Rapid detection of *Aspergillus* within 6 hours with the PCR method

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The Fight Against Hospital-Acquired Infections

- The prevention and control of **nosocomial infections is a major public health issue**.
- **Monitoring of the hospital environment** is based on environmental measures and notably on microbiological air control to assess the risk of airborne infections.
- In hospitals, air is a contamination vector responsible for the transmission of nosocomial infections.
- The search for aerobic and fungal flora has become mandatory in healthcare establishments over the past several years (NFS 90 351: 2003).
- These controls carried out within controlled environment zones are one of the tools for the preservation of a microbial environment, a defined zone where microbiological contamination is controlled using specified means[1].

An innovative sampling method for a 6-hour result

- The microbiological tests of air and surfaces using the Petri dish culture method **take up to 7 days[2] to search for fungal flora and total flora**.
- During the COVID-19 pandemic, the teams of EOLIA group, specialized in the management of the quality of the air since 2009, have developed **sampling techniques for SARS-CoV-2 screening** of specific areas:
  - → air samples using a Coriolis Micro air collector[3],
  - → surfaces using swabs and a preservative solution.
- The samples are analyzed using the polymerase chain reaction (PCR) technique[4]. This technique enables the identification and quantification of all microorganisms: molds, wine yeasts, contamination yeasts, lactic acid bacteria or acetic acid bacteria. The main **benefit of the PCR technique is that the results are obtained within 6 hours**, from the reception of the sample at C4Diagnostics laboratory.
- **EOLIA** has teamed up with **C4Diagnostics**, a biotech specialized in the development of *in vitro* **diagnostic tests for human infectious diseases**, to implement the PCR technique for the detection of *Aspergillus* and any other pathogen.

→ EOLIA offer: a new method designed to address emergency situations in the search of *Aspergillus*

- Based on this innovative procedure, EOLIA group has set up a similar research method for the detection of molds and *Aspergillus*.

[1] This definition is taken from the NF EN 17141 August 2020 standard. "Cleanrooms and related controlled environments - Biocontamination control"
[2] 7 days incubation at a temperature of 30 to 35 degrees Celsius
[3] A sampling plan is carried out in accordance to the client's needs.
[4] This technique allows the amplification of a DNA sequence to make it detectable

- This **method is perfectly suited for emergency situations**: in the event of a contamination in a hospital context, notably in intensive care units, it is of major importance to promptly get the test results so as to proceed to emergency decontaminations.
- EOLIA offers hospitals sampling kits for *Aspergillus* to help them transition to the PCR technique and thus benefit from a **detection within 6 hours**.

Specific features of the protocol implemented

- The sampling approach developed by EOLIA is based on the **EN 17141 norm**[5] which indicates the possibility to use in addition to "*alternative methods that can improve the understanding of the conditions of control of the environment or provide other benefits for certain applications*".
- The samples are sent to C4Diagnostics, the partner laboratory, to be processed on the same day and at the latest 24 hours later, taking all necessary isothermal precautions.

→ C4Diagnostics offer: a 6-hour operating procedure, from sample reception to analysis

- At **C4Diagnostics**, samples are forward going in the lab, from the reception room to the analysis room, a process common to medical biology activities in controlled areas.
- Once collected, the air or surface samples are treated in a conservative medium, wrapped in a three-layer packaging within a biohazard transport box, and then shipped in compliance with optimal temperatures and storage conditions.
- The whole process, from sample reception to result delivery, is completed in 6 hours (**Figure 1**).

