A new multiplex real time PCR for detection and identification of Zygomycetes and *Aspergillus* spp. in human biosamples

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Introduction

The success of treatment of fungal infection of the lungs depends to a large extent on early etiological diagnostics, which is often problematic when conventional methods of analysis are used.

Objectives

The objective of this study was to develop a PCR for simultaneous detection and identification of Zygomycetes and *Aspergillus* spp. in human biological samples.

Materials and Methods

A multiplex real time PCR with High Resolution Melt analysis (HRM-Zygo-Asp-PCR) was developed for detection and identification of Zygomycetes and *Aspergillus* spp. using fungi isolates from Russian collection of pathogenic fungi of Kashkin Research Institute of Medical Mycology (Saint-Petersburg). Identification of the fungi isolates was carried out using morphological criteria and by sequencing of ITS and D1/D2 regions of rRNA gene and β-tubulin gene fragment of micromycetes. The HRM-Zygo-Asp was tested on clinical samples (sputum, bronchoalveolar lavage, blood, autopsy matherial) obtained from patients with mycotic infection of the lungs. Samples from the patients were also analyzed using direct DNA sequencing, a TaqMan PCR for detection of *Aspergillus* spp., microbiological (microscopy and culture) and Platelia-Aspergillus EIA (BioRad).

Results

Specificity of the HRM-Zygo-Asp-PCR

22 strains of Zygomycetes	22 strains of *Aspergillus* spp.	20 strains of other filamentous and yeast-like fungi
YES	**YES**	**NO**

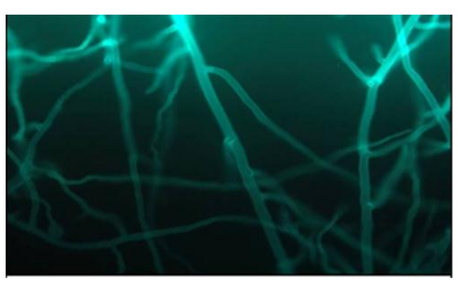
Specific reproducible amplification of proper DNA fragments was achieved for aspergillus- and zygomycetes-specific primer pairs separately and in the multiplex PCR setting.

The HRM-Zygo-Asp-PCR allows to identify the representatives of *Aspergillus* and *Absidia* to the genus level, *Rhizomucor pusillus*, *Rhizopus microsporus*, *Mucor circinelloides*, *Cunninghamella echinulata*, *Syncephalastrum racemosum*, *Lichtheimia corymbifera* to the species level and *Rhizopus arrhizus* / *Rhizopus stolonifer* and *Mucor racemosus* / *Mucor plumbeus* to the pair of species level.

Evaluation of the HRM-Zygo-Asp-PCR on clinical material

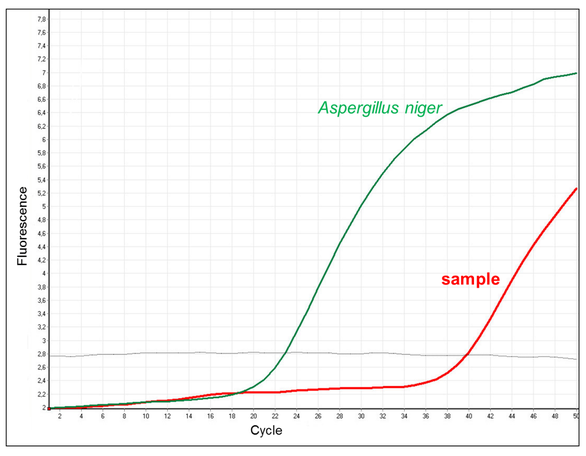
Detection of mixed infection by *Aspergillus* spp. and Zygomycetes in BAL sample

A - Luminescent microscopy of BAL sample using calcofluor white, x400



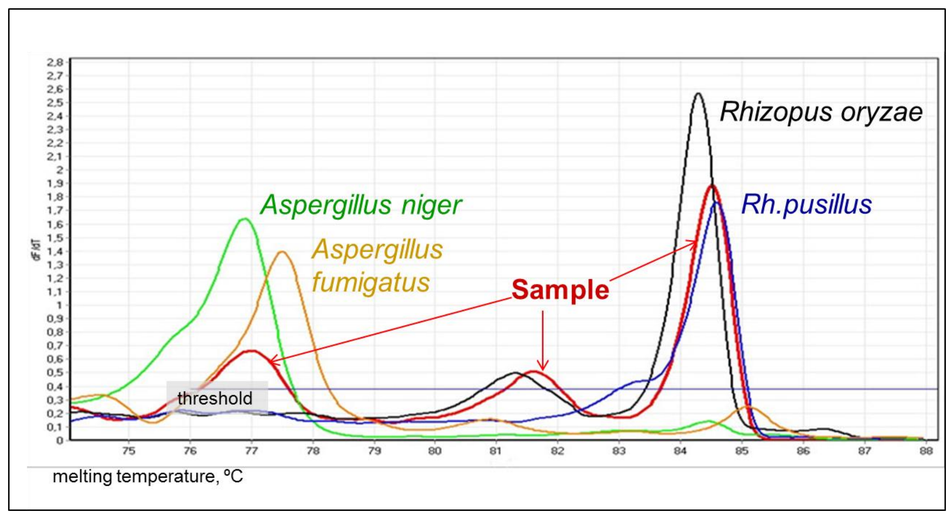
nonseptate mycelium

B – Real-time PCR of BAL sample using TaqMan PCR for detection of *Aspergillus* spp.



Aspergillus sp.

C – HRM-Zygo-Asp-PCR of BAL sample



- *Rhizopus arrhizus*
- *Aspergillus* sp.

D – Direct sequencing – *Rhizopus arrhizus*
E - Platelia-Aspergillus EIA (BioRad) – *Aspergillus* sp.

A, B, C, D, E

The HRM-Zygo-Asp-PCR allows the detection of mixed infection by *Aspergillus* spp. and Zygomycetes

Conclusions

We have developed a multiplex HRM-Zygo-Asp-PCR for detection and identification of Zygomycetes and *Aspergillus* spp. in human biological samples.The preliminary results of the evaluation indicate that the HRM-Zygo-Asp-PCR may be a very useful tool for revealing of etiologic agent of mycotic infection of the lungs, particularly in the case of a mixed infection by *Aspergillus* spp. and Zygomycetes.