

Optimizing the detection of environmental fungal contamination by comparing sample collection and detection methods



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Background

- Healthcare-associated invasive fungal infections (HA-IFIs) cause devastating morbidity and mortality and are commonly linked to environmental sources.
- No established threshold values or regulatory levels for these pathogens in the healthcare setting due to a lack of practice standards for assessment and remediation fungal contamination of the environment.

Objectives:

- To compare a) the sampling efficacy of commonly used healthcare sampling techniques and b) the detection efficacy of culture-based and qPCR quantification of fungi on common healthcare surface materials.

Methods

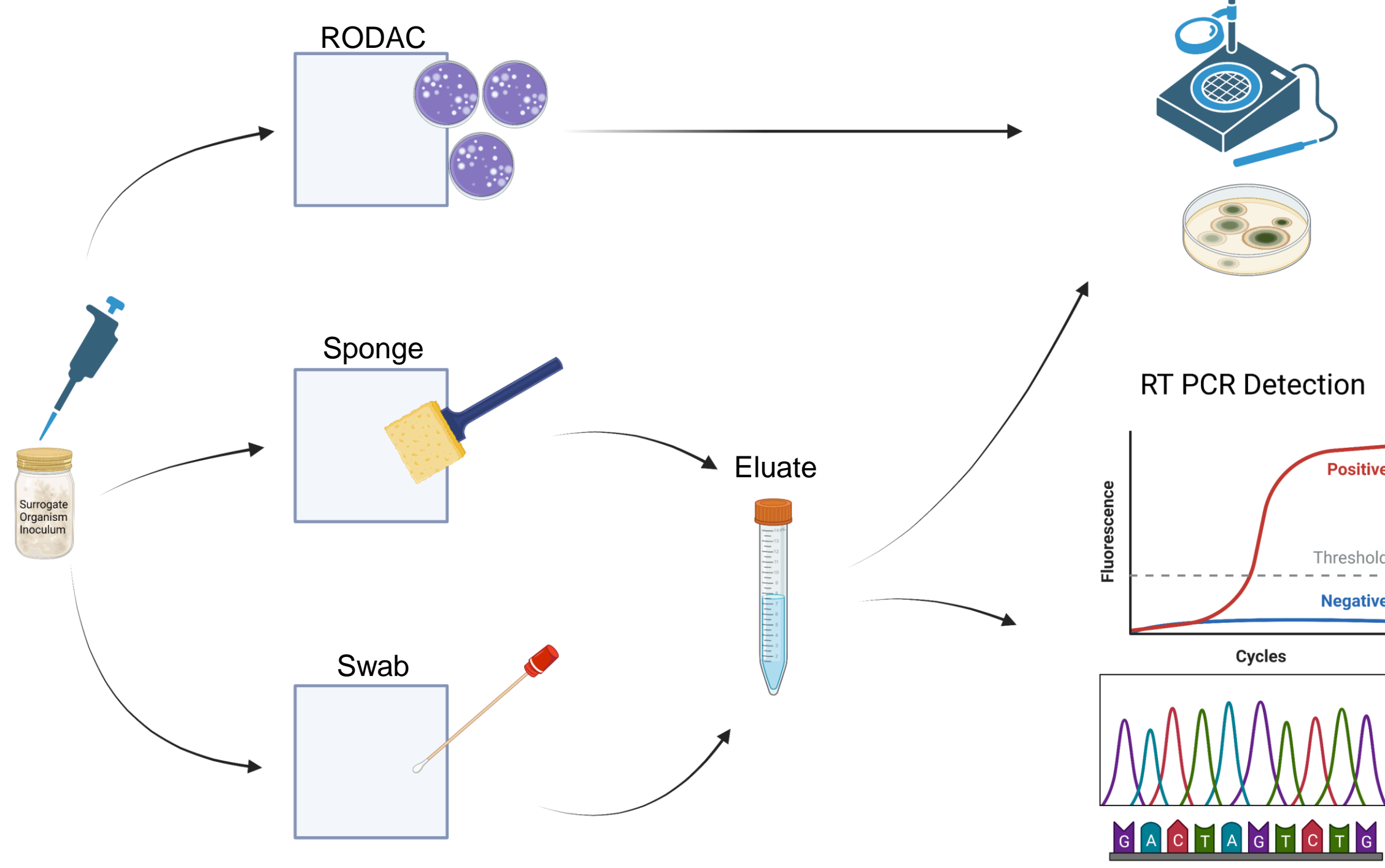
Definitions

- Laboratory experimental study
