***Supplementary Table***

**Supplementary Table 1. Summary of conventional cultivation and commercial rapid detection methods.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Method | Rapidness (hours)\* | VBNC\* | Portability\* | Viability\* | Multiplex w/o customization\* |
| Most probable number | **24-48** | **No** | Yes | Yes | Yes |
| Viable counts (e.g., PetrifilmTM) | **24-72** | **No** | Yes | Yes | Yes |
| Lateral flow | <1 | **No** | Yes | **No** | **No** |
| DEFT/SPC | <1 | **No** | **No** | **No** | **No** |
| Immunoassay | 1-4 | **Enrichment required** | Yes | **Enrichment required** | **No** |
| Flow cytometry | <1 | Yes | **No** | Yes | **No** |
| MALDI-TOF | 1 | **No** | **No** | Yes | Yes |
| DNA/PCR | ~4 | Yes / **False positive** | Yes | **No** / **False positive** | **No** |
| RNA/RT-PCR | ~4 | Yes / **False positive** | Yes | Yes / **False positive** | **No** |
| Next generation sequencing (NGS) | 1-4 | Yes | **No** | Yes | Yes |
| **Nanopore sequencing** | **1-4** | **Yes** | **Yes** | **Yes** | **Yes** |

\* Bold indicates major limitations.

**Supplementary Table 2. Nanopore sequencing input amount of RNA and RT-PCR amplicon (cDNA).** CFU number was measured by plating count and nucleotide concentration was tested by Qubit assay. *Ec*, *Se* and *Lm* indicated *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes*, respectively. The bacteria culture condition was 37°C incubation.

|  |  |  |  |
| --- | --- | --- | --- |
| Sequencing | Nanopore sequencing - Direct metatranscriptome RNA-seq | RT-PCR amplicon | Next generation sequencing - Direct metatranscriptome RNA-seq |
| Sample | BHI 336 24h  | LJE 336 24h  | BHI 334 4h  | LJE 334 4h  | BHI 336 24h  | LJE 336 24h  |
| Initial culture conc. (Log CFU/mL) | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* |
| 2.9 | 3.1 | 4 | 3.5 | 3.5 | 6 | 3.6 | 3.5 | 5 | 3.6 | 3.3 | 6 | 2.3 | - | 5 | 2.9 | 3.3 | 5 |
| 24h/4h culture conc. (Log CFU/mL) | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* | *Ec* | *Se* | *Lm* |
| 8.7 | 9.2 | 7 | 8.6 | 8.0 | 5 | 5.3 | 5.0 | 6 | 4.5 | 3.6 | 5 | 9.4 | 7.7 | 9 | 5.2 | 5.2 | 6 |
| Sample size (mL) | 5.3 | 5.0 | 0.2 | 0.2 | 0.04 | 0.24 |
| RNA input for multiplex RT-PCR (ng) | - | - | 72.0 | 36.5 | - | - |
| Yield of cDNA from multiplex RT-PCR (ng) | - | - | 1267.5 | 705.2 | - | - |
| RNA/DNA input for library prep (ng) | 3490.2 | 1338.0 | 33.8 | 165.0 | 100.0 | 100.0 |
| Yield after Poly A tailing and purification (ng) | 1824.0 | 617.5 | - | - | - | - |
| Yield after library prep and sequencing input (ng) | 744.0 | 129.2 | <30.0Too low to detect | 125.0 | <1 (1 nM) | <1 (1 nM) |

**Supplementary Table 3. Primer information for qPCR, RT-qPCR and multiplex RT-PCR.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species**  | **Target Gene\*** | **Primer (5'-3')** | **Product Size (bp)** | **Reference** |
| *Ec* | *stx* | F: GAGCGAAATAATTTATATGTG  | 520 | (Zhang et al., 2000; Toma et al., 2003; Nguyen et al., 2016) |
| R: TGATGATGGCAATTCAGTAT |
| *stx1A* | F: TGACAGGATTTGTTAACAGGAC | 294 | (Zhang et al., 2000; Toma et al., 2003; Nguyen et al., 2016) |
| R: TCTGTATTTGCCGAAAACGT |
| *Se* | *invA* | F: ACAGTGCTCGTTTACGACCTGAAT  | 244 | (van der Velden et al., 2000; Nguyen et al., 2016) |
| R: AGACGACTGGTACTGATCGATAAT |
| *Lm* | *inlA* | F: GATTAACACGAGTAACGG R: TAGATCTGTTTGCGAGAC  | 153 | (Vázquez-Boland et al., 2001; Xiao et al., 2012; Nguyen et al., 2016) |

\* *stx1A* was used in qPCR and RT-qPCR of *E. coli* O157:H7. *stx*, *invA* and *inlA* were used in multiplex RT-PCR of 4-hour BHI and 4-hour LJE cocktail cultures.

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