**Supplementary Materials**

**Supplementary Table ST1**

Primers, probes and cycling parameters used in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Assays | Oligonucleotides sequences (5' - 3') | Primers and probes (nM) | Cycling parameters | References |
| Sketa22 | F: GGT TTC CGC AGC TGG G  R: CCG AGC CGT CCT GGT CTA  P: FAM-AGT CGC AGG CGG CCA CCG T-TAMRA | 500  500  400 | 95 °C for 10 min and 45 cycles of 95 °C for 15 s, 63 °C for 45 s | Haugland et al., 2005 |
| MHV | F: GGA ACT TCT CGT TGG GCA TTA TAC T  R: ACC ACA AGA TTA TCA TTT TCA CAA CAT A  P: FAM-ACA TGC TAC GGC TCG TGT AAC CGA ACT GT-BHQ | 300  300  300 | 50 °C for 10 min, 95 °C for 5 min, and 40 cycles of 95 °C for 15 s, 60 °C for 1 min | Besselsen et al., 2002 |
| CrAssphage | F: CAG AAG TAC AAA CTC CTA AAA AAC GTA GAG  R: AT GAC CAA TAA ACA AGC CAT TAG C  P: FAM-AAT AAC GAT TTA CGT GAT GTA AC-TAMRA | 1000  1000  100 | 95°C for 10 min and 45 cycles of 95°C for 15 s, 60°C for 60 s. | Stachler et al., 2017 |
| HPyV | F: AGT CTT TAG GGT CTT CTA CCT TT  R: GGT GCC AAC CTA TGG AAC AG  P: FAM-TCA TCA CTG GCA AAC AT-MGBNFQ | 240  240  160 | 10 min at 95°C, 45 cycles of 30 s at 95°C, 20 s at 55°C and 60 s at 60°C | McQuaig et al., 2009 |
| EV | F: CCC TGA ATG CGG CTA AT  R: TGT CAC CAT AAG CAG CCA  P: ACG GAC ACC CAA AGT AGT CGG TTC | 300  900  100 | 50°C for 10 min, 95°C for 10 min and 45 cycles of 95°C for 15 s, 60°C for 1 min. | Cashdollar et al., 2013 |
| HAdV | F: GCC ACG GTG GGG TTT CTA AAC TT  R: GCC CCA GTG GTC TTA CAT GCA CAT C  P: FAM-TGC ACC AGA CCC GGG CTC AGG TAC TCC GA-TAMRA | 200  200  200 | 10 min at 95°C, 45 cycles of 15 s at 95°C, 60 s at 60°C | Heim et al., 2003 |
| HAV | F: TC AC CGC CGT TTG CCT AG  R: GGA GAG CCC TGG AAG AAA G  P: FAM-TTA ATT CCT GCA GGT TCA GG-BHQ1 | 900  500  250 | 50°C for 10 min, 95°C for 10 min and 45 cycles of 95°C for 15 s, 60°C for 1 min, 70°C for 1 min | Costafreda et al., 2006 |
| HNoV GII | F: ATG TTC AGR TGG ATG AGR TTC TCW GA  R: TCG ACG CCA TCT TCA TTC ACA  P: FAM-AGC ACG TGG GAG GGC GAT CG-ZEN | 250  250  100 | 50°C for 10 min, 95°C for 10 min and 45 cycles of 95°C for 15 s, 60°C for 1 min | Jothikumar et al., 2005 |
| BoV | Not known | Not known | 95°C for 10 min, 45 cycles of 95°C for 15 s, 60°C for 60 s. | Thermofisher Scientific |
| EBV, IAV, IBV, PeV, RhV A, RhV B, RSV A, RSV B | Not known | Not known | 50°C for 10 min, 95°C for 10 min and 45 cycles of 95°C for 15 s, 60°C for 1 min. | Thermofisher Scientific |

MHV: Murine hepatitis virus; CrAssphage: Cross-assembly phage; HPyV: human polyomavirus; EV: Enterovirus; HAdV: Human adenovirus; HAV: Hepatitis A virus; HNoV GII: Human norovirus GII, BoV: Bocavirus; EBV: Epstein-Barr Virus; IAV: Influenza A virus; IBV: Influenza B virus; PeV: Parechovirus; RhV A: Rhinovirus A; RhV B: Rhinovirus B; RSV A: Respiratory syncytial virus A; RSV B: Respiratory syncytial virus B.

