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Short Communication

Can *Aspergillus fumigatus* conidia cause false-positive results in the galactomannan enzyme immunoassay test?

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Abstract

Introduction: Several factors can cause false-positive results in the galactomannan (GM) test; however, others remain unknown. Presently, the impact of airborne contamination by *Aspergillus* conidia during enzyme-linked immunosorbent assay (ELISA) remains uninvestigated. **Methods:** We studied 12 *A. fumigatus* isolates. Fungal conidia were serially diluted and tested for GM detection using the Platelia® *Aspergillus* enzyme immunoassay (EIA). **Results:** The conidia concentration required for an EIA-positive result was 4.8×10^3 (median). **Conclusions:** This is the first study to evaluate the impact of environmental contamination on the Platelia® *Aspergillus* EIA assay. Only massive contamination can interfere with GM optical readings, suggesting that environmental contamination does not cause false-positive test results.

Keywords: Invasive aspergillosis. Diagnosis. Platelia® *Aspergillus* EIA. Galactomannan. Air quality.

Galactomannan (GM) is a hydrosoluble polysaccharide derived from the fungal cell wall. Accordingly, GM detection plays an important role in the diagnosis of invasive fungal diseases, particularly invasive aspergillosis, since *Aspergillus* species produce large amounts of this antigen[1,2]. One of the main limitations of GM testing, however, is the frequent occurrence of false-positive results (up to 10%), mostly due to the use of antibiotics from fungal origin, mucositis, and dialysis, in addition to cross-reaction with other fungal infections[3-5].

Platelia® *Aspergillus* enzyme immunoassay (EIA) GM assay is highly sensitive (limit of detection in serum of 0.5ng/mL)[6,7]. However, when considering that the air quality of indoor facilities, which may contain dozens to hundreds of *Aspergillus* conidia per square meter[8], we wonder whether environmental contamination could be an additional source of false-positive results in the GM test. Thus, the aim of this study was to determine whether low-concentration contamination with *Aspergillus fumigatus* conidia could result in positive GM readings in the Platelia® *Aspergillus* EIA assay.

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Twelve *A. fumigatus* isolates from the Mycology Laboratory of the Faculty of Medicine of the Federal University of Rio Grande (FURG, Brazil) were used in the study. Isolates were obtained from patients with invasive aspergillosis (n = 3) and Magellanic penguins (n = 3). In addition, environmental isolates (n = 3) and reference *Aspergillus* strains were obtained (AF10, AF71 and AF13073, kindly provided by Prof. David W. Denning, National Aspergillosis Centre, UK).

To obtain young colonies, subcultures of the isolates were carried out in potato dextrose agar (PDA) at 25°C for 48 hours. Sterile saline solution (0.85%) supplemented with 200µL of Tween 80 were added to the cultures, and a scraping of the surface of the colonies was carried out to obtain the solution of fungal propagules. After 30 min, the suspension was filtered using a sterile double layer of gauze to retain the higher particles, ensuring that only conidia remained. Conidia suspensions were adjusted to 80-82% transmittance (absorbance of 0.09-0.11) by spectrophotometry (700S FEMTO®) at 530nm. Subsequently, a 1:50 dilution in sterile saline solution was performed according to the protocol described by the Clinical & Laboratory Standard Institute (CLSI)[9]. The amount of conidia in each inoculum was determined by the Pour Plate technique, in which results were expressed in colony forming units/mL (CFU/mL).

Three serial dilutions (1:10) of the standardized inoculum were tested for GM using a commercial kit (Platelia®

Aspergillus EIA, according to the manufacturer's instructions). Positive, negative, and cut-off controls were incorporated into each assay. GM results were expressed as optical densities (ODs), and samples were considered positive if the GM index was > 0.5. All experiments were performed in duplicate. Data were compiled, and statistical analysis (descriptive analyses and Kruskal-Wallis) was performed using the SPSS® 20.0 program.

Table 1 shows the inoculum standardization in solution, ranging from 1.6×10^6 to 6.7×10^7 CFU/mL. Considering the volume of inoculum used in each well (300µL) for the test, these concentrations were calculated to determine the conidia amount in each tested well by multiplying by 0.3 and the corresponding dilution (10^{-1} to 10^{-3}). Therefore, the median conidia concentration required to generate a positive result in the Platelia® *Aspergillus* EIA test was determined as 4.8×10^3, ranging from 4.8×10^2 to 2×10^6. The amount of conidia required for positive GM readings did not correlate with the origin of the isolates (kw = 0.082), with medians of 3.6×10^3 (humans), 6×10^3 (penguins), 1.2×10^3 (environmental) and 5.4×10^4 (reference strains). GM indices for those isolates showed minimal conidia concentrations, ranging from 0.519 in an environmental strain (concentration = 1.2×10^3) to 3.57 in a penguin aspergillosis strain (concentration = 1.2×10^6) (**Figure 1**).

