Contribution of a Cyclonic-Based Liquid Air Collector for Detecting *Aspergillus Fumigatus* by QPCR in Air Samples

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Analytical Performance Issues
Contribution of a Cyclonic-Based Liquid Air Collector for Detecting Aspergillus Fumigatus by QPCR in Air Samples

INTRODUCTION

Fungal infections represent a constant threat in hospitals, especially for high-risk patients hospitalized in hematology and bone marrow transplant units. While routine air sampling to predict the fungal contamination risk is discussed by some authors,1,2 French hospital guidelines specify that each hospital is responsible for the air it provides to patients and that air control efficiency must be monitored by specific methodologies.3

Traditional monitoring involves impacted culture media that are incubated for several days so that the fungal species may grow and be identified on the basis of macroscopic and microscopic criteria.4 A variety of portable air impactors are commercially available and validated,5,6 but these systems are limited by their link with culture techniques, involving a time-lapse to results of around 7 days and by a maximal sampling rate of 100 L/min.

As optimal environmental monitoring requires large sample sizes and fast, accurate detection of microorganisms contained within the samples,7,8 new techniques concurring with these characteristics are regularly evaluated. Quantitative polymerase chain reaction (QPCR) represents a very attractive alternative to culture techniques as it allows Aspergillus fumigatus, which is the preponderant etiologic agent of invasive aspergillosis, to be specifically detected in under 2 days. Reducing the time to results is key to permit intervention to prevent Aspergillus exposure in immunocompromised patients.

In this way, QPCR was previously used to amplify Aspergillus fumigatus DNA on various substrates, such as tap water,9 carpets,7 air filters,10 and impacted low-melt agar plates.11 A new device, the Coriolis µ air sampler (Bertin Technologies, Montigny, France), based on a cyclonic system, was recently proposed for collecting large volumes of air on liquid medium quickly. This device was previously assessed for different areas other than fungal aero-contamination monitoring: analysis of pollen grain distribution12,13 and detection of Pneumocystis jiroveci DNA by QPCR.14

A preliminary study was carried out to test this cyclonic-based liquid device, combined with QPCR, and to assess its performance for detecting A. fumigatus DNA.

MATERIAL AND METHODS

Comparison of the Two Sampling Devices

The characteristics (weight, battery autonomy, sound level, and ease of use) of the two air samplers (the MAS 100 impactor and the Coriolis µ air sampler) were compared to assess their respective practicality. Both devices allow sampling of both...
conidia (under 5 \( \mu \)m in size) and hyphal fragments, which vary markedly in size (5–100 \( \mu \)m).

**Air Sampling**

Air sampling was carried out in the corridors of the hematology units and in non-hematology areas during a period of 30 days with two devices:

1. 100 L of air was collected using a MAS 100 impactor (Merck, Darmstadt, Germany) on culture media, which is the reference technique used in standard routine environmental controls in our university hospital. With this system, the sampled air is blown through a perforated plate (400 holes of 0.7 mm in diameter) and is propelled on to a media plate with an impact speed of 10.8 m/s. Several other portable battery-powered air impactors are commercially available, and all present similar operational capability.[6]

2. 1.5 m\(^2\) of air was collected using a Coriolis \( \mu \) air sampler in 15 mL of sterile phosphate buffered saline (PBS) + 0.002% Tween 80. With this device, the air is drawn into the liquid in a whirling motion, pulling the particles against the wall of the tube by centrifugal force and separated from air to be concentrated into the liquid. For now, there is no other commercially available device that provides the same operational capability.

Thirty-three samples were taken simultaneously with the two devices (same time/same place). Fourteen samples were taken in corridors of the hematology units, and 19 samples were taken in non-hematology hospital areas. In all cases, both devices were placed 1 m from the floor.

**Detection of A. fumigatus by Culture Technique**

Impacted culture media were dichloran-18% glycerol (DG18) (Oxoid, Basingstoke, UK), incubated at 30\( ^\circ \)C, and malt agar extract (3%) (Difco, Detroit, Mich.) incubated at 37\( ^\circ \)C. Cultures were checked after 3 and 7 days of incubation. Fungal species were identified by macroscopic and microscopic examinations. Results were expressed in colony forming unit (CFU) per 100 L of air.

**Detection of A. fumigatus DNA by QPCR**

**DNA Extraction**

Rapid DNA extraction was performed as described previously,[15] with the following modifications: each liquid sample was centrifuged at 1300 g for 10 min, and the supernatant was carefully removed to leave a 1-mL pellet that was subsequently transferred into 2-mL screw-cap tubes prefilled with 1.4-mm ceramic beads. Samples were placed in a mini-bead beater (Magna Lyser; Roche Diagnostic, Meylan, France) three times for 10 sec, then heated in a boiling water bath for 10 min. Tubes were stored on ice for 10 min and then centrifuged in a micro-centrifuge (12000 g for 2 min). The supernatants above the beads were carefully removed and stored at \(-20^\circ\)C before QPCR analysis.

**QPCR**

DNA from *A. fumigatus* was detected using primers and Taqman probes as described previously by Haugland et al.[16,17] QPCR was carried out in a 20 \( \mu \)L final volume using the LightCycler FastStart Master HybProbe (Roche): 2 \( \mu \)L of Master Mix 1a/1b, 2.2 \( \mu \)L of MgCl\(_2\) (25 mM stock solution), 1.6 \( \mu \)L probe (AFUMI P1, 1 pm), 4 \( \mu \)L of each primer (AFUMI F1/R1, 5 pm), 0.2 \( \mu \)L of uracil DNA glycosylase (USB, Cleveland, Ohio), and 10 \( \mu \)L of sample extract. The thermal cycling conditions were: a denaturation step at 95\( ^\circ \)C for 10 min followed by amplification for 45 cycles at 60\( ^\circ \)C for 1 min.

