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# Comparative Testing and Evaluation of Nine Different Air Samplers: End-to-End Sampling Efficiencies as Specific Performance Measurements for Bioaerosol Applications — Source link

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**Supplemental Information**

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## MATERIALS AND METHODS

### Cultivation analysis

The collected samples were diluted as needed with PBSTA before triplicate plating on appropriate growth medium plates. NA plates were used for BG and SM, while TSA plates were used for KR. MS2-containing samples were analyzed on NA plates by a pour-plate method using 1.0 x $10^7$ cfu of log-phase *Escherichia coli* (DSM 4230) cells in soft NA (0.7% agar). The cultivation plates were incubated (18 hr) at 30°C (BG and SM) or 37°C (KR and MS2) and plates containing between 30 and 300 cfu or pfu were manually counted.

### Molecular analysis

The qPCR assay for KR was designed using Primer-Blast with standard parameters for primer search and specificity checking (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The query sequence was *gyrB* from the genome sequence of *Kocuria rhizophila* DC2201 (NC_010617). Specificity checking was performed towards the GenBank nr nucleotide collection database.

Nucleic acids were purified using NucliSENS isolation kits (bioMérieux, Marcy l'Etoile, France) and the EasyMAG instrument (bioMérieux). Samples containing SM, KR and MS2 were purified directly, while BG samples were homogenized (1 ml sample, 60 s, max intensity) with a Mini Beadbeater-8 (BioSpec, Bartlesville, OK) using bead beating tubes (2ml, Sarstedt, Nümbrecht, Germany) containing 0.5 g 100 µm and 0.5 g 500 µm zirconium-silica beads (BioSpec). Purified nucleic acids were analyzed as triplicate reactions in 96-well PCR plates (Roche Diagnostics, Indianapolis, IN) using a LightCycler 480 instrument (Roche Diagnostics). The primers and probes were purchased from Invitrogen (Table S1).

Each qPCR reaction (20 µl) contained; 2X SYBR Green master mix (10 µl, Roche Diagnostics), PCR grade water (6 µl, Roche Diagnostics), 10 mM forward and reverse primers (1 µl each) and template (2 µl). The qPCR program consisted of denaturation (95°C, 5 min) and 40 cycles of denaturation (95°C, 20 s), annealing (60°C, 20 s) and extension (72°C, 20 s).

Each qRT-PCR reaction (20 µl) contained; 5X Reaction buffer (4 µl) and 50X Enzyme blend (0.4 µl) from the Real-time Ready RNA Virus Master kit (Roche Diagnostics), PCR grade water (10.6 µl), 10 mM forward and reverse primers (1 µl each), 10 mM probe (1 µl) and template (2 µl). The qRT-PCR program consisted of reverse transcription (50 °C, 8 min), denaturation (95°C, 5 min) and 40 cycles of denaturation (95°C, 20 s), annealing (60°C, 20 s) and extension (72°C, 20 s).

Standard curves were constructed for each test agent by purifying nucleic acids from serial dilutions of the respective spray solutions. PCR grade water was used as negative

amplification controls, while standards from the standard curves were used as a positive amplification controls and internal calibrators. Melting curves were constructed to verify specific amplifications when SYBR Green was used. Possible PCR inhibition due to differences in the collection liquids were investigated by analyzing nucleic acids purified from collection liquids spiked with standards from the standard curves. No inhibition was observed for any of the collection liquids. Possible false positive PCR amplification was addressed by testing each PCR assay to all test agents, and no cross-reactivity was observed.

**Direct count analysis**

The collected samples were diluted as needed with PBSTA and filtered onto black polycarbonate membrane filters (Isopore, 25mm diameter, 0.2 µm pore-size, Millipore) housed in polypropylene filter holders (Swinnex-25, Millipore). The membrane filters were mounted on microscopy slides and analyzed using a fluorescence microscope (AxioSkop 2, Carl Zeiss, Thornwood, NY) fitted with a digital camera (AxioCam HRc, Carl Zeiss). Yellow-green FS (1 µm) were viewed using 488nm and 525nm band pass filters (filter set 52, Carl Zeiss), while red Fluospheres (4 µm) were viewed using 546nm and 575-640nm band pass filters (filter set 20, Carl Zeiss). Photomicrographs were captured (AxioVision, Carl Zeiss) from random field-of-views (>20 fields or >1000 FS) and processed using image analysis software (ImageJ, http://rsbweb.nih.gov/ij/index.html).

