**Respiratory viruses in the patient environment**

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**SUPPLEMENTAL MATERIALS**

**Supplementary Material 1. Control patient rooms**

Environmental surfaces of five clean empty patient rooms in four different hospital units were randomly chosen to sample for viruses on two different days. Surfaces tested for the presence of Influenza A, Influenza B, and Rhinovirus included: bed rail, IV pole, tray table, bed control, telephone, call button, glove, gown, mask with eye shield, sharp box, light switch, computer mouse, computer keyboard, chair, vital cart handle, and isolation stethoscope.

**Supplementary Material 2. Air Sampling**

Air sampling used the NIOSH 2-stage cyclone air sampler, which is size-selective sampling device that operates at 3.5 L/min to collect: particles with aerodynamic diameter, d­a < 1 μm on a 37 mm polytetrafluoroethylene filter with 2 µm pores (225-27-07; SKC) in a filter cassette (225-309, SKC); particles with 1 < d­a < 4 μm in a 1.5 mL micro centrifuge tube (02-681-339; Fisher Scientific); and particles with d­a > 4 μm in a 15 mL micro centrifuge tube (35-2096; Falcon). In order to avoid carry-over contamination, the air samplers and sampling accessories were rinsed with deionized water, followed by isopropanol, and air dried after each experiment.

**Supplemental Material 3: Surface sampling protocol**

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| --- | --- | --- |
| **Sampling**  **Surface** | **Baseline**  **swabbing instructions** | **Post-experiment**  **swabbing instructions** |
| Bed rails | Randomly swab one side of the bed rails (40 cm2) | Swab the other side of the bed rails (40 cm2) |
| Monitors on IV pole | Randomly swab the top or bottom of the monitor (125 cm2). This area applies for experiments before 10/19/17  Since 10/19/17, hospital changed a different IV monitor screen, which area is 336 cm2 (32 x 21 cm) | Swab the top/ or bottom of the (125 cm2) for experiment before 10/19/17  After that, the swab area is 336 cm2. Sampling area changed due to hospital equipment upgrade |
| Computer mouse (if present) | Randomly swab right or left side of the mouse (27.5 cm2) | Swab the other side of the mouse (27.5cm2) |
| Computer keyboard (if present) | Randomly swab right or left of the keyboard (252 cm2) | Swab the other side of the keyboard (252 cm2) |
| Tray table | Randomly swab the right half side of the tray table (100 cm2) | Swab the other side of the tray table (100 cm2) |
| Exterior bed control area |  | Swab the entire button area (15 x 8 = 120 cm2) |
| IV pole hangers |  | Swab one pole hanger (20 cm long x 1 cm diameter of the circle) = 64.37 cm2 |
| Telephone |  | Swab the top of the telephone handset (20 x 5 = 100 cm2) |
| Call button/ TV remote control |  | Swab entire of the call button area (20x7 = 140 cm2) |
| Isolation stethoscope (**if present**) |  | Swab entire of the stethoscope  Area = 102.67 cm2 |
| Other medical equipment used |  | Vital sign machine handle (54 cm x 3 cm = 162 cm2)  Chair rail: 100 cm2 |

a Surface Area of a Stethoscope is estimated as below: 2 V-shaped cords: 19 cm length and 1 cm diameter (cylinder shape); Black rubber cord: 56 cm length and 3 cm diameter (cylinder shape); Large bell circle shape: 4.5 cm diameter; Small bell circle shape: 2.9 cm diameter; A = 102.67 cm2

**Supplemental Materials 4: Sample Processing and Analysis**

All swabs and air sampling collection devices were placed in a cooler with dry ice for transportation to the UIC DNA Services facility for sample processing. RNA was extracted from swabs and air filters using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Promega, WI), according to the manufacturer’s instructions but with modifications specific for air filters. Briefly, after incubation, air filters were processed by bead-beating in tubes with glass beads (0.1 mm) for 30 seconds at 6 m/s using FastPrep-24™ 5G (MP Bio). Subsequently, tubes were spun down for 1 min at 13,000 g. Lysate was transferred into cartridges for extraction. RNA was eluted in 50 µL of RNase-free water, and stored at -80°C until used for cDNA synthesis and qPCR. The isolated viral RNA was reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit with random primers (Applied Biosystems, CA). The cDNA synthesis was performed according to the manufacturer’s instructions. The reverse transcribed viral RNA (cDNA) was processed next using pre-amplification with *TaqMan* PreAmp Master Mix (Applied Biosystems), according to the manufacturer’s instructions. Reactions were conducted with 5 µL of cDNA, 10 µl of pre-amplification master mix and 5 µl of pooled *TaqMan* assays (see below), and amplified using a T100 thermocycler (BioRad, CA) with the following conditions: 10 min at 95°C, and 14 cycles of 15 s at 95°C and 4 min at 60°C. Pre-amplified samples were diluted in 1:10 ratio with TE buffer (1X Tris-EDTA Solution, IDT) and used for qPCR.