**Supplementary Table ST2**

qPCR and RT-qPCR performance characteristics and assay limit of detection (ALOD).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Assays | qPCR/RT-qPCR performance characteristics | | | | qPCR/RT-qPCR GC/reaction | References |
|  | Efficiency (%) | Correlation coefficient (r2) | Slope | Y-intercept |  |  |
| CrAssphage | 100.4 | 0.99 | -3.31 | -40.23 | 6.02 | Ahmed et al., 2023c |
| HPyV | 102.6 | 0.98 | -3.26 | -39.40 | 8.23 | Ahmed et al., 2023c |
| EV | 99.9 | 0.98 | -3.32 | -38.65 | 9.30 | Ahmed et al., 2023c |
| HAdV | 99.6 | 0.99 | -3.33 | -38.40 | 9.00 | Ahmed et al., 2023c |
| HAV | 94.6 | 0.99 | -3.31 | 38.23 | 12.2 | This study |
| HNoV GII | 99.3 | 0.97 | -3.34 | 35.40 | 18.3 | Ahmed et al., 2023d |
| BoV | 97 | 0.99 | -3.41 | 38.38 | 7.30 | Ahmed et al., 2023b |
| EBV | 105 | 1.00 | -3.22 | 37.54 | 5.60 | Ahmed et al., 2023b |
| IAV | 95 | 1.00 | -3.45 | 38.68 | 8.40 | Ahmed et al., 2023b |
| IBV | 97 | 0.97 | -3.39 | 39.21 | 7.32 | Ahmed et al., 2023b |
| PeV | 99 | 0.99 | -3.33 | 38.37 | 8.10 | Ahmed et al., 2023b |
| RhV A | 101 | 1.00 | -3.10 | 36.13 | 9.30 | Ahmed et al., 2023b |
| RhV B | 110 | 1.00 | -3.10 | 35.75 | 7.41 | Ahmed et al., 2023b |
| RSV A | 103 | 0.99 | -3.26 | 37.16 | 7.32 | Ahmed et al., 2023b |
| RSV B | 91 | 0.98 | -3.56 | 40.68 | 6.80 | Ahmed et al., 2023b |

CrAssphage: Cross-assembly phage; HPyV: human polyomavirus; EV: Enterovirus; HAdV: Human adenovirus; HAV: Hepatitis A virus; HNoV GII: Human norovirus GII, BoV: Bocavirus; EBV: Epstein-Barr Virus; IAV: Influenza A virus; IBV: Influenza B virus; PeV: Parechovirus; RhV A: Rhinovirus A; RhV B: Rhinovirus B; RSV A: Respiratory syncytial virus A; RSV B: Respiratory syncytial virus B.