Quantitative results were expressed as cycle quantification (Cq), which indicates the cycle during which fluorescence of the sample became exponential in comparison with the baseline signal. The Cq was inversely proportional to the amount of DNA in the sample: the higher the Cq, the less DNA in the sample. All samples were tested in duplicate and differences between two replicates never exceeded 1 Cq.

**Standard Curve for A. fumigatus**

A suspension containing \( 10^6 \) *A. fumigatus* conidia/mL (BCCM/IHEM 23670) was prepared after counting in Malassez cells. Six ten-fold dilutions of the initial suspension were prepared, and the dilutions were counted again. This process was repeated three times. The standard curve was established using the mean Cq of the three extracts obtained for each conidia/mL concentration.

**Specificity of the QPCR Assay**

The specificity of the QPCR assay was determined using conidia suspensions of *Lichteimia corymbifera*, *Aspergillus versicolor*, *Penicillium chrysogenum*, *Alternaria alternata*, *Stachybotrys chartarum*, *Eurotium* sp., *Aspergillus sydowii*, *Aspergillus penicillioides*, *Wallemia sebi*, *Penicillium brevicompactum*, *Penicillium olsoni*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus flavus*, *Penicillium variotii*, *Penicillium citrinum*, and *Penicillium crustosum* (\( 10^4 \) conidia/mL for each species).

**RESULTS**

**Comparison of the Two Sampling Devices**

The different characteristics of the two disposals used are presented in Table I. The Coriolis \( \mu \) air sampler was slightly less practical to use than the MAS 100; it was heavier, was not furnished with an adapted transport case, made more noise, and had shorter battery autonomy.

**Standard Curve for A. fumigatus**

QPCR results obtained for the *A. fumigatus* conidia suspensions showed that *A. fumigatus* conidia were detected at a concentration of less than 10 conidia/mL with a Cq of approximately 40 (Figure 1).
TABLE I. Differential Characteristics of the Two Disposals Used for Air Sampling

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MAS 100 Impactor</th>
<th>Coriolis µ Air Sampler</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>≈2 kg</td>
<td>≈3 kg</td>
</tr>
<tr>
<td>Physical resistance</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Practicality</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Sound level</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maximal airflow rate</td>
<td>100 L/min</td>
<td>300 L/min</td>
</tr>
<tr>
<td>Battery autonomy</td>
<td>7 hr</td>
<td>2 hr</td>
</tr>
<tr>
<td>Easiness to use</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

Specificity of QPCR

The QPCR was specific to *A. fumigatus*; no false-positive results were observed when using conidia suspensions from other fungal species.

Detection of *A. fumigatus* in Air Samples from Hematology Units

The 14 air samples from hematology units were negative for *A. fumigatus* with both techniques (culture and QPCR).

Detection of *A. fumigatus* in Air Samples from Non-Hematology Hospital Areas

**After Sampling with the MAS 100**

Ten of the 19 air samples from non-hematology hospital areas were culture positive for *A. fumigatus* after impaction with the MAS 100 with a mean and standard deviation of 2.11±3.13 CFU/100 L of air (Table II).

After Sampling with the Coriolis µ

Nine of the 19 air samples from non-hematology hospital areas were QPCR positive for *A. fumigatus* after sampling using the Coriolis µ with a mean and standard deviation of Cq of 39.38±1.09 (Table II).

Comparison of the Results Obtained Using the MAS 100 and the Coriolis µ

Twenty-three of the 33 samples (70%) were negative for *A. fumigatus* with both techniques (culture and QPCR). Nine of the 33 samples (27%) were positive with both techniques (detailed results in Table II). One of the 19 samples was positive in culture after impaction (with one CFU/100 L of air), whereas it was negative after liquid-based collection and QPCR analysis.

![Standard curve A. fumigatus](image)

**FIGURE 1.** Standard curve obtained for *A. fumigatus* conidia detection using the mean Cq of three independent extracts for each conidia/mL concentration.
DISCUSSION

The aim of this study was to evaluate the performance of a new air sampling device, the Coriolis µ, which is able to sample large volumes of air over a short period, uses a higher airflow rate than the MAS 100, and provides substrates for analysis techniques such as QPCR.

In this experiment, a good concordance was observed, in terms of positive and negative results for the detection of A. fumigatus, between the two devices. Although the number of samples of this preliminary assay was limited, the Coriolis µ seemed promising and could be an alternative for routine monitoring of fungal aero-contamination. Using QPCR makes it possible to react more quickly in terms of prevention and remediation and is particularly useful in cases of hospital building works.\(^{(10)}\)

It may be that by sampling larger air volumes (100 L with the MAS 100 compared with 1500 L with the Coriolis µ), more samples would have been positive with the Coriolis µ. This could be explained by the fact that 15 mL of liquid is necessary for sampling with the Coriolis µ to generate the cyclonic reaction. The liquid was centrifuged to concentrate the particles, but fungal spores are light and some may have remained in the supernatants. One solution for improving the quantification of A. fumigatus conidia in the air could be to reduce the volume of liquid used for sampling so that the totality of the air sampled would be extracted. However, such a solution is not yet possible because the cyclonic reaction cannot be created with less than 15 mL of liquid medium. Another solution to concentrate the sample could be to either filter the 15 mL sampled and to extract the filter or to use ultra-centrifugal filter units.

In conclusion, the Coriolis µ could be an interesting alternative to culture techniques and allow A. fumigatus DNA to be detected quickly in air sampled by QPCR.

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REFERENCES


