



Monitoring *Aspergillus fumigatus* aerosols from a composting facility

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Abstract

The large, outdoor Islip Yard Waste Composting Facility on Long Island, New York was investigated as a source of airborne fungus spores. The Burkard-Hirst volumetric spore trap was used for the first extensive sampling of small mold spores for this application. Samplers were operated continuously from 21 August to 30 November 1992 in the facility and in a suburban community about 540 m from the facility. A control site approximately 10 000 m from the facility was also sampled to establish background levels of fungus spores. The facility site had higher average readings of *Aspergillus fumigatus* spores than did the community and both were higher than the control. *A. fumigatus* was the only fungus among 30 categories tracked that differed significantly between the facility and control sites. It was also isolated repeatedly from the compost. Higher average levels of *A. fumigatus* were measured in the community when winds blew from the facility, and also during times when the compost was moved or mixed at the facility. No correlation was found between wind direction or work times and *A. fumigatus* conidia at the control site. The study shows that this compost facility can produce a measurable increase in the number of airborne *A. fumigatus* conidia both at the edge of the facility and at 540 m downwind. It also demonstrates that the Burkard spore trap can be used for monitoring small, airborne mold spores, but it is a difficult and labor intensive task.

Keywords: Airborne spores; *Aspergillus fumigatus*; Burkard sampler; Hirst sampler; Compost; Fungi

1. Introduction

This study is to determine the kinds and amounts of fungus spores released by The Islip Yard Waste Composting Facility on Long Island, New York, and to see if the spores reach a nearby residential community, and if wind velocity, wind direction, and agitation of the compost affect their concentrations. It is also a test of using the Burkard-Hirst spore trap for quantifying small, airborne mold spores. The study presented here was part of a larger project attempting to correlate airborne fungus and actinomycete spore fluctuations with symptoms recorded by volunteers (New York State Department of Health, 1994). The present paper is restricted to the measurement and identification of airborne fungus spores from compost, and it concentrates on the most prevalent of compost fungi, *A. fumigatus*.

Composting is increasingly utilized by municipalities to reduce the amount of material sent to landfills. The densely populated township of Islip on Long Island operates one of the largest outdoor composting facilities

in New York. When it commenced operations in 1988, it processed 50 000 tons of lawn clippings and leaves into soil for landscaping. It soon became a victim of its own success when residents of a nearby community complained of odors. By 1992, the amount of material processed at the facility was reduced to 25 000 tons by encouraging home composting, but the odor problem persisted. The residents of the nearby community became worried about health effects from the compost, and numerous complaints of fevers, rashes, headaches and other symptoms were received by authorities.

The site available to us for the study is almost 200 miles from our laboratory and it was decided that living samples on agar media like those generated by Andersen samplers would have to be changed several times a day. They would be too awkward to transport, and would require greater laboratory equipment than we had at our disposal, so the decision was made to use a Hirst-type spore trap sampler, the Burkard. Several good studies of airborne spores from different kinds of compost have been made with Andersen samplers (Millner et al., 1977, 1980; Passman, 1983), but to our knowledge, the Burkard had not been used specifically for the small

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mold spores encountered from compost. The advantages of the Burkard are that it does not require media or the growth of hazardous fungi in the laboratory, the sample is changed weekly, samples can be mailed or stored before analysis, and the sample is a long-lasting slide preparation that can be reexamined at any time. The sample is representative of the kinds of particles capable of being inhaled in that it includes both biological and non-biological, viable and non-viable particles. Disadvantages of the Burkard are that skill and practice are needed to identify small spores and that analysis is very labor intensive.

Kane and Mullins (1981) and Millner et al. (1977)

examined sewage sludge compost fungi and found *Aspergillus fumigatus* Fresenius to be among the most frequently isolated fungi, and Millner et al. (1980) measured high levels of *A. fumigatus* immediately downwind from a Beltsville, MD sewage sludge compost facility by using six stage, Andersen viable samplers running for 2–30 min per sample. Millner et al. also found airborne *A. fumigatus* to be strongly elevated when the compost was mechanically moved.

Our study differs in having a continuous record of all airborne particulates over a 100-day period at two sites near a yard waste composting facility plus a control site 10 000 m downwind to determine background fungi.