To our knowledge, this is the first study to experimentally determine the required amount of *A. fumigatus* conidia to produce a positive test result in a GM reaction. We demonstrated that at least 500 conidia (4.8×10^2 to 2×10^6) of *A. fumigatus*

TABLE 1: Results of the inoculum standardization following the CLSI protocol for 12 *Aspergillus fumigatus* isolates used in this study.

Strain	Origin	Inoculum (CFU/mL)
AF13013	Reference strain	6.7×10^7
AF71	Reference strain	1.8×10^7
AF10	Reference strain	2.8×10^6
M1270	Human aspergillosis	1.2×10^7
M1437	Human aspergillosis	2.6×10^6
M1834	Human aspergillosis	3.1×10^7
C33	Penguin aspergillosis	1.1×10^7
C90	Penguin aspergillosis	2.0×10^6
C272	Penguin aspergillosis	4.1×10^7
PL2	Environmental isolate	2.0×10^6
PL3	Environmental isolate	9.0×10^6
PL63	Environmental isolate	1.6×10^6

CLSI: Clinical and Laboratory Standard Institute; **CFU**: colony forming unit.

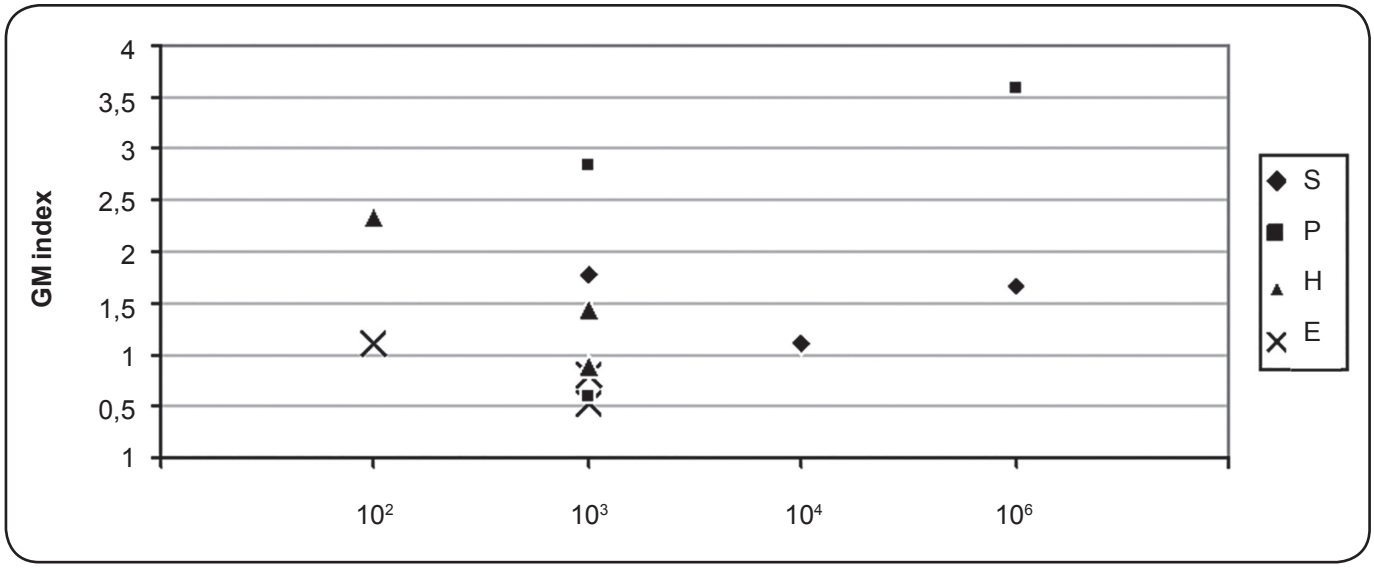

FIGURE 1: GM index at the lowest *Aspergillus fumigatus* conidial concentration, which causes positive results in the Platelia® *Aspergillus* EIA (n = 12). **S**: standard strains; **P**: strains from penguin aspergillosis; **H:** strains from human aspergillosis; **E**: environmental strains; **GM**: galactomannan; **EIA**: enzyme immunoassay.

are necessary to generate a positive result in the test (GM index above 0.5). Therefore, false-positive GM results due to this factor would require massive environmental contamination, which is not likely to occur in most clinical laboratories[10].

We observed a wide variation in the amount of conidia required for a positive GM test result. Since *Aspergillus* hyphae release far more GM than *Aspergillus* conidia, strain-related differences in germination could explain these findings[11,12]. Differences in GM release have already been described between and within *Aspergillus* species, including in the same strains of *A. fumigatus* used in our study[13,14].

Data found in our study contribute to the interpretation of Platelia® *Aspergillus* EIA results, demonstrating that the risk of a GM false-positive test result due to environmental contamination is low when performed following basic laboratory safety standards. Data of our study refer only to contamination by *Aspergillus* conidia, which is a limitation as other anemophily fungi (such as *Penicillium* and *Fusarium*) can also produce GM[15] and were not tested in this context. Further studies are required to confirm or discharge the interference of other microorganisms in the Platelia® *Aspergillus* EIA.

Conflict of interest

The authors declare that there is no conflict of interest.

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