 | Negative | Whole blood | / | / |
| S20 | TP | Serum | / | 30.61 |
| S21 | Negative | Whole blood | / | / |
| S22 | Negative | Whole blood | / | / |
| S23 | TP | Serum | / | 34.09 |
| S24 | Negative | Whole blood | / | / |
| S25 | Negative | Whole blood | / | / |
| S26 | Negative | Whole blood | / | / |
| S27 | Negative | Whole blood | / | / |

| | | | | |
|---|---|---|---|---|
| S28 | HIV | Serum | 31.41 | / |
| S29 | Negative | Whole blood | / | / |
| S30 | Negative | Whole blood | / | / |
| S31 | HIV | Serum | 29.44 | / |
| S32 | Negative | Whole blood | / | / |
| S33 | TP | Serum | / | 31.1 |
| S34 | TP | Serum | / | 35.36 |
| S35 | Negative | Whole blood | / | / |
| S36 | Negative | Whole blood | / | / |
| S37 | TP | Serum | / | 36.18 |
| S38 | Negative | Whole blood | / | / |
| S39 | Negative | Whole blood | / | / |
| S40 | Negative | Whole blood | / | / |
| S41 | TP | Serum | / | 32.49 |
| S42 | Negative | Whole blood | / | / |
| S43 | Negative | Whole blood | / | / |
| S44 | Negative | Whole blood | / | / |
| S45 | Negative | Whole blood | / | / |
| S46 | Negative | Whole blood | / | / |

**Supplementary Table 4. Comparison of the existing multiplex CRISPR assays.**

| | Name | Assay | Detection unit | Target | Sensitivity | Time R:Reaction time W: Whole time | Lyophilization | Extraction-free | Reference |
|---|---|---|---|---|---|---|---|---|---|
| **Two-step assay** | / | Duplex RPA, Cas12a/Cas13a | Handheld device | SARS-CoV-2 orf1ab and S gene | 8 copies/μL of Orf1ab and N genes | 30 min (R) | × | × | [1] |
| | MPT-Cas 12a/13a | Duplex PCR, Cas12a/Cas13a | Plate reader | CaMV35S and T-nos | 11 copies of T-nos and 13 copies of CaMV35S | ~2 h (R) | × | × | [2] |
| | SHERLOCKv2 | Duplex RPA, LwaCas13a/PsmCas 13b/CcaCas13b/As Cas12a | Plate reader | DENV, ZIKV, DNA1, and RNA1 | 1 aM | ~1 h (R) | × | × | [3] |
| | / | Duplex RPA, Cas12a/Cas13a | Plate reader | Monkeypox virus and HIV/SARS-CoV-2 | 40 copies | 40 min (R) | × | × | [4] |
| | MRRDC | Duplex RPA, Cas12a/Cas13a | LFA | Enterovirus 71 and Coxsackievirus A16 | 10 copies/μL | 40 min (W) | × | √ | [5] |
| **Single-step assay** | SHINE.v2 | Duplex RPA-Cas12a/Cas13a | Plate reader | SARS-CoV-2 and RNase P | 1000 copies/μL | 1.5 h (W) | √ | √ | [6] |
| | CRISPRD | Multiplex LAMP-AapCas12b/TccCas 13a/HheCas13a | Plate reader | HPV16, HPV18 and internal control | 10 copies/μL | 1 h (R) | × | × | [7] |
| | OPTIMA-dx | RT-LAMP-TccCas1 3a/AapCas12b | Plate reader | SARS-CoV-2 and RNase P | 10 copies/μL | 1 h (R) | × | × | [8] |
| | DIAL | Duplex RPA-Cas12a/Cas13a | LFA | HIV and TP | 100 copies/μL | 45 min (W) | √ | √ | This work |

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