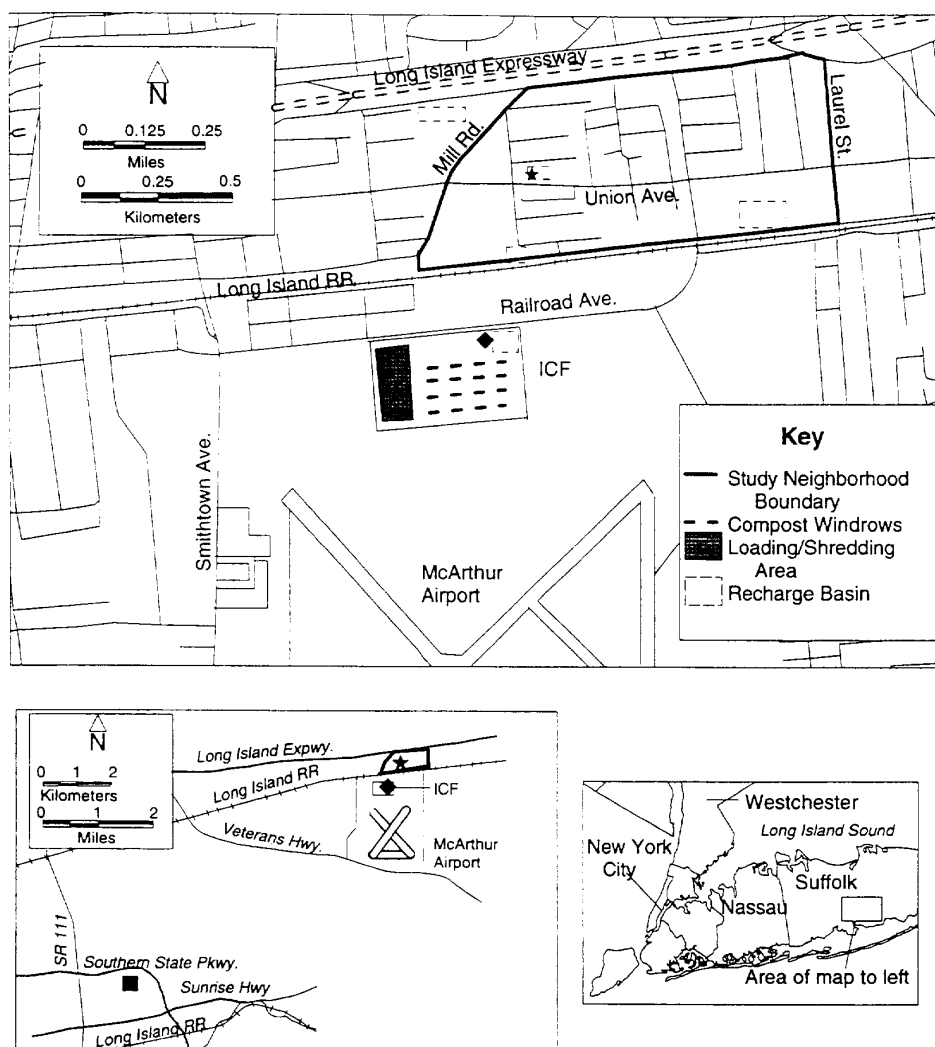


Fig. 1. Long Island, New York sites showing location of samplers; ♦, Isip Yard Waste Composting Facility, 'compost facility'; ★, Union Avenue in the village of Ronkonkoma, 'study community'; ■, Fisher Avenue in the village of Islip Terrace, 'control site.' From NYSDOH report (1994).

2. Materials and methods

2.1. Compost

The Islip Yard Waste Composting Facility (Fig. 1) is located at the northern edge of the partially abandoned MacArthur Airport near the southern shore of Long Island. It took in about 25 000 tons of organic material during 1992, the year of the sampling. About two-thirds of it was leaves and brush and about one-third was grass clippings. Natural composting without seeding, inoculation, or additives takes place in open windrows occupying approximately 43 acres. The piles reach 60°C through self heating from microbial growth. Aeration is accomplished by mechanically mixing the piles when their internal temperatures become elevated. Mixing is accomplished by large machines that move along the pile and mix the compost with rotating blades like those of a garden cultivator. Though efficient because the machines can operate almost continuously, the process creates a visible organic aerosol. The composting area on the facility is surrounded by an earthen berm about 30 feet high, on the outside of which is planted a thin row of pines.

