Supplementary data

Figure S1. Location of penA-LAMP3 primer in *Neisseria gonorrhoeae* wild-type penA from the WHO Reference strain F (J Antimicrob Chemother 2016; 71: 3096–3108. GenBank: LT591897.1). Each number indicates the position from the start codon of the WHO Reference strain F penA. □: Solid boxes show each selected primer. ------:

Dashed lines indicate loop primer.

```
gacgtctccgtgcgcataccatgttacccccttttgaatgtgtgcggcccatagtc
1019
agaaatcgttccacgctgcccagagccatctgtctggccgttgggcccgaagaaatgt
f1
atgacttctatcatgaattgctcgcgttcgtgcgtatgcactggctttcggccgaaa
b2
ctgcagtttggtag
1213
```
## Supplemental Table S1. LAMP primers used in this study

| Name           | Sequence (5’ → 3’)                                                                 | Final concentration (µM) | Reference                  |
|----------------|-----------------------------------------------------------------------------------|--------------------------|----------------------------|
| NG-penA-LAMP1  |                                                                                   |                          |                            |
| penA-F3        | TGACCAGTAACGGACGGATT                                                              | 0.2                      | Antimicrob Agents          |
| penA-R3        | CTCAACGCCAAAAACCGCA                                                              | 0.2                      | Chemother 2020; 64: e01663-19. |
| penA-FIP       | TACCGTCTGCAGATACAGCGCAATGGCGGTCTTGTGGGGCTCTT                                      | 1.2                      |                            |
| penA-BIP       | TGAAAGAAACGGCGACAACCCGGCGGTAGCGCGGCAGTG                                          | 1.2                      |                            |
| penA-LF        | CGGGCAATCACCCAGGCAA                                                               | 0.8                      |                            |
| penA-LB        | GATTGTGGGACTCAAGCAT                                                                | 0.8                      |                            |
| NG-penA-LAMP3  |                                                                                   |                          |                            |
| NonMosaic-F3   | GACCGTCTCCGTGCGC                                                                  | 0.27                     |                            |
| NonMosaic-R3   | TCAACAAACCTGCAGTTCC                                                               | 0.27                     | Current study              |
| NonMosaic-FIP  | TTGCTTGTGCGACGTGGAGTATGGCGGTGCGGCGATT                                            | 1.2                      |                            |
| NonMosaic-BIP  | GGCAACCGAAGAATGTATGACACCGACACCGATGCCAGATT                                        | 1.2                      |                            |
| NonMosaic-LF   | CATTTCCTGCATAATGCC                                                                | 0.8                      |                            |

Note: The FIP primer was composed of the F2 region at the 3' end and a sequence
complementary to the F1 region at the 5' end. The BIP primer was composed of the B2 region at the 3' end and a sequence complementary to the B1 region at the 5' end. An amplification refractory mutation system (ARMS) strategy (Nucleic Acids Research 1989; 17:2503–2516) was employed to suppress false-positive reactions owing to the presence of a mosaic penA type; some nucleotide sequences improved the primer (Table S1). Thus, an intentional mutation was introduced at the fourth or second nucleotide, which is underlined in bold, at the 5’ and/or 3’ terminus of the BIP (B1c+ B2) primers.

**Supplemental Table S2. Distribution of penA genes in culture-confirmed N. gonorrhoeae strains from NAAT-positive clinical specimens**

| penA NG-STAR                  | CFM MIC (mg/L) | >0.064 | ≤0.064 |
|-------------------------------|----------------|--------|--------|
| (n= no. of strains)           |                |        |        |
| 1.001 _NonMosaic (n=2)        |                | 0      | 2      |
| 2.001 _NonMosaic (n=17)       |                | 1      | 16     |
| 2.002 _NonMosaic (n=10)       |                | 0      | 10     |
| 5.002 _NonMosaic (n=8)        |                | 0      | 8      |
| 10.001 _Mosaic (n=38)         |                | 36     | 2      |
| 10.008 _Mosaic (n=1)          |                | 1      | 0      |
| 18.001 _NonMosaic (n=1)       |                | 0      | 1      |
| 34.001 _Mosaic (n=4)          |                | 2      | 2      |
| 34.006 _Mosaic (n=2)          |                | 1      | 1      |
|                           | n=4 | n=1 |
|---------------------------|-----|-----|
| 34.007 _ Mosaic           | 3   | 1   |
| 72.001 _ Mosaic           | 1   | 1   |
| 101.001 _ Mosaic          | 4   | 0   |
| 106.001 _ NonMosaic       | 0   | 1   |
| 215.001 _ Mosaic          | 0   | 1   |
| **Total strains**         | 49  | 46  |

*penA type
(n= no. of strains)*

|                           | n=39 | n=56 |
|---------------------------|------|------|
| NonMosaic (n=39)          | 1    | 38   |
| Mosaic (n=56)             | 48   | 8    |
| **Total strains**         | 49   | 46   |