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Comparison of PCR protocols for detecting Histoplasma capsulatum and Coccidioides spp. DNA through a multi-center study

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Introduction: In-house real-time PCR (qPCR) is increasingly used for the diagnosis of endemic mycoses and diverse assays are in use in specialized laboratories. External quality control is currently lacking.

Objective: To compare the performance of different molecular detection protocols for the detection of Histoplasma capsulatum and Coccidioides spp. in a multicenter study involving five European laboratories.

Methods: Two test sample panels were sent to each laboratory which performed the analysis with their in-house assay. Recipients were blinded to sample content. The Histoplasma panel included 14 samples representing a range of Histoplasma DNA (n = 7), as well as a negative control and DNA from other fungi to test for specificity (Paracoccidioides brasiliensis n = 1, Blastomyces dermatitidis n = 1, Aspergillus fumigatus n = 1, Exserohilum rostratum n = 2, and Candida albicans n = 1). The Coccidioides panel included 10 samples representing a range of DNA concentrations of Coccidioides posadensis (n = 6), as well as a negative control and DNA from other fungi to test specificity (Uncinocarpus reesii n = 1, Phialophyton parasiticum n = 1, and Candida albicans n = 1). Molecular identification was performed for all samples using real-time PCR and one laboratory performed a conventional PCR and a broad-range PCR (bPCR) for fungal DNA. Four laboratories used different Coccidioides qPCRs and one laboratory a bPCR to detect Coccidioides DNA.

Results: Concerning the Histoplasma panel, qPCR assays were the most sensitive and agreement in the lowest detected amount of Histoplasma DNA was very satisfying, ranging from 3 pg to 4 pg/μl (genomic equivalent mean sensitivity: 96.4%). The lowest detected amount of Histoplasma DNA by qPCR (sensitivity 71.4%) and the bPCR (sensitivity 42.9%) was 0.1 and 10 pg, respectively. Overall, specificity ranged from 42.9-100% (mean 83.3%). Overall, specificity ranged from 76.6-100%, with false positive results occurring with high DNA concentrations (200 pg/μl) of Blastomyces spp. in two laboratories that used qPCR. Enzyme immunoassay (EIA) in one laboratory and Aspergillus in one laboratory that used qPCR. Concerning the Coccidioides panel, sensitivity ranged from 53.3-100% (mean 76.6%), and agreement of the lowest detected amount of Coccidioides DNA by qPCR ranged from 1-16 pg/μl (genomic equivalent) (mean sensitivity: 87.5%) and in the bPCR 10 pg (sensitivity: 33.3%). Specificity was between 87.5-100%, with one false positive result occurring with high DNA concentrations (20 pg/μl) of Uncinocarpus in one laboratory using qPCR.

Conclusions: Specific protocols based on qPCR showed better sensitivity than conventional and bPCR. These methods are useful for the rapid and sensitive detection Histoplasma and Coccidioides. Application of these tools on clinical samples may speed-up diagnosis and potentially limit laboratory exposure to these fungi. Comparisons of in-house tests are essential to ensure the performance and detect potential cross-reactivities and achieve a consensus.

Figure 1. Diagram of the Dynamiker Aspergillus specific IgG antibody (LFA)