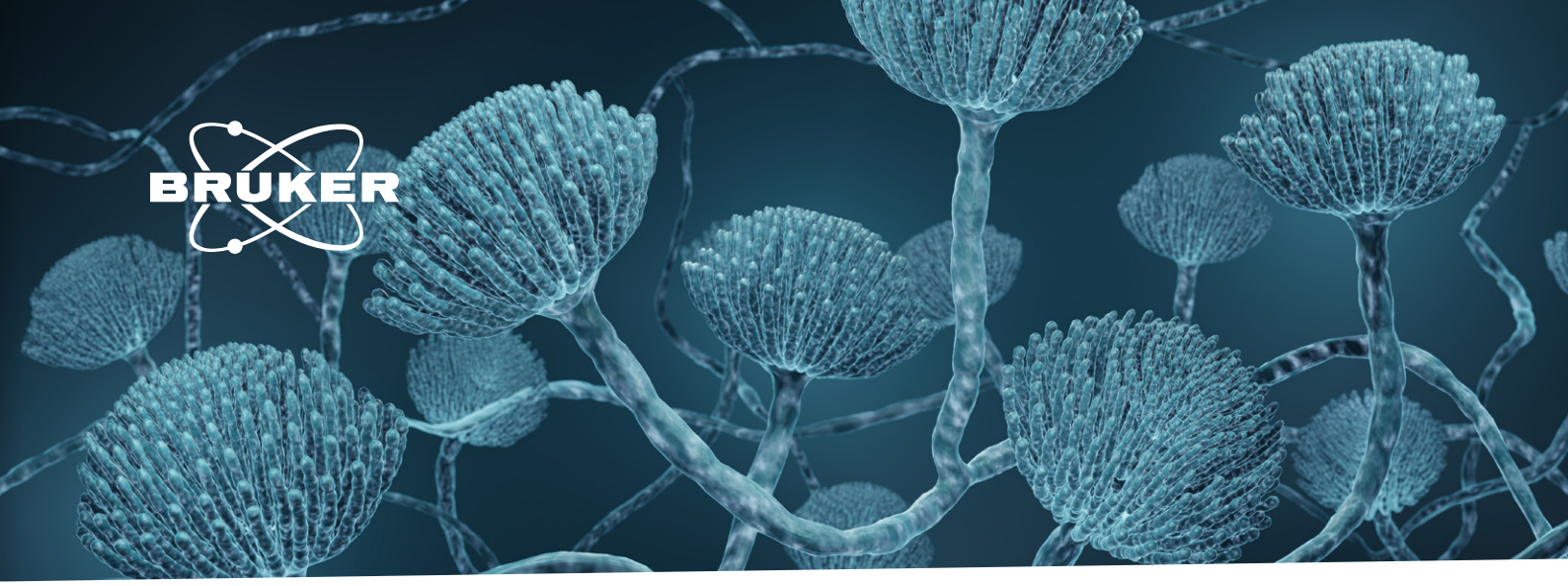

Optimizing antifungal therapy by PCR detection of *Aspergillus*: a diagnostic workflow

Author: Dr. Jennifer A. Dougan, Head of Business Unit Syndromic LiquidArrays, Bruker Microbiology & Diagnostics

Abstract

Invasive fungal diseases (IFDs) such as invasive aspergillosis (IA) pose a significant health threat to immunocompromised individuals, with patients suffering high morbidity and mortality if the infection is not detected and treated quickly. A factor that exacerbates the already challenging clinical picture of IA is co-infection with SARS-CoV-2, which can lead to the development of COVID-19 associated pulmonary aspergillosis (CAPA). Implementing polymerase chain reaction (PCR) as a complementary method to traditional microbiological IFD tests can improve diagnostic accuracy and help clinicians initiate appropriate therapies sooner, improving overall health outcomes, reducing the risk of antifungal resistance, and lowering patient management costs. A diagnostic workflow was developed incorporating *Aspergillus* PCR with IFD and CAPA clinical assessment. This workflow provides a robust diagnostic approach for detecting *Aspergillus* infection and is a valuable tool for clinicians looking to optimise antifungal drug use.

Introduction

Invasive aspergillosis (IA) is caused by certain species of fungi belonging to the *Aspergillus* genus, most commonly *Aspergillus fumigatus*. *Aspergillus* spores are rarely pathogenic in healthy individuals, but IA is an opportunistic infection that impacts immunosuppressed people, such as patients that have received stem cell transplants, chemotherapy, or organ transplants. IA typically affects the lung, with non-specific clinical symptoms that make diagnosis difficult. Without timely treatment, IA mortality rates can reach nearly 100%.[1]

Conventional diagnostic methods such as microscopic analysis or blood culture have poor sensitivity and require significant expertise. Other commonly used methods have been associated with a high level of false positive results.

IA diagnosis has been further complicated by the COVID-19 pandemic. Immunosuppressed patients that are already susceptible to IA are also prone to severe disease caused by SARS-CoV-2. COVID-19 associated pulmonary aspergillosis (CAPA) is hard to diagnose as the symptoms and clinical features of COVID-19 and IA overlap considerably.