Primer sets and probes for qPCR were based on assays for each target virus (Table S2). Their speciﬁcity was examined using the basic local alignment search tool.1 When optimal designs were verified, primers and probes were synthesized (Thermo Fisher Scientific, CA). Synthetic DNA templates, serving as qPCR standards, were designed and synthesized by IDT. Synthetic templates were generated by concatenating sequences of target viruses. (Table S3) Absolute quantification of each template was performed using fluorimetry using a Qubit fluorometer (Life Technologies), and based on DNA concentration, a copy number was calculated based on known sequence length. Each assay was validated with a dilution series of the appropriate synthetic DNA template to verify reaction efficiency and presence of background contamination.

After assay efficiency and optimization, quantitative PCR (qPCR) analyses were performed by generating dilution series of standards and conducting pre-amplification of both standards and cDNA from samples. Subsequently, all pre-amplified templates were assayed using qPCR in triplicate in 10 µL reaction volumes. Each reaction contained 2.5 µL of cDNA, 5 µl of 2X TaqMan Fast Advanced Master Mix (Applied Biosystems), 0.5 µl of custom designed 20X TaqMan Gene Expression Assay with specific minor groove binding probe labeled with either the 5’ reporter dye 6-carboxy-ﬂuorescein (FAM) or 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) ﬂuorogenic dyes. Ampliﬁcation and detection were performed with an ViiA7 Real Time PCR System (Applied Biosystems), under the following conditions: 2 min at 50°C to attain optimal AmpErase uracil-N-glycosylase activity, 2 min at 95°C to activate the DNA polymerase, and 40 cycles of 1 s at 95°C and 20 s at 60°C allowed for denaturation, annealing and extension. The reporter dyes (FAM and VIC) signals were measured relative to the internal reference dye (ROX) to normalize for non-PCR-related ﬂuorescence ﬂuctuations occurring from well to well. The automatic threshold cycle (Ct) number generated after each run was used for all analyses.

**Primers and probes for the quantitative PCR amplification of viral RNA**

|  |  |  |  |
| --- | --- | --- | --- |
| **Virus type** | **Primer or Probe** | **Sequence** | **References** |
| HCoV - HKU1 | HKU1 – F | 5’ CCCGCAAACATGAATTTTGTT |  |
|  | HKU1 – R | 5’ CATTCATTCGCAAGGCGATA | In house design |
|  | HKU1 – probe | 6FAM-AATCTATCACCATGTGAA-MGBNFQ |  |
| HCoV - NL63 | NL63 - F | 5’ AACCTCGTTGGAAGCGTGTT |  |
|  | NL63 - R | 5’ CGAGGACCAAAGCACTGAATAA | In house design |
|  | NL63 - probe | VIC-ATTTTCCTCTCTGGTAG-MGBNFQ |  |
| HCoV - 229E | 229E - F | 5’ CTGCCAAGAYTCTTGCTCGTT |  |
|  | 229E - R | 5’ TCTTTTCCACCGTGGCTTTT | In house design |
|  | 229E - probe | VIC-AGAACAAAAGCATGAAATG-MGBFQ |  |
| HCoV - OC43 | OC43 - F | 5’ GACATGGCTGATCAAATTGCTAGT |  |
|  | OC43 - R | 5’ GCTGAGGTTTYGTGGCATCCTT | In house design |
|  | OC43 - probe | 6FAM-TCTGGCAAAACTTGG-MGBNFQ |  |
| Influenza A | InflA - F | 5’ GACCRATCYTGTCACCTCTGAC | 2 |
|  | InflA - R | 5’ GAAACACGGACACCCAAAGTAGT |
|  | InflA - probe | 6FAM- TGCAGTCCTCGCTCACTGGGCACG |
| Influenza B | InflB - F | 5’ TCCTCAAYTCACTCTTCGAGCG | 2 |
|  | InflB - R | 5’ AGGGCATTYTGGACAAAKCGTCTA |
|  | InflB - probe | 6FAM- CCAATTCGAGCAGCTGAAACTGCGGTG |
| PIV 1 | PIV1 - F | 5’ CAAAGAGARAATGCRGATCTAG |  |
|  | PIV1 - R | 5’ AGCTCCGAGACATGCAGGAT | In house design |
|  | PIV1 - probe | 6FAM-TCCATATGTCTGAAGCAAT-MGBNFQ |  |
| PIV 2 | PIV2 - F | 5’ ATTCCAGATGCTCGATCAACTATG |  |
|  | PIV2 - R | 5’ TCYTCAGCTAATGCTTCRAARGC | In house design |
|  | PIV2 - probe | 6FAM-AGCACYTCTCCTCTGG-MGBNFQ |  |
| PIV 3 | PIV3 - F | 5’ CGCGCYCCWTTYATCTGTATC |  |
|  | PIV3 - R | 5’ TTGCCTGGTGCGAACTCA | In house design |
|  | PIV3 - probe | 6FAM-TCAGAGATCCYATACATG-MGBNFQ |  |
| PIV 4a | PIV4a - F | 5’ CACACACACAATGGGCACAA |  |
|  | PIV4a - R | 5’ GCGTATTTGGTGAGAGTTTTGAGTT | In house design |
|  | PIV4a - probe | VIC-CAATCCCACACTACAAC-MGBNFQ |  |
| PIV 4b | PIV4b - F | 5’ CGCACACATACAATGAAAGCAA |  |
|  | PIV4b - R | 5’ TGGCGGGATTTCTGAGTTG | In house design |
|  | PIV4b - probe | VIC-CACTCCTGTCCCACATC-MGBNFQ |  |
| Rhinovirus | RhinV - F | 5’ TCCTCCGGCCCCTGAAT | 3 |
|  | RhinV – R | 5’ GAAACACGGACACCCAAAGTAGT |
|  | RhinV - probe | 6FAM - YGGCTAACCYWAACCC |
| RSV A | RSV A - F | 5’ AGATCAACTTCTGTCATCCAGCAA | 4 |
|  | RSV A - R | 5’ ATTGATACTCCTAATTATGATGTGC |
|  | RSV A - probe | 6FAM - CACCATCCAACGGAGCACAGGAGAT |
| RSV B | RSV B – F | 5’ AAGATGCAAATCATAAATTCACAGGA | 4 |
|  | RSV B – R | 5’ ACTATAAAGATACTTAAAGATGCTGGATATCA |
|  | RSV B – probe | 6FAM - TTCCCTTCCTAACCTGGACATAGCATATAACATACCT |
| MS2 | MS2 - F | 5’ CCGTTAGCGAAGTTGCTTGG | 5 |
|  | MS2 - R | 5’ GTCCATACCTTAGATGCGTTAGC |
|  | MS2 - probe | 6FAM - AGTCACGTCGCCAGTTCCGCCA |

