

# Development of a novel and standardised real-time PCR assay for the detection of *Aspergillus* spp. in immuno-compromised patients

N. Finnström<sup>1</sup>, T. Eriksson<sup>1</sup>, E. K. Pisa<sup>1</sup> and J. Loeffler<sup>2</sup>

1) Sangtec Molecular Diagnostics, Bromma, Sweden, 2) Universitaet Wuerzburg, Medizinische Klinik II, Wuerzburg, Germany

## Background

Invasive aspergillosis (IA) is a leading cause of mortality in the growing immunocompromised patient population which comprises transplant recipients, individuals with hematological malignancies and a range of diseases arising from decreased immune function. The mortality rate is extremely high in these neutropenic patient groupings with reports as high as 90%. This is partly attributable to the lack of standardised molecular tests for the early diagnosis and optimal therapeutic intervention by clinicians to manage disease. Real time polymerase chain reaction (PCR)-based methodologies for the detection of DNA from *Aspergillus* spp is a promising technology for the diagnosis of invasive aspergillosis. The method can be made both extremely specific and detect pathogen nucleic acids with a high degree of sensitivity. However, until now no standardised PCR diagnostic assay was commercially available.

## Method

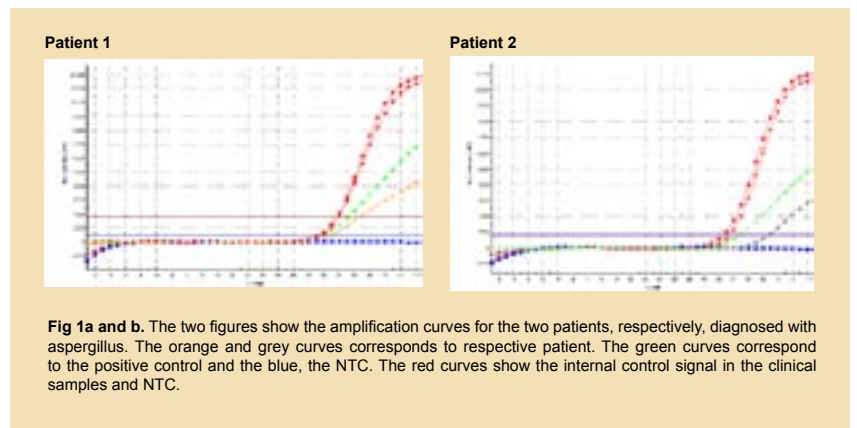
For description of the procedure see fig 2. Five ml EDTA blood was collected from BMT and leukaemia patients (n = 33) weekly. In total, 145 specimens were analysed for the presence of *Aspergillus* spp genomic DNA. Nucleic acid was prepared by an initial erythrocyte lysis step and subsequent lymphocyte enrichment by employing the HighPure PCR Template Preparation Kit (Roche). An internal control (IC) was introduced at the lysis step of the sample preparation to control for extraction efficiency and the presence of inhibitors. *Aspergillus* DNA and IC were amplified and detected in parallel using the affigene® aspergillus tracer assay (Sangtec Molecular Diagnostics, Sweden) on the MX3000P instrument (Stratagene, USA) according to the manufacturer's instructions. The real time-PCR assay has been standardised according to the EC IVD directive. At the time of blood collection, patients were clinically assessed for symptoms of aspergillosis. Prestanda of the assay is shown in table 1.

**Table 1.** Prestanda of the affigene® aspergillus tracer

Parameters	Value
Limit of detection	0.5 genome eq/ul
Inclusivity	- <i>A. versicolor</i> - <i>A. usufus</i> - <i>A. terreus</i> - <i>A. oryzae</i> - <i>A. clavatus</i> - <i>A. flavus</i> - <i>A. fumigatus</i> - <i>A. nidulans</i> - <i>A. niger</i>
Exclusivity	- <i>C. albicans</i> - <i>C. glabrata</i> - <i>C. neoformans</i> - <i>E. dermatidis</i> - <i>S. cerevisiae</i> - <i>M. furfur</i> - <i>F. solani</i> - <i>H. capsulatum</i> - <i>T. beigelii</i>

## Results

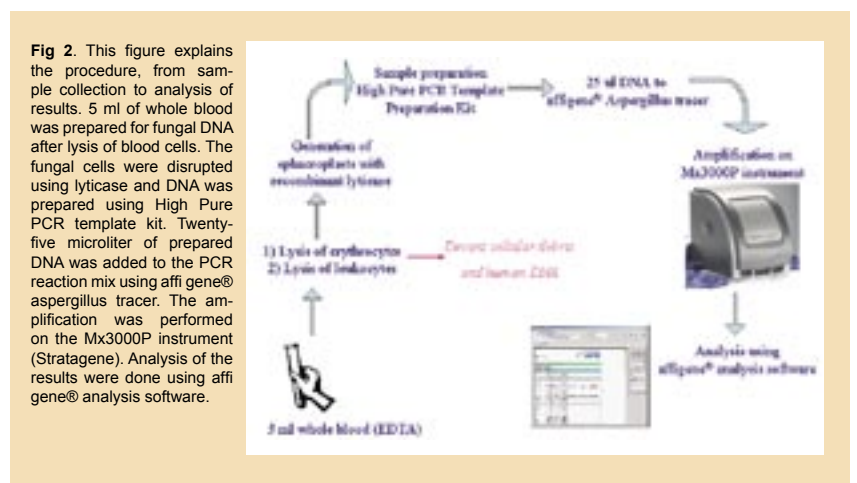
Of the 33 patients, two were diagnosed as having a proven or probable aspergillosis according to the EORTC criteria. They showed fever during neutropenia, had pulmonary infiltrates and were positive for aspergillus in BAL by microscopical studies. These two patients were the only ones that showed a positive result when employing the affigene® aspergillus tracer kit (see fig 1a and b). None of the other patient samples were found to be positive for *Aspergillus* using this real-time PCR method.



**Fig 1a and b.** The two figures show the amplification curves for the two patients, respectively, diagnosed with aspergillus. The orange and grey curves corresponds to respective patient. The green curves correspond to the positive control and the blue, the NTC. The red curves show the internal control signal in the clinical samples and NTC.

## Conclusion

The affigene® aspergillus tracer standardised real-time PCR assay aspergillus tracer standardised real-time PCR assay serves as a valuable molecular tool, in combination with defined EORTC criteria for assessment of clinical symptoms, for the diagnosis of aspergillosis in immunocompromised patients.



**Fig 2.** This figure explains the procedure, from sample collection to analysis of results. 5 ml of whole blood was prepared for fungal DNA after lysis of blood cells. The fungal cells were disrupted using lysozyme and DNA was prepared using High Pure PCR template kit. Twenty-five microliter of prepared DNA was added to the PCR reaction mix using affigene® aspergillus tracer. The amplification was performed on the Mx3000P instrument (Stratagene). Analysis of the results were done using affigene® analysis software.