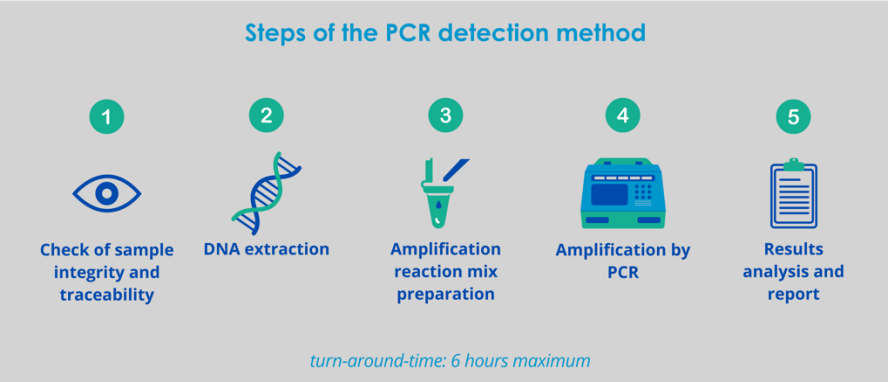

Figure 1. Steps of the PCR detection method at C4Diagnostics laboratories

1. Upon samples arrival at **C4Diagnostics**, the first step is to **check their integrity and compliance**. The data is recorded for traceability purposes. Duration: 60 minutes.
2. The samples are then transferred to the extraction room for unpacking, preprocessing, and extraction. The **extraction of *Aspergillus'* DNA** is performed with a specific extraction kit for bacteria, yeast and fungi. Duration: 30 minutes (unpacking and preprocessing) + 40 minutes (extraction).
3. During the extraction phase, which comprises 36 minutes of incubation, the multi-well plate where the batch of samples will be simultaneously amplified is prepared. The **preparation of the PCR reaction mix and amplification plate** is carried out in a dedicated molecular biology room, in which no sample enters to avoid cross-contamination. The amplification kit includes three target genes to ensure the high specificity of the PCR result: one gene specific to *Aspergillus t*erreus, one gene common to the 12 other species of *Aspergillus* (*Aspergillus spp.*), and one internal control gene. Duration: 30 minutes.

[5] NF EN 17141 August 2020 norm. "Cleanrooms and related controlled environments - Biocontamination control"

4. The DNA extracts and the amplification plate are transferred to the amplification room. Under the PCR hood, the extracts are dispatched into the multi-well amplification plate, and then the **DNA is amplified** in a thermocycler. Duration: 20 minutes (preparation) + 100 minutes (amplification).

5. The last step is the **analysis and interpretation of the results**. The real time PCR Ct curves go through qualitative and quantitative analysis using a software program to assess the presence of *Aspergillus* for each sample tested. A report is then produced and validated by **C4Diagnostics**. Duration: 60 minutes.

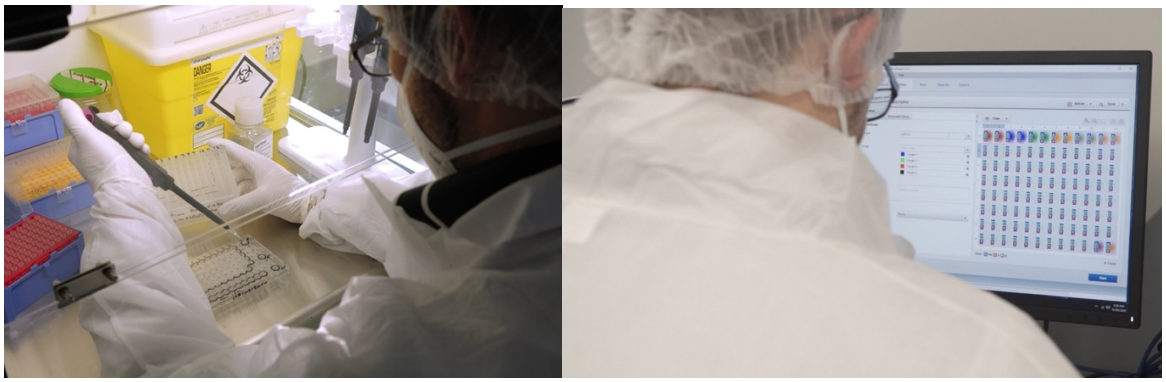

Figure 2: Preparation of amplification plates (left) and analysis of results (right) by Dr Gabriel Martin (credit: C4Diagnostics)

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