# Evaluation of a new commercial real-time PCR for the detection of Aspergillus spp. in serum and respiratory samples

Hayette M.P., Meex C., Boreux R., Huynen P., Melin P. and P. De Mol

Medical Microbiology department, Univ. Hosp., Liège, Belgium

## Abstract

### Objectives

Diagnosis of invasive aspergillosis is still disappointing and often delayed because of the lack of sensitivity of diagnostic tools. Galactomannan detection methods have been developed, but differ widely and comparisons are difficult to assess. The objective of the study is to compare a new commercial real-time PCR kit, affigene® Aspergillus tracer assay, with an "in house" nested PCR targeting 18S rRNA Aspergillus spp. gene.

### Background

Diagnosis of invasive aspergillosis is still disappointing and often delayed because of the lack of sensitivity of diagnostic tools. Galactomannan detection in serum is widely used for the diagnosis of IA in neutropenic patients with haematological malignancies. DNA detection-based methods have been developed, but differ widely for a centre to another and comparisons are difficult to assess.

It is important to develop commercial techniques in order to develop the use of molecular tests by the laboratories involved in the diagnosis of fungal opportunistic infection as invasive aspergillosis.

### Methods

**DNA extraction.** The DNA extraction was performed by using the QiAamp DNA mini kit (Qiagen, Germany). All samples were tested by both PCR assays and respiratory samples were cultured.

**Galactomannan detection test (Platelia® Aspergillus, Biorad)**

The test was performed on serum samples following the manufacturer's recommendations.

**PCR methods**

**DNA extraction.** The DNA extraction was performed by using the QiAamp DNA mini kit (Qiagen, Germany). All samples were tested by both PCR assays and respiratory samples were cultured. For both serum and respiratory samples, the input volume was 200 µl and the elution volume 100 µl.

**In house** PCR. This method is a nested PCR adapted from Yamakami et al. (2). The targeted sequence is the 18S rRNA gene of several Aspergillus species. Briefly, the PCR reactions were performed for both PCR except for McgC. The PCR were carried out in a 50 µl volume containing 10 mM Tris-HCl at pH 8.3, 50 mM KCl and 1.5 mM MgCl₂, 2.25 mM for the nested PCR (GeneAmp 10 x PCR buffer II, Applied Biosystems, USA) with 0.4 µM of both primers (Eurogentec, Belgium), 12.5 U Taq polymerase (Ampliqaq DNA polymerase, ABI), 5 µl volume of DNA was added to the mixture. Positive and negative controls were amplified in parallel to validate the run. Thermal cycling conditions (Thermohydaid, UK) were as follows: 5 min at 94°C; 30 cycles of 1 min at 94°C; 1 min at 50°C (65°C for the nested PCR) and 1 min at 72°C followed by a final extension step of 10 min at 72°C. For the nested PCR, 2 µl of the first amplified product was added to the new reaction mixture. The reaction mixture was amplified (35 cycles) were analyzed on 2% agarose gels (Amresco, USA) and visualized by UV transillumination. Each sample was investigated for the presence of inhibitors by amplification of the β-globin gene. All samples were performed in duplicate. The equivocal results (one positive test, one negative test) were re-run.

**affigene® Aspergillus tracer kit**

The kit utilizes the real-time PCR technology. Low requirement reagents are included for the amplification: a mastermix containing nucleotides, primers/probes, UDG and DNA polymerase in a buffered solution. Positive, negative and internal control are also included.

The amplification has been conducted on the Mx3000P® instrument (Stratagene, La Jolla, USA). The fluorescence was collected in the FAM (targeted gene) and ROX (control) channels. The results are automatically calculated by a specific software and reported as negative or positive (with a Ct value). Only qualitative results are obtained. The run is completed after 2.5 hours.

The kits and the RT-PCR instrument were provided by Sangtec molecular diagnostics.

## Results

### Sensitivity comparison between the two PCR methods

**DNA in serum.** affigene® assay

- **87% agreement between the two PCR assays.**

**DNA detection based-methods have been developed, but differ widely and comparisons are difficult to assess.** Folow E.O.R.T.C. criteria (1). Fifteen serum and respiratory paired samples were collected in total. They were frozen at –20° C until performing the assays.

**Fungal cultures**

The cultures were performed on sabouraud agar supplemented with antibiotics (Biopharm, France). The tubes were incubated for 28 days at 30°C.

#### Galactomannan detection test (Platelia® Aspergillus, Biorad)

The test was performed on serum samples following the manufacturer's recommendations.

### Conclusions

- **Both PCR are in good agreement for the detection of Aspergillus DNA in clinical samples.**

- **Both PCR assays are able to detect at least three different Aspergillus species: A. fumigatus, A. flavus and A. niger.** These tests allow the detection of aspergillosis due to other species than A. fumigatus the most frequently targeted species.

- **affigene® test is more sensitive than the presence of inhibitors in serum than the conventional test.**

- **None of these PCR methods could detect Aspergillus DNA in serum even in proven aspergillosis.** Despite the positivity of the galactomannan detection test, they believe that blood sample is not an appropriate material to be tested by PCR for the diagnosis of invasive aspergillosis.

- **affigene® assay can easy replace the “in house” assay:** it allows a fast and standardized detection of Aspergillus sp. DNA in clinical samples.

## References