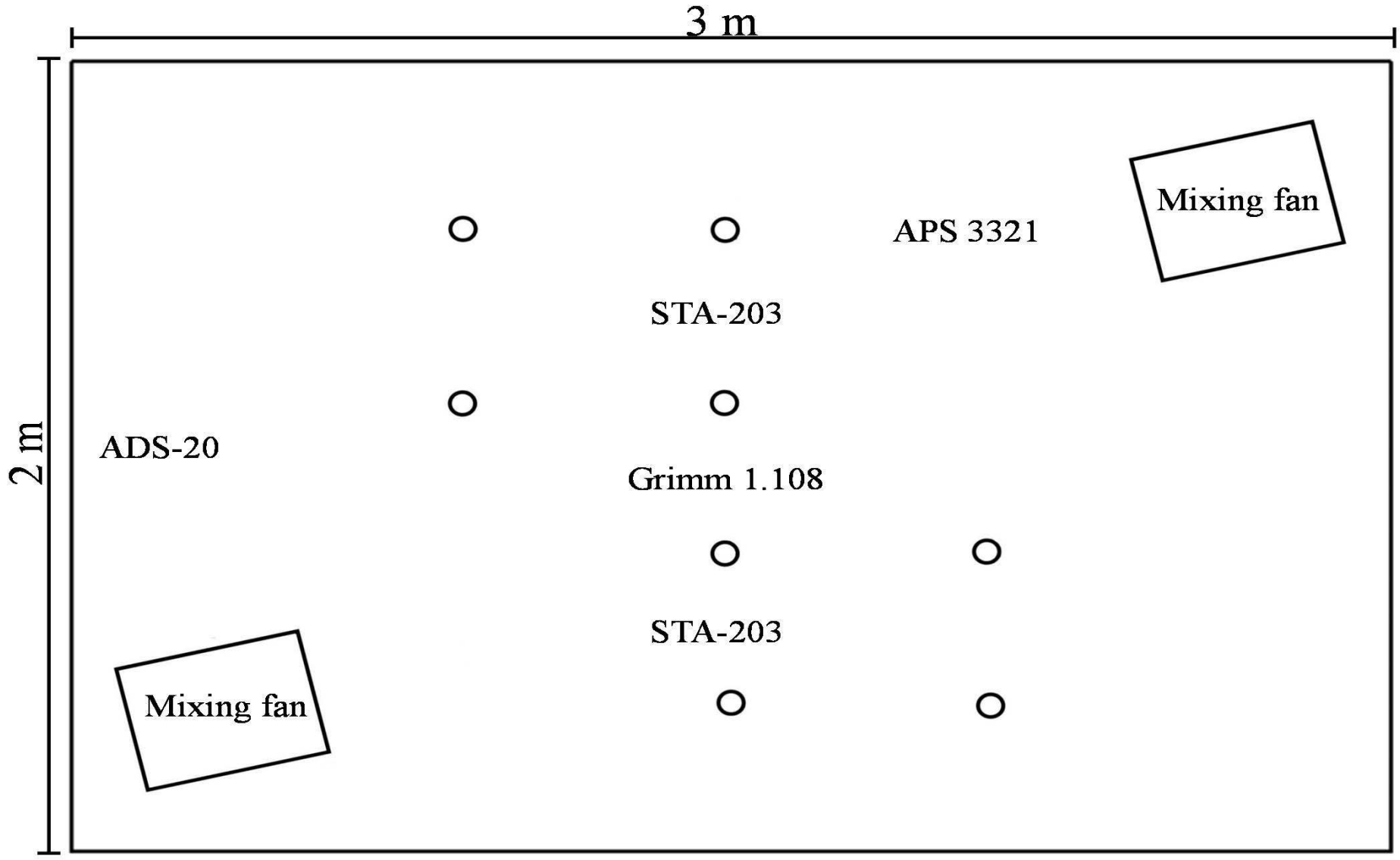


Figure S1. Top-down schematic layout of the 12 $m^3$ aerosol test chamber (ATC).The sampling positions used for air sampler testing are designated with open circles. Mixing fan; two chamber-mounted mixing fans (120 mm), ADS-20; injection port for test aerosols from the ADS-20 aerosol dilution system, STA-203; two chamber-mounted slit-to-agar samplers, APS3321; aerodynamic particle sizer, Grimm 1.108; optical particle counter.


Figure S2. Air sampling equipment subjected to testing and evaluation (T&E) in this study. From the left: OMNI-3000, SASS 2300, Coriolis FR, BioSampler (reference sampler), BioCapture 650, SASS 3100, XMX-CV, and ESP (prototype). The gelatin filters are not shown in this picture.