**Sequences of synthetic DNA templates used for standards**

|  |  |
| --- | --- |
| **Name** | **Sequence** |
| gBlock 1:  Influenza A  Influenza B  RVS A  RSV B | GAGACTGGAAGTGTCTTATACACTCAACAAAGATCAACTTCTGTCATCCAGCAAATACACCATC  CAACGGAGCACAGGAGATAGTATTGATACTCCTAATTATGATGTGCAGAAACACATTATTAATC  ACTGAAGATGCAAATCATAAATTCACAGGATTAATAGGTATGTTATATGCTATGTCCAGGTTAG  GAAGGGAAGACACTATAAAGATACTTAAAGATGCTGGATATCATGTTAAGCTATGGCCATCGG  ATCCTCAACTCACTCTTCGAGCGTCTTAATGAACATTCAAAGCCAATTCGAGCAGCTGAAACT  GCGGTGGGAGTCTTATCCCAATTTGGTCtAAGAGCACCGATTATCACCTGTAAGACAAGACCAA  TCCTGTCACCTCTGACTAAGGAATTGTTCACGCTCACCGTGCCCAGTGAGCGAGGACTGCAGC  GTAGACGCTTTGTCCAAAATGCCCTGAATGGGAATGGTTAAACTATACAAGAAGCT |
| gBlock 2:  HKU1  NL63  22E  OC43  PIV1  PIV2  PIV3  PIV4a  PIV4b  RhinoV | AAATATTTTGCGTATTGTTAGTAGTTTAGTTTTGGCCCGCAAACATGAATTTTGTTGTTCACA  TGGTGATAGATTTTATCGCCTTGCGAATGAATGTGCTCAAGTTTAAGAAACCTCGTTGGAAGC  GTGTTCCTACCAGAGAGGAAAATGTTATTCAGTGCTTTGGTCCTCGTGATTTTAATTCAATCT  GCTGCCAAGATTCTTGCTCGTTCTCAGAGTTCTGAAACAAAAGAACAAAAGCATGAAATGCAA  AAGCCACGGTGGAAAAGACAGCCTAACGATACACCTGACATGGCTGATCAAATTGCTAGTCTT  GTTCTGGCAAAACTTGGCAAGGATGCCACCAAACCTCAGCAAGTAAAAGGACAAAGAGAGAAT  GCGGATCTAGAAGCATTGCTTCAGACATATGGATATCCTGCATGTCTCGGAGCTATAATTCCG  AGTTATTCCAGATGCTCGATCAACTATGTCCAGAGGAGAAGTGCTGGCCTTCGAAGCATTAGC  TGAGGACATTCCTGATACAAAGGGACCACGCGCCCCATTCATCTGTATCCTCAGAGATCCCAT  ACATGGTGAGTTCGCACCAGGCAACTATCCTGCAAAAAATCCCCAATACCCGCAATCGCACAC  ACACAATGGGCACAATCAATCCCACACTACAACATAACTCAAAACTCTCACCAAATACGCCAT  CACAACTCACAATCGCACACATACAATGAAAGCAATCACTCCTGTCCCACATCACAACTCAGA  AATCCCGCCAAACGCAATTGTGAGTCCTCCGGCCCCTGAATGCGGCTAATCCTAACCCTGCAG  CCATTGCAAACATTCCAGTTTGTGGGTGGTCGTAACGAGTAATTGCGGGATGGAACCGACTAC  TTTGGGTGTCCGTGTTTCTCTTTTTAACTTGATGAGTGTCTTATGGTTACAATTATACAGTAA  CCAT |