- Primary outcome: recovery of organisms, defined as total recovered CFU compared to known inoculum CFU.
- Sampling techniques: Foam sponges, swabs, RODAC plates
- Study surfaces: aluminum, formica, linen, and HEPA material
- Surrogate species: Filamentous fungi (*Aspergillus fumigatus*) and yeast (*C. parapsilosis*)

Study Protocol

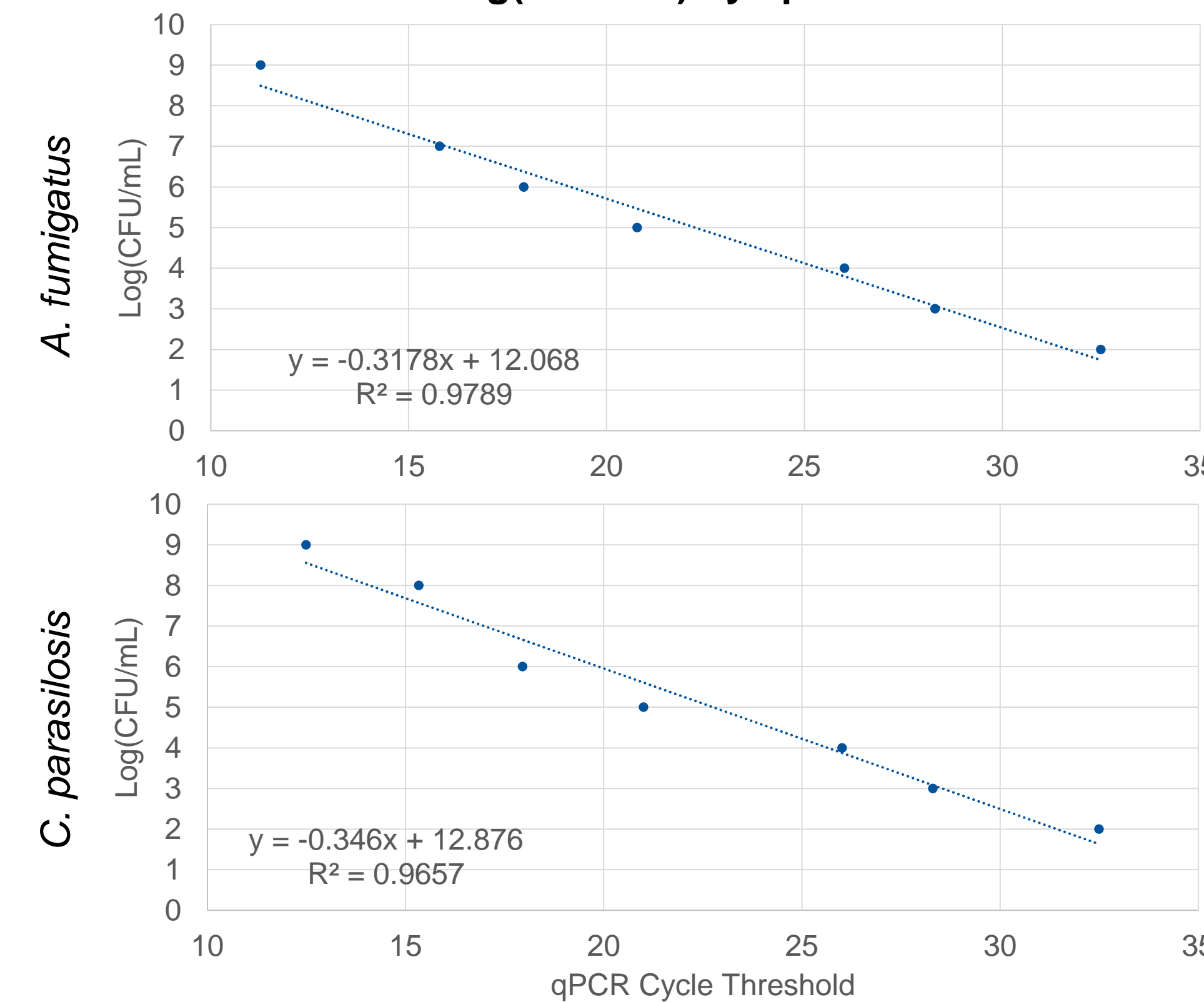
- Mock 10x10cm study surfaces were inoculated with ~10⁴ CFU of a surrogate species which was spread evenly and allowed to air dry.
- Sponges and swabs pre-moistened with neutralizing buffer were used to sample the complete surface area of study surfaces.
- RODAC plates filled with species-specific nutrient buffer were pressed to the middle of the study surface and removed.
- Eluents from sponges and swabs were used for culture-based quantification via serial dilution and for qPCR using the FungiQuant primers and probe for the fungal 18S rRNA gene.

