Supplemental Table 3. *Escherichia coli* isolates (ST131 and non-ST131) used for blinded, independent validation of the novel 3-pool multiplex PCR assay.

| Code | Isolate | ST (phylogroup) | ST131 PCR profile number | Subclone<sup>d</sup> (fimH allele or O:H type) / clade<sup>d</sup> |
|------|---------|-----------------|--------------------------|--------------------------------------------------|
| Ec-01 | H17     | ST131 (B2)      | 4                        | H22 subclone (fimH27) / clade B0                  |
| Ec-02 | JJ1897  | ST131 (B2)      | 5                        | H22 subclone / clade B0                           |
| Ec-03 | JJ1969  | ST131 (B2)      | 6<sup>e</sup>            | H22 subclone / clade B1                           |
| Ec-04 | CD306   | ST131 (B2)      | 9                        | H30S subclone / clade C0<sup>e</sup>              |
| Ec-05 | JJ2193  | ST131 (B2)      | 11                       | H30R1 subclone / clade C1                          |
| Ec-06 | JJ1886  | ST131 (B2)      | 13                       | H30Rx subclone / clade C2                          |
| Ec-07 | U004    | ST131 (B2)      | 14                       | H30Rx subclone (fimH35) / clade C2                 |
| Ec-08 | U024    | ST131 (B2)      | 12                       | H30R1 subclone / clade C1-M27                      |
| Ec-09 | U4      | ST144 (B2)      | n.a.<sup>f</sup>         | n.a.<sup>f</sup>                                  |
| Ec-10 | BS488   | ST131 (B2)      | 2                        | H41 subclone (O25b:H4) / clade A                   |
| Ec-11 | M670745 | ST131 (B2)      | 3                        | H41 subclone (O25b:H5) / clade A                   |
| Ec-12 | MVAST20 | ST131 (B2)      | 1                        | H41 subclone (O16:H5) / clade A                    |
| Ec-13 | CD400   | ST131 (B2)      | 6<sup>e</sup>            | H22 subclone / clade B1                           |
| Ec-14 | 2H16    | ST10 (A)        | n.a.<sup>f</sup>         | n.a.<sup>f</sup>                                  |
| Ec-15 | V27     | ST73 (B2)       | n.a.<sup>f</sup>         | n.a.<sup>f</sup>                                  |
| Ec-16 | 2H25    | ST95 (B2)       | n.a.<sup>f</sup>         | n.a.<sup>f</sup>                                  |
| Ec-17 | USVAST306 | ST1193 (B2)   | n.a.<sup>f</sup>         | n.a.<sup>f</sup>                                  |
| Ec-18 | V31     | ST12 (B2)       | n.a.<sup>f</sup>         | n.a.<sup>f</sup>                                  |
| Ec-19 | V10     | ST69 (D)        | n.a.<sup>f</sup>         | n.a.<sup>f</sup>                                  |

<sup>a</sup>Ec numbers served as code labels during blinded validation testing in a separate laboratory. Actual strain identities were revealed to the separate laboratory after their PCR assay results had been recorded in the interactive Excel file and validated by the initial laboratory.

<sup>b</sup>The ST131 isolates represented 11 of 15 defined PCR assay profiles (Figure 1, Table 5).

<sup>c</sup>Unique ST131 PCR assay profiles not included in this isolate set: profiles 7 (clade B1, fimH30), 8 (clade B1, fimH-negative), 10 (clade C0, fluoroquinolone-resistant), and 15 (clade C2, O-nontypable, H30Rx).

<sup>d</sup>Subclone labels are per Price et al., clade labels per Petty et al. and Matsumura et al.

<sup>e</sup>ST131 profile 6 was represented by two isolates (JJ1969 and CD400).

<sup>f</sup>n.a., not applicable (non-ST131).