**Supplementary Table ST3**

Concentrations (log10 GC/12.5 mL) of indicator, enteric and respiratory viruses in 24 aircraft wastewater samples determined using the AE workflow.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Aircraft wastewater samples | Log10 GC/12.5 mL | | | | | | | | | |
|  | CrAssphage | HPyV | EV | HNoV GII | HAdV | BoV | PeV | RhV A | RhV B | RSV B |
| AWS 1 | 7.23 | 4.93 | - | - | 2.10 | - | - | 3.61 | 4.06 | 3.53 |
| AWS 1 | 5.37 | 5.54 | - | - | - | - | - | 4.35 | 3.93 | 3.46 |
| AWS 3 | 7.37 | 6.00 | - | - | - | - | - | 2.71 | 2.84 | - |
| AWS 4 | 6.77 | 4.41 | - | 2.89 | - | - | - | 2.87 | 2.90 | - |
| AWS 5 | 7.61 | 5.49 | - | - | 2.89 | - | - | 2.86 | 3.01 | - |
| AWS 6 | 7.08 | 4.95 | - | - | - | - | - | 3.82 | 3.01 | - |
| AWS 7 | 7.55 | 5.51 | - | - | - | - | - | 2.71 | 2.88 | - |
| AWS 8 | 2.98 | 4.66 | - | - | - | - | - | 2.66 | 2.79 | - |
| AWS 9 | 6.10 | 4.99 | - | - | - | 3.53 | - | 3.33 | 3.31 | - |
| AWS 10 | 7.24 | 5.56 | - | - | - | - | - | 2.89 | 3.10 | - |
| AWS 11 | 7.09 | 5.78 | - | - | - | - | - | 2.90 | 2.99 | - |
| AWS 12 | 7.50 | 6.04 | - | - | - | - | - | 3.18 | 3.23 | - |
| AWS 13 | 6.91 | 5.09 | - | - | - | - | - | 3.38 | 3.45 | - |
| AWS 14 | 7.40 | 5.53 | - | - | - | - | - | 3.27 | 3.72 | - |
| AWS 15 | 7.62 | 5.51 | - | - | - | - | - | 2.81 | 2.84 | - |
| AWS 16 | 6.71 | 5.39 | - | - | - | - | - | 2.71 | 2.97 | - |
| AWS 17 | 7.83 | 6.41 | - | - | - | - | - | 2.66 | 3.23 | - |
| AWS 18 | 7.70 | 5.06 | - | - | - | - | - | 3.32 | 3.79 | - |
| AWS 19 | 6.77 | 6.55 | 3.49 | - | 3.47 | - | - | 2.16 | 2.89 | - |
| AWS 20 | 7.55 | 4.94 | - | - | - | - | - | 2.94 | 2.84 | - |
| AWS 21 | 7.33 | 5.67 | - | - | - | 2.95 | - | 2.97 | 2.80 | - |
| AWS 22 | 3.26 | 5.02 | - | - | 3.34 | - | 2.48 | 3.38 | 3.41 | - |
| AWS 23 | 7.18 | 5.94 | 3.76 | - | 4.02 | - | - | 3.49 | 3.61 | - |
| AWS 24 | 6.13 | 6.13 | - | - | - | - | - | 3.68 | 3.88 | - |

CrAssphage: Cross-assembly phage; HPyV: human polyomavirus; EV: Enterovirus; HNoV GII: Human norovirus GII; HAdV: Human adenovirus; BoV: Bocavirus; PeV: Parechovirus; RhV A: Rhinovirus A; RhV B: Rhinovirus B; RSV B: Respiratory syncytial virus B; -: samples were either negative or positive but did not quantify.

**Supplementary Table ST4**

Concentrations (log10 GC/12.5 mL) of indicator, enteric and respiratory viruses in 24 aircraft wastewater samples determined using the NMAP workflow.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Aircraft wastewater samples | Log10 GC/12.5 mL | | | | | | | | | |
|  | CrAssphage | HPyV | EV | HNoV GII | HAdV | BoV | PeV | RhV A | RhV B | RSV B |
| AWS 1 | 5.21 | 3.25 | - | - | - | - | - | 3.15 | 2.99 | - |
| AWS 2 | 3.42 | 3.02 | - | - | - | - | - | 3.00 | 3.01 | - |
| AWS 3 | 5.84 | 4.79 | - | - | - | - | - | 2.80 | 3.58 | - |
| AWS 4 | 5.47 | 3.37 | 2.85 | 2.54 | - | - | - | 3.97 | 3.82 | - |
| AWS 5 | 5.51 | 3.71 | - | - | - | - | - | 3.93 | 4.05 | - |
| AWS 6 | 5.87 | 4.89 | - | - | - | - | - | 4.44 | 4.20 | - |
| AWS 7 | 5.87 | 3.80 | - | - | - | - | - | 3.12 | 3.69 | - |
| AWS 8 | 3.41 | 4.83 | - | - | - | - | - | 2.68 | 3.95 | - |
| AWS 9 | 5.15 | 4.25 | - | - | - | - | - | 2.64 | 4.12 | - |
| AWS 10 | 5.41 | 4.40 | - | - | - | - | - | 3.83 | 3.74 | - |
| AWS 11 | 5.16 | 4.16 | - | - | - | - | - | 3.80 | 3.76 | - |
| AWS 12 | 5.46 | 4.22 | - | - | - | - | - | 3.04 | 3.81 | - |
| AWS 13 | 5.92 | 5.10 | - | - | - | - | - | 3.95 | 3.76 | - |
| AWS 14 | 5.50 | 4.31 | - | - | - | - | - | 3.87 | 3.80 | - |
| AWS 15 | 5.89 | 4.34 | - | - | - | - | - | 3.85 | 5.13 | - |
| AWS 16 | 6.17 | 5.74 | - | - | - | - | - | 4.02 | 3.91 | - |
| AWS 17 | 5.55 | 4.56 | 2.40 | - | - | - | - | 4.00 | 4.06 | - |
| AWS 18 | 5.71 | 3.72 | 2.44 | - | - | - | - | 3.96 | 4.13 | - |
| AWS 19 | 4.50 | 4.59 | 2.99 | - | - | - | - | 3.29 | 3.21 | - |
| AWS 20 | 5.10 | 3.36 | - | - | - | - | - | 3.10 | 3.07 | - |
| AWS 21 | 5.75 | 4.24 | - | - | - | - | - | 3.27 | 3.18 | - |
| AWS 22 | 3.74 | 3.40 | 2.20 | - | - | - | - | 3.36 | 3.27 | - |
| AWS 23 | 5.06 | 4.14 | 2.23 | - | - | - | - | 3.51 | 3.48 | - |
| AWS 24 | 3.47 | 4.62 | 2.29 | - | - | - | - | 3.48 | 3.91 | - |

