Analysis of *Aspergillus spp*. burden by culture- based and molecular methods in different occupational environments: what needs to be done?

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Purpose

Fungal burden has traditionally been detected by conventional culture analysis, which despite its limitations, is widely used by the scientific community. Alternatively, quantitative real-time PCR (qPCR), based on the amplification of genomic regions specific to certain fungal species, has been associated with increased sensivity, allowing the detection of dormant forms of fungi, such as spores.

We present several studies where both methods were used to detect the presence of toxigenic fungi, namely *Aspergillus*, particularly from the *Fumigati*, *Flavi* and *Circumdati* sections.

Materials and Methods

Several matrices, such as air and surfaces were either subject to culture analysis or molecular biology detection. For culture analysis, extracted material was streaked onto MEA and DG18. After incubation at 27 ºC for 5 to 7 days, *Aspergillus* spp. densities (colony-forming units, CFU/m^3/ m^2) were calculated, and *Aspergillus* sections were identified through macro and microscopic characteristics. The molecular detection of the *Aspergillus* sections *Fumigati, Flavi* (only the toxigenic strains) and *Circumdati* was performed by Real Time PCR (RT-PCR).

Table I - Sequence of primers and TaqMan probes used for qPCR

Aspergillus sections targeted	Sequences
***Flavi* (Toxigenic Strains)**	
Primer Forward	5'-GTCCAAGCAACAGGCCAAGT-3'
Primer Reverse	5'-TCGTGCATGTTGGTGATGGT-3'
Probe	5'-TGTCTTGATCGGCGCCCG-3'
Fumigati	
Primer Forward	5'-CGCGTCCGGTCCTCG-3'
Primer Reverse	5'-TTAGAAAAATAAAGTTGGGTGTCGG -3'
Probe	5'-TGTCACCTGCTCTGTAGGCCCG -3'
Circumdati	
Primer Forward	5'-CGGGTCTAATGCAGCTCCAA-3'
Primer Reverse	5'-CGGGCACCAATCCTTTCA-3'
Probe	5'-CGTCAATAAGCGCTTTT-3'

Results and discussion

Air samples were isolated from different settings, including 2 wastewater plants, 1 wastewater elevation plant, 4 waste treatment plants, 3 cork industries, 5 slaughter houses, 4 feed industries, 1 poultry pavilion and 2 swine pavilions. 125 air samples were subject to conventional analysis, while 100 air samples were analysed by real-time PCR detection of *Aspergillus* sections *Circumdati, Flavi and Fumigati.*

All settings presented sampling sites where detection of specific species/ strains was possible but in some cases could not be identified by conventional methods. qPCR analysis successfully amplified DNA from the *Aspergillus* section *Fumigati* in 18 sampling sites where culture base-methods could not identify this species (1). Table II sumarises the results obtained in some of the settings analysed.

Table II - Isolates quantification and molecular detection from *Aspergillus* section *Fumigati* in the assessed occupational environments.

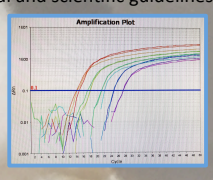
WWTP2	Air (CFU/m^3)	Surfaces (CFU/m^2)	Real-time PCR (Ct, cycle threshold)
Lift station	-	-	n.d.
Flotation sludge	-	-	32.85
Sludge dewatering	-	-	n.d.
Screening	-	10000	n.d.
Cogeneration	-	-	31.42
Aerobic digestion	-	-	35.91
Cantine	40	10000	-
Control room	-	-	-
WTP sorting1	**Air (CFU/m3)**	**Surfaces (CFU/m2)**	**Real-time PCR (Ct, cycle threshold)**
Package reception	980	500	29.07
Sorting cabinet 1	100	30000	28.69
Sorting cabinet 2	120	500	27.49
Sorting cabinet 3	-	500	27.78
Sorting cabinet 4	-	40000	27.21
Sorting cabinet 5	-	500	27.89
Sorting cabinet 6	-	150000	32.02
Circulation zone	-	-	30.33
WTP sorting2	**Air (CFU/m3)**	**Surfaces (CFU/m2)**	**Real-time PCR (Ct, cycle threshold)**
Alveoli	6	-	36.97
Waste without Sorting cabinet	-	-	38.11
Waste with sorting cabinet	-	-	n.d.
WTP composting	**Air (CFU/m3)**	**Surfaces (CFU/m2)**	**Real-time PCR (Ct, cycle threshold)**
Maturation park	-	30000	29.67
Waste screw	60	-	34.97
Workshop	-	-	37.07
Room process control	-	10000	33.02
Pretreatment	160	-	32.56
Centrifugues	20	300000	37.41
WTP incineration	**Air (CFU/m3)**	**Surfaces (CFU/m2)**	**Real-time PCR (Ct, cycle threshold)**
Boiler - 1	140	80000	32.38
Boiler - 2	-	50000	30.82
Control room	-	20000	34.8
Elevator	60	-	38.74
Weighbriges room	100	30000	31.04
Worksop	260	30000	34.51
Briges room	40	-	36.1
Slaughterhouse swine1	**Air (CFU/m3)**	**Surfaces (CFU/m2)**	**Real-time PCR (Ct, cycle threshold)**
Swine bleeding	-	-	-
Meat cutting	-	-	35.0
Swine gutting	-	-	-
Slaughterhouse large animal	**Air (CFU/m3)**	**Surfaces (CFU/m2)**	**Real-time PCR (Ct, cycle threshold)**
Bovine line	-	-	n.d.
Paws room	-	-	35.85
Heads room	-	-	n.d.
Gut room	-	-	n.d.
Expedition	-	-	34.47
Barn	-	-	-
Slaughterhouse poultry	**Air (CFU/m3)**	**Surfaces (CFU/m2)**	**Real-time PCR (Ct, cycle threshold)**
Birds hanging	-	-	n.d.
Reception	-	-	n.d.
Stacker	10	-	35.7
Bleeding	30	-	35.92
Evisceration	10	-	36.52
Cutting	-	-	n.d.

Conclusions

Upon comparing conventional and molecular analysis of *Aspergillus* spp. detection, we came to the conclusion that the ideal scenario is to use these two methods in parallel, as they complement each other to provide useful information for the assessment of exposure to *Apsergillus* spp.

Future work

Molecular analysis has de added value of quantifying specific *Aspergillus* species. This can be achieved by performing calibration curves, which are currently under development (Figure 1A and 1B). Furthermore, the establishment of these standard curves will ultimately allow the correlation between copy number (obtained by qPCR) and CFU/m^3, the reference measure used by technical and scientific guidelines.



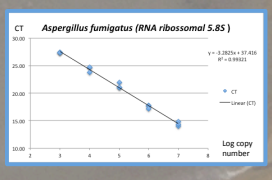


Figure 1- A) Amplification curves of 5.8S ribossomal RNA from serial diluted DNA from *Aspergillus fumigatus spp*. B) Linear regression of log copy number versus CT

References

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