2.2. Sampling

To determine the fungal content of the compost, eight samples were taken from 10- to 20-cm depths from the compost windrows at different stages in the composting process. The samples were diluted with sterile, distilled water and plated onto Sabouraud's media with cycloheximide added and incubated at 44°C.

Air sampling was done using four Burkard 7-day recording volumetric spore traps run on 110-volt house current. Each was mounted on a wooden platform so that the sampling orifice was 1.5 m above the ground. All the samplers were placed in open areas as far from trees, herbs, and shrubs as possible. One sampler, referred to as 'compost facility,' was located on top of the 10-m high berm at the northeast (downwind) corner of the compost facility approximately 100 ft from the nearest windrow (Fig. 1). This location was as close as we could place a sampler to the compost without interfering with the facility machinery. A second sampler, referred to as 'study community,' was placed in the nearest community at about 540 m north-northeast of the facility in the direction of the prevailing winds. The sampler used to establish background levels of spores, referred to as 'control,' was placed in a neighborhood similar to the study community but located about 10 000 m to the southwest of the facility.

Once every 7 days, each sampler drum was replaced with one with a fresh sample tape. Melinex tape provided by the Burkard Company was used with Thomas Lubri-Seal® as an adhesive. The adhesive was laid on as thin as possible with a laboratory tissue moistened with toluene so that the grease was just capable of showing a fingerprint when touched lightly.

Extra sampler drums were used so that the completed samples could be shipped to the laboratory by express mail and newly prepared drums could be sent to the sample site for installation in the Burkard. When a new drum was installed, a mark was left at the beginning of the tape by placing a scribe through the sampling orifice and drum number and exact time recorded on a log sheet that accompanied each drum. Once at the laboratory, the tapes were removed, cut into seven 24-h segments, mounted on microscope slides with unstained lactophenol mountant, and sealed with fingernail polish. By finding the starting mark on the tape under the microscope and aligning it with the center line in the eyepiece reticule, the number on the vernier scale on the microscope stage could be used to find the exact locations of 06:00, 12:00, 18:00, and 00:00 h by adding 2 mm for each hour onto the start time recorded on the sample from the log sheet. The sample at each of those four times was examined by making one lateral transect of the tape with the $\times 100$ phase objective of a good quality microscope. By counting all the spores in the optical field of the microscope of known diameter, the area can be calculated and transformed to spores per m^3 using a formula supplied by the Burkard Company. As a check on the lateral transect counts, several days were counted at hourly intervals to find how the counts fluctuated throughout the day. As many as five counters were employed during the course of the study, but most of the work was done by two employees. The best counters were not necessarily trained in mycology, but were careful observers. Frequent comparisons with known samples and close cooperation, frequent cross checks, and second opinions between counters are essential to obtain consistent results. All counts were made without the counter knowing the origin or significance of the sample so as to avoid bias.

Recognition of the spores (conidia) of *A. fumigatus* was done by comparing the spores in the sample with those from a known culture. The criteria used to make a positive identification are that it must be a spherical spore close to 2.5 μm , and may have faint cinereous pigmentation, slight surface ornamentation which appears as minute dots at the perimeter, and sometimes a large, flat projection where the spore attaches to the spores below it. Many times the spores are in short chains or in small groups adhered together. More extensive information of the identification of *A. fumigatus* spores in air samples will be the topic of a future publication.

The sample was recorded on a digital counter with 30 categories able to accommodate all types of fungus spores. Some categories were very broad and inclusive, like 'hyaline basidiospores,' while others were specific, like '*Aspergillus fumigatus*' and '*Ganoderma*.' All records were made as individual spores and translated to spores/ m^3 . Spores/ m^3 is not the same unit of measurement as colony forming units (CFU)/ m^3 used to quote the results of live trap sampling, in which each colony