Table S1. Primers and probes.

| Organism | Gene | Forward primer (5'-3') | Reverse primer (5'-3') | Detection probe (5'-3') | Product size (bp) | References |
|---|---|---|---|---|---|---|
| *Serratia marcescens* | gyrB | AGTGCACGAACAAACTTACAG | GTCGTACTCGAAATCGGTCACA | n.a. | 138 | Saikaly et al. 2007 |
| *Bacillus atrophaeus* spores | recA | ACCAGACAATGCTCGACGTT | CCCTCTTGAAATTCCCGAAT | n.a. | 131 | Buttner et al. 2004 |
| *Kocuria rhizophila* | gyrB | CCGTGGACATGCACCCCACC | CGGGACAGGGCGTTGACCAC | n.a. | 148 | This study |
| Bacteriophage MS2 | A protein | GTCGCGGTAATTGGCGC | GGCCACGTGTTTTGATCGA | FAM-AGGCGCTCCGCTACCTTGCCCT-BBQ | 77 | O'Connell et al. 2006 |

n.a.; not applicable, bp; base pair.

Table S2. Particle size distributions for the test aerosols based on APS 3321 aerodynamic particle sizer measurements. The size distributions were calculated based on all aerosol experiments and reported as the mass median aerodynamic diameter (MMAD) with geometric standard deviation (GSD), and a MMAD size range for the individual experiments.

| **Aerosol test agent** | **Particle size distribution based on APS 3321 measurements from all aerosol experiments** | | | | | |
|---|---|---|---|---|---|---|
| | **1 µm MMAD targeted** | | | **4 µm MMAD targeted** | | |
| | **MMAD (µm)** | **GSD** | **Range (µm)** | **MMAD (µm)** | **GSD** | **Range (µm)** |
| FluoSpheres (FS) | 1.04 | 1.09 | 1.02-1.20 | 3.84 | 1.10 | 3.67-3.96 |
| *Bacillus atrophaeus* spores (BG) | 1.22 | 1.31 | 1.03-1.46 | 4.04 | 1.34 | 3.83-4.20 |
| *Kocuria rhizophila* (KR) | | | | 3.92 | 1.40 | 3.61-4.37 |
| *Serratia marcescens* (SM) | | | | 4.38 | 1.53 | 4.08-4.63 |
| Bacteriophage MS2 (MS2) | | | | 3.87 | 1.36 | 3.66-4.35 |

MMAD; mass median aerodynamic diamater, GSD; geometric standard deviation

Table S3. Summary of the evaluated air samplers' end-to-end cultivation-based biological sampling efficiencies (BSEs) and qPCR- or fluorescence microscopy-based physical sampling efficiencies (PSEs) relative to the reference sampler (BioSampler).

| Air sampler | Relative sampling efficiency (average ± standard deviation)[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 µm MMAD aerosols | | | 4 µm MMAD aerosols | | | | | | | | |
| | Fluospheres (FS) | *Bacillus atrophaeus* spores (BG) | | Fluospheres (FS) | *Bacillus atrophaeus* spores (BG) | | *Serratia marcescens* (SM) | | *Kocuria rhizophila* (KR) | | Bacteriophage MS2 (MS2) | |
| | FM | Cultivation | qPCR | FM | Cultivation | qPCR | Cultivation | qPCR | Cultivation | qPCR | Cultivation | qRT-PCR |
| **ESP** | 0.24 ± 0.04 | 0.35 ± 0.04 | 0.36 ± 0.13 | 0.47 ± 0.06 | 0.55 ± 0.14 | 0.52 ± 0.09 | 0.13 ± 0.09 | 0.39 ± 0.07 | 0.48 ± 0.23 | 0.62 ± 0.22 | 0.03 ± 0.03 | 0.30 ± 0.05 |
| **SASS 2300** | 0.05 ± 0.05 | 0.10 ± 0.01 | 0.08 ± 0.02 | 0.13 ± 0.02 | 0.43 ± 0.10 | 0.39 ± 0.09 | 1.60 ± 0.39 | 0.57 ± 0.11 | 0.85 ± 0.20 | 0.39 ± 0.16 | 0.63 ± 0.17 | 0.57 ± 0.21 |
| **SASS 3100** | 0.70 ± 0.10 | 0.73 ± 0.12 | 0.83 ± 0.15 | 0.63 ± 0.11 | 0.83 ± 0.18 | 0.77 ± 0.11 | 0.01 ± 0.01 | 0.52 ± 0.06 | 0.78 ± 0.17 | 0.57 ± 0.28 | 0.02 ± 0.02 | 0.62 ± 0.09 |
| **Gelatin filter** | 1.27 ± 0.12 | 1.28 ± 0.09 | 1.18 ± 0.09 | 1.25 ± 0.16 | 1.17 ± 0.30 | 1.03 ± 0.08 | 0.03 ± 0.04 | 1.07 ± 0.17 | 1.18 ± 0.32 | 0.92 ± 0.17 | 1.06 ± 0.27 | 0.22 ± 0.08 |
| **XMX-CV** | 0.05 ± 0.01 | 0.22 ± 0.05 | 0.19 ± 0.06 | 1.07 ± 0.27 | 1.26 ± 0.20 | 1.11 ± 0.17 | 2.63 ± 0.56 | 0.98 ± 0.17 | 1.45 ± 0.32 | 1.00 ± 0.31 | 0.21 ± 0.10 | 0.16 ± 0.08 |
| **BioCapture 650** | 0.24 ± 0.05 | 0.22 ± 0.17 | 0.22 ± 0.06 | 0.74 ± 0.21 | 0.78 ± 0.13 | 0.60 ± 0.14 | 0.18 ± 0.05 | 0.46 ± 0.13 | 0.90 ± 0.27 | 0.57 ± 0.11 | 0.72 ± 0.17 | 0.68 ± 0.11 |
| **Coriolis FR** | 0.47 ± 0.08 | 0.49 ± 0.25 | 0.53 ± 0.08 | 0.88 ± 0.08 | 0.69 ± 0.09 | 0.67 ± 0.07 | 0.72 ± 0.18 | 0.53 ± 0.16 | 0.88 ± 0.17 | 0.62 ± 0.19 | 0.70 ± 0.19 | 0.49 ± 0.14 |
| **OMNI-3000** | 0.02 ± 0.01 | 0.16 ± 0.10 | 0.20 ± 0.09 | 0.08 ± 0.05 | 0.16 ± 0.05 | 0.21 ± 0.07 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