**Supplemental Material 5: Potential determinants of virus concentrations in stationary and personal air samples**

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| **Mean (75thP, Max) Virus Concentration in Sample (copies/m3)** | **Predictors**  **Mean (Median, Max)** | **Spearman Correlation** |
| Stationary  7.3×103  (1.2×103 ; 1.4×105) | Number of observed coughs  6.8 (3; 52) | -0.12  (p = 0.40) |
| Distance from stationary air sampler to patient head (cm)  79.5 (80; 160) | - 0.26  (p = 0.07) |
| Total number of encounters with HCW  6.1 (6; 10) | 0.08  (p = 0.57) |
| Personal air samples (copies/m3)  3.0×106 (0; 6.8×107) | -0.13  ( p = 0.56) |
| Personal  3.0×106  (0; 6.8×107) | Number of observed coughs  8.3 (4; 45) | 0.03 (  p = 0.89) |
| Total number of encounters with HCW   * 1. (7; 10) | -0.19  (p = 0.37) |

**Supplemental Material 6: Correlation between the virus concentration on surfaces after the observation period and the number of surface contacts by patients**

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| --- | --- | --- | --- | --- |
| **Number of contacts by patient on surface** | **Spearman’s Correlation Coefficient (p-value)** | | | |
| **Virus concentration at the end of the experiments** | | | |
| **Tray table** | **Bed rail** | **Call button** | **Telephone** |
| **Tray table** | -0.1  (p = 0.43) |  |  |  |
| **Bed rail** |  | 0.0  (p = 0.99) |  |  |
| **Call button** |  |  | 0.0  (p = 0.81) |  |
| **Telephone** |  |  |  | 0.1  (p = 0.37) |
| **Other bed surface** |  | 0.0  (p = 0.97) |  |  |
| **All surfaces** | -0.1  (p = 0.46) | -0.1  (p = 0.58) | -0.1  (p = 0.73) | 0.0  (p = 0.90) |

**Supplemental Material 7: Correlations between virus concentrations on surfaces measured at baseline and after the experiment**

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| --- | --- | --- | --- | --- | --- |
| **Virus concentration**  **on surface after**  **the experiment (copies/cm2)** | **Spearman’s correlation (p-value)** | | | | |
| **Virus concentration on surfaces at the baseline (copies/cm2)** | | | | |
| **Tray table** | **IV Monitor** | **Mouse** | **Keyboard** | **Bed rail** |
| **Tray table** | 0.14  (p = 0.35) |  |  |  |  |
| **IV monitor** |  | 0.18  (p = 0.28) |  |  |  |
| **Mouse** |  |  | -0.07  (p =0.78) |  |  |
| **Keyboard** |  |  |  | **0.99**  **(p < 0.05)** |  |
| **Bedrail** |  |  |  |  | -0.0  (p = 0.92) |

**References**

1. Altschul SF, Gish W, Miller W, et al. Basic local alignment tool. *J Mol Biol*. 1990; 215(3): 403-410.
2. Selvaraju SB, Selvarangan R. Evaluation of three influenza A and B real-time reverse transcription-PCR assays and new 2009 H1N1 assay for detection of influenza viruses. *J Clin Microbiol.* 2010; 48(11);3870-3875.
3. Do DH, Laus S, Leber A, et al. A one-step, real-time PCR assay for rapid detection of rhinovirus. *J Mol Diagn*. 2010; 12(1):102-108.
4. Van Elden LJR, van Loon AM, van der Beek A, et al. Applicability of a real-time quantitative PCR assay for diagnosis of respiratory syncytial virus infection in immunocompromised adults. *J Clin Microbiol* 2005; 43(8): 4308-4308.
5. Usachev EV, Pankova AV, Rafailova EA, et al. Portable automatic bioaerosol sampling system for rapid on-site detection of targeted airborne microorganisms. *J Environ Monit.* 2012; 14(10): 2739.