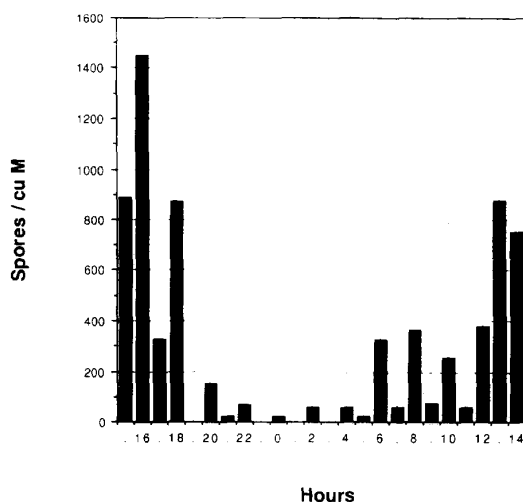


Fig. 2. Levels of *Aspergillus fumigatus* in spores/m³ at the Islip Compost Facility for the period from 15:00 h Thursday, 15 October to 14:00 h Friday, 16 October 1992.

may be derived from a single spore or a cluster of spores and non-viable spores are not measured.

All of the spore counts were entered into a database along with the date of the sample, starting time of the sample, sampler location, date of the count, and the

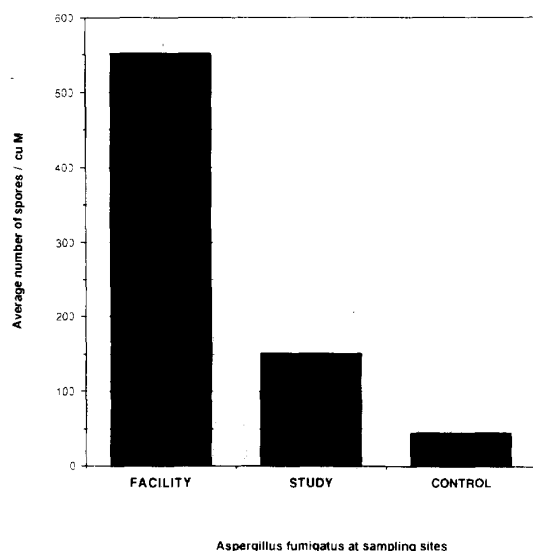


Fig. 3. Average of all spore counts of *Aspergillus fumigatus* expressed in spores/m³ for three Long Island sites over the period 21 August–30 November 1992. 'Compost', Northeast corner of Islip Yard Waste Compost Facility; 'Study', Residential area 600 m northeast (direction of the prevailing wind) of the facility; 'Control', Residential area 10 000m southwest of the facility.

name of the person making the count. A separate database of wind direction and wind speed entered as a four-digit code where the first two numbers indicate compass direction of the wind and the last two the speed in mph was compiled. A weather code to indicate rain and visibility was also entered.

3. Results

Direct plating of compost onto Sabouraud's agar demonstrated *A. fumigatus* to be the predominant fungus in all eight samples. *A. Rhizomucor* species was also present in two of the samples and *Humicola* species in one.

Airborne fungal spore categories found at all three sites listed in order of magnitude, include: 'hyaline basidiospores,' *Cladosporium*, *Ganoderma*, *Coprinus*, *Aspergillus fumigatus*, and *Alternaria*, plus many others in lesser amounts. Although *A. fumigatus* was found at all three sites, it had a much higher average concentration at the facility site (552 spores/m³) than at the control site (44 spores/m³) and the concentration at the study community (151 spores/m³) was in between the two (Fig. 3). Only *A. niger* had a similar pattern of occurrence, but in much lower concentrations. *Humicola* species were found in the air samples, but in too small numbers to compare between the sample sites.

To find whether work days at the facility, or when the piles of compost were turned, had greater concentrations of airborne *A. fumigatus* spores than Sunday, average counts were separated by day of the week (Fig. 4). Sunday had lower concentrations than other days at the study community and the compost facility, but not at the control site.

A distinctive diurnal pattern of *A. fumigatus* at the compost facility and the study community was observed. The pattern observed for 15–16 October 1992 (Fig. 2) with highest levels during daylight (working) hours, as opposed to night-time (nonworking) hours, is typical.