IVD

There is not only a need to improve diagnostic speed and accuracy to ensure the best patient outcomes, but there is also a widely acknowledged overuse of antifungal drugs, due to historically poor diagnostic performance and a lack of confidence in diagnosing IA.[2] Reducing the unnecessary use of antifungals could not only decrease healthcare costs, but also minimize side effects and alleviate some of the risk of antifungal resistance, which is becoming a serious threat to IA treatment.

A diagnostic strategy that can rule out disease and reduce the unnecessary use of antifungal drugs, while allowing for the targeted treatment using the right drug at the right time, is therefore crucial and could help to improve patient management and cut healthcare costs.

An integrated diagnostic and treatment strategy

Bruker's Fungiplex® Aspergillus IVD Real-Time PCR Kit rapidly detects *Aspergillus* spp., and its proven clinical performance[3] makes it an ideal ancillary test for early IA diagnosis. With its high sensitivity and speed, the test is a powerful tool for detecting *Aspergillus*, contributing to the clinical assessment of disease. The *Aspergillus* PCR assay can be used as part of a diagnostic workflow, together with other microbiological tests and blood culture, to contribute to patient assessment.

As a culture-free method, the Fungiplex Aspergillus IVD Real-Time PCR Kit uses routinely collected samples that are used for other microbiological tests, including serum, plasma, and bronchoalveolar lavage (BAL). As it is designed to run on widely used laboratory equipment, incorporating the Fungiplex Aspergillus PCR assay into existing workflows is straightforward and requires minimal training.

An important element of this workflow is the prompt implementation of effective antifungal treatments when *Aspergillus* infection has been detected. Antifungal stewardship programs are a vital element of successful treatment of fungal diseases, and will play an important role in IA and CAPA management. The aim of such programs is to reduce inappropriate antifungal use, improve patient outcomes, and limit the emergence of resistance.

Tackling resistance

The azole class of antifungal drugs is the primary option for the prophylaxis and treatment of IA. Bruker's Fungiplex® Aspergillus Azole-R IVD Real-Time PCR Kit can identify patient samples that are resistant to azoles by detecting the presence of mutations in the *Cyp51* gene of *A. fumigatus*. The test can be run alongside Fungiplex Aspergillus PCR for an early indication of azole resistance in high-risk patients, or in patient samples testing positive for *Aspergillus* with the Fungiplex Aspergillus PCR.

The CAPA challenge: adopting Fungiplex Aspergillus

Clinical scientists at Health Services Laboratories (HSL), in association with the University College London Hospitals (UCLH), have demonstrated the potential power of using Fungiplex Aspergillus PCR to help identify CAPA patients quickly and initiate therapy as soon as possible (Figure 1).

Dr. Rebecca Gorton, Principal Clinical Scientist at HSL, explains how her laboratory uses PCR in its diagnostic workflow:

"We implemented *Aspergillus* PCR during the second COVID-19 peak. The ability to perform PCR in-house from endotracheal (ET) aspirates transformed the way we were screening for invasive aspergillosis (IA) compared with the first peak. In our COVID-19 patients we observed a clear association between high positive PCR results (C_T<30) for *Aspergillus* spp. and upper respiratory tract and pulmonary IA. It was incredibly useful to be able to identify those patients quickly and proceed to the most appropriate treatment. We also utilized the negativity of PCR to exclude IA in the vast majority of patients."

Dr. Neil Stone, Consultant in Infectious Disease Microbiology at UCLH, elaborates:
"We wanted to come up with a manageable diagnostic algorithm for trying to diagnose fungal infections in COVID-19 patients. Whenever we received a respiratory sample from a COVID-19 patient on the intensive care unit (ICU), we would, according to this algorithm, perform a panel of tests including galactomannan, fungal and respiratory culture, *Pneumocystis* PCR, and Fungiplex Aspergillus PCR as a matter of routine. I think bringing in Fungiplex Aspergillus PCR was a huge help to us. Dr. Rebecca Gorton has led the collaboration with clinicians and has been able to provide advice in real time, which makes a genuine impact on clinical decision making."