Figure 1. Study Flow Diagram



Results

Figure 2. Standard Curve for qPCR Cycle Threshold and Log(CFU/mL) by species



Results (cont.)

- 20 experiments for each combination of species (n=2), study surface (n=4), sampling method (n=3) and quantification method (n=2) resulting in 960 total samples. Overall, median percent recovery for culture-based and qPCR-based detection methods were 6.4% and 26.7%, respectively, (p<0.01).
- Median percent recovery for sponges and swabs were 17.9% (11.4-30.0) and 3.8% (1.9-6.7) via culture and 36.2% (25.7-78.4) and 10.5% (7.7-36.0) via qPCR; culture vs qPCR percent recovery for sponges and swabs (p<0.01), culture and qPCR percent recovery for sponges vs swabs (p<0.01).
- RODAC median percent recovery via culture was 3.4% (1.0-7.1).

Table 1. Culture recovery proportions by sample methodology and surface material

| | | RODAC | | Swab | | | Sponge | | | | |
|-----------------|----------|--------------------------|-----------------------------------|--------------------------|-----------------------------------|--------------|--------------------------------|--------------------------|-----------------------------------|--------------|--------------------------------|
| | | Culture Median CFU (IQR) | Culture Recovery Proportion (IQR) | Culture Median CFU (IQR) | Culture Recovery Proportion (IQR) | qPCR CT (SD) | qPCR Recovery Proportion (IQR) | Culture Median CFU (IQR) | Culture Recovery Proportion (IQR) | qPCR CT (SD) | qPCR Recovery Proportion (IQR) |
| Both species | Overall | 552 (261-1007) | 3.4 (0.8-7.1) | 300 (200-700) | 3.8 (1.9-6.7) | 30.8 (1.1) | 10.5 (7.7-36.0) | 2850 (900-4800) | 17.9 (11.4-30.0) | 29.5 (0.9) | 36.2 (25.7-78.4) |
| | Linen | 144 (65-215) | 2.8 (1.5-3.6) | 200 (100-300) | 3.3 (2.8-6.8) | 32.0 (1.0) | 13.7 (7.4-26.1) | 750 (525-975) | 13.3 (10.0-20.5) | 30.3 (0.9) | 54.6 (25.7-117.4) |
| | Formica | 964 (864-1086) | 10.6 (2.6-13.6) | 1000 (500-1850) | 5.8 (3.8-7.1) | 31.0 (0.7) | 9.8 (7.3-12.8) | 5850 (2883-9350) | 29.8 (27.3-38.1) | 29.0 (0.6) | 40.2 (32.1-62.3) |
| | Aluminum | 914 (540-1149) | 5.1 (3.4-9.4) | 300 (200-475) | 1.9 (0.6-6.3) | 29.6 (0.8) | 30.6 (7.9-130.5) | 1900 (725-4675) | 13.3 (8.6-16.6) | 29.1 (0.4) | 47.8 (20.7-110.9) |