The influence of wind direction on the prevalence of *A. fumigatus* spores at the study community site was examined by comparing wind direction with spore counts (Table 1). The greatest concentration of spores was found in the winds from the south, south-west and west when the wind vector was from the compost to the study community. Generally only background concentrations of < 100 spores/m³ were seen in winds from the north and east. Occasionally high concentrations of *A. fumigatus* were seen in north winds in gusty conditions, possibly due to mixing from turbulence. Concentrations of *A. fumigatus* from the sampler on the north-east corner of the compost facility (Table 1) demonstrated an even higher correlation with south, south-west, and west winds than at the study community. *A. fumigatus* at the

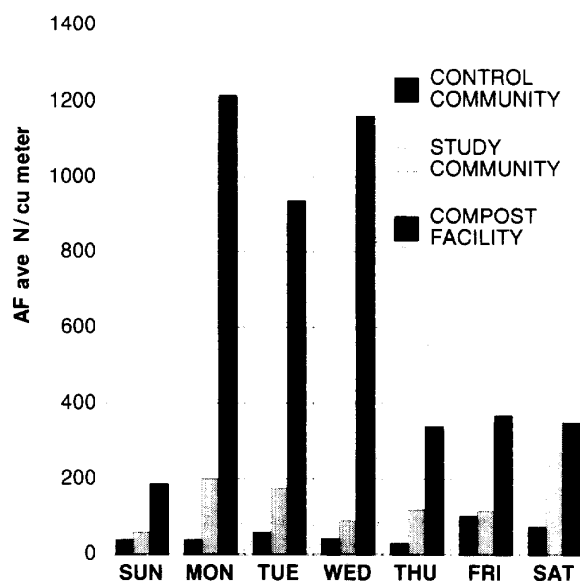


Fig. 4. Average concentrations of *Aspergillus fumigatus* expressed in spores/m³ by day of the week for the period 21 August–30 November 1992 for the 'compost,' 'study,' and 'control' sites.

control community did not differ significantly with wind direction.

When the winds were from the north and east, the spore populations were more similar than when the winds were from the south and south-west because the spores from the north and east are background spores only and not compost generated spores (Table 3). The low *P*-values for the south and south-west winds are due

Table 1
Variation in *Aspergillus fumigatus* spores/m³ vs. wind direction

	Wind direction				
	North	East	South	South-west	West
Compost facility					
Average	111.5	119.7	884.1	941.2	919.4
S.D.	279.8	242.2	1960.9	2210.8	3009.65
Maximum	2119	1048	9429	16 833	18 548
N	87	39	63	94	70
Study community					
Average	88.1	28.6	205.9	191.8	131.2
S.D.	273.5	79.4	393.9	362.2	269.7
Maximum	1595	310	2452	2452	1500
N	86	39	64	93	68
Control community					
Average	64.1	36.6	49.5	74.7	75.0
S.D.	172.3	69.4	81.2	167.2	194.8
Maximum	1000	214	310	1111	1111
N	59	28	49	66	45

Table 2

Variation in *Aspergillus fumigatus* spores/m³ vs. wind velocity

	Wind velocity (mph)			
	1–5	6–10	11–15	> 15
Compost facility				
Average	211	530	813	2139
S.D.	513	1815	2883	4097
Maximum	2278	16 833	18 548	9429
N	70	139	43	5
Study community				
Average	83	117	524	168
S.D.	167	326	2218	159
Maximum	778	2452	14 204	333
N	68	141	41	5
Control community				
Average	62	62	12	119
S.D.	171	145	25	168
Maximum	1000	1111	71	238
N	54	105	19	2

to more *A. fumigatus* spores arriving at the Facility and Study samplers from the compost which is located south and south-west from both samplers' location. The influence of wind velocity on the prevalence of *A. fumigatus* was studied by comparing weather data and spore concentrations (Table 2). At the compost sampling site, the concentration of *A. fumigatus* was double when winds were 6–10 mph as compared to when winds were 1–5 mph, and increased about 10-fold when the winds were >15 mph. It is not yet clear whether high winds facilitate spore take-off or delivery to the samplers.