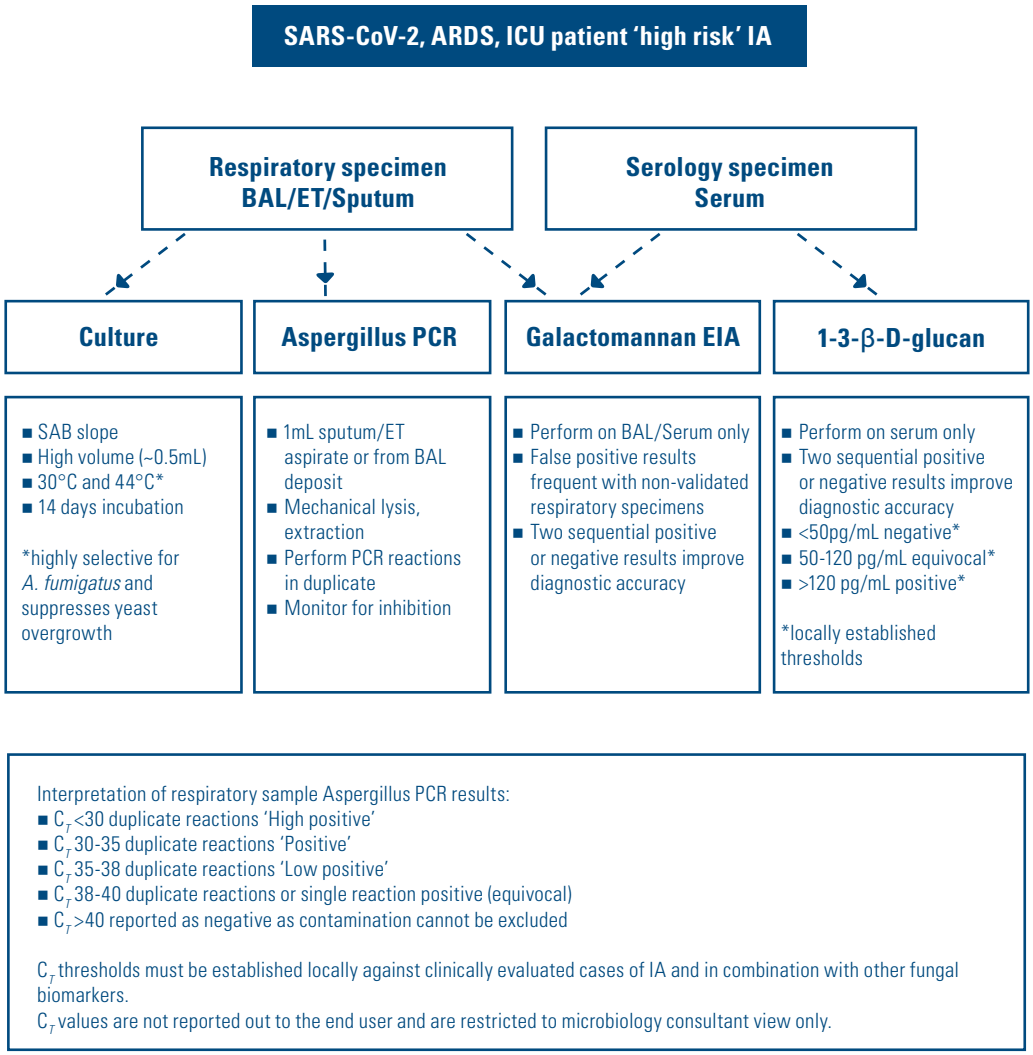

Figure 1
Diagnostic driven strategy for screening and confirming fungal infection in CAPA patients.

Interpretation of respiratory sample Aspergillus PCR results:
- C_T<30 duplicate reactions 'High positive'
- C_T 30-35 duplicate reactions 'Positive'
- C_T 35-38 duplicate reactions 'Low positive'
- C_T 38-40 duplicate reactions or single reaction positive (equivocal)
- C_T>40 reported as negative as contamination cannot be excluded

C_T thresholds must be established locally against clinically evaluated cases of IA and in combination with other fungal biomarkers.
C_T values are not reported out to the end user and are restricted to microbiology consultant view only.

ARDS = acute respiratory distress syndrome
ICU = intensive care unit
BAL = bronchoalveolar lavage
ET = endotracheal
EIA = enzyme immunoassay
SAB = Sabouraud dextrose agar

Summary

PCR tests such as Bruker's Fungiplex Aspergillus and Fungiplex Aspergillus Azole-R PCR assays are playing an important role in transforming diagnostic-driven treatment strategies for IA and CAPA patients. Advantages of these assays include:

- Culture-free identification of *Aspergillus* spp.
- Results in under two hours from extracted DNA
- High sensitivity and specificity contributes to clinical assessment to confirm or rule out IA
- Can identify mutations associated with azole resistance directly from clinical specimens

Incorporating Fungiplex Aspergillus PCR into IA diagnostic workflows can provide clinical mycologists with the information clinicians need to make confident diagnoses and initiate targeted therapeutic strategies. Patients benefit from timely therapeutic intervention when needed or are spared unnecessary use of antifungal drugs when infection is ruled out. This supports antifungal stewardship, improved patient outcomes and optimized healthcare efficiency.

For more information about the Bruker Fungiplex Aspergillus IVD Real-Time PCR Kit, please visit:
https://www.bruker.com/en/products-and-solutions/microbiology-and-diagnostics/molecular-diagnostics/fungiplex-aspergillus.html

And to learn more about the Fungiplex Aspergillus Azole-R IVD Real-Time PCR Kit, please visit:
https://www.bruker.com/en/products-and-solutions/microbiology-and-diagnostics/molecular-diagnostics/fungiplex-aspergillus-azole-r.html

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Online information
bruker.com/microbiology

Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0) 421-2205-0

info.md@bruker.com