MOLECULAR TECHNIQUES APPLIED TO AEROBIOLOGICAL MONITORING

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PURPOSES AND INTRODUCTION

DOUBLE PURPOSE FOR OUR MICROBIOLOBY LABORATORY:
Use of target DNA amplification (Real Time PCR) for

- Qualitative and quantitative determination of pollen grains and spores (*Alternaria* spp and *Cladosporium* spp)
- Biodiversity determination on the aerobiological monitoring slides

Real Time PCR ADVANTAGES:

- ✓ Less time needed for the analysis;
- ✓ Potentially high sensitity and specificity;
- ✓ It shows the presence of microorganisms in the sample, regardless of their ability to grow on a cultural medium or not (fungal spores).

coltural techniques do not allow all fungal species to grow

WHEREAS

AND

through microscopical analysis, identification is possible at genus level only

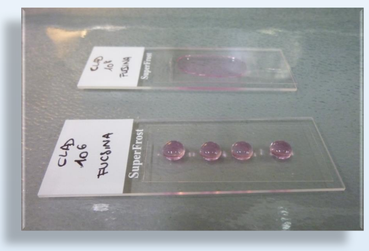
MATERIALS AND METHODS

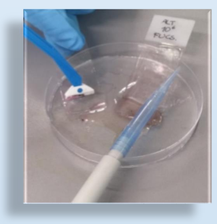
PCR was carried out

on *Alternaria* spp and *Cladosporium* spp samples, cultured on Petri dishes

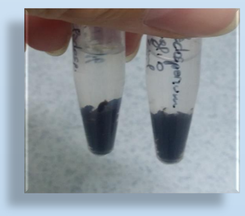
on hazel (*Corylus avellana*) pollen, directly collected from the inflorescences

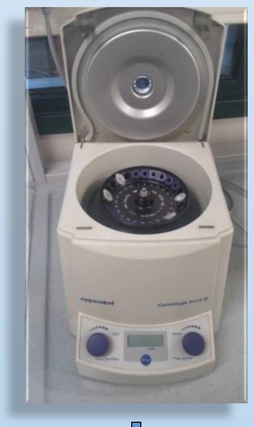
on aerobiological monitoring slides

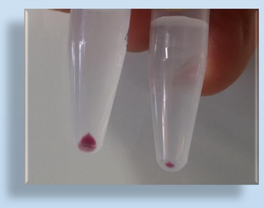




DNA extraction using CTAB technique



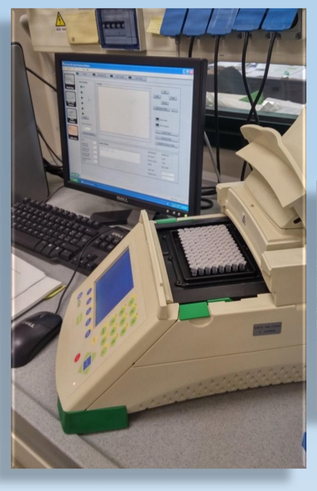




DNA amplification
(SYBR Green Supermix and BIO-RAD primers)

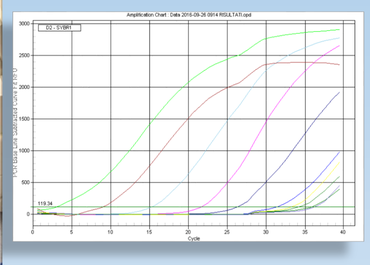
Fungi

- **ITS1** and **ITS4** primers to verify the presence of fungal DNA (ITS = Internal Transcribed Spacer rDNA; White *et al*., 1990; Gardes and Bruns, 1993)
- **ALT** primers to verify the presence of *Alternaria* spp. (ITS; Crespo-Sempere *et al*., 2013)
- **CLAD** primers to verify the presence of *Cladosporium* spp. (SSU = Small Sub Unit rDNA ; Qing-Yin Zeng *et al.,* 2006)



Pollen

- **tRNA-LEU** gene sequence (Laube I. *et al*., 2010)



RESULTS

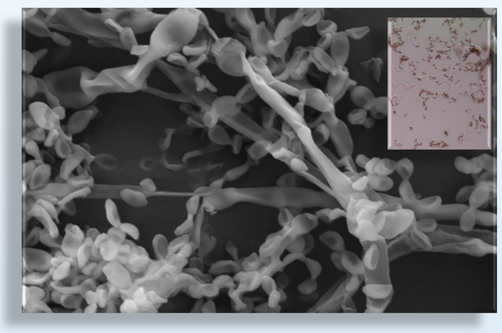
PCR

ITS1 and **ITS4** low Efficiency and low Performance;
ALT Efficiency = 91,9%;
Sensitivity = 13,5 spores/reaction;
Good Specificity (melting curve analysis);
Reproducibility = CV% 8,77)
CLAD Efficiency = 92,5%;
Sensitivity = 0,56 spores/reaction;
Good Specificity (melting curve analysis);
Reproducibility = CV% 11,98)

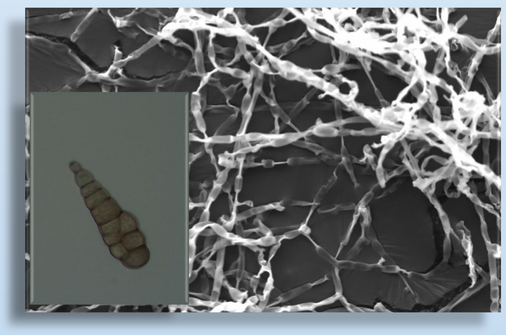
Whole method (Extraction + PCR)

ALT Extraction Efficiency = not calculable;
Sensitivity = 10^5 spores/reaction;
Reproducibility = 17.40 CV%
CLAD Extraction Efficiency = 24%;
Sensitivity = 10^2 spores/reaction;
Reproducibility = 16.43 CV%

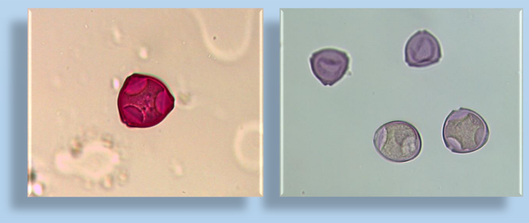
Good efficiency, reproducibility and sensitivity, particularly for ***Cladosporium* spp** detection.



As regards ***Alternaria* spp**, we pointed out some critical issues, probably due to the nucleic acid extraction and purification phase.



It is possible to detect pollen and spores from a **simulated monitoring slide** (in contact with fuchsine during a few hours)



Its applicability on the **aerobiological monitoring slides** has not been verified.

CONCLUSIONS

For more details scan the QR Code, or visit the page www.arpa.vda.it in the section dedicated to the publications.

All pictures shown were taken in ARPA Valle d'Aosta laboratories