Even though the average spores/m³ of *A. fumigatus* at all the sampler sites ranged between 44 and 552 for the period 21 August–30 November 1992 (Fig. 3), many of the four-time daily spore counts revealed no *A. fumigatus* at all. This occurred in 133 out of a total of 202 or 66% of the counts from the control community, 170 out of a total of 281 or 61% of the counts from the study community, and 119 out of a total of 280 or 43%

Table 3
P-values for *t*-test comparisons

Wind direction	Facility–Study	Facility–Control	Study–Control
North	0.377	0.244	0.339
East	0.108	0.205	0.674
South	0.017	0.009	0.018
South-west	0.001	0.002	0.015
West	0.015	0.011	0.181
Total data	0.003	0.002	0.039

of the counts for the compost facility. The facility had the lowest number of samples in which no *A. fumigatus* was detected. Conversely, high counts were more prevalent from the compost facility, lowest from the control community with the study community in between. *A. fumigatus* counts of > 100 spores/m³ occurred in less than 14% of the control community, 22% of the study community, and 39% of the compost facility samples. *A. fumigatus* counts > 500 spores/m³ demonstrated an even more pronounced difference between the sites with 17% of the compost facility, 7% of the study, and only 2% of the control community counts measuring more than 500 spores/m³. It is the greater number of high counts that accounts for the major differences in the averages between the sites.

4. Discussion

Growth of *A. fumigatus*, *Rhizomucor*, and *Humicola* from compost is not surprising, as they have all been reported before (Kane and Mullins, 1973; Millner et al., 1980). Concentration on *A. fumigatus* in the study is justified on several grounds. It is the most prevalent species isolated from the compost. It is the only fungus spore strongly correlated with the compost facility location and providing sufficiently large numerical samples to perform analyses, and it is a well-known human pathogen.

A. fumigatus is an extremely widespread fungus, found in air samples almost everywhere but usually in small concentrations. Large amounts of total average airborne *A. fumigatus* spores were measured at the facility site (552 spores/m³ as compared with the control site 44 spores/m³) (Fig. 3). This difference implicates the facility as the source of the spores. The counts from the sampler at the northeast corner of the facility were much higher when winds were from the southwest (Table 1), when the winds passed over the compost and blew towards the sampler. This also suggests the facility as the source. Samples at the study community site had a similar pattern but were at lower levels of concentration (151 spores/m³) (Fig. 3). There was no strong correlation between wind direction and *A. fumigatus* at the control site (Table 1) which may indicate that there is no large, nearby source and that there is no large source of *A. fumigatus* coming to the general region from the southwest. The higher amounts of *A. fumigatus* on work days (Monday–Saturday) as opposed to non-work days (Sundays) provide further evidence that the facility is the source and that the mechanism for the spores getting into the air is mechanical agitation.

Use of the Burkard or similar Hirst-type (continuous, volumetric) air sampler has some very distinct advantages over viable samplers, but the samples are still very difficult and time-consuming to analyze. The size of *Aspergillus* spores and the differences in color, size, and

ornamentation between the spores of different species make it very difficult to identify *A. fumigatus* with absolute accuracy. Fortunately, the samples in the present study contained an abundance of *A. fumigatus* so it was easy enough to pick out. Another advantage of the Burkard-Hirst is that it is possible to scan the image of the sample trace from the tape and convert it into a database which represents the changes in total particulates over the time of the sample.

The relevance of our counts of *A. fumigatus* to public health is published in the State of New York Department of Health Report (1994). Although the study demonstrated 2–4 times the level of *A. fumigatus* spores in the study community as compared with the control community, no positive relationship between spores and allergy and asthma symptoms reported by questionnaire was demonstrated.

During this study, 21 August–1 December 1992, the compost facility was supplied with leaf and twig material. This was mixed with wood chips and was the principle nutrient for fungal growth. Seasonal temperature changes from the warmth of late summer to the cold weather of early winter moderated growth and fungal spore production.

5. Conclusions

The Burkard-Hirst type sampler proved to be useful to measure *A. fumigatus* for this study of a large outdoor composting facility. It had the additional advantages of quantitatively capturing other spores and particles, and requiring very little maintenance and easy once a week sample changes. Its distinct disadvantage is the large amount of labor and skill needed to analyze the samples and the fact that spore recognition is ultimately a subjective process. The evidence that we did, indeed, correctly recognize the spores of *A. fumigatus* is in the reasonably good fit of our spore counts to our predictions of where we thought spore concentrations would be the highest. There were more *A. fumigatus* spores counted in our samples on workdays as opposed to Sundays, and more when the wind was blowing from the compost to the sampler. In conclusion the Hirst-type sampler is very easy and convenient to take samples at compost sites, but the samples are analyzed only with great effort and high cost. If immunofluorescent antibody or other molecular techniques could be developed to identify *A. fumigatus* spores on the sample tapes this could become the preferred technique.

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