CrAssphage: Cross-assembly phage; HPyV: human polyomavirus; EV: Enterovirus; HNoV GII: Human norovirus GII; HAdV: Human adenovirus; BoV: Bocavirus; PeV: Parechovirus; RhV A: Rhinovirus A; RhV B: Rhinovirus B; RSV B: Respiratory syncytial virus B; -: samples were either negative or positive but did not quantify.

**Supplementary Table ST5**

Descriptive statistics of indicator, enteric and respiratory viruses in aircraft wastewater samples using the AE workflow

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Descriptive statistics | Log10 GC/12.5 mL | | | | | | | | | |
|  | CrAssphage | HPyV | EV | HNoV GII | HAdV | BoV | PeV | RhV A | RhV B | RSV B |
| Number of quantifiable samples | 24 | 24 | 2 | 1 | 5 | 2 | 1 | 24 | 24 | 2 |
| Minimum | 2.98 | 4.41 | 3.49 | 2.89 | 2.40 | 2.95 | 2.48 | 2.16 | 2.79 | 3.46 |
| Maximum | 7.83 | 6.55 | 3.76 | 2.89 | 4.02 | 3.53 | 2.48 | 4.35 | 4.06 | 3.53 |
| Mean | 6.76 | 5.46 | 3.63 | 2.89 | 3.22 | 3.24 | 2.48 | 3.11 | 3.23 | 3.50 |
| Std. Deviation | 1.26 | 0.54 | 0.19 |  | 0.61 | 0.41 |  | 0.47 | 0.40 | 0.05 |
| Lower 95% CI of mean | 6.23 | 5.23 | 1.91 |  | 2.47 | -0.44 |  | 2.91 | 3.06 | 3.05 |
| Upper 95% CI of mean | 7.29 | 5.69 | 5.34 |  | 3.98 | 6.92 |  | 3.31 | 3.40 | 3.94 |

**Supplementary Table ST6**

Descriptive statistics of indicator, enteric and respiratory viruses in aircraft wastewater samples using the NMAP workflow

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Descriptive statistics | Log10 GC/12.5 mL | | | | | |
|  | CrAssphage | HPyV | EV | HNoV GII | RhV A | RhV B |
| Number of quantifiable samples | 24 | 24 | 7 | 1 | 24 | 24 |
| Minimum | 3.42 | 3.02 | 2.20 | 2.55 | 2.64 | 2.99 |
| Maximum | 6.17 | 5.75 | 2.99 | 2.55 | 4.45 | 5.13 |
| Mean | 5.18 | 4.20 | 2.49 | 2.55 | 3.51 | 3.74 |
| Std. Deviation | 0.84 | 0.66 | 0.31 |  | 0.49 | 0.48 |
| Lower 95% CI of mean | 4.82 | 3.93 | 2.20 |  | 3.30 | 3.54 |
| Upper 95% CI of mean | 5.53 | 4.48 | 2.78 |  | 3.72 | 3.94 |