n.d; not determined, FM; fluorescence microscopy-based direct count analysis, Cultivation; plate count analysis, q(RT-)PCR; quantitative (reverse transcriptase) polymerase chain reaction analysis.

[1]The air samplers' end-to-end BSEs and PSEs were determined relative to a reference sampler (BioSampler) and reported as an averaged value (± standard deviation) based on a minimum of five aerosol experiments.

Color coding:

| |
|---|
| **<0.10** |
| **<0.25** |
| **<0.50** |
| **<0.75** |
| **≤1.00** |
| **>1.00** |

Table S4. Summary of the evaluated air samplers' end-to-end biological concentration factors (BCFs) and physical concentration factors (PCFs) relative to the reference sampler (BioSampler).

| Air sampler | Relative concentration factors[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 µm MMAD aerosols | | | 4 µm MMAD aerosols | | | | | | | | |
| | Fluospheres (FS) | *Bacillus atrophaeus* spores (BG) | | Fluospheres (FS) | *Bacillus atrophaeus* spores (BG) | | *Serratia marcescens* (SM) | | *Kocuria rhizophila* (KR) | | Bacteriophage MS2 (MS2) | |
| | Microscopy | Cultivation | qPCR | Microscopy | Cultivation | qPCR | Cultivation | qPCR | Cultivation | qPCR | Cultivation | qRT-PCR |
| **ESP** | 19.7 | 28.7 | 29.5 | 38.6 | 45.1 | 42.7 | 10.7 | 32.0 | 39.4 | 50.9 | 2.5 | 24.6 |
| **SASS 2300** | 7.4 | 14.8 | 11.9 | 19.3 | 63.7 | 57.8 | 237.1 | 84.5 | 126.0 | 57.8 | 93.4 | 84.5 |
| **SASS 3100** | 42.6 | 44.4 | 50.5 | 38.3 | 50.5 | 46.8 | 0.6 | 31.6 | 47.4 | 34.7 | 1.2 | 37.7 |
| **Gelatin filter** | 2.9 | 2.9 | 2.7 | 2.9 | 2.7 | 2.3 | 0.1 | 2.4 | 2.7 | 2.1 | 2.4 | 0.5 |
| **XMX-CV** | 10.1 | 44.3 | 38.3 | 215.5 | 253.8 | 223.6 | 529.7 | 197.4 | 292.0 | 201.4 | 42.3 | 32.2 |
| **BioCapture 650** | 14.6 | 13.4 | 13.4 | 45.0 | 47.4 | 36.5 | 10.9 | 28.0 | 54.7 | 34.7 | 43.8 | 41.3 |
| **Coriolis FR** | 23.8 | 24.8 | 26.9 | 44.6 | 35.0 | 33.9 | 36.5 | 26.9 | 44.6 | 31.4 | 35.5 | 24.8 |
| **OMNI-3000** | 0.9 | 7.3 | 9.1 | 3.6 | 7.3 | 9.6 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

n.d; not determined, Microscopy; fluorescence microscopy-based direct count analysis, Cultivation; plate count analysis, q(RT-)PCR; quantitative (reverse transcriptase) polymerase chain reaction analysis.

[1]The air samplers' end-to-end biological and physical concentration factors relative to the reference sampler (BioSampler) were calculated by multiplying the theoretical relative concentration factors (Table 1) with the end-to-end relative biological or physical sampling efficiencies, BSEs and PSEs, respectively (Table S3).

Color coding:

| |
|---|
| **<1** |
| **<10** |
| **<25** |
| **<50** |
| **≤100** |
| **>100** |