| | HEPA | 276 (197-332) | 0.9 (0.6-5.5) | 600 (200-1175) | 3.1 (1.8-5.5) | 30.6 (0.3) | 19.2 (4.8-36.4) | 3800 (2925-4976) | 20.2 (9.4-59.5) | 29.6 (1.0) | 32.3 (21.1-39.6) |
| A. fumigatus | Overall | 406 (242-822) | 7.0 (4.2-11.0) | 300 (200-500) | 5.0 (3.3-8.3) | 31.1 (1.5) | 24.5 (10.4-55.3) | 1473 (700-3184) | 23.5 (11.7-48.4) | 30.1 (0.9) | 44.8 (30.7-78.6) |
| | Linen | 214 (200-246) | 3.6 (3.3-4.1) | 200 (100-300) | 3.3 (1.7-5.0) | 32.8 (0.5) | 7.5 (5.4-10.2) | 700 (525-800) | 11.7 (8.8-13.3) | 31.1 (0.2) | 25.8 (21.9-27.7) |
| | Formica | 1032 (941-1119) | 12.9 (11.8-14.0) | 500 (225-675) | 6.3 (2.8-8.4) | 31.7 (0.3) | 12.0 (10.6-14.1) | 2968 (2318-3750) | 37.1 (29.0-46.9) | 29.5 (0.4) | 61.6 (46.8-76.6) |
| | Aluminum | 552 (451-635) | 9.2 (7.5-10.6) | 350 (225-500) | 5.8 (3.8-8.3) | 29.0 (0.7) | 121.0 (74.9-176.6) | 750 (500-900) | 12.5 (8.3-15.0) | 29.2 (0.4) | 110.9 (79.4-134.5) |
| | HEPA | 299 (238-350) | 5.4 (4.3-6.4) | 300 (100-600) | 5.5 (1.8-10.9) | 30.7 (0.2) | 36.4 (33.2-42.3) | 3250 (2220-4675) | 59.1 (40.0-85.0) | 30.6 (0.2) | 39.5 (35.1-44.0) |
| C. parapsilosis | Overall | 592 (94-1060) | 2.4 (0.8-3.2) | 500 (200-1475) | 2.5 (1.4-4.8) | 30.5 (0.5) | 7.9 (6.3-14.5) | 4350 (1650-7325) | 16.1 (9.9-26.8) | 28.9 (0.5) | 29.7 (21.1-70.4) |
| | Linen | 66 (52-83) | 1.5 (1.2-1.9) | 200 (100-300) | 4.5 (2.3-6.8) | 31.1 (0.6) | 25.5 (20.2-43.4) | 900 (525-1175) | 20.5 (11.9-26.7) | 29.4 (0.3) | 116.3 (90.8-137.4) |
| | Formica | 874 (840-966) | 2.6 (2.5-2.9) | 1800 (1425-2075) | 5.5 (4.3-6.3) | 30.3 (0.3) | 7.3 (6.3-8.7) | 9300 (8750-9900) | 28.2 (26.5-30.0) | 28.5 (0.2) | 33.4 (26.8-37.2) |
| | Aluminum | 1142 (1094-1180) | 3.5 (3.3-3.6) | 200 (100-400) | 0.6 (0.3-1.2) | 30.2 (0.1) | 7.9 (7.7-8.3) | 4650 (3800-5650) | 14.1 (11.5-17.1) | 29.0 (0.4) | 20.9 (15.7-30.3) |
| | HEPA | 246 (189-319) | 0.6 (0.4-0.7) | 1100 (650-1550) | 2.5 (1.5-3.5) | 30.4 (0.4) | 4.8 (4.0-6.9) | 4150 (3450-5175) | 9.4 (7.8-11.8) | 28.6 (0.2) | 21.1 (17.9-25.6) |

Conclusions

- qPCR-based detection and sampling by foam sponges were each associated with highest fungal pathogen recovery
- Future studies are needed to assess our study's identified optimized sample collection and detection techniques in a real-